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Vienna, Austria, June 11 - 14, 2015

ABSTRACT BOOK

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About EHA

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

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

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

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

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

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Einsele H, *Germany*
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Fehse B, *Germany*
Fenaux P, *France*
Fielding A, *United Kingdom*
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Garcia-Sanz R, *Spain*
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Germing U, *Germany*
Gibson B, *United Kingdom*
Gisselbrecht C, *France*
Gobhrial I, *USA*
Goker H, *Turkey*
Goodeve A, *United Kingdom*
Greenfield D, *United Kingdom*
Gribben J, *United Kingdom*
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Halkes CJ, *the Netherlands*
Halter J, *Switzerland*
Hansen JB, *Norway*
Harrison C, *United Kingdom*
Hasselbalch H, *Denmark*
Hellström-Lindberg E, *Sweden*
Hill A, *United Kingdom*
Hochhaus A, *Germany*
Höchsmann B, *Germany*
Hoskin PJ, *United Kingdom*
Huang XJ, *China*
Hughes T, *Australia*
Huguet F, *France*
Huisman MV, *the Netherlands*
Huntly B, *United Kingdom*
Izraeli S, *Israel*
Jäger U, *Austria*
Jansen J, *the Netherlands*
Jantunen E, *Finland*
Jilma B, *Austria*
Karlsson S, *Sweden*
Kastritis S, *Greece*
Kim D, *Canada*
Kindler T, *Germany*
Kluin-Nelemans JC, *the Netherlands*
Kouskoff V, *United Kingdom*
Kremer Hovinga J, *Switzerland*
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Kuball JK, *the Netherlands*
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Louw VJ, *South Africa*
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Ludwig H, *Austria*
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Rees D, *United Kingdom*
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Socié G, *France*
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Somervaille T, *United Kingdom*
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Soverini S, *Italy*
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Word of Welcome

We are proud that this year we can, together with you, celebrate 20 years of the annual congress of the European Hematology Association. On behalf of the EHA Board and the Scientific Program Committee we are therefore pleased to introduce to you this year's anniversary Abstract Program.

The Scientific Program Committee has compiled an exciting and topical program of Simultaneous Oral and Poster Sessions from over 2200 submitted abstracts. The poster sessions have a slightly different setup this year. A selection of posters will be presented in the traditional Poster Walks. More time will be dedicated to discussion of the important research presented there, so look out for the Poster Walk Moderators in their fashionable red baseball caps! There will also be E-posters available on the E-poster screens, for which a specific time is allocated during the Poster Browsing Time at the end of each Walk. The simultaneous oral sessions have now been spread over three days (Friday to Sunday), with the five Best Abstracts to be presented during the Presidential Symposium on Friday afternoon.

For the second year running, we have invited "late breaking abstracts" for "hot" data that were not available by the time of the regular submission deadline. The most exciting results have been selected and will be presented in the two dedicated sessions on Sunday morning. There are also late breaking posters that will be included in the poster walk of the relevant topic.

All posters can be viewed on the E-poster screens from Friday morning to Saturday evening and are also available on the EHA Learning Center, for which delegates have complimentary access after the congress: learningcenter.ehaweb.org.

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

David Grimwade
Chair Scientific Program Committee 20th Congress

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Table of Contents

Simultaneous sessions

Poster session

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Table of Contents

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Table of Contents

Simultaneous sessions

E-posters

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Table of Contents

Publication Only

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the hematology journal

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

Ancient Greek

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

Scientific Latin

haematologicus (adjective) = related to blood

Scientific Latin

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

Modern English

the hematology journal
2012 JCR® Impact Factor = 5.935

Haematologica/The Hematology Journal, as the official organ of the European Hematology Association (EHA), aims not only to serve the scientific community, but also to promote European cultural identity.

SIMULTANEOUS SESSIONS

Multiple myeloma: Clinical studies 1

S101

IMPROVED OVERALL SURVIVAL WITH AUTOLOGOUS TRANSPLANTATION *VS* CYCLOPHOSPHAMIDE-LENALIDOMIDE-DEXAMETHASONE IN NEWLY DIAGNOSED MYELOMA: A PHASE 3 TRIAL

F. Gay[1,*] M. Genuardi[1], R. Hajek[2], M.T. Petrucci[3], L. Pour[4], A.P. Falcone[3], M. Offidani[3], F. Patriarca[3], P. Corradini[3], M. Gilestro[1], N. Pescosta[3], A.M. Carella[3], P. Finsinger[3], A.M. Liberati[3], D. Petrò[3], S. Pulini[3], S. Oliva[1], F. Di Raimondo[3], A. Spencer[5], M. Boccadoro[1], A. Palumbo[1]
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Background: High-dose chemotherapy followed by autologous stem cell transplantation (ASCT) improves survival in multiple myeloma (MM) patients. The introduction of novel agents has challenged the role of ASCT at diagnosis.
Aims: We conducted a multicentre international randomized phase 3 trial to compare ASCT with conventional chemotherapy plus lenalidomide in patients ≤65 years with newly diagnosed MM. The primary endpoint was progression-free survival (PFS), the secondary endpoints included safety and overall survival (OS).
Methods: Eligible patients ≤65 years of age with newly diagnosed MM were enrolled. All patients received lenalidomide-dexamethasone induction (four 28-day cycles of lenalidomide 25 mg day 1–21 and low-dose dexamethasone 40 mg day 1, 8, 15, 22) followed by stem cell mobilization. Patients were randomized to receive consolidation with 2 cycles of MEL200-ASCT (melphalan 200 mg/m^2 with stem-cell support) or cyclophosphamide-lenalidomide-dexamethasone (CRD) [six 28-day cycles of cyclophosphamide (300 mg/m^2 day 1, 8, 15), dexamethasone (40 mg days 1, 8, 15, 22) and lenalidomide (25 mg days 1–21)].
Results: Three-hundred and eighty-nine patients were enrolled in the trial. Patient characteristics were well balanced between MEL200-ASCT and CRD. After a median follow-up of 4 years, the median PFS was 42 months for MEL200-ASCT and 28 months for CRD (HR 0.67, 95% CI 0.48-0.93, P=0.014). The 4-year OS was 87% for MEL200-ASCT and 71% for CRD (HR 0.51, 95% CI 0.28-0.93, P=0.028). The advantage in PFS and OS for MEL200-ASCT *vs* CRD was noticed in most of the analysed subgroups. The rate of grade 3-4 hematologic (84% *vs* 26%, P<0.001) and non-hematologic (39% *vs* 22%, P=0.008) adverse events (AEs) was higher in the MEL200-ASCT arm compared with the CRD arm. The main non-hematologic AEs were infections (19% with MEL200-ASCT *vs* 6% with CRD, P=0.004) and gastrointestinal AEs (20% with MEL200-ASCT *vs* 5% with CRD, P<0.001). Toxicities were however manageable. Despite the increase in grade 3-4 AEs with MEL200-ASCT, the rate of serious hematologic (0% *vs* 2%, P=0.49) and extra-hematologic AEs (7% *vs* 10%, P=0.393) was similar between MEL200-ASCT and CRD arms. No toxic deaths were reported in the MEL200-ASCT arm; 1 patient died of septic shock in the CRD arm. Four patients who went off protocol before consolidation developed a second primary malignancy (SPM): 1 renal cancer, 1 breast cancer, 1 squamous cell carcinoma, 1 gastrointestinal cancer. Eight patients randomized to MEL200-ASCT developed a SPM: 6 squamous cell carcinomas, 1 melanoma, and 1 prostate cancer. Five patients randomized to CRD developed an SPM: 1 squamous cell carcinoma, 1 renal cancer, 1 breast cancer, 1 gastrointestinal cancer and 1 glioblastoma.
Summary and Conclusions: MEL200-ASCT significantly prolonged PFS and OS in comparison with CRD. No increase in serious AEs, toxic deaths, and SPMs were reported with MEL200-ASCT.

S102

SUBGROUP ANALYSIS BY PRIOR TREATMENT AMONG PATIENTS WITH RELAPSED OR RELAPSED AND REFRACTORY MULTIPLE MYELOMA IN THE PANORAMA 1 STUDY OF PANOBINOSTAT OR PLACEBO PLUS BORTEZOMIB AND DEXAMETHASONE

H. Einsele[1,*] P. Richardson[2], V. Hungria[3], S.S. Yoon[4], M. Beksac[5], M. Dimopoulos[6], A. Elghandour[7], W. Jedrzejczak[8], A. Guenther[9], T. Na Nakorn[10], N. Siritanaratkul[11], R. Schlossman[2], J. Hou[12], P. Moreau[13], S. Lonial[14], J.H. Lee[15], M. Sopala[16], B.R. Bengoudifa[16], C. Corrado[16], J. San-Miguel[17]
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Background: Panobinostat (PAN), a potent pan-deacetylase inhibitor (pan-DACi), targets aberrations in multiple myeloma (MM) biology, including epigenetics and protein metabolism. In the PANORAMA 1 phase 3 clinical trial, the addition of PAN to bortezomib (BTZ) and dexamethasone (Dex; PAN-BTZ-Dex) led to a clinically relevant and statistically significant increase in median progression-free survival (PFS) of ≈ 4 months compared with placebo (Pbo) + BTZ and Dex (Pbo-BTZ-Dex) in patients (pts) with relapsed or relapsed and refractory MM.
Aims: To better characterize the efficacy and safety of PAN-BTZ-Dex within pt populations with limited treatment options by presenting a subgroup analysis of pts from the PANORAMA1 trial based on prior treatment.
Methods: The study design was described previously (San-Miguel JF, *et al. Lancet Oncol.* 2014;15:1195-1206). For this subanalysis, pts were analyzed for efficacy and safety based on prior treatment characteristics, including receipt of prior immunomodulatory drugs (IMiDs); BTZ + IMiDs; and BTZ + IMiDs and ≥2 prior lines of therapy.
Results: A summary of efficacy based on prior treatment is provided in the Table. Consistent or better efficacy benefit for the PAN arm was observed across all subgroups analyzed. The benefit in median PFS was particularly noteworthy among the largest subgroup in this analysis, pts who received prior IMiDs. A total of 485 pts (63%) received prior IMiDs (PAN-BTZ-Dex [n =245] or Pbo-BTZ-Dex [n =240]). Among these pts, the median PFS as determined by investigator assessment for the PAN arm was 12.3 months (95% CI, 10.25-13.83) *vs* 7.4 months (95% CI, 6.01-7.89) for the Pbo arm (hazard ratio [HR] 0.55; 95% CI, 0.44-0.70). The overall response rate for these pts was 62% (95% CI, 55.6%>68.1%) *vs* 50% (95% CI, 43.3%>56.5%), and ≥near complete response (nCR) rate was 29% (95% CI, 23%>34.7%) *vs* 13% (95% CI, 8.9%>17.8%) in the PAN and Pbo arms, respectively. Common grade 3/4 adverse events and laboratory abnormalities in each arm included thrombocytopenia (67% *vs* 36%), lymphopenia (54% *vs* 41%), neutropenia (37% *vs* 13%), diarrhea (26% *vs* 8%), and asthenia/fatigue (25% *vs* 12%). The percentage of on-treatment deaths in each arm was 7.1% *vs* 4.2%. The safety profile was consistent among the other subgroups analyzed.

Tabella 1. Efficacy of PAN-BTZ-Dex and Pbo-BTZ-Dex among patient subgroups classified by prior treatment history

	Prior IMiD (n = 485)		Prior BTZ + IMiD (n = 193)		Prior BTZ + IMiD and ≥ 2 prior lines (n = 147)	
	PAN-BTZ-Dex (n = 245)	Pbo-BTZ-Dex (n = 240)	PAN-BTZ-Dex (n = 94)	PAN-BTZ-Dex (n = 99)	PAN-BTZ-Dex (n = 73)	Pbo-BTZ-Dex (n = 74)
PFS, months (95% CI)	12.3 (10.3-13.8)	7.4[a] (6.0-7.9)	10.6 (7.6-13.8)	5.8[b] (4.4-7.1)	12.5 (7.3-14.0)	4.7[c] (3.7-6.1)
ORR, % (95% CI)	62 (55.6-68.1)	50 (43.3-56.5)	58.5 (47.9-68.6)	41.4 (31.6-51.8)	58.9 (46.8-70.3)	39.2 (28.0-51.2)
nCR/CR, % (95% CI)	29 (23.0-34.7)	13 (8.9-17.8)	22.3 (14.4-32.1)	9.1 (4.2-16.6)	21.9 (13.1-33.1)	8.1 (3.0-16.8)

[a] HR, 0.55; 95% CI, 0.44-0.70
[b] HR, 0.52; 95% CI, 0.36-0.76
[c] HR, 0.47; 95% CI, 0.32-0.72

Summary and Conclusions: PAN-BTZ-Dex demonstrated efficacy, with consistent increases in median PFS and nCR/CR rates compared with the Pbo arm among pt subgroups with a clear unmet need, including an increase in PFS of nearly 5 months among pts who received prior IMiDs. The safety profile across these subgroups was consistent with that in the overall PANORAMA 1 population, with the exception of the frequency of on-treatment deaths, which was more similar between treatment arms across each subgroup.

S103

A RANDOMIZED, OPEN-LABEL, PHASE 2 STUDY OF BORTEZOMIB AND DEXAMETHASONE WITH OR WITHOUT ELOTUZUMAB IN PATIENTS WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA

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Background: Elotuzumab, an immunotherapeutic monoclonal antibody (mAb), recognizes Signaling Lymphocytic Activation Molecule F7 (SLAMF7), a protein highly expressed on myeloma cells and natural killer cells. Elotuzumab targets and kills SLAMF7-expressing myeloma cells by direct activation of natural killer cells with minimal effect on normal tissues. Elotuzumab demonstrated enhanced activity when combined with bortezomib in a preclinical myeloma model,[1] and elotuzumab/bortezomib showed encouraging clinical activity in a Phase 1 study.[2]

Aims: This Phase 2 open-label study (NCT01478048, CA204-009) aimed to investigate the efficacy and safety of elotuzumab + bortezomib/dexamethasone (Bd) compared with Bd alone in patients (pts) with relapsed/refractory multiple myeloma (RRMM).

Methods: Pts with RRMM who had received 1–3 prior therapies were given elotuzumab + Bd (EBd) or Bd in 21-day (Cycles 1–8) or 28-day Cycles (9+) until disease progression or unacceptable toxicity. Dosing schedule: elotuzumab (10 mg/kg IV) weekly Cycles 1–2, Day 1 and 11 Cycles 3–8, then Day 1 and 15; bortezomib (1.3 mg/m^2 IV/SC) Day 1, 4, 8, and 11 Cycles 1–8, then Day 1, 8, and 15; dexamethasone 20 mg on non-elotuzumab days, 8 mg PO + 8 mg IV on elotuzumab days. The primary endpoint was progression-free survival (PFS; ITT population) according to IMWG criteria. In this proof-of-concept study, a 2-sided 0.30 significance level was specified to test for PFS difference between arms; p≤0.3 was considered significant. The study had 80% power to detect a hazard ratio (HR) of 0.69 with 103 events. Informed written consent was obtained for all participants.

Results: In total, 152 pts (median age 66 years) were randomized to either EBd (77) or Bd (75). At data cut-off (12 Sep 2014), 18% of pts treated with EBd *vs* 10% of pts treated with Bd remained on therapy. The median number of treatment cycles was 12 with EBd and 7 with Bd. Discontinuation was mainly for disease progression (52%). HR for PFS was 0.71 (70% CI 0.58, 0.87; p=0.08). One-year PFS rate was 39% (95% CI 28%, 50%) in the EBd group *vs* 32% (95% CI 21%, 44%) in the Bd group, and 2-year PFS rate was 24% (95% CI 13%, 36%) in the EBd group and 6% (95% CI 1%, 19%) in the Bd group (Figure). PFS HR (EBd *vs* Bd), adjusting for prognostic factors, was 0.58 (70% CI 0.47, 0.72; p=0.01). The median PFS observed with EBd was 9.7 months, *vs* 6.9 months with Bd. Overall response rate was 66% with EBd *vs* 63% with Bd. Early overall survival (OS) results revealed a HR of 0.61 (70% CI 0.43, 0.85); 1-year OS rate was 85% (95% CI 75%, 92%) in the EBd group *vs* 74% (95% CI 62%, 83%) in the Bd group (Figure). Forty deaths (17 EBd, 23 Bd) were observed at the time of analysis, mainly due to disease progression. Follow-up for OS continues. Grade 3/4 adverse events (AEs) were reported in 51 (68%) pts with EBd treatment *vs* 45 (60%) with Bd. AEs ≥Grade 3 that occurred in ≥15% of pts in each group were thrombocytopenia (7 [9%] in EBd group; 13 [17%] in Bd group) and infections (14 [19%] in EBd group; 11 [15%] in Bd group). Infusion reactions (IRs; all Grade 1–2) occurred in 7% of pts treated with EBd. There were no IRs at the maximum planned 5 mL/min infusion rate.

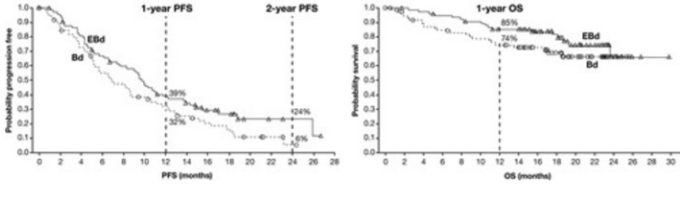

Figure 1. Progression-free survival (PFS) and overall survival (OS)

Summary and Conclusions: This study met the primary endpoint: PFS was longer with EBd than with Bd. More pts continued on EBd than Bd and early OS data favor EBd. Rate of IRs was low with EBd, and IRs were manageable with premedication. In pts with RRMM, elotuzumab, an immunotherapeutic mAb, provides clinical benefit with limited added toxicity when combined with Bd *vs* Bd alone.

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S104

NEOD001 DEMONSTRATES CARDIAC AND RENAL BIOMARKER RESPONSES IN A PHASE 1/2 STUDY IN PATIENTS WITH AL AMYLOIDOSIS AND PERSISTENT ORGAN DYSFUNCTION

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Background: Light chain (AL) amyloidosis results from an accumulation of misfolded proteins that cause the dysfunction of vital organs (eg, heart and kidneys). Current therapeutic approaches target the plasma cells that produce the pathogenic light chain proteins and are typically associated with significant adverse effects. There is a substantial need for a safe and effective therapy that specifically targets the misfolded proteins responsible for the underlying organ dysfunction. NEOD001, a monoclonal antibody that targets these misfolded proteins, is hypothesized to neutralize circulating soluble protein aggregates and to clear insoluble aggregates from organs. We report data from a phase 1/2 dose-escalation/expansion study of NEOD001 in patients with AL amyloidosis and persistent organ dysfunction (NCT01707264).

Aims: The primary aims of this study were to determine the maximum tolerated dose/recommended phase 2 dose (RP2D) and the safety and tolerability of single-agent NEOD001 when administered to patients with AL amyloidosis. Secondary and exploratory objectives included pharmacokinetics (PK), immunogenicity, and hematologic and best organ responses based on consensus criteria.

Methods: Patients who completed ≥1 previous anti–plasma cell systemic therapy, had partial response or better, did not require additional chemotherapy, and had persistent organ dysfunction received NEOD001 intravenously every 28 days (q28d). Dose levels of 0.5, 1, 2, 4, 8, 16, and 24 mg/kg were evaluated in a 3+3 study design. Informed consent was obtained from all patients.

Results: As of September 30, 2014, 27 patients in 7 cohorts received 209 infusions. Mean treatment duration was 8 months. No deaths, drug-related serious adverse events (AEs), discontinuations due to drug-related AEs, dose-limiting toxicities, or antidrug antibodies were reported. The most frequently reported AEs were fatigue, cough, and dyspnea. 24 mg/kg was selected as the RP2D. PK data support intravenous dosing q28d. Of the 14 patients evaluable for cardiac biomarker assessment, 50% met criteria for cardiac response (NT-proBNP: 30% reduction), and 50% achieved disease stabilization (Figure). Of the 14 renal evaluable patients, 43% met criteria for renal response (24-hour urine protein: 30% reduction), and 57% achieved disease stabilization (Figure).

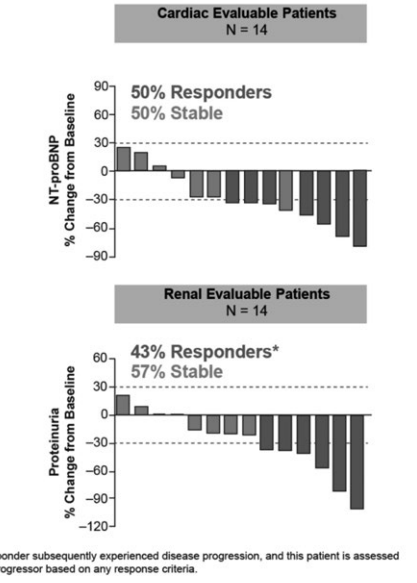

Figure 1. Change from baseline of cardiac response (NT-proBNP) and renal response (24-hour urine protein) in organ-evaluable patients.

Summary and Conclusions: Monthly infusions of NEOD001 were safe and well tolerated. 24 mg/kg was the RP2D. The cardiac response rate was 50%, and the renal response rate was 43%. These organ response rates compare favorably to those reported with traditional chemotherapy. The phase 2 expansion phase is ongoing. A phase 3 study has been initiated. Antibody therapy may represent a new therapeutic platform for the management of AL amyloidosis.

S105

UPDATED OVERALL SURVIVAL ANALYSIS OF THE FIRST STUDY: CONTINUOUS LENALIDOMIDE PLUS LOW-DOSE DEXAMETHASONE VS MELPHALAN, PREDNISONE, AND THALIDOMIDE IN PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA

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Background: The combination of melphalan, prednisone, and thalidomide (MPT) is considered a standard treatment (Tx) option in many countries for patients (pts) with newly diagnosed multiple myeloma (NDMM) who are ineligible for stem cell transplant (SCT). The FIRST trial is the largest prospective phase 3 trial conducted in pts with NDMM ineligible for SCT. In this study, patients were treated with lenalidomide plus low-dose dexamethasone administered until disease progression or unacceptable toxicity (Rd continuous), Rd for 18 cycles (Rd18), or MPT for 12 cycles. At the planned primary analysis for OS, Rd continuous was associated with improved progression-free survival (PFS) and an overall survival (OS) advantage *vs* MPT (Benboubker, *N Engl J Med*, 2014).
Aims: This abstract presents an updated analysis of OS and other efficacy measures that was not initially planned but was requested by regulatory authorities.
Methods: Pts were randomized 1:1:1 to 1 of 3 Tx arms: Rd continuous (28-day cycles), Rd18 (28-day cycles), or MPT for 12 cycles (42-day cycles). Eligible pts (aged ≥18 yrs) had symptomatic NDMM and an Eastern Cooperative Oncology Group performance status of 0-2 and were ineligible for SCT. Pts were excluded if they had received prior anti-myeloma Tx, had specified laboratory abnormalities, or had a history of malignancy, other than multiple myeloma, within the past 3 yrs. The primary endpoint was PFS (Rd continuous *vs* MPT; primary comparison). Secondary endpoints included OS, overall response rate, and safety. Time from randomization to second disease progression (PFS2) or death was included as an additional analysis.
Results: A total of 1623 pts were randomized; 535 pts received Rd continuous, 541 received Rd18, and 547 received MPT. At the time of data cutoff (March 3, 2014), 91 pts remained on Tx with Rd continuous. Across all Tx arms, 697 pts (42.9%) died; 38.9% of pts treated with Rd continuous died compared with 42.1% and 47.7% of pts treated with Rd18 and MPT, respectively. With a median follow-up of 45.5 mos, median OS was 58.9 mos (95% CI, 56.0-not evaluable [NE]) with Rd continuous *vs* 56.7 mos (95% CI, 50.1-NE) for pts treated with Rd18 and 48.5 mos (95% CI, 44.2-52.0) for pts treated with MPT. For comparison of OS with Rd continuous *vs* MPT, the hazard ratio (HR) was 0.75 (95% CI, 0.62-0.90). For the updated PFS analysis based on investigators' assessment, the median was 26.0 mos for pts treated with Rd continuous compared with 21.0 mos with Rd18 and 21.9 mos with MPT. The HR between Rd continuous and MPT was 0.69 (95% CI, 0.59-0.80). The majority of second-line Tx were bortezomib-based (55.7%). PFS2 events occurred in 58% of pts, and the median PFS2 was extended with Rd continuous *vs* Rd18 and MPT (42.9 *vs* 40.0 and 35 mos, respectively), with an HR for Rd continuous *vs* MPT of 0.74 (95% CI, 0.63-0.86). The mean durations of Tx for pts who received Rd continuous, Rd18, and MPT were 22.5, 12.6, and 11.9 mos respectively. Updated safety data will be presented.
Summary and Conclusions: The OS and PFS benefits observed in the original analysis were maintained with Rd continuous. Rd continuous was better tolerated than MPT. The safety profile remained consistent with the interim analysis. Improvements in PFS2 suggest that the benefit of Rd continuous is retained through second-line therapy without inducing resistance. These findings confirm Rd continuous as a new standard of care for pts with NDMM ineligible for SCT.

Treatment and outcome in non-Hodgkin lymphomas

S106

HIGH DOSE SEQUENTIALCHEMOTHERAPY WITH RITUXIMAB AND ASCT AS FIRST LINE THERAPY IN ADULT MCL PATIENTS: CLINICAL AND MOLECULARRESPONSE OF THE MCL0208 TRIAL, A FIL STUDY

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Background: In spite of the improvement of the disease control obtained with the intensive chemo-immunotherapy in adult patients with MCL with or without autograft (ASCT) the rate of relapse and death is still high. Recent data showed that a therapeutic strategy including a maintenance in responding patients to chemoimmunotherapy can prolong the response duration and clinical outcome of MCL patients
Aims: In 2008 the Fondazione Italiana Linfomi (FIL) designed the phase III trial MCL0208, to evaluate the efficacy and safety of lenalidomide as maintenance therapy in patients with MCL achieving at least a Partial Response (PR) after an upfront intensive chemotherapy with rituximab (R) and ASCT (NCT02354313). This trial was approved by the Ethical Committee of all partecipating centers. Herein, we present the analysis of clinical and molecular response after the chemotherapy with Rituximab (R) and ASCT, one of the secondary objectives of MCL0208 study.
Methods: Adult patients aged<66 years, with advanced stage MCL without clinically significant comorbidities are enrolled. The primary end-point of the study is the 2-year PFS from randomization. Patients receive 3 cycles of R-CHOP-21, followed by R-HDS which includes: R-high-dose cyclophosphamide (R-HD-CTX) (4g/m2), 2 cycles of R-high-dose Ara-C (R-HD-Ara-C) (2g/m2 q12x3 d), followed by BEAM and ASCT. CD34+ cell harvest is performed after the first course of R-HD-Ara-C. A second harvest will be performed after the second course of R-HD-Ara-C, if prior harvest is PCR+. After ASCT, responding patients are randomized between maintenance with lenalidomide (15 mg days 1-21 every 28 days) or observation for 24 months. Minimal Residual Disease (MRD) is examined at diagnosis, after R-HDCT, before and after ASCT, during maintenance/observation and during follow-up every six months. The total number of patients to be enrolled is 300.
Results: From May 2010 to November 2014, 260 patients have been enrolled by 48 italian and 1 international (Lisboa, Portugal) cancer centers. The median age was 57 years (IQR 51-61), predominantly male (80%) and the majority of patients presented with adverse features such as: advanced stage (98%), poor ECOG-PS (24%), bulky disease (>5 cm) (33%), elevated LDH (31%), BM infiltration (76%) and intermediate-high MIPI (53%). Nine percent of patients had blastoid variant. Among the 260 enrolled patients, 187 completed R-HDS (72%). Ultimately, 168 patients (65%) proceed to ASCT and 146 (56%) have been randomized between lenalidomide or observation. At the time of the present analysis according to Cheson (JCO 2007) of 202 patients evaluable for final response

137 (68%) reached CR after RHDS and 156 (77%) after ASCT. Regarding MRD a molecular marker was found in 87% of cases. Before ASCT a complete molecular response (CMR) on PB and BM were 72% and 53% by nested PCR and 80% and 67% by RQ-PCR. After ASCT CMR on PB and BM were 79% and 50% by nested PCR and 86% and 73% by RQ-PCR. After a median follow-up of 19 months the 2-year PFS and OS were 77% and 88%, respectively. As expected with intensive regimens there was an hematological toxicity, particularly CTC grade 3-4 neutropenia (38% of cycles) and thrombocytopenia (31% of cycles), but the infections were recorded only in 17% of patients and the treatment-related deaths (TRD) were 1.6%.
Summary and Conclusions: RHDS with ASCT is a feasible regimen with limited toxicity in a multicenter setting and produces an high rate of durable responses. These promising results are supported by the high rate of molecular responses by RQ-PCR.

S107

IMPACT OF PRIOR TREATMENT ON PFS FOR RELAPSED/REFRACTORY MANTLE CELL LYMPHOMA PATIENTS RANDOMIZED TO LENALIDOMIDE VS INVESTIGATOR'S CHOICE: A SUBGROUP ANALYSIS OF THE PHASE II MCL-002 (SPRINT) STUDY

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Background: Clinically meaningful and statistically significant improved activity was shown for lenalidomide, an immunomodulator with antineoplastic and antiproliferative effects, over single-agent investigator choice (IC) treatment in the MCL-002 (SPRINT) study of relapsed/refractory (R/R) patients with mantle cell lymphoma (MCL).
Aims: Evaluate the potential impact of prior therapy on progression-free survival (PFS) in R/R MCL patients randomized to lenalidomide *vs* IC.
Methods: Patients were randomized to lenalidomide (25 mg/day PO on days 1-21/28 days) or single-agent IC (rituximab, gemcitabine, fludarabine, chlorambucil, or cytarabine). The primary endpoint of this phase II study was PFS; prespecified exploratory analyses of PFS by subgroups were conducted.
Results: Following a median of 2 prior therapies, 254 R/R MCL patients were randomized 2:1 to lenalidomide (n=170) or IC (n=84). The preferred single-agent IC therapy was selected for each patient prior to randomization. Overall, the median PFS was significantly improved for lenalidomide *vs* IC (8.7 *vs* 5.2 months; HR=0.61, *P*=0.004). Exploratory analysis of PFS by central review based on selected IC treatment showed that lenalidomide provided a reduction in the risk of progression or death *vs* each IC treatment. Compared with lenalidomide and taking into account small patient numbers per IC group, the risk reduction in PFS was 22% *vs* rituximab (n=27), 56% *vs* gemcitabine (n=20), 42% *vs* fludarabine (n=18), 43% *vs* chlorambucil (n=11), and 8% *vs* cytarabine (n=8). Subgroup analysis of PFS by central review based on prior treatment-related subgroups favored the use of lenalidomide overall. Several subgroups showed statistically improved PFS for lenalidomide over IC, including patients with ≥2 prior systemic antilymphoma therapies, >1 prior relapse, refractory to last prior treatment, prior rituximab exposure, no prior hyperCVAD±rituximab or stem cell transplantation, ≥6 months time from last therapy (≥230 days from last prior rituximab), and <3 years from diagnosis to study treatment. The only category without risk reduction (but statistically insignificant) was ≥4 prior systemic antilymphoma therapies, partly explained by low patient numbers in each arm. Treatment group was the main effect associated with significantly better PFS by univariate Cox regression analysis (HR=0.619; *P*=0.004), and was highly significant in the multivariate analysis (HR=0.384). Other factors associated with significantly better PFS by both univariate and multivariate analysis were <3 prior systemic antilymphoma therapies, and ≥230 days from last prior rituximab.
Summary and Conclusions: Subgroup and regression analyses of the primary study endpoint PFS showed superiority for lenalidomide over IC therapy in providing consistent clinical benefit in patients with R/R MCL irrespective of prior treatment history.

S108

LONG-TERM OUTCOME OF 490 PATIENTS WITH EARLY-STAGE EXTRA-NODAL MARGINAL ZONE LYMPHOMA

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Background: Localized early-stage extra-nodal marginal zone lymphoma (MZL) presents with heterogeneous organ involvement and is treated with various modalities, including resection, radiotherapy, and, infrequently, systemic therapy. We report the long-term outcome of a large cohort of extra-nodal MZL and assess the impact of patient and disease characteristics, organ site, and treatment strategy on disease control and survival.
Aims: To study patient characteristics and long-term outcome of a large cohort of patients with early-stage localized extra-nodal marginal zone lymphoma
Methods: We identified 490 consecutive patients with stage IE or IIE MZL referred between 1992 and 2012 to Memorial Sloan Kettering Cancer Center. Pathology was confirmed by hematopathologists at our institution. Patient and disease factors as well as treatment types were analyzed for association with relapse-free survival (RFS), overall survival (OS), and cumulative incidence of relapse.
Results: Median follow-up was 4.8 years. Median patient age was 60 years and 57% were female. Ann-Arbor stage was IE in 89%. Most common sites were stomach (32%), orbit (14%), lung (12%), skin (12%), and parotid (5%). Radiotherapy alone (RT) was the initial treatment in 50% of patients, followed by surgical resection (30%), observation (9%), immunotherapy (4%), and chemotherapy (2%). Five-year OS and RFS were 90% and 64%, respectively; 10-year OS and RFS were 73% and 45%. Disease-specific death was 1.3% at 5 years and 1.8% at 10 years. Cumulative incidence of progression/relapse was 29% by 5 years and 39% by 10 years. Amongst the 384 patients with complete response (CR), 99 patients experienced relapse. On multivariable analysis, initial treatment type and primary disease site were independently associated with RFS and relapse (all $p<0.005$). All disease sites (HR>2.0, $p\leq0.01$) except for thyroid ($p=0.8$) had worse RFS relative to stomach. Compared with RT, chemotherapy or immunotherapy had worse RFS (HR 2.2, $p=0.004$) while surgery was no different ($p=0.52$). After RT, only 11 patients experienced in-field failure, with a 5-year cumulative incidence of 2.4%. Most common location of relapse after CR was distant; relapses were also observed in paired untreated organs, such as the orbit, salivary gland, and breast. Crude rate of transformation to pathologically-confirmed large-cell lymphoma was 2% (11 patients). Second tumors in irradiated sites developed in 3 patients: 2 of these were breast ductal carcinoma *in situ* cured with surgical resection.
Summary and Conclusions: Overall and cause-specific survival are excellent in early-stage extra-nodal MZL. Treatment with RT or surgery was associated with longer RFS and reduced the need for salvage. Relapses are common after initial remission, and most frequently occur in distant sites. Transformation to large cell lymphomas is rare. Stomach cases are less likely to relapse than other anatomic primary sites, perhaps in part because the entire organ is irradiated, *versus* other sites that are either bilateral or where only part of the organ is treated, such as the skin and lung. This study supports the use of local therapies to treat stage IE and IIE MZL.

S109

A DOSE-ESCALATION STUDY OF THE BCL-2 INHIBITOR VENETOCLAX (ABT-199/GDC-0199) PLUS BENDAMUSTINE (B) AND RITUXIMAB (R) IN PATIENTS WITH RELAPSED/REFRACTORY (R/R) NON-HODGKIN'S LYMPHOMA (NHL)

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Background: Venetoclax is a selective, potent, orally bioavailable BCL-2 inhibitor that has shown single agent activity in patients with R/R NHL. The current study evaluates venetoclax in combination with an immunochemotherapy regimen, BR, an active regimen widely used to treat patients with NHL.
Aims: The primary objectives were to assess safety, pharmacokinetics (PK), and determine the maximum tolerated dose (MTD) and recommended Phase 2 dose of the combination. Secondary objectives evaluated preliminary efficacy.
Methods: Dose escalation used a 3+3 design on a 28 day (d) cycle (C) with 3 venetoclax schedules: 3, 7, and 28 d/C. The BR regimen was 6 C: B (2 d/C, 90mg/m^2) and R (1 d/C, 375mg/m^2). DLTs for dose escalation were assessed during C1. Responses were first assessed on C3 d1. Patients who completed venetoclax + BR with continued tolerability and without disease progression could continue venetoclax monotherapy up to 2 yrs.

Results: As of January 9 2015, 33 patients were treated: 20 (61%) FL, 10 (30%) DLBCL, and 3 (9%) MZL. The median age was 62 (29-90) yrs. All had prior R or R-combination, of which 32 (97%) had R-based chemotherapy and 8 (24%) had prior B or BR. Sixteen (48%) patients are active; 17 discontinued (12 PD, 2 AE, 1 withdrew consent, 1 non-compliance, and 1 completed the induction regimen). Median time on study was 90 d (1–876); 15 (45%) completed 6 C of the combination. The most common AEs (in >25%) were nausea (58%), anemia, neutropenia (each 42%), thrombocytopenia, diarrhea (each 39%), hyperglycemia (36%), and vomiting, hypokalemia, fatigue (each 27%). The most common grade 3/4 AEs (in >10%) were neutropenia (30%), leukopenia, thrombocytopenia, lymphopenia (each 21%), and anemia (18%). The most frequent SAE was febrile neutropenia (9%). There were no drug-related AEs that led to death. DLTs are summarized in the table. Preliminary PK results suggest that co-administration of BR did not significantly impact venetoclax PK. Twenty-nine patients were evaluable for objective response: 6 (21%) CR and 13 (45%) PR. The ORR was 66% in all patients and 74% in patients with FL.

Tabella 1.

Cohort	1	2	3	4	5	6	7[a]	8[a]
Patients, n	4	4	4	3	3	4	5	6
Venetoclax, mg	50	100	100	100	200	200	400	400
Schedule, d/C	3	3	7	28	28	7	7	28
DLTs, n								
Thrombocytopenia					1			
Febrile neutropenia					1			
Stevens-Johnson Syndrome[b]								1

[a]Post-amendment to G-CSF prophylaxis and DLT criteria; [b]Primary reasonable possibility due to allopurinol; pt discontinued

Summary and Conclusions: Preliminary data demonstrate a tolerable safety profile of venetoclax + BR. Early responses were seen across all cohorts in this heavily pretreated patient population. The MTD has not been reached; cohort 9 is enrolling at 600 mg 28 d/C.

S110

INTERNATIONAL EXTRANODAL NK/T-CELL LYMPHOMA PROJECT: PROGNOSTIC FACTORS IN THE ERA OF NONANTHRACYCLINE-BASED TREATMENT

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Background: Extranodal natural killer/T-cell lymphoma (ENKTL), nasal type, is a rare type of non-Hodgkin lymphoma with poor prognosis because tumor cells are frequently resistant to anthracycline-containing chemotherapy such as CHOP due to expression of the multidrug-resistant p-glycoprotein. As a result, the treatment strategy for ENKTL has changed to non-anthracycline based regimens such as SMILE (steroid, methotrexate, ifosfamide, L-asparaginase, and etoposide) with or without radiotherapy. Thus, concurrent chemoradiotherapy followed by non-anthracycline based chemotherapy is recommended for localized disease whereas intensified chemotherapy such as SMILE is for advanced disease. However, there is no proven prognostic model for ENKTL in the era of this new treatment strategy because previous prognostic models were developed by analyzing patients who were treated with CHOP or CHOP-like regimens.
Aims: This study is to explore risk factors for poor progression-free survival (PFS) and overall survival (OS) in ENKTL, and establish a prognostic model for ENKTL patients treated with non-anthracycline based treatment.
Methods: This is a retrospective cohort study using anonymized information from patients with ENKTL. The following criteria are required: (1) Patients diagnosed with ENKTL, nasal type between January 1, 1995 and December 31, 2012; (2) Patients treated with non-anthracycline based therapy as an initial treatment. The pathology of initial diagnosis was reviewed by designated pathologists.
Results: The data of 557 patients were from 32 hospitals from six Asian countries (Korea, Japan, Hong Kong China, Singapore, Taiwan, and Malaysia) and 6 hospitals from five Western countries (France, Germany, Denmark, Sweden, and USA). Thirty cases were excluded from the analysis due to following reasons: Missing follow-up data, different pathology from ENKTL, and different treatment regimens containing anthracycline. Thus, 527 patients were analyzed, and male (n =341) was predominant compared to female (n =186). 70% of patients were ≤60 years at diagnosis whereas 30% was older than 60 years. Two-thirds of patients had stage I/II (n =346) and nasal tract was most commonly involved extranodal site (n =421, 80%). Bone marrow involvement was observed in 83 patients (16%), and distant lymph nodes were involved in 86 patients (16%). With a median follow-up of 45 months (IQR: 22-65 months), 306 patients (58%) were alive at the time of analysis and the event of progression-free survival occurred in 272 patients including relapse or progression (n =187). The median OS was 76 months (95% CI: 51-101 months) and PFS was 32 months (95% CI: 20-45 months). The multivariate analysis for OS and PFS showed a significant association of four parameters with survival outcomes: age >60, stage III/IV, distant lymph node involvement, and non-nasal tract involvement (P <0.001). Thus, the prognosis of patients with age >60, stage III/IV and distant lymph node involvement was poor, and patients who did not involve nasal tract also showed worse OS and PFS than patient with nasal tract involvement. Thus, we gave one score for each parameter. According to the sum of scores, patients with score 0 or 1 were grouped as low risk, score 2 was intermediate, and score 3 or 4 was high risk. This new risk model showed a strong association with OS and PFS (Figure 1). Among 328 patients who were initially evaluated for EBV DNA in blood, 189 patients (58%) showed detectable level of EBV DNA. The multivariate analysis including EBV DNA titer focused on these 328 patients showed that aforementioned four parameters and the presence of EBV DNA were independently associated with OS and PFS. Thus, we proposed another risk model for patients who had EBV DNA data with these five parameters consisting of low (score 0/1), low-intermediate (score 2), high-intermediate (score 3), and high risk (score 4/5).

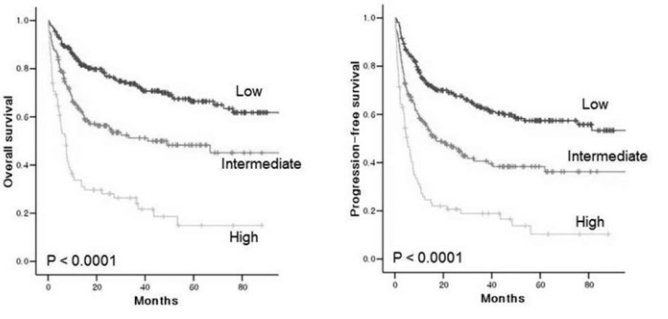

Figure 1.

Summary and Conclusions: Our multinational, multicenter retrospective study proposed a new prognostic model for newly diagnosed ENKTL patients who were treated with non-anthracycline based treatment. It consists of age >60, stage III/IV, distant lymph node involvement, and no involvement of nasal tract. Our finding will be validated by currently ongoing study with an independent cohort including China.

ALL clinical trials

S111

CHIMERIC ANTIGEN RECEPTOR (CAR)-MODIFIED T CELLS TARGETING CD19 INDUCE SUSTAINED REMISSIONS IN CHILDREN AND YOUNG ADULTS WITH RELAPSED/REFRACTORY ALL

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Background: Relapsed/refractory pediatric acute lymphoblastic leukemia (ALL) poses a substantial therapeutic challenge. Targeted immunotherapy using chimeric antigen receptor (CAR)-modified T cells combines the specificity of an antibody's single chain variable fragment (scFv) with intracellular T cell signaling domains, delivering T cells with potent cytotoxicity to antigen-expressing tumor cells. We previously reported complete remissions and prolonged persistence in children and adults with ALL treated with CD19-specific CART cells (CTL019). We now report on outcomes and longer follow-up of the first 40 patients with relapsed/refractory ALL treated on the pediatric trial of CTL019 in the largest cell therapy experience reported to date.
Aims: Establish the safety and efficacy of CTL019 for patients with relapsed/refractory CD19+ ALL.
Methods: After informed consent, T cells collected from the patient were transduced with a lentiviral vector encoding a CAR composed of anti-CD19 scFv, CD3z, and 4-1BB domains, activated/expanded *ex vivo* with anti-CD3/anti-CD28 beads, cryopreserved, and then infused. 35/40 patients received lymphodepleting chemotherapy the week prior to cell infusion.
Results: Of 40 patients aged 5-22y (median 11y) with CD19+ ALL, 33 had detectable disease prior to CTL019 cell infusion, while 7 were negative for minimal residual disease (MRD). 28 had relapsed after prior stem cell transplantation (SCT). A median of $3.6x10^6$ CTL019 cells/kg ($0.98\text{-}17x10^6$/kg) were infused in 1-3 fractions. At assessment 1 month after infusion, 37/40 (93%) were in a complete remission (CR). MRD <0.01% by flow cytometry was achieved in 34 patients. A CR rate of 86% was achieved in 22 patients with an M3 marrow (>25% marrow lymphoblasts) at infusion. With median follow-up 7 mo (1-31 mo) as of January 1, 2015, 26 patients had ongoing CR, with only 5 receiving subsequent therapy (1 donor lymphocyte infusion [DLI], 4 SCT), 6-month EFS was 72% (95% CI, 59-89%), and OS was 77% (95% CI, 64-92%). CTL019 cells were detected in the CSF and 4 patients with CNS2a disease at infusion have experienced ongoing CRs in CSF. Ten patients subsequently relapsed, 5 with CD19(-) disease. CTL019 persistence was accompanied by B cell aplasia, which continued up to 30 months in patients with ongoing CR. Cytokine release syndrome (CRS) was seen in almost all (37) patients. Dramatic elevations in ferritin were observed, suggesting an association of macrophage activation syndrome with CRS. Severe CRS requiring hemodynamic or respiratory support occurred in 33% of patients, was associated with high pre-treatment disease burden and with elevations in CRP and IL6 after infusion. Severe CRS was rapidly reversed in each case with the anti-IL6R agent tocilizumab, demonstrating the importance of IL6 in driving CRS.
Summary and Conclusions: Single-agent CTL019 immunotherapy can induce potent and durable responses in patients with relapsed/refractory ALL. CRS was effectively controlled with IL6 blockade. Long-term disease control is possible without subsequent stem cell transplantation.

S112

EFFICACY AND SAFETY OF CD19-TARGETED 19-28Z CAR MODIFIED T CELLS IN ADULT PATIENTS WITH RELAPSED OR REFRACTORY B-ALL

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Background: Adult patients with relapsed or refractory (R/R) acute lymphoblastic leukemia (ALL) have dismal prognosis. We previously reported high anti-tumor activity of autologous T cells genetically modified to express 19-28z chimeric antigen receptor (CAR) targeting CD19 in adult patients with ALL.
Aims: Herein, we report the long-term outcome of our phase I clinical trial in adults with R/R ALL (NCT01044069).
Methods: Adult patients with R/R B-ALL underwent leukapheresis, and T cells were transduced with a gammaretroviral vector encoding a CAR construct composed of anti-CD19 scFv linked to CD28 and CD3ζ signaling domains (19-28z). All patients received conditioning chemotherapy followed by $1–3x10^6$ 19-28z CAR T cells/kg.
Results: 33 patients have been treated, and 32 patients are evaluable for response. The median age was 54 years (range, 22-74). 12 patients (36%) had Ph+ ALL, 11 patients (33%) had prior allogeneic stem cell transplant (allo-SCT), and 14 patients (42%) had ≥3 prior lines of therapy. At the time of CAR T cell infusion, 16 had morphologic disease (>5% blasts in BM) and the remaining 16 patients had minimal residual disease (MRD). 13/16 patients with morphologic disease (81%) and 16/16 patients with MRD (100%) were in complete remission (CR) after 19-28z CAR T cell infusion, yielding an overall CR rate of 91% (29/32). Of the 28 MRD evaluable patients, MRD negative CR rate was 82%. 11 patients underwent allo-SCT following the CAR T cells. As of 1/25/15, the median follow-up was 5.1 months (range, 1.0-37.6+), with 14 patients having ≥6 months of follow-up. 6-month overall survival (OS) rate of all patients was 58% (95% CI: 36-74). Among the patients who achieved CR, OS rate at 6 months for patients who had allo-SCT *vs* no allo-SCT following CAR T cells was 70% (95% CI: 33-89) *vs* 61% (95% CI: 29-82; p=0.30). Severe cytokine release syndrome (sCRS) requiring vasopressors or mechanical ventilation for hypoxia was observed in 7 patients, effectively managed with IL-6R inhibitor and/or corticosteroids.
Summary and Conclusions: 19-28z CAR T cells can induce a high CR rate of 91% in adult patients with R/R ALL. The risk of sCRS correlates with disease burden and can be effectively managed. These findings strongly support the use of 19-28z CAR T cells in adults with R/R ALL and warrants investigation in a phase 2 trial.

S113

NILOTINIB IN COMBINATION WITH CHEMOTHERAPY FOR FIRST-LINE TREATMENT IN ELDERLY PATIENTS WITH PHILADELPHIA-POSITIVE ALL: RESULTS OF THE EUROPEAN WORKING GROUP FOR ADULT ALL(EWALL-PH-02)

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Background: Tyrosine kinase inhibitors such as imatinib induce a high rate of complete hematologic remission (CHR) in Philadelphia positive (Ph+) acute lymphoblastic leukaemia (ALL), but the overall prognosis particularly in elderly patients (pts.) remains poor, primarily due to relapse. Nilotinib is a potent ABL kinase inhibitor (TKI) approved for treatment of CML. Data on its efficacy in Ph+ ALL are limited.
Aims: The EWALL (European Working Group for Adult ALL) initiated a prospective, investigator-initiated multicenter European clinical trial to examine the efficacy and safety of nilotinib in conjunction with a chemotherapy backbone in elderly patients (above 55 years of age) with newly diagnosed Ph+ ALL.
Methods: Male or female pts. >55 years with untreated Ph+ and/or BCR-ABL1 positive ALL were eligible if they had a WHO performance status of 0-2, adequate organ function and had signed written informed consent. The trial was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the ethics committees of all participating centres (registered under NCT01528085). After a pre-phase with dexamethasone (Dex) and cyclophosphamide (optional), nilotinib was administered at 400 mg BID and given continuously thereafter. During induction, nilotinib was combined with vincristine (VCR) and Dex, repeated weekly for 4 weeks. Consolidation cycles consisted of nilotinib 400 mg BID, methotrexate (MTX) and asparaginase for cycles 1, 3 and 5 and cytarabine for cycles 2, 4 and 6. Maintenance phase consisted of nilotinib, 6-MP, MTX and Dex/VCR. The primary end-point is the rate of pts. without an event (defined as relapse, death, SAE or study treatment discontinuation) at 12 months, secondary endpoints were the rate of CHR after

induction, death during induction or in CHR, event free (and overall) survival, the rate of molecular response defined by BCR-ABL/ABL ratios <0.1% (MMR) and <0.001% (CMR), respectively.

Results: As of December 2015, 56 pts. (25 male, 31 female) have been enrolled. Median age is 65 years (55-85 years), twelve pts. are older than 70 years of age. To date, all pts. are evaluable for safety and 47 pts. are evaluable for efficacy. The CHR rate is 87 %, one pt. was refractory (2%), one pt. had a partial remission (2%). One pt. died during induction therapy (2%), three pts. discontinued therapy before CR evaluation. With a median follow-up of 5.5 months, 34 of the 41 pts. who achieved CR are in CCR and 3 pts. relapsed, two of whom had discontinued study treatment to undergo allogeneic SCT. 11 pts. with documented induction response have discontinued study treatment prematurely because of transfer to allogeneic SCT, as explicitly permitted by the protocol. 9 pts. of them are transplanted. 11 pts. discontinued for various other reasons. The rate of molecular remission (MMR) after induction (25 pts. evaluable) was 45.5 %, with 5 pts. having undetectable BCR-ABL1 transcripts. During consolidation, 30 of 40 pts. (80%) had a MMR, and BCR-ABL transcripts were undetectable in 10 of 40 pts. (20%). Tolerability has been acceptable. Infectious events and neutropenic fever predominated, individual SAEs included metabolic, cardiovascular, neurologic, renal and hepatic events.

Summary and Conclusions: Nilotinib in conjunction with chemotherapy according to the EWALL-PH-02 protocol is highly effective and well tolerated in elderly pts. with newly diagnosed Ph+ ALL. Molecular response rates are high and MRD levels in responding pts. continue to decrease with time.

S114

INOTUZUMAB OZOGAMICIN IN COMBINATION WITH LOW-INTENSITY CHEMOTHERAPY (MINI-HYPER-CVD) FOR THE FRONTLINE THERAPY IN ELDERLY PATIENTS (≥60 YEARS) WITH ACUTE LYMPHOBLASTIC LEUKEMIA (ALL)

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Background: Older patients (pts) with ALL have a significantly worse outcome. This is primarily due to poor tolerance of intensive chemotherapy. Addition of targeted non-myelosuppressive therapy to effective low-intensity chemotherapy might improve outcome. CD22 expression occurs in >90% of pts with ALL. Inotuzumab ozogamicin (INO) is a CD22 monoclonal antibody bound to a toxin, calecheamicin, and has shown single-agent activity in relapsed/refractory ALL (Kantarjian *et al.* Lancet Oncology 2012).

Aims: To determine the efficacy of INO in combination with mini-hper-CVD assessed by objective response rate, progression-free, and overall survival and to assess the side effects of this treatment.

Methods: Pts ≥60 years (yrs) with newly-diagnosed B-cell ALL were eligible. The chemotherapy was lower intensity than conventional hyper-CVAD and referred to as mini-hyper-CVD (cyclophosphamide and dexamethasone at 50% dose reduction, no anthracycline, methotrexate at 75% dose reduction, cytarabine at 0.5 g/m^2 x 4 doses). Rituximab and intrathecal chemotherapy were given for first 4 courses. INO was given on Day 3 of each of the first 4 courses. The first 6 pts received 1.3 mg/m^2 for cycle 1 followed by 0.8 mg/m^2 for subsequent cycles; Pts 7 onwards received 1.8 mg/m^2 for Cycle 1 followed by 1.3 mg/m^2 for subsequent cycles.

Results: Thirty-three pts (20 men, 13 women) have been treated so far. Pts characteristics and outcome are summarized in Table 1. Median age is 69 yrs (range, 60-79). Median follow-up is 15 months (mos) (range, 2-35). Of the 30 pts evaluable for response (three pts started in CR; two achieved with single-agent steroids and one with one course of HCVAD), 29 pts (97%) achieved CR/CRp (24 CR, 5 CRp). All pts achieving CR have also achieved flow-cytometric MRD negative status, in 79% at the time of CR achievement. Grade 3-4 toxicities included infections (n=29; 88%), prolonged thrombocytopenia (n=25; 76%), hyperglycemia (n=17; 52%); hypokalemia (n=11; 33%); increased bilirubin (n=8; 24%); increased ALT (n=7; 21%), and intracranial hemorrhage (n=4; 12%). Grade 2 veno-occlusive occurred in 2 (7%) pts (7%). At the last follow-up, 24 (73%) pts are alive, and 23 (70%) are in CR. Nine (27%) pts died: 1 had primary refractory ALL and died after the first salvage; 2 relapsed after receiving 3 and 2 courses only due to prolonged myelossuppression and died of disease progression; and 6 died in CR from pneumonia complications (n=1), sepsis and multiple organ failure (n=1), gun-shot wound (n=1), renal failure and metabolic encephalopathy (n=1), complications due to dementia (n=1), and unknown (n=1). One pt received allogeneic stem cell transplantation. The 2-year progression-free survival and overall survival rates were 85% and 70%, respectively. The mini-hyper-CVD (n=33) appears superior to the historical HCVAD +/-rituximab (n=46) in similar patient population (2-year survival rates 78% and 38%, respectively; Figure 1).

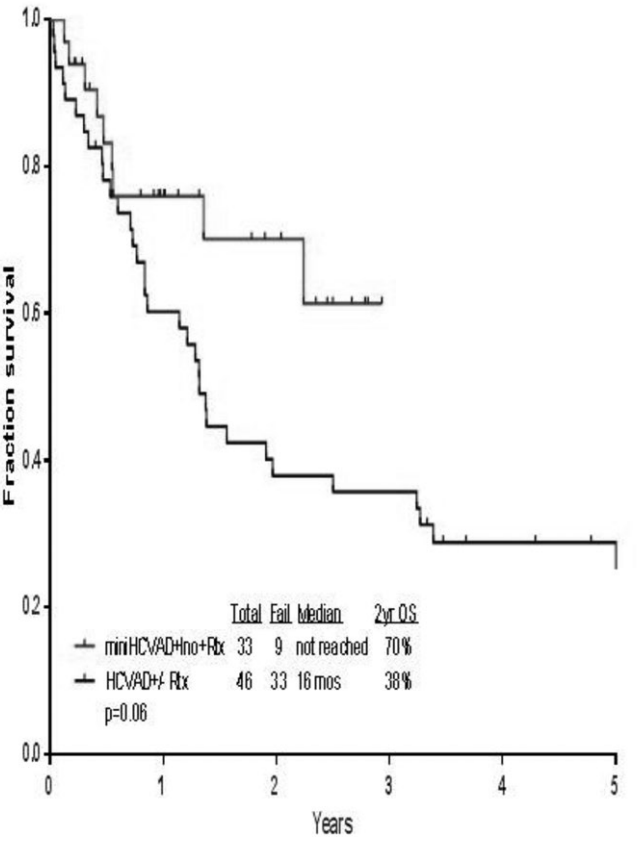

Figure 1. Survival with mini-HCVD-INO *vs* HCVAD +/-Rituximab in frontline ALL

Tabella 1. Patient characteristics and outcome

Parameter	Category	N (%)/Median [Range]
Follow-up (mos)		15 [2-35]
Age (yrs)		69 [60-79]
Performance Status (ECOG)	0-1	29 (88)
WBC		3.5 [0. 6- 111.0]
Karyotype	Diploid	9 (33)
	Miscellaneous	18 (55)
	Insufficient metaphases/ Unknown	4 (12)
CD22		98 [72-100]
CD20	≥ 20%	23/33 (70%)
CR		24 (80)
CRp		5 (17)
No response		1 (3)
ORR		29 (97)
Cytogenetic CR	17 abnormal at start	17 (100)
Neg MRD at D21 overall	(5 not done / 3 CR at start)	19 (79) 32 (100)
Early death		0
2-year PFS %		85
2-year OS %		70

Summary and Conclusions: The combination of INO with low-intensity mini-hyper-CVD chemotherapy is safe and shows encouraging results (96% CR/CRp) in the frontline setting in older pts with ALL. These results appear to be better than those achieved with a chemotherapy alone approach and may become the new standard of care for frontline treatment of older pts with ALL.

S115

BLINATUMOMAB SAFETY AND ACTIVITY IN OLDER PATIENTS WITH RELAPSED/REFRACTORY B-PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA IN TWO PHASE 2 STUDIES

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Background: Treatment options for older patients with relapsed/refractory acute lymphoblastic leukemia (ALL) are limited. Blinatumomab is a bispecific T-cell engager (BiTE®) antibody construct that directs cytotoxic T cells to CD19-expressing B cells, and is approved in the US for treatment of Ph-negative relapsed/refractory ALL. In two phase 2 adult studies of blinatumomab (Topp MS, *et al.* J Clin Oncol. 2014;32:4134-40; Topp MS, *et al.* Lancet Oncol. 2015;16:57-66), 69% and 43% of patients, respectively, achieved complete response (CR) or CR with partial hematologic recovery (CRh*).
Aims: We report pooled data for the combined subsets of older patients (≥65 years).
Methods: Patients with relapsed/refractory, Ph-negative B-precursor ALL received open-label blinatumomab by continuous intravenous infusion (4 weeks on/2 weeks off). Patients achieving CR or CRh* after two cycles could receive three consolidation cycles. Response was assessed by bone marrow aspiration and complete blood count with differential. CR required blasts <5%, ANC >1000/μL and platelets >100,000/μL. CRh* required blasts <5%, ANC >500/μL and platelets >50,000/μL. Minimal residual disease (MRD) was detected by ASO-PCR of Ig heavy chain loci.
Results: A total of 36 older patients (median age 70 years, range 65-79) received blinatumomab for a median (range) of 2 (1-6) cycles. Twenty (56%) patients achieved best response of CR/CRh* within two cycles, including 14 (39%) CR and 6 (17%) CRh*. Among patients who responded to blinatumomab, 16 (80%) had an MRD response; of these, 12 (60%) had complete MRD response (undetectable MRD) and 4 (20%) others had detectable MRD but with $<10^{-4}$blasts. With median follow-up of 18.2 months, median (range) relapse-free survival was 7.4 (1.0-34.0) months. With median follow-up of 29.4 months, overall survival among patients was 5.5 (0.3-41.9) months. Ten (28%) patients were alive at last follow up, including 6 in sustained remission. Two (10%) of the patients who responded to blinatumomab underwent allogeneic hematopoietic stem cell transplantation (HSCT) after blinatumomab therapy. Treatment-emergent adverse events (AE) CTCAE grade ≥3 were reported for 31 (86%) patients, most commonly febrile neutropenia (22%) and neutropenia (19%). Neurologic AE occurred in 26 (72%) patients, including grade ≥3 events for 28%. One (3%) patient had grade ≥3 cytokine release syndrome. Of 7 fatal AEs reported in patients, none were considered related to treatment.
Summary and Conclusions: Older patients (≥65 years) with relapsed/refractory ALL in two phase 2 studies of single-agent blinatumomab had similar treatment responses and tolerability compared with patients in the overall study populations.

Molecular pathogenesis of AML

S116

CLONAL ARCHITECTURE DEFINES DISTINCT MECHANISMS FOR ACCUMULATION OF GENOMIC OR CHROMOSOMAL LESIONS IN ACUTE MYELOID LEUKEMIA

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Background: Mutations in epigenetic regulators such as *DNMT3A, TET2, and ASXL1* have been shown to occur with ageing, leading to clonal pre-leukemic hematopoiesis, which may evolve into acute myeloid leukemia (AML) once additional lesions have accumulated. Studies of clonal architecture and pre-leukemic hematopoietic stem cells (HSCs) of AML with normal karyotype suggest that *DNMT3A* mutations, but not other sub-clonal mutations, are the pre-leukemic initiating events and the hallmark of relapse reservoir.
Aims: It is unknown whether AML carrying other mutations or chromosomal lesions have a clonal architecture and reservoirs of pre-leukemic HSCs similar to those of *DNMT3A* mutant AML. To address this point we analyzed the clonal architecture and evolution at relapse of a series of AML with both normal and abnormal karyotype, *i.e.* MLL rearrangement, t(8;21), inv(16), del(20q), +8, del(7), and complex karyotype (CK).
Methods: Molecular and cytogenetic analyses were performed at time of diagnosis (n=58 patients) and relapse (n=20). Exome analysis (n=9) and targeted sequencing (122 genes, n=53) were performed using Illumina platforms. Variants were validated by Sanger sequencing. Somatic mutations were assessed using remission samples or T cells. Cells from 14 patients (8 with abnormal karyotype) were grown in methylcellulose and individual colonies were picked for genotyping and FISH analysis.
Results: We found 234 lesions (median=4/patient) including 190 mutations in 60 genes. 36% of lesions target epigenetic regulators (DNMT3A, TET2/3, IDH1/2, MLL, polycombs, del(20q)), with a frequent (34%) occurrence of multiple epigenetic events. 24 % of lesions involve proliferative events (FLT3, RAS signalling, +8), and 17% affect transcription or splicing factors, NPM1, and ubiquitin ligases. Mutations in other pathways (NOTCH, WNT, DNA repair, cohesin), CBF translocations, and other chromosomal abnormalities account for 22% of the total. FISH and genotyping analysis of 1,873 individual colonies revealed that lesions involving epigenetic regulators, including MLL and 20q rearrangements, occur frequently as first events (11/14). Analysis of variant allele frequencies (VAF), FISH results, and relapse samples allowed us to build the clonal phylogeny for 38 of the 44 remaining AML. Overall, a recurrent order of events was observed, with CBF translocations and epigenetic abnormalities as first events (37/52) and signalling or proliferation lesions as last ones (29/52). In 17 cases this order was not observed, suggesting a distinct mechanism of leukemogenesis, especially in patients with CK or chromosome 7 abnormalities (n=7). Clonal composition changed in 14/20 samples at relapse. In one patient, a second AML occurred on donor transplanted cord blood HSCs. In another patient, a chronic myelo-monocytic leukemia developed after 9 years on an *ASXL1*-mutant minor clone already detected at time of diagnosis. Changes in relative VAF of *IDH1, IDH2, TET3, WT1, NF1,* and *ZRSR2* variants were observed in 5 patients. Seven samples carried events not detected at diagnosis (t(1;6), gain of chromosome 8, and *ZRSR2, SETBP1, RUNX1, PTPN11, KDM6A, CEBPA* variants), while in 9 cases, lesions were lost at relapse (*KDM6A, NPM1, PTPN11, WT1, DSCAM, POLR2A, TET2, UBEJ1, RUNX1, IDH1, BCOR, FLT3,* trisomy8). In all 14 patients harbouring an initial epigenetic lesion, the event persisted at relapse.
Summary and Conclusions: Our results identify 2 groups of AML suggesting 2 ways for leukemogenesis. The 1st group includes cases with CK and chromosome 7 lesions, which may result from chromosome or DNA maintenance defects. For instance, *TP53* mutations are frequent in CK AML. The 2nd group is characterized by early epigenetic events, including *DNMT3A, TET2,* and *ASXL1* mutations, which always persist at relapse. These events may lead to an over-expansion of mutant HSCs. In these pre-leukemic cells the increased mitotic rate may result in a faster accumulation of replication errors and mutations than in normal HSCs.

S117

IDENTIFICATION OF RUNX1/ETO TARGETS REQUIRED FOR LEUKAEMIC PROPAGATION

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Background: RUNX1/ETO is a driver leukaemic fusion protein produced by the translocation t(8;21) and accounts for 10-12% of Acute Myeloid Leukaemia (AML) cases. RUNX1/ETO is required for maintaining the leukaemic phenotype influencing both leukaemic clonogenicity and growth. Currently the molecular mechanism(s) by which it regulates leukaemic self-renewal and propagation are just beginning to be unravelled. In our previous work, we characterised the core-transcriptional network driven by RUNX1/ETO required to maintain the t(8;21) AML phenotype (Ptasinska *et al.*, 2014).
Aims: We have now functionally characterised the significance of individual members of this network for leukaemic self-renewal by performing targeted RNAi screens both in tissue culture and in a xenotransplant setting.
Methods: To that end, we intersected RNAseq, CHIPseq and microarray data and identified a set of 103 genes comprising both direct RUNX1/ETO target genes and genes potentially cooperating with such targets. For the RNAi screens, we used a Doxycycline (Dox)-inducible lentiviral RNAi library covering each gene of this set with at least 3 shRNAs. We transduced two t(8;21)-positive AML cell lines (Kasumi-1 and SKNO-1) with this library and performed parallel screens employing colony formation and long-term suspension culture assays in the *in vitro* arm, and intrafemoral transplantation of highly immunodeficient NSG mice for the *in vivo* screen. Both test series contained Dox and no Dox groups. RNA and genomic DNA were isolated from transduced cells and subjected to targeted Next Generation Sequencing. Changes in shRNA pool compositions were identified by comparison of the corresponding Dox and no Dox groups using DESEQ.
Results: The analysis of the *in vitro* screen demonstrated that l RUNX1/ETO shRNA constructs rapidly disappeared upon induction under all *in vitro* conditions, confirming the central role of RUNX1/ETO in maintaining t(8;21) AML, while levels of non-targeting control shRNA genes did not change during the course of the experiments. Moreover, *RUVBL1* (Pontin) shRNAs were depleted in agreement with our previous finding of a dependence of t(8;21) AML cells on this factor. In addition, shRNAs targeting *KIT* which in both cell lines carries the activating mutation N822K, disappeared. These combined results demonstrate the functionality of this targeted screen. Most importantly, the top hits of this *in vitro* screen identified a group of self-renewal genes including those encoding the ubiquitin ligase gene *SKP2* and Cyclin D2 (*CCND2*). The more stringent *in vivo* screen confirmed *CCND2* as a RUNX1/ETO target gene relevant for leukaemic propagation. Notably, this screen also identified several new genes, which had not scored in the *in vitro* setting. This included several genes involved in ubiquitination (*UBASH3B, UBE2L6*), regulation of G proteins (*GPRC5C, ARHGEF12, ARHGEF3*), transcriptional control (*ID1, KLF2, ERG*) or glycolysis (*SCL2A3, PFKP*). Importantly, several shRNAs targeting genes such as *NOTCH2, SLA* or *LAPTM5*, which are repressed by RUNX/ETO, were also depleted in the *in vivo* screen emphasising our previous observation that leukaemic self-renewal and propagation is driven by a dynamic equilibrium between RUNX1 and RUNX1/ETO.
Summary and Conclusions: We are now further characterising the contribution of these different target genes to the leukaemic self-renewal and propagation both *in vitro* and *in vivo*. The ultimate aim of our studies is the identification of RUNX1/ETO dependent pathways, whose combined inhibition may phenocopy the loss of this hard-to-target initiator and driver of leukaemogenesis.

S118

THE ROLE OF 2-OXOGLUTARATE DEPENDENT DIOXYGENASES IN NORMAL HAEMATOPOIESIS AND ACUTE MYELOID LEUKEMIA

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Background: The finely tuned regulation of haematopoietic stem and progenitor cells (HSPCs) is crucial to ensure a normal haematopoiesis. Its dysregulation can generate leukaemic stem cells (LSCs) which are difficult to eliminate with current therapies, placing a focus on identifying novel therapeutic targets to eradicate LSCs. Emerging studies have described that key oncometabolites (such as succinate and fumarate) are main inhibitors of 2-oxoglutarate dependent dioxygenases (2-OGDO), a family of enzymes that have been reported to play an important role in leukaemogenesis. Here two of these 2-OGDO, Jmjd6 (a jumonji protein associated with alternative splicing) and Phd2 (a prolyl hydroxylase that controls hypoxic response), were investigated in normal haematopoiesis and acute myeloid leukaemia (AML).
Aims: The aim of this study is to investigate the role of two 2-OGDO (*Phd2* and *Jmjd6*) in the development and/or maintenance of AML LSCs.
Methods: To elucidate the role of *Jmjd6* in normal haematopoiesis, we generated a *Jmjd6* conditional knock-out within the haematopoietic system (*Jmjd6$^{fl/fl}$;Vav-iCre*) and fully characterized it. To study the role of *Jmjd6 and Phd2* in leukaemogenesis, purified HSPCs from *Jmjd6$^{fl/fl}$;Vav-iCre* and *Phd2$^{fl/fl}$;Vav-iCre* mice were transduced with retrovirus expressing respectively *Mll-AF9* and *Meis1/Hoxa9*. These transformed cells were characterized *in vitro* (*e.g.* colony forming cell assay) and their leukemic potential was assessed by transplantation. The *Mll-AF9* knock-in (*Mll-AF9$^{KI/+}$*) transgenic model was also used to determine levels of mRNA expression.
Results: In normal haematopoiesis studies, mice lacking Jmjd6 specifically within the haematopoietic system presented hyposplenism and reduced spleen cellularity. Although these mice have normal numbers of stem and progenitor cells (namely Lin$^-$Sca-1$^+$c-Kit$^+$ (LSK)), the distribution within this compartment is perturbed, leading to an accumulation of more committed progenitor cells. This phenotype functionally correlates with the reduced engraftment exhibited by mice transplanted with cells lacking *Jmjd6* upon transplantation with either *Jmjd6$^{fl/fl}$;Vav-iCre* HSCs or total bone marrow (BM) when compared to controls. Preliminary data from the leukaemic studies showed that the absence of Jmjd6 does not impact on the generation of pre-leukaemic stem cells (pre-LSC). Conversely, LSCs from the transgenic *Mll-AF9$^{KI/+}$* mouse model have decreased *Jmjd6* mRNA transcript levels. *in vitro, Meis1/HoxA9* transformed *Phd2*-deficient cells showed increased proliferation and lower apoptosis however, *in vivo*, mice transplanted with cells lacking *Phd2* developed disease later than the respective controls.
Summary and Conclusions: Deletion of *Jmjd6* in the haematopoietic system leads to an accumulation of committed progenitor cells that have a decreased self renewal capacity and, therefore, a compromised repopulating capacity. The deletion of Jmjd6 did not affect the *in vitro* generation of pre-LSC cells. With regard to *Phd2*, it was observed a delay in AML initiation in mice transplanted with *Phd2* KO pre-LSCs. Taken together, these data unravel a new role for *Jmjd6* as a player in normal haematopoiesis and suggest that *Phd2* may act as tumor suppressor in AML.

S119

CONVERGENCE OF SOMATIC MUTATIONS WITHIN THE JAK-STAT SIGNALLING PATHWAY IN A NOVEL RUNX1-MUTATED PEDIGREE

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Background: Germline mutations in the transcription factor *RUNX1* confer an autosomal dominant predisposition to familial platelet disorder (FPD) and myelodysplasia/acute myeloid leukaemia (MDS/AML). The penetrance of mutations varies with approximately 40% of carriers developing overt malignancy, whilst others remain asymptomatic or manifest mild to moderate FPD. We describe a novel *RUNX1*-mutated family, where 3 young siblings presented with secondary AML, providing a rare opportunity to compare the molecular events initiating disease (*Figure 1A*).
Aims: To genetically profile somatic aberrations in multiple cases of MDS/AML from a novel FPD/AML pedigree
Methods: We performed whole exome sequencing (WES) on bone marrow (BM) DNA from 4 siblings with MDS/AML with an average exonic coverage of 96x. Peripheral blood (PB) DNA samples from both healthy parents were also sequenced to enable exclusion of inherited variants in the 4 children. Acquired mutations, copy number aberrations (CNA) and loss of heterozygosity (LOH) were then defined across the four siblings. Key mutations were confirmed with Sanger sequencing, whilst further verification of CNA was performed using multiplex ligation-dependent probe amplification (MLPA).
Results: Direct Sanger sequencing of the 4 siblings (II.1-II.4) and their mother (I.1) revealed the germline *RUNX1* mutation, p.R201X. *Figure 1B* summarises the clinical timeline, with the key molecular and cytogenetic lesions detected in MDS/AML from each sibling. Their mother (45y) remains an asymptomatic carrier, with no peripheral cytopenias. The dizygotic twins (II.1 and II.2) presented within a period of 2 weeks at 5y, both with hepatosplenomegaly and pancytopenia. BM morphology of each twin revealed AML with dysplastic and myelomonocytic features. Significant somatic chromosomal aberrations included gain of 21q (II.1), monosomy 7 and deletion of chromosome 9q (II.2). Ten years later, sibling II.4 also presented at 5y with monocytosis. BM examination revealed myelomonocytic AML with dysplastic features and a normal cytogenetic profile. Sibling II.3 is now 14y, her BM examination revealed multi-lineage dysplasia and normal cytogenetics. WES revealed molecular addiction to JAK2 signalling in 3 siblings (II.1, II.2 and II.4). II.2 and II.4 both acquired *JAK2* V617F mutations, with homozygosity of the mutant allele observed in II.2 due to 9p acquired uniparental disomy (aUPD). In II.1, we detected a somatic mutation in *SH2B3 (p.R392Q),* with apparent homozygosity caused by aUPD of 12q. The p.R392Q mutation was localised to the SH2 domain, which normally binds both mutant and WT isoforms of JAK2, inhibiting their phosphorylation. Further somatic mutations occurred in *CDC27* (anaphase promoting complex, II.2 and II.4), RBBP8 (DNA double-strand break repair, II.2) and *U2AF2* (spliceosome complex, II.4). Notably, all 3 siblings with somatic JAK2-signalling lesions had aggressive disease. Both twins died within a year of presentation, II.1 due to relapse and II.2 from chemotherapy-refractory disease. Sibling II.4 relapsed after 13 months and is currently in CR2 following allogeneic HSCT.

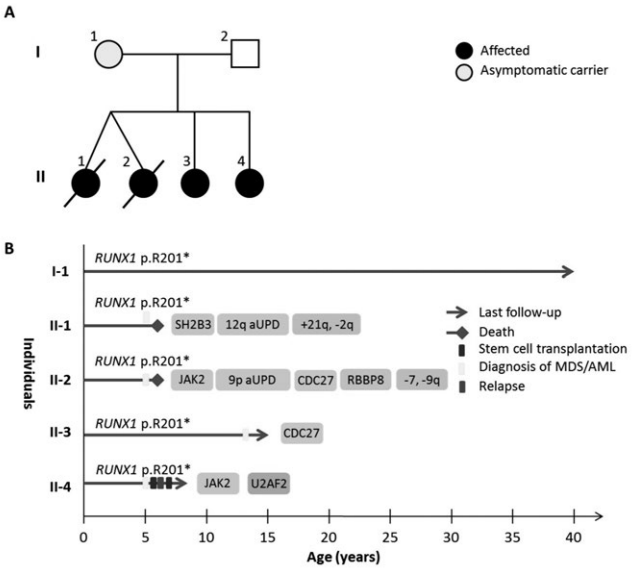

Figure 1.

Summary and Conclusions: We describe a novel FPD-AML pedigree demonstrating convergence of lesions within the JAK-STAT signalling pathway in 3 siblings with MDS/AML. Since *JAK2* mutations are reported in <5% of sporadic *RUNX1*-mutated AML, our findings suggest somatic mutations in FPD/AML may be enriched within distinct signalling pathways, often associated with aUPD to increase the mutant allele burden within tumours.

S120

HIF-2A IS A TUMOUR SUPPRESSOR IN AML

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Background: Normal and malignant haematopoiesis occur under hypoxic conditions. Hif-1α and Hif-2α regulate gene expression to facilitate adaptation to low oxygen tension. Several studies investigated the role of Hif-1α and Hif-2α in acute myeloid leukaemia (AML). These studies used shRNA knockdown in human AML samples to show that *HIF-1α* and *HIF-2α* knockdown compromised the ability of AML samples to reconstitute AML upon transplantation into recipient mice, suggesting that HIF-1 and HIF-2 are potential therapeutic targets for AML. However, a recent study demonstrated that conditional genetic deletion of *Hif-1α* did not compromise the development and maintenance of mouse leukaemic stem cells (LSCs) and on the contrary, accelerated the development of AML, indicating that *Hif-1α* is a tumour suppressor in AML. To date, the effect of conditional deletion of *Hif-2α* has not been examined in AML.
Aims: To investigate the requirement of *Hif-2α* in the development and maintenance of AML leukaemic stem cells.
Methods: We generated *Hif-2α*$^{fl/fl}$;*Vav-iCre* mice that lack *Hif-2α* specifically within the hematopoietic system. We retrovirally co-transduced BM c-Kit$^+$ cells with oncogenes *Meis1a* and *Hoxa9* and serially re-plated them to establish pre-leukaemic stem cells (pre-LSCs). Retrovirally transduced cells were subjected to *in vitro* assays in normoxia and hypoxia or transplanted into lethally irradiated primary recipients, and subsequently into secondary recipients. We next generated *Mll-AF9*$^{KI/+}$*; Hif-2α*$^{fl/fl}$;*Vav-iCre* mice and controls and transplanted Lin$^-$Sca-1$^+$c-Kit$^+$(LSK) cells from these mice into primary recipients, and Lin$^-$Sca-1$^-$c-Kit$^+$(LK) cells into secondary recipients.
Results: *Hif-2α*-deficient and control cells displayed similar re-plating capacity and generated comparable numbers of colonies, but had increased proliferative capacity. Thus, *Hif-2α* is not required for *in vitro* transformation and generation of pre-LSCs but suppresses their proliferation. Transplantation of pre-LSCs to primary recipients demonstrated that a smaller proportion of recipients of *Hif-2α*-deficient pre-LSCs remained leukaemia-free, and therefore succumbed to AML faster than recipients of control pre-LSCs. However, secondary recipients of both *Hif-2α*-deficient and control LSCs generated aggressive AML with similar latency. In concordance with these data, recipients of *Mll-AF9*$^{KI/+}$*; Hif-2α*$^{fl/fl}$;*Vav-iCre* succumbed to AML faster compared to recipients of control LSK cells, whereas, secondary recipients of both genotypes generated AML with similar latency.
Summary and Conclusions: Deletion of *Hif-2α* in pre-LSCs accelerates development of LSCs and shortens AML latency induced by *Mll-AF9* and its downstream effectors *Meis1* and *Hoxa9*. Surprisingly, established LSCs lacking *Hif-2α* efficiently propagate aggressive AML. We conclude that while *Hif-2α* suppresses the development of AML, it is not required for LSC maintenance. Therefore, HIF-2 is unlikely to be a broad therapeutic target in AML and the benefit of HIF inhibition should be carefully re-examined in all subsets of AML.

CLL - Biology: Interacting determinants of CLL ontogeny and evolution

S121

DISSECTING RESISTANCE MECHANISMS IN CHRONIC LYMPHOCYTIC LEUKEMIA USING WHOLE-EXOME SEQUENCING: IMPACT OF RECURRENT RPS15 MUTATIONS ON P53 DYSREGULATION

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Background: Fludarabine, cyclophosphamide and rituximab (FCR) is the gold-standard first-line regimen in medically fit patients with chronic lymphocytic leukemia (CLL); however, despite good response rates most patients will eventually relapse. Besides *TP53* aberrations, the mechanisms leading to relapse after FCR treatment are currently poorly understood.
Aims: To characterize the genetic mechanisms underlying relapse following treatment with FCR using whole-exome sequencing (WES).
Methods: Forty-one CLL patients receiving FCR with either a partial response (PR, with ≥4 cycles of treatment completed) or a complete response (CR, ≥1 cycle of treatment completed) were selected. Pre-treatment and relapse samples (mean time to relapse 3.2 years, range 0.7-10.9), together with matched germline DNA for 28 patients, were analyzed by WES. Well-established bioinformatics tools and pipelines were used to process raw sequencing reads, enabling the identification of somatic mutations and also facilitating the analysis of copy-number aberrations (CNA) and absolute cancer cell fractions (CCF).
Results: Amongst the 28 patients with matched germline DNA, 1191 somatic variants (>10% allele frequency) were found in the pre-treatment samples and 1334 in the relapse samples, with an average of 15.2 (range, 3-24) and 17.6 (range, 2-32) non-silent mutations per case, respectively. Mutations were predominantly missense substitutions (81%) and less frequently frameshift or in-frame insertions/deletions (14%) or nonsense mutations (5%). As expected, at relapse, a high proportion of cases harbored mutations in genes previously linked to adverse prognosis in CLL: *TP53* (n=8; 19.5%), *NOTCH1* (n=8; 19.5%), *ATM* (n=7; 17%), *SF3B1* (n=6; 14.6%), *NFKBIE* (n=4; 9.8%), *EGR2* (n=4; 9.8%) and *BIRC3* (n=3; 7.3%). Intriguingly, a large proportion of cases also harbored mutations in *RPS15* (n=8; 19.5%), a gene encoding a component of the 40S ribosomal subunit. High allele frequencies were observed for *RPS15* mutations at both time points (range, 29%-56%), and all mutations were missense variants residing within a 7 amino-acid evolutionarily conserved region. Besides its role in protein translation, RPS15 has been shown to stabilize p53 by interfering with the MDM2-p53-MDMX network and inhibiting MDM2-mediated p53 degradation. Characterization of two recurrent *RPS15* mutations in the HCT116 colorectal cancer cell line transiently expressing either wild-type (wt) or mutant RPS15 revealed impaired ability of RPS15^{P131S} and RPS15^{G132A} in regulating endogenous p53. As both mutations map within the region that interacts with MDM2, this finding strongly suggests that binding of RPS15^{P131S} and RPS15^{G132A} to MDM2 is less efficient compared to wt protein thus leading to more pronounced p53 degradation. Finally, by calculating the absolute CCF for all mutations at both time points allowed monitoring of clonal heterogeneity over time. All 24 cases with available exome-derived CNA data showed mutations expanding ≥0.3 in CCF between the time points (mean 7.4 mutations, range 1-21). Among recurrently mutated genes, i) *RPS15* remained stable over time, ii) *TP53*, *EGR2*, *NOTCH1* and *BIRC3* mutations expanded or remained stable, and iii) for *SF3B1* and *ATM* mutations both increasing and decreasing CCFs were observed.

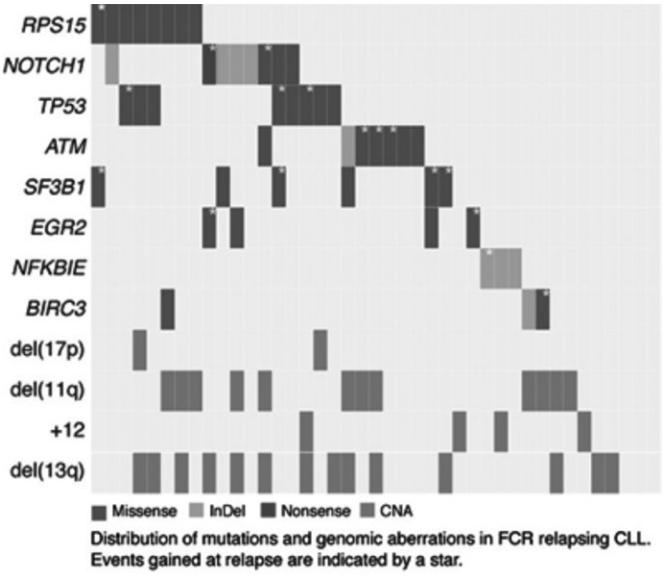

Figure 1.

Summary and Conclusions: We provide novel insights into the heterogeneous genetic landscape of CLL relapsing after FCR treatment with our most prominent finding being recurrent *RPS15* mutations (19.5%) and with *in vitro* studies of *RPS15* mutations pointing to a novel mechanism for p53 dysregulation in CLL.

S122

IN VITRO AND *IN VIVO* ANTILEUKEMIC ACTIVITY OF BEPRIDIL IN CHRONIC LYMPHOCYTIC LEUKEMIA IS ASSOCIATED WITH INHIBITION OF THE NOTCH1 PATHWAY

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Background: In chronic lymphocytic leukemia (CLL), NOTCH1 is constitutively expressed promoting leukemia cell survival and resistance to apoptosis (Rosati et al, Blood 2009). Additionally, *NOTCH1* PEST mutations have emerged as one of the most frequent somatic alterations in CLL, affecting up to 20% of patients (Sportoletti et al, Leukemia 2014). Thus, inhibiting NOTCH1 activity represents a potential therapeutic opportunity for this disease and the incorporation of NOTCH1 pathway antagonists may improve standard CLL treatment. Targeting NOTCH1 has been a therapeutic strategy of interest in many cancers. However, the use of gamma secretase inhibitors (GSI) evaluated in clinical trials showed on target toxicities suggesting the need for the discovery of more selective NOTCH1 pathway antagonists that preferentially target NOTCH1 *versus* NOTCH2 or that target mutated receptors compared to wild-type. Recently, an expression-based screen identified several calcium modulators as a potential strategy to target NOTCH1 (Roti et al, Cancer Cell 2013). Among numerous ion flux modulators validated to induce a NOTCH1 off signature, one of the top hits was the clinically relevant calcium channel blocker, bepridil, used to treat patients with cardiac disease. Bepridil demonstrated anti NOTCH1 modulating activity in T-ALL by a mechanism unique from GSI (Roti et al, ASH 2009).
Aims: The goal of the study was to evaluate whether bepridil exerts antitumor activity in primary CLL cells *in vitro* and in a xenotransplant model and whether these effects are associated with NOTCH1 inhibition.
Methods: *in vitro*, we evaluated NOTCH1 expression in primary CLL cells after 24 hours of bepridil treatment using western blot and flow cytometric analysis. We also measured apoptosis using annexin V/propidium iodide staining and by assessing PARP, MCL-1 and NOXA expression. *in vivo*, CLL cells were transplanted into NSG mice and engraftment was evaluated after 28 days of bepridil treatment.
Results: Bepridil treatment reduced the viability of primary CLL cells at a 2.5 μM *concentration.* In treated CLL cells, viability significantly decreased to 23.8±20.2% compared to 41.5±21.8% of the vehicle control (N=40, p <0.0001). Conversely, bepridil did not affect viability of normal T cells from CLL patients nor the viability of B and T cells from healthy donors, demonstrating that bepridil selectively impairs the viability of B neoplastic cells compared to normal hematopoietic cells. Bepridil significantly increased the percentage of annexin V/propidium iodide positive apoptotic CLL cells compared to vehicle treated cells (49.2±20.9% *vs* 29.3±15.3% N=34 p<0.0001). These apoptotic effects were supported by the detection of increased PARP degradation (p<0.05), significant reduction of MCL-1 protein expression (p<0.001) and a 5-fold up-regulation of *NOXA* transcript levels. Induction of apoptosis with bepridil treatment in cultured CLL cells was not correlated with ZAP-70 expression, *IGVH* rearrangement or *NOTCH1* mutation status. However, the NOTCH1 pathway was inhibited at concentrations of bepridil that induced apoptosis. Specifically, flow cytometric analysis of cultured CLL cells (N=15) demonstrated that bepridil significantly reduced the surface expression of NOTCH1 compared to vehicle (32.8±19.2% *vs* 50.6±18.9% respectively, p<0.05). As previously reported in T-ALL bepridil treatment lead to down regulation of the trans-membrane bound portion of NOTCH1. Notably, NOTCH2 protein level remained unchanged in bepridil treated CLL samples compared to vehicle (N=3), supporting a preferential effect of bepridil on NOTCH1. Interestingly, we observed that CLL cells co-cultured with different stromal layers (including primary mesenchymal cells, HS5 and OP9 cell lines) fail to apoptose upon bepridil treatment. The addition of a CXCR4 antagonist restored bepridil efficacy, suggesting a synergistic effects against the survival stimuli of the stroma. Finally, we established CLL NSG primagrafts and tested bepridil in this leukemia model. Strikingly, flow cytometry analysis revealed a statistically significant decrease in human CD45+CD19+CD5+ percentage in the spleen of bepridil-treated mice compared to vehicle (1.9±1% *vs* 10.8±10%, N=13, p<0.05). As anticipated by our results *in vitro* bepridil treatment did not have a major effect in the neoplastic bone marrow cell population of treated animals.
Summary and Conclusions: In conclusion, we showed that bepridil reduced viability and increased apoptosis in primary CLL cells *in vitro*. This antileukemic effect is associated with the inhibition of the NOTCH1 signal. Moreover, this clinically relevant drug demonstrated efficacy in controlling splenic disease in a mouse model of human CLL, suggesting a potential for rapid translation to clinical testing.

S123

HIGH-THROUGHPUT T-CELL RECEPTOR GENE REPERTOIRE PROFILING IN CHRONIC LYMPHOCYTIC LEUKEMIA SUBSET #4: FURTHER EVIDENCE OF ANTIGENIC STIMULATION

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Background: Chronic lymphocytic leukemia (CLL) is characterized by a remarkably restricted immunoglobulin (IG) gene repertoire, mainly attributable to the existence of subsets of patients with stereotyped B-cell receptor (BcR) IGs strongly implying clonal selection by a restricted set of antigens. Our preliminary high-throughput, next-generation sequencing studies of the T-cell receptor beta chain (TRB) gene repertoire in CLL cases from various subsets indicated repertoire skewing, pointing to antigenic selection of the T cells as well, which is relevant in view of the bidirectional CLL-T cell interactions.
Aims: We performed in-depth profiling of the TRB gene repertoire in CLL subset #4 which became our focus for the following reasons: (i) it is the most indolent subgroup of CLL patients identified thus far; (ii) previous studies have strongly supported ongoing antigenic stimulation attested by the intraclonal diversification of the clonotypic IG.
Methods: We studied 11 untreated CLL subset #4 cases and a healthy control. RNA was isolated from peripheral blood mononuclear cells (n=12) or purified $CD4^+$ and $CD8^+$ T cells (n=2 CLL cases). Two patients were studied overtime. TRBV-TRBD-TRBJ gene rearrangements were amplified on cDNA according to the BIOMED2 protocol and subjected to paired-end NGS (MiSeq Illumina Platform). The experimental design allowed sequencing of the complementarity determining region 3 (CDR3) twice/read, so as to increase the accuracy of results. Computational processing of raw data was performed using a purpose-built algorithm and a bioinformatics platform was developed for IMGT/HighV-QUEST metadata clustering and analysis.
Results: Overall, 12,261,280 TRBV-TRBD-TRBJ reads were produced (median 397,035 reads/sample, median Q-score 38.4). Poor quality, incomplete, out-of-frame and unproductive rearrangements were filtered out. For repertoire analyses, clonotypes (*i.e.* TRB rearrangements with identical TRBV gene usage and amino acid CDR3 sequence) rather than single rearrangement reads were considered (median 77020 distinct clonotypes/sample, 54733 singletons *versus* 22287 expanded). Among the 53 functional TRBV genes identified, 5 predominated: *TRBV12-3/12-4* (9.9%), *TRBV29-1* (8.6%), *TRBV19* (7.5%), *TRBV5-1* (5.5%), and *TRBV6-5* (4.9%), collectively accounting for 36.4% of the TRBV repertoire. Comparison of the TRBV gene repertoire of $CD8^+$*vs* $CD4^+$ cells showed that *TRBV19* was overrepresented in the CD4+ compartment (9.4% *versus* 6.9%, p<0.001). The TRB repertoire was significantly more oligoclonal in CLL compared to the healthy control (median frequency of the predominant clonotype: 3.6% *versus* 0.47%, respectively, p<0.001), and this skewing stemmed mainly from the $CD8^+$ rather than the $CD4^+$ compartment (median

frequency of the predominant clonotype 10.7% *versus* 1.0%, respectively, p<0.001). Cluster analysis of all CLL cases identified 37303 different clonotypes (excluding singletons) shared by different patients and not present in the healthy control. The longitudinal analysis of 2 cases showed contrasting results, with 14.6% of all expanded clonotypes persisting over time in one case, but only 0.06% in the other. In the former case, 5 of the persisting clonotypes ranked among the 10 most expanded within the patient's T cell repertoire, whereas in the latter case only minor clonotypes persisted.

Summary and Conclusions: Our study provides large-scale evidence of TR repertoire skewing and oligoclonality in CLL subset #4, strongly supporting antigenic selection. The nature of selecting antigens remains to be elucidated.

S124

CLL SUSCEPTIBILITY SNPS IN MBL SIBLINGS OF CLL PATIENTS

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Background: Genome-wide association studies have identified 37 SNPs in 26 loci that confer risk of CLL. Here, we analyse genomic DNA from a prospective cohort of CLL patients that have an MBL sibling for the presence of these CLL risk alleles.
Aims: To investigate whether CLL risk loci are also prevalent in MBL cells found in a sibling.
Methods: Whole blood of siblings of patients with confirmed CLL patients was screened with informed consent and ethical approval for presence of a distinct $CD19^{+}CD5^{+}CD20^{low}CD79b^{low}$ population by flow cytometry. If such an MBL population was present, genomic DNA from granulocytes of both CLL patient and MBL sibling was isolated from the same blood sample. All cases with sufficient available DNA were subjected to whole exome sequencing (WES) based on SureSelect Human All Exon V4 kit (Agilent) capture on the HiSeq2000 (Illumina) platform to an average coverage of 40x. Sequence reads were filtered and mapped to the human reference genome (GRCh37). Analysis of gene copy numbers and heterozygosity was performed by either 10K, 250K, SNP6, or CytoscanHD arrays (Affymetrix). In addition, PCR was performed on 35 reported CLL susceptibility alleles and analyzed by direct Sanger sequencing of gel-purified amplicons (PCR Clean-Up System, Promega). The frequency of all risk alleles was compared to healthy individuals from the literature by Fisher's exact test.
Results: Screening of 160 siblings of CLL patients identified 19 MBL siblings (MBL prevalence: 11.9%) from 16 CLL patients. In all but 2 MBL, the clonal B-cell count was <100/ul. Genomic data from 13 CLL and 14 MBL cases, including 12 complete CLL/MBL pairs are described in this report. Nine of total of 37 CLL susceptibility SNPs could be called on WES data obtained from 5 CLL and 4 MBL cases. 18 CLL risk SNPs could be analyzed on SNP arrays as indicated by a median call rate of at least 98%. The combined SNP array and WES data permitted analysis of 23 SNP of 15 loci. By targeted re-sequencing on 10 CLL and 6 MBL cases, the total number of analyzed risk SNPs was increased to 35 of the 37 known CLL risk alleles. 16 risk alleles were statistically overrepresented in combined CLL and MBL compared to the healthy population (Table). Out of these 16 SNPs, 11 were significantly overrepresented in CLL and nine in MBL, respectively. SNP rs11083846 was significantly overrepresented in MBL only but not in CLL or in the combined CLL and MBL cases.

Tabella 1.

Table				CLL		MBL		CLL-MBL combined	
Chromosome	Nearest gene(s)	SNP	Position	RAF	P-value (Fisher's)	RAF	P-value (Fisher's)	RAF	P-value (Fisher's)
4q26	CAMK2D	rs6858698	4:114683844	0.83	<0.0001	1.00	<0.0001	0.91	<0.0001
6q25.2	IPCEF1	rs2236256	6:154478440	0.89	0.0013	0.93	0.0018	0.91	<0.0001
11p15.5	C11orf21, TSPAN32	rs7944004	11:2311152	0.89	0.0005	0.57	0.7325	0.75	0.0057
2q33.1	CASP10/CASP8	rs3769825	2:202111380	0.67	0.1074	0.86	0.0024	0.75	0.0012
18q21.32	PMAIP1	rs4368253	18:57622287	0.94	0.0194	0.79	0.5706	0.50	0.0034
15q15.1	BMF	rs8024033	15:40403657	0.89	0.0014	0.57	0.8474	0.75	0.0112
2p22.2	QPCT, PRKD3	rs3770745	2:37596089	0.50	0.0164	0.33	0.3118	0.43	0.0149
2q13	ACOXL, BCL2L11	rs13401811	2:111616104	1.00	0.0547	1.00	0.0871	1.00	0.0037
2q13	ACOXL, BCL2L11	rs17483466	2:111797458	0.63	<0.0001	0.43	0.0726	0.53	<0.0001
6p21.3	HLA-DQA1	rs9272219	6:32602269	0.75	0.0035	0.86	0.0004	0.80	<0.0001
16q24.1	IRF8	rs391525	16:85944439	0.64	0.0022	0.64	0.0022	0.70	<0.0001
16q24.1	IRF8	rs2292982	16:85944823	0.56	0.0069	0.43	0.2199	0.50	0.0025
16q24.1	IRF8	rs2292980	16:85945076	1.00	<0.0001	1.00	<0.0001	0.50	<0.0001
6p25.3	IRF4	rs872071	6:411064	0.80	0.1205	0.86	0.0277	0.83	0.0036
6p25.3	IRF4	rs9378805	6:417727	0.57	0.8492	0.86	0.0131	0.71	0.0498
11q24.1	GRAMD1B	rs735665	11:123361397	0.40	0.2335	0.60	0.0084	0.50	0.0039
19q13.32	PRKD2, STRN4	rs11083846	19:47207654	0.06	0.2207	0.57	0.0046	0.28	0.4075

Summary and Conclusions: Our data provide an independent validation of 16 of 35 previously reported CLL risk loci using combined SNP array, WES, and Sanger sequencing data on a unique prospectively assembled cohort of co-occurrence of CLL and MBL in siblings. No discrepancies were observed between the various analysis platforms. Besides providing genetic correlates for a familial susceptibility in CLL, the shared presence of these risk loci in CLL and their MBL siblings indicate a causal role for clonal premalignant B-cell expansion. These risk alleles therefore appear to contribute to the initiation of a CLL-like phenotype, but further endogenous or exogenous triggers are required to drive MBL to CLL.

S125

GENETIC PROFILE OF CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS WITH ULTRA-STABLE DISEASE

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Background: Chronic lymphocytic leukemia (CLL) shows an extremely heterogeneous clinical course. Beside cases with aggressive disease requiring immediate treatment and those with an initial indolent phase followed by progression, there are patients who do not progress for decades. Next generation sequencing (NGS) technologies have allowed a further understanding of the molecular complexity of CLL. In the present study, we characterized 41 ultra-stable CLL cases by whole exome sequencing (WES), ultra-deep NGS and copy number aberration (CNA) analysis. Ultra-stable disease was defined as absence of progression for at least 10 years from diagnosis.
Aims: To investigate the so far unexplored mutational profile and CNA load of ultra-stable CLL patients.
Methods: Peripheral blood samples from 20 ultra-stable CLL patients were used for WES analysis (Illumina HiSeq2000), including paired germline DNA in 14. Sanger sequencing (ABI PRISM 3100) was used to validate WES mutations and to screen the recurrency of mutated genes identified in ≥2 cases by WES, in a second cohort of 21 ultra-stable CLL samples. Bad prognosticator genes (*NOTCH1-BIRC3-SF3B1-TP53)* were also investigated. Subclonal *TP53* mutations were examined by ultra-deep NGS (Roche-454 GS Junior) in 36 cases. CNA analysis (Affymetrix Cytoscan HD arrays) was performed in 30 cases.
Results: WES analysis of the 14 cases having paired germline DNA predicted 83 non-silent somatic mutations in 81 genes, with a mutation load of 6 mutations/case (range: 1-12). The remaining 6 cases without germline DNA were analyzed to assess the recurrence of mutations identified in the former cohort and to investigate those with known significance. Three genes were recurrently mutated: *RBM46, KLHL6, UBR5.* Since *RBM46* was the most recurrent among ultra-stable CLL (3 cases, 15%) and never reported in other CLL WES studies, Sanger sequencing of the whole codifying region was performed on the screening cohort with no additional mutated case identified. Interestingly, none of the genes with known adverse prognostic impact was found mutated in 41 ultra-stable CLL, including *ATM* and *MYD88* in the WES cohort. Unexpectedly, ultra-deep-NGS of *TP53* revealed subclonal mutations in 2/36 cases (5.5%). One case after 19 years from diagnosis showed 4 mutations with a median allele frequency (AF) of 1.55% (range 0.9-1.79), corrected for tumor representation, and developed a clinical progression soon after the inclusion in the study; after therapy, a clonal *TP53* mutation expansion occurred. The second case showed 1 subclonal mutation (AF 5.2%) after 29 years from diagnosis; for the subsequent 4 years she remained in clinical spontaneous regression of CLL but developed a breast cancer. AS-PCR validation of these subclonal mutations is ongoing. CNA analysis identified 31 lesions represented by 90% of losses and 10% of gains, giving a CNA load of 1/case. Twelve cases (40%) showed no lesion, 9 (30%) showed isolated del(13q), 5 cases (17%) del(13q) with additional non-canonical CNAs and 4 cases (13%) a median of 1 non-canonical CNAs. There was no recurrent CNA beside 13q deletion, as well as no CNA with known poor prognostic significance.

Tabella 1. Biological and clinical features of 41 ultra-stable CLL patients

Gender	25 M/16 F
Age (years)	51 (range: 29-73)
Binet stage	40 A; 1 B
CD38 positive (cut-off 30%)	0/0
IGHV mutated	41/41
Follow-up from diagnosis (years)	16 (range: 10-34)

Summary and Conclusions: Ultra-stable CLL show no driver mutations or CNAs. No new recurrent lesion associated to a highly stable course was identified. We found two cases with *TP53* subclonal mutations with an extremely divergent clinical history, which could be due to the subclonal architecture complexity and to the chemotherapy selective pressure in the case with clonal evolution.

Stem cell transplantation: Clinical 1

S126

OUTCOMES OF OLDER PATIENTS EXPERIENCING REDUCED INTENSITY CONDITIONING ALLOGRAFT: RESULTS OF THE NCRI AML16 TRIAL

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Background: Reduced Intensity conditioning (RIC) is increasingly offered to older patients who are to undergo a stem cell transplant. We have previously shown that a RIC allograft, particularly from a sibling donor is beneficial in intermediate risk patients between the ages of 45-64, and may have advantages over myeloablative conditioning in patients aged 35-44. Based on this analysis of the UK NCRI AML15 trial in younger patients, the current recommendation within the NCRI AML trials is to transplant intermediate risk patients with a RIC allograft if a sibling is available excluding those who are NPM1+/ FLT3 wt.
Aims: We here present similar analyses from the UK NCRI AML16 trial extending this experience to older patients.
Methods: The UK NCRI AML16 trial ran from 2006-2012 and randomised patients, generally aged 60+, suitable for intensive chemotherapy between Daunorubicin/Ara-C (DA) and Daunorubicin/Clofarabine (DClo), both with or without Mylotarg, or between DA with or without etoposide and with or without ATRA. Patients could be randomised between 2 or 3 courses of therapy and maintenance or not with azacitidine. Because only 4/225 transplants took place in patients over the age of 70, attention was restricted to patients who were aged less than 70 years, achieved remission, and did not have core binding factor leukaemia. A total of 963 patients were studied, with transplant in first remission given to 162 patients (sibling allograft n=51, MUD n=93; other/unknown n=18). Follow-up is complete to 1st January 2014 with median follow-up for survival from CR of 46.3 months. Comparisons of confirmed allogeneic transplant in 1st remission *versus* not are carried out using Mantel-Byar analysis to allow for time to transplant, with patients censored at the time of non-RIC allo transplant.
Results: Among the 144 allografts, 93 had intermediate risk cytogenetics, 19 adverse risk, and 32 were unknown. In transplanted patients, survival from transplant was 36% at 5 years, and while the survival for sibling allografts (42%) was better than that for MUDs (37%) this did not reach statistical significance (p=0.2), and 84% of transplants in adverse risk cytogenetics were from an unrelated donor. In analyses adjusted for Wheatley risk group there was no significant difference in outcome (HR 1.27 (0.80-2.03) p=0.3). When comparing allograft *versus* no allograft, survival was significantly improved (35% *vs* 20%, HR 0.75 (0.61-0.93) p=0.006). When stratified by Wheatley risk group, there was no evidence of any interaction (p-value for trend 0.5), and the adjusted hazard ratio, allowing for differences in Wheatley group between transplant and no transplant was 0.76 (0.61-0.94), p=0.008 reflecting the consistent benefit. When considering type of transplant, sibling allograft performed consistently better than MUD in Mantel-Byar analyses across the risk groups (overall sibling 41%, MUD 35%, no SCT 20%; good risk 47% *vs* 42% *vs* 26%; standard risk 38% *vs* 34% *vs* 25%; poor risk, not reached *vs* 23% *vs* 8%).
Summary and Conclusions: The results are consistent with those seen in our analysis of AML15 While, particularly as patients get older, there will be selection for transplant on the basis of fitness, the analysis adjusted for clinical features of the disease shows that RIC transplant in first remission appears an attractive option for older patients with AML. While no significant difference was seen between sibling and MUD allograft, even after adjusting for Wheatley risk, outcomes are better in all Wheatley groups with sibling allografts.

S127

REDUCED INTENSITY CONDITIONING (RIC) ALLO TRANSPLANTATION IS ASSOCIATED WITH SUPERIOR LONG-TERM DISEASE CONTROL IN RELAPSED/REFRACTORY GRADE I/II (G-I/II) FOLLICULAR LYMPHOMA

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Background: We compared long-term outcomes of allogeneic transplantation (alloHCT) *vs* autologous (auto) HCT in pts with G-I/II follicular lymphoma (FL) in the rituximab-era.
Aims: To compared survival outcomes following autoHCT *vs* alloHCT in G-I/II FL, when either modality is used as the *first* transplantation approach.
Methods: Adult pts with relapsed/refractory G-I/II FL undergoing 1st RIC alloHCT or 1st autoHCT reported to Center for International Blood and Marrow Transplant Research during 2000-12 were eligible. Pts with large cell transformation and those not receiving rituximab before HCT were excluded.
Results: Characteristics of 518 pts included in this analysis are shown in **Table 1**. AlloHCT pts were younger, more heavily pretreated, had more advanced stage disease, had longer interval between diagnosis and HCT, more extranodal involvement and were chemotherapy resistant compared with auto-HCT pts. The 5-year adjusted probabilities of non-relapse mortality (NRM), relapse/progression, progression-free survival (PFS) and overall survival (OS) of autoHCT *vs* alloHCT groups were 5% *vs* 26% (p<0.0001); 54% *vs* 20% (p<0.0001); 41% *vs* 58% (p<0.001) and 74% *vs* 66% (p=0.05), respectively. Cumulative incidence of second malignancies at 5 years did not differ significantly (alloHCT=8%; autoHCT=5%). On multivariate analysis (MVA) autoHCT was associated with reduced NRM (RR=0.21; p<0.0001) and time varying effects were seen on other outcomes. Within the first 5 and 11months post HCT, auto-and alloHCT had similar relapse/progression and PFS. AutoHCT was associated with a higher risk of relapse/progression beyond 5 months post HCT (RR=4.4; p<0.0001), and worse PFS (RR=2.9; p<0.0001) beyond 11 months post HCT, respectively. In the first 24 months post HCT, autoHCT was associated with improved OS (RR=0.41; p<0.0001), but beyond 24months with inferior OS (RR=2.2; p=0.006). A landmark analysis of pts alive and progression-free at 2-years post HCT confirmed these observations, showing no difference in NRM between the auto-and alloHCT groups, but significantly higher risk of relapse/progression (RR=7.3; p<0.0001) and inferior PFS (RR=3.2; p<0.0001) and OS (RR=2.1; p=0.04) following autoHCT.

Tabella 1.

	AlloHCT N=268	AutoHCT N=205	P
Median age, years	52 (27-74)	54 (22-79)	0.01
KPS ≥90	193 (72)	168 (67)	0.06
Stage III-IV at diagnosis	217 (81)	185 (74)	<0.0001
Extranodal disease at HCT	10 (26)	40 (16)	0.002
Prior rituximab-resistance	118 (44)	161 (64)	<0.001
Chemosensitive at HCT	202 (75)	226 (90)	<0.001
Median lines of therapy	4 (1-5)	3 (1-5)	0.001
Duration of 1st response			0.76
<1 year	79 (29)	68 (27)	
≥1 year	173 (65)	164 (66)	
Time from diagnosis to HCT	43mon (4-352)	34mon (6-315)	0.001
Matched sibling Donor	143 (53)	N/A	-
≥7/8 unrelated donor	125 (46)	N/A	-
Median follow up	61 (3-154)	61 (3-169)	-

S128

UNRELATED DONOR (UD) ALLOGENEIC STEM CELL TRANSPLANTATION (ALLO-SCT) IN PRIMARY REFRACTORY ACUTE MYELOID LEUKEMIA (AML): REPORT OF 381 PATIENTS FROM THE ACUTE LEUKEMIA WORKING PARTY OF EBMT

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Background: Primary refractory AML is associated with a dismal prognosis. Approximately one third of patients younger than 60 years, and 50 % of older patients, with newly diagnosed AML fail to achieve complete remission (CR) with standard induction chemotherapy. Allo-SCT in the setting of active disease is an alternative but highly debatable strategy. The increased availability of UD and the use of reduced-intensity conditioning (RIC) regimens have opened the possibility for transplantation to a larger number of patients in comparison to standard myeloablative regimens (MAC).
Aims: The current study aimed to assess outcomes in a cohort of 381 primary refractory AML patients who received allo-SCT from an UD (10/10 or 9/10). Primary refractoriness was defined as failure to achieve CR within 60 days after starting induction.
Methods: Patients with primary refractory AML reported between 2000 and 2013 to the registry of the Acute Leukemia Working Party of the EBMT were included in this study. The major endpoints were to assess overall survival (OS), leukemia-free survival (LFS), relapse incidence (RI), and non relapse mortality (NRM).
Results: Median age was 50.5 (range, 18-74) years and 56% were males. Median time from diagnosis to allo-SCT was 111 (range, 60-178) days. 51 % received a MAC regimen, and 49% a RIC regimen. Peripheral blood stem cell (PBSC) was the main stem cell source (94.8%). The median follow-up was 18 months(range, 1.2-153). 296 patients received a matched UD (10/10) and 85 a mismatched UD (9/10). Engraftment was achieved in 95.2% of cases. 70.4% patients reached CR after allo-SCT. At 2 years, the cumulative incidences of acute GVHD≥2 and chronic GVHD (cGVHD) were 35.5% and 25.8%, respectively. At 2 years, OS and LFS rates were 34.3% and 28.3%. RI was 46.4% and NRM 25.1%. In multivariate analysis, 2 predictive factors were associated with lower OS: cytogenetics (poor *vs* intermediary; HR=2.00, 95%CI,1.25-3.18, p=0.004) and positive CMV status of the recipient (HR=1.52, 95%CI,1.09-2.11, P=0.01), whereas Karnofsky status at transplant ≥80% (KS) was associated with better OS (HR=0.65, 95%CI,0.43-0.98, p=0.04) (Fig1). The same factors were predictive for LFS: cytogenetics (HR=1.86, 95%CI,1.19-2.92, p=0.01) and positive CMV status of the recipient (HR=1.46, 95%CI, 1.07-1.99, p=0.02) were negative predictive factors, whereas KS was a positive one (HR=0.61, 95%CI,0.41-0.91, p=0.02). In multivariate analysis for RI, cytogenetics was the only risk factor associated with increased relapse (HR=1.92, 95%CI,1.15-3.19, p=0.001). As for NRM, patient gender (female *vs* male) and KS were factors associated with lower NRM (HR=0.49, 95%CI,0.29-0.84, p=0.01; HR=0.41, 95%CI,0.22-0.76, p=0.004), while CMV positive status was the only factor associated with higher NRM (HR=1.96, 95%CI=1.11-3.43, p=0.02).

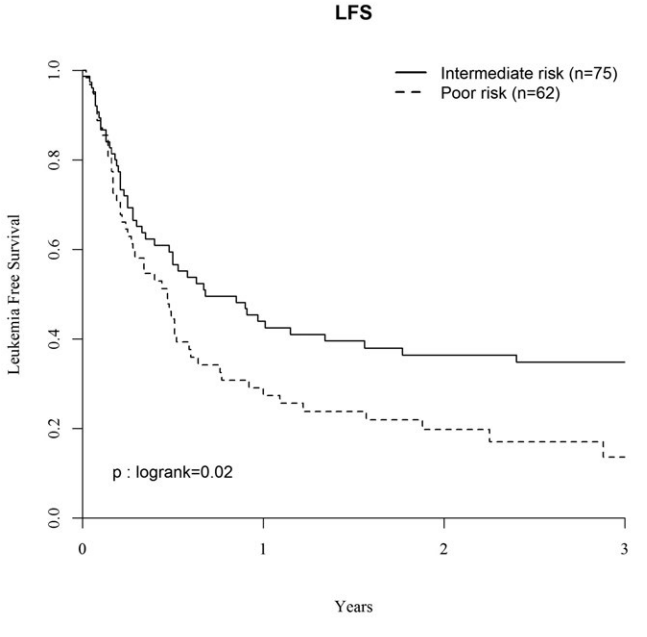

Figure 1.

Summary and Conclusions: Unrelated donor transplantation (10/10 or 9/10) may rescue about one third of the patients with primary refractory AML. Moreover, this study identifies cytogenetics, KS, and CMV status as major prognostic factors. Finally, these data pave the way not only for improving patients' selection, but also for investigating more intensive additional approaches relying on sequential conditioning regimens (debulking phase followed by RIC) and/or post-transplant treatments such as 5-azacytidine, prophylactic donor lymphocytes infusions, or targeted therapy which further improve results in this devastating group of patients.

S129

ALLOGENEIC STEM CELL TRANSPLANTATION IN ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) PATIENTS OLDER THAN 60 YEARS: A SURVEY FROM THE ACUTE LEUKEMIA WORKING PARTY OF EBMT

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Background: ALL is rare in patients older than 60 years, and is associated with poor prognosis with chemotherapy alone, with reported leukemia-free survival (LFS) rates below 20%**.** When feasible, allogeneic stem cell transplantation (allo-SCT) is an attractive treatment option for those patients. However due to comorbidites, non-relapse mortality (NRM) can be a limiting factor.
Aims: This study aimed to evaluate the results and risk factors associated with outcome of allo-SCT in ALL patients older than 60 years, and who received a reduced-intensity conditioning (RIC) regimen prior to allo-SCT between 2001 and 2012.
Methods: 117 patients with fully documented data could be identified and analyzed. Median age at time of allo-SCT was 63 (range, 60-75) years, with 34 patients (29%) being older than 65. 57% of patients had a Karnosky performance status ≥90%. Median year of allo-SCT was 2009. Median follow up was 37 (range, 10-134) m. 82 patients (70%) were transplanted in CR1, 19 (16%) in CR2 and CR3, and 16 (14%) in more advanced disease. Of 109 patients with available cytogenetics data, 53 (49%) harboured t(9;22) at diagnosis. Conditioning regimen included fludarabine and busulfan (Bu-Flu) for 34% of patients, 26% had a low-dose TBI-based regimen, while 20% received fludarabine and melphalan. ATG was used in 41% of cases. Fifty-two (45%) of patients were transplanted with an HLA-identical-sibling donor (MSD). Graft source was peripheral blood stem cells in 95% of cases.
Results: At 2 years the probabilities of LFS, and overall survival (OS) were 35% and 47%, respectively. The cumulative incidences (CI) of NRM, relapse incidence (RI), and chronic GVHD were 22%, 43%, and 38%, respectively. In univariate analysis, disease status was associated with LFS, RI and OS: LFS rate was 25% for CR2 and 13% for advanced *vs* 41% for CR1 (p<0.01), RI rate was 70% for CR2 and 63% for advanced *vs* 33% for CR1 (p<0.01), OS rate 40% for CR2 and 19% for advanced *vs* 54% for CR1 (p<0.01). Patients transplanted from a MSD had a higher RI (56% *vs* 33%) compared to those transplanted from unrelated donors (UD, p<0.01). Factors associated with NRM were the use of Bu-Flu (NRM was 13% for Bu-Flu *vs* 26% in other regimens) (p=0.05)) and Karnofsky-score ≥90% (NRM was 14% for KPS<90% *vs* 31% KPS ≥90%) (p<0.05)). In multivariate analysis, factors associated with LFS were disease status (advanced disease, HR=3.6, p<0.01) and CR2, HR=1.90, p=0.04), and the use of unrelated donors (HR=0.60, p=0.03). Disease status was associated with better OS (HR=0.28, p<0.001). On the other hand, advanced disease status, use of a MSD, performance status <90% was associated with a higher RI (HR=4.64, p<0.01, HR=2.38, p=0.06, HR=2.25, p<0.05 respectively). NRM was lower for patients with performance status ≥90% and for those receiving the Bu-Flu regimen (HR=0.48, p=0.06 and HR=0.33, p<0.05, respectively).
Summary and Conclusions: Allo-SCT after RIC is a feasible and effective option for patients with ALL older than 60 years with LFS of 35% and OS of 47%. The results were better for patients transplanted in CR1, and with good performance status.

S130

ALLO-HSCT FOLLOWING RIC FOR ELDERLY PATIENTS (60 YEARS AND OLDER) WITH HEMATOLOGICAL MALIGNANCIES USING UNRELATED DONORS: A RETROSPECTIVE STUDY OF THE SFGM-TC

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Background: The use of unrelated donors (URD) in patients aged of 60 years or more has drastically increased in the past few years. To date, there are only limited data on URD allo-HSCT in elderly patients (60 years or more).
Aims: The purpose of the current study is to describe outcomes in a large cohort of patients aged 60 years or older, who received a RIC URD allo-HSCT in recent years.
Methods: Between 2008 and 2012, 516 consecutive patients aged of 60 years or more, who received a first allo-HSCT for hematological malignancies from an URD after a RIC regimen in France were included. Conditioning regimen was fludarabine-based in 91% of the patients. Two groups of patients were defined: patients with age at allo-HSCT less than 65 years old ("URD<65 group", n=374) and patients who were aged of 65 years old or more ("URD ≥65 group", n=142).
Results: Patient characteristics were similar between the 2 groups. The median follow-up was 36 months (range, 0.36-73.5) for URD<65 group and 32 months (range, 0.03-72) for URD≥65 group. During evolution, the cumulative incidence (CI) of grade II–IV acute GvHD was 32% in URD<65 group and 32% in URD≥65 group (p=0.975) while the CI of chronic GvHD at 2 years was 25 % and 26%, respectively (p=0.701). CI of non-relapse mortality (NRM), disease free survival (DFS) and overall survival (OS) were not different between the 2 groups Multivariate analysis for NRM, DFS and OS show that age by itself has no influence on outcomes.
Summary and Conclusions: These data suggest equivalence of outcome between URD<65 group and URD≥65 group after RIC URD allo-HSCT. Age by itself thus appears not to be a limitation in this particular population of elderly patients.

Biology in MDS

S131

CLONAL ARCHITECTURE AND EVOLUTION IN MYELODYSPLASTIC SYNDROMES

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Background: Myelodysplastic syndromes (MDS) are a heterogeneous group of hematopoietic neoplasms characterized by bone marrow dysplasia and one or more peripheral blood cytopenias. Underlying this heterogeneity is a variety of genetic abnormalities. Within the bone marrow and blood of an individual patient, several clones of cells with their own set of mutations may be present simultaneously. During the course of the disease, the clonal composition may change and additional genetic defects can be acquired. Patient-specific (sub)clonal architectures may contribute to the heterogeneity of MDS, both in terms of clinical manifestations and response to treatment.
Aims: We studied the clonal composition of MDS on the basis of somatic mutation profiles, the long-term diachronous changes in clonal architecture throughout the course of the disease, and the correlations between clonal architecture/evolution and clinical parameters and treatment response.
Methods: Bone marrow and blood samples from 12 patients with low-to intermediate-risk MDS with long follow-up times (2.5-11 years) were collected at regular intervals (5-19 sampling moments/patient). Whole-exome sequencing was applied on the first and last and several intermediate time points for each patient. In total, 64 samples were used for whole-exome sequencing, to an average depth of 110x. Cultured T-cell DNA was used as reference. In addition, the same samples were analyzed using high resolution SNP-arrays. From this, in total 348 different acquired somatic mutations were identified in 300 different genes. In order to be able to quantify the presence of these mutations with high accuracy, for all mutations specific assays were developed for amplicon-based deep sequencing (IonTorrent, with a coverage of ≈ 10,000 x). This was used to measure the mutational burden at the different sampling time points.
Results: The median number of validated cancer-associated gene mutations per patient was 14 (range 10-26) and 3 (range 0-5) for well-known, recurrently mutated driver mutations in MDS. Integrated analysis revealed the clonal architecture and its evolution over time. Both linear and branched evolution were present in different patients. Diverse patterns of clonal evolution, ranging from a single clone remaining stably dominant for many years, to cases with highly dynamic shifts in the subclonal composition were observed. Five patients were treated with lenalidomide, four of which achieved complete remission. In one of these patients, clinical complete remission was accompanied by molecular remission (mutations <1%), followed by a relapse after acquisition of a *TP53* and *RELN* mutation in the original clone, containing six other mutations. One doubly-*TET2*-mutated patient, treated with EPO and G-CSF displayed a branched evolutionary process with two competitive subclones: an initially major *NRAS*-mutated subclone and a minor *RRAS*-mutated subclone. Over the course of two years, the *RRAS*-mutated subclone became dominant with subsequent transformation to AML.
Summary and Conclusions: Our study demonstrates that great diversity in clonal composition and evolution underlies the clinical heterogeneity of MDS. In some patients, clonal composition was very stable over time, whereas in others, a highly dynamic pattern was observed. Our findings emphasize the importance of genetically unbiased disease monitoring and the development of therapeutic strategies aiming to eradicate multiple different clones.

S132

INITIAL ANALYSIS OF THE PHENOTYPIC CONSEQUENCES OF SF3B1 K700E MUTATION EXPRESSION *IN VITRO* AND *IN VIVO*

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Background: Myelodysplastic syndromes (MDS) are clonal hematopoietic stem cell disorders characterized by dysplastic hematopoiesis in the bone marrow and peripheral blood cytopenias. The splicing factor *SF3B1* is the most commonly mutated gene in MDS and is mainly found in refractory anemia with ring sideroblasts (RARS). *SF3B1* mutations are missense, heterozygous and clustered, suggesting they are gain-of-function with a causal link to the development of RARS. Nevertheless, *in vitro* studies are hampered by the inability

to clone the full length *SF3B1* cDNA and model the effect of its mutation in cell lines. Few reports on *Sf3b1* knock-down mice have not convincingly recapitulated the features of its somatic mutations in RARS.
Aims: We therefore targeted the *Sf3b1* locus of mouse embryonic stem cells (ESC) to create a conditional allele encoding the most common mutation *Sf3b1*^{K700E} to assess the phenotypic consequences of its expression *in vitro* and *in vivo*.
Methods: The C57BL/6N ESC line JM8 was targeted by homologous recombination. Albino pseudopregnant females were injected allowing for chimera screening based on coat color. One mouse carrying germline transmission was selected and its heterozygous offspring was crossed to Tg(*Mx1*:Cre) mice. *Sf3b1*^{K700E} expression was then elicited in the bone marrow with pIpC injection to activate the Cre recombinase. In parallel, Cre was overexpressed in ESC to generate clones with constitutive *Sf3b1*^{K700E} expression to perform *in vitro* work. WT and mutant ES cells were analyzed in basal conditions and after differentiation into haematopoietic cells. For the latter procedure, cells were allowed to grow into embryoid bodies that were subsequently dissociated with trypsin and cultured in a hematopoietic cytokines mix.
Results: WT and mutant *Sf3b1* ESC showed proliferation rates and colony morphologies similar to native ESC. Upon differentiation, >80% of cells expressed the pan-hematopoietic marker Cd45, with no difference between genotypes. Erythroid differentiation in particular was efficient as confirmed by an increase in transcription levels of the globin genes, that was reduced in *Sf3b1* mutant cells. Nevertheless, gross morphology was similar in WT and mutant cells. Flow cytometry showed a marginal decrease in the percentage of Cd71^{low}/Ter119^{hi} cells for the mutant genotype, but no major differences in the frequency of expression markers of stem and progenitor, myeloid and megakaryocytic cells. By unsupervised clustering of gene expression profiles, we found that WT and mutant cells cluster closely together in the undifferentiated state, whereas differentiation causes mutant cells to acquire a dramatically different expression profile than WT cells. Mutant differentiated cells showed an excess of downregulated genes, among which known players in congenital sideroblastic anemias (*Abcb7, Glrx5*, and *Scl25A38*). Gene ontology analysis showed downregulation of several biological processes, including the respiratory electron transport chain and regulators of mitochondrial ion transport as shown previously, but also processes involved in RNA metabolism like translation initiation and pre-mRNA splicing. Initial analysis of conditional *Sf3b1*^{K700E} expression in mice showed that heterozygous animals display no overt sign of disease at our median follow-up of 116 days. In turn, peripheral blood counts post-pIpC injection show that hemoglobin is significantly decreased at one (17.2 *vs* 14.2 gr/dl, p=0.001) and two months (16.7 *vs* 15, p=0.006) in mutant animals, with no difference in white cell and platelet counts. Analysis of bone marrow cells by flow cytometry showed a 2-fold decrease in Cd71^{low}/Ter119^{hi} (p=0.02) cells for the mutant genotype, suggestive of a late maturation defect. Lastly, iron stain of bone marrow cells showed an increment of iron-laden macrophages and occasional ringed sideroblasts in the mutant animals.
Summary and Conclusions: Our data closely recapitulates initial observations in human samples and suggests mutated *Sf3b1* expression leads to defective erythroid maturation. Our mouse model will allow better characterization of the molecular pathways altered by the mutation and the analysis of modifiers of this phenotype.

S133

IN VIVO TREATMENT BY LENALIDOMIDE CHANGES THE CLONAL EVOLUTION OF HEMATOPOIETIC STEM PROGENITOR CELLS OF NON DEL(5Q) LOW RISK MDS PATIENTS

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Background: Recurrent somatic mutations are thought to support MDS initiation and progression. Recently, studies have highlighted the clonal evolution of MDS which could be affected by treatments. Lenalidomide (Len) is designated as a second line treatment in non-del (5q) transfusion-dependent (TD) ESA-resistant low/int-1 MDS patients, which achieve an erythroid response in 30% of cases.
Aims: To investigate whether mutations may represent molecular markers of sensitivity to Len, we used next generation sequencing to examine 26 recurrently mutated genes in non-del(5q) MDS patients treated by Len. We also investigated the clonal evolution of hematopoietic stem progenitor cell (HSPC) following this therapy.
Methods: Bone marrow mononuclear cells (BMMC) were collected in 99 patients at screening, in 15 patients (6 non responder and 9 responder) after 4 cycles of treatment within 4 to 8 months after treatment initiation. For 8 responder, 3 to 5 sequential samples were collected until the 26th month of follow-up. Samples were genotyped by next generation and Sanger sequencing approaches. Clonal architecture of CD34^{+}CD38^{-}HSPC was determined by the genotyping of single cell-derived clones expanded on murine MS5 stromal cells, in 5 patients at screening and was also monitored after 4 cycles of treatment.
Results: On the global cohort, four genes had a mutation frequency over 10%: *SF3B1* (73%), *TET2* (46%), *ASXL1* (20%), *DNMT3A* (20%). As judged on variant allele frequencies (VAF), *SF3B1* and *DNMT3A* mutations were mostly clonal while *TET2* and *ASXL1* mutations were clonal or subclonal. The presence of *DNMT3A* mutations was linked to the response to Len. Among the 15 patients with a follow-up after 4 cycles, VAF were stable in 5/6 non-responder, while a subclonal *NRAS* mutation became visible in one case. Among responder patients, a significant decrease of VAF was observed in 5/9 cases. VAF detected in total BMMC mirrored the mutation representation in HSPC compartment. Furthermore, genotyping of recurrent mutations in individual HSPC described the clonal hierarchy. In some cases, the founding clone was the dominant clone, while in other cases it preceded its appearance. Subclonal mutations either colonised the dominant clone or defined a new independent clone. After 4 cycles of treatment, the dominant clone collapsed in 3/5 patients. In two responder patients, the dominant *SF3B1/DNMT3A*^{mut} clone either completely disappeared in favor of the founding *DNMT3A*^{mut} pre-leukemic clone or obviously decreased in favor of a minor *SF3B1ex14*^{mut}*/DNMT3A*^{mut}clone. In a 3rd case, the dominant *SF3B1/TET2*^{mut} clone decreased in favor of founding *SF3B1*^{mut} clone. In the two other cases, dominant clones *SRSF2/TET2*^{mut} or *SRSF2/TET2/ASXL1*^{mut} remained stable. Long term follow-up of VAF in 8 responder until 26 months after the beginning of treatment identified an increase of the dominant mutations and/or the emergence of mutations in *EZH2*, *TP53* or *ASXL1* genes in 4/5 patients concomitantly with the loss of response.
Summary and Conclusions: Len is able to modify the clonal evolution of non del(5q) low/int-1 MDS by targeting the dominant clone in HSPC compartment, in cases affected by *DNMT3A* and *SF3B1* mutations. Conversely, emergence of subclones can be observed before or at the time of treatment failure. Extended analysis to a larger number of patients will confirm that the clonal evolution under treatment must be monitored.

S134

SRSF2 P95H MUTATION RESULTS IN IMPAIRED STEM CELL REPOPULATION AND COMPROMISED HEMATOPOIETIC DIFFERENTIATION IN MICE

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Background: Recent genetic studies have revealed frequent and specific pathway mutations involving multiple components of the RNA splicing machinery in myelodysplasia. Among these, *SRSF2* mutations are more prevalent in CMML subtype and are associated with poor prognosis. Mutations showed a prominent hotspot involving proline 95, causing either P95H, P95L, or P95R conversion. The molecular mechanism by which *SRSF2* mutations lead to myelodysplasia remains largely unknown.
Aims: This study aimed to clarify the role of *SRSF2* mutations in the development of myelodysplasia through the analysis of *Srsf2 P95H* conditional knock-in mice.
Methods: We first generated a heterozygous conditional knock-in mouse model of *Srsf2 P95H* mutation and crossed them with *Vav1-Cre* transgenic mice. We then performed detailed analysis of hematological phenotype of *Srsf2 P95H* knock-in mice and also evaluated their reconstitution ability in competitive transplantation experiments.
Results: Heterozygous *Srsf2 P95H* conditional knock-in mice exhibited no significant change in total peripheral blood (PB) counts compared to wild-type mice at 8-15 weeks after birth. Bone marrow (BM) cellularity and spleen weight showed no obvious difference between *Srsf2 P95H* and wild-type mice. Analysis of hematopoietic stem and progenitor cells fractions showed significant decrease of the frequency of HSCs in *Srsf2 P95H* mice compared to wild-type mice. On the other hand, there were no significant differences in the number of more differentiated progenitor cells including multipotent progenitor (MPP) cell fractions, myeloid progenitors (MEPs, CMPs, and GMPs) and lymphoid progenitors between *Srsf2 P95H* and wild-type mice. We next performed non-competitive transplantation experiments to assess the cell intrinsic effects of *Srsf2 P95H* mutations. 3 months after transplantation, recipient mice transplanted with *Srsf2 P95H* BM cells showed significant leukopenia due to impaired lymphopoiesis and anemia. Flow cytometrical analysis revealed decreased numbers of HSCs and MPPs fractions, whereas there were no sig-

nificant changes in MEPs, CMPs, and GMPs in BM. The frequency of erythroid progenitor populations was significantly reduced in *Srsf2 P95H* mice in BM and spleens. The population of B cell lineage also decreased at as early as pre-pro B cell stages. These observations suggested that *SRSF2 P95H* mutations lead to impaired stem cell functions and ineffective hematopoiesis, resembling the phenotype of MDS. Subsequently, we assessed the reconstitution capacity of whole BM cells from *SRSF2* mutant mice in competitive transplantation experiments. The donor chimerism of *Srsf2 P95H*-derived cells in PB was significantly lower than that of wild-type cells. At 4 months post transplantation, chimerism of *Srsf2 P95H*-derived cells was remarkably lower than that of wild-type cells in the fractions of HSCs, MPPs, CMPs, MEPs, GMPs and CLPs. Furthermore, the reduced donor chimerism for *Srsf2 P95H* mutants was recapitulated in secondary transplantation experiments. Finally, we have not observed overt MDS phenotypes in none of these *Srsf2*-mutant mice or transplantation models.
Summary and Conclusions: Our results demonstrated that heterozygous P95H mutation of *Srsf2* lead to deregulation of hematopoietic stem cells that was evident from reduced competitive repopulation in lethally irradiated mice and impaired hematopoietic differentiation. *SRSF2* mutation by itself does not seem to be sufficient to develop MDS but to require additional genetic and/or epigenetic events for overt MDS phenotype.

S135

CSNK1A1 BEHAVES LIKE A TUMOR SUPPRESSOR GENE AND MUTATIONS IN CSNK1A1 ASSOCIATE WITH MUTATIONS IN SPLICEOSOMAL GENES IN MYELODYSPLASTIC SYNDROME WITH ISOLATED DEL(5Q)
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Background: Deletion of 5q is one of the most frequent cytogenetic aberrations in myelodysplastic syndromes (MDS). The WHO category of myelodysplastic syndrome with isolated del(5q) describes an own entity showing good prognosis and also demonstrating response to specific treatment such as lenalidomide. However, in some patients the disease evolves to secondary AML. The underlying pathogenic mechanisms are still under debate. Recently, *CSNK1A1* was found to be frequently mutated in MDS with del(5q) irrespective of the presence of additional cytogenetic lesions. *CSNK1A1* is located on 5q32 in the commonly deleted region.
Aims: To analyze mutations in *CSNK1A1* in correlation to other gene mutations as well as to clinical data and prognostic information in MDS with isolated del(5q).
Methods: We investigated 115 patients (82 female, 33 male) presenting with MDS with isolated del(5q) that were strictly diagnosed according to WHO classification 2008 with respect to cytomorphology and cytogenetics (blasts below 5% in the bone marrow and 5q deletion sole). All patients were analyzed for mutations in *ASXL1*, *BCOR*, *BRAF*, *CBL, CSNK1A1*, *DNMT3A*, *ETV6*, *EZH2*, *FLT3*-TKD, *GATA1*, *GATA2*, *IDH1*, *IDH2*, *JAK2*, *KIT*, *NRAS*, *KRAS*, *MPL*, *NPM1*, *PHF6*, *RUNX1*, *SF3B1*, *SRSF2*, *TET2*, *TP53*, *U2AF1*, and *WT1*. The libraries were generated with the ThunderStorm (RainDance Technologies, Billerica, MA) and the Access Array System (Fluidigm, San Francisco, CA) and both libraries were sequenced on a MiSeq instrument (Illumina, San Diego, CA). Missense variants not yet described in any public database were excluded from statistical analyses.
Results: In the total cohort the most frequently mutated genes were *DNMT3A* and *TP53* (21/115, 18% each), followed by *SF3B1* (18/115, 16%), *TET2* (13/113, 12%), *CSNK1A1* (11/115, 10%), *ASXL1* (9/115, 8%), and *JAK2* (7/115, 6%). The mutation frequencies of all other analyzed genes were below 5%, respectively. In only 35/115 patients (30%) no gene mutation was identified. Therefore, *CSNK1A1* is the fifth most frequently mutated gene in MDS with isolated del(5q). All *CSNK1A1* mutations were missense mutations in either amino acid Glu98 (n=9) or Asp140 (n=2). Investigating concomitant gene mutations with *CSNK1A1* mutations resulted in two cases showing no additional gene mutation. The other nine cases showed mutations in *ASXL1* (n=2), *DNMT3A* (n=2), *SF3B1* (n=3), *SRSF2*, *TET2*, or *U2AF1,* respectively. Interestingly, five of the eleven cases (46%) presented with an additional mutation in one of the spliceosomal genes *SF3B1* (n=3), *SRSF2* (n=1), or *U2AF1* (n=1) (*vs* only 16% in *CSNK1A1* wildtype, p=0.034). No correlation of *CSNK1A1* mutations with age, sex, white blood cell count, hemoglobin level, platelet count, as well as bone marrow blast count were found. There was also no prognostic impact of *CSNK1A1* mutations on overall survival. Furthermore we raised the question if the *CSNK1A1* mutations occur more likely in the del(5q) cell clone or in the remaining cells. Therefore, we compared the percentage of del(5q) positive cells by evaluation of the corresponding FISH data with the mutation load of *CSNK1A1* and found a significant correlation (r=0.761, p=0.006), suggesting that *CSNK1A1* mutations occur more likely in the del(5q) cell clone and is therefore affected on both alleles.
Summary and Conclusions: 1) *CSNK1A1* is the fifth most frequently mutated gene in MDS with isolated del(5q). 2) Cases with mutations in *CSNK1A1* frequently associate with mutations in one of the spliceosomal genes *SF3B1*, *SRSF2*, or *U2AF1*. 3) *CSNK1A1* mutations are most likely present in the del(5q) clone.

Red cells: Novel clinical aspects

S136

LUSPATERCEPT (ACE-536) INCREASES HEMOGLOBIN AND DECREASES TRANSFUSION BURDEN AND LIVER IRON CONCENTRATION IN ADULTS WITH BETA-THALASSEMIA: PRELIMINARY RESULTS FROM A PHASE 2 STUDY
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Background: Luspatercept, a fusion protein containing modified activin receptor, is being developed for the treatment of beta-thalassemia (β-thal). Luspatercept binds to GDF11 and other ligands in the TGF-β superfamily to promote late-stage erythroid differentiation. Luspatercept corrected the effects of ineffective erythropoiesis in a thalassemia mouse model (Suragani R, Blood 2014) and was well tolerated and increased hemoglobin (Hb) in a phase 1 clinical study (Attie K, Am J Hematol 2014).
Aims: This is an ongoing, phase 2, multicenter, open-label, dose-finding study to evaluate luspatercept in adults with transfusion-dependent (TD) or non-transfusion dependent (NTD) β-thal. Efficacy outcomes include Hb increase in NTD patients, reduced RBC transfusion burden in TD patients, and liver iron concentration (LIC) by MRI.
Methods: Inclusion criteria included age ≥18 yr and either being TD or NTD β-thal with baseline Hb <10.0 g/dL. Luspatercept was administered SC every 3 wks for up to 5 doses with a 2-month follow-up. Six sequential cohorts (n=up to 6 each) were treated at escalating doses from 0.2 to 1.25 mg/kg. An expansion cohort (n=30) is ongoing; patients who complete the core study may be eligible to enroll in a 12-month extension study.
Results: Preliminary data (as of 16-Jan-2015) were available for 35 patients treated for 3 months. Median age was 35 yr, ranging from 20-57 yr, and 86% had prior splenectomy. TD β-thal patients (n=11). Transfusion burden prior to treatment ranged from 4 to 8 units/12 weeks. All 10 (100%) evaluable patients achieved the primary endpoint of >20% decrease in transfusion burden over 12 weeks (includes 1 patient with only 8 weeks) compared with the 12 weeks prior to treatment (median 67%, ranging from 43 to 100%). The 5 TD β-thal patients in the higher dose groups with baseline LIC ≥5 mg/g dw who were on chronic iron chelation therapy (mean duration 2.5 yr) had a mean LIC decrease of 17% by week 16; this decrease in LIC correlated with a decrease in transfusion burden. NTD β-thal patients (n=24). Mean baseline Hb was 8.3 g/dL, ranging from 6.5 to 9.6 g/dL. Three of 7 (43%) patients in the higher dose groups achieved the primary endpoint of increase in Hb >1.5 g/dL sustained for ≥2 weeks (mean duration 9 weeks) compared with 0 of 17 (0%) patients in the lower dose groups. The 5 NTD β-thal patients in the higher dose groups with baseline LIC ≥5 mg/g dw (includes 3 patients on iron chelation therapy) had a mean LIC decrease of 18%; this decrease in LIC correlated with an increase in hemoglobin. All 3 β-thal patients with chronic leg ulcers at baseline (2 NTD, 1 TD) had substantial, rapid healing, beginning 4-6 weeks after the first dose of luspatercept. Luspatercept was generally well tolerated, with no related serious adverse events reported to date. Adverse events were mostly mild-moderate and the most frequent related adverse events (>10% patients) were bone pain, headache, myalgia, and pain in extremity.
Summary and Conclusions: Luspatercept treatment for up to 3 months was well-tolerated, increased Hb levels in NTD patients, and decreased transfusion requirement in TD patients with β-thalassemia. Both TD and NTD β-thal patients had decreases in LIC at therapeutic doses, and healing of leg ulcers occurred in 3 of 3 patients. These changes represent a significant reduction in disease burden for patients with β-thalassemia. Phase 3 studies of luspatercept in β-thalassemia are planned.

S137

INTERIM RESULTS FROM A PHASE 2A, OPEN-LABEL, DOSE-FINDING STUDY OF SOTATERCEPT (ACE-011) IN ADULT PATIENTS (PTS) WITH BETA-THALASSEMIA
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Background: Beta-thalassemias are characterized by ineffective erythropoiesis leading to anemia, iron overload, and organ failure. Sotatercept (ACE-011) is a novel and first-in-class activin type IIA receptor fusion protein that acts on late-stage erythropoiesis to increase the release of mature erythrocytes into circulation (Carrancio S, *et al.* Br J Haematol 2014;165:870-82). RAP-011, a murine version of sotatercept, was effective in a mouse model of beta-thalassemia, thereby supporting the clinical development of sotatercept for beta-thalassemia (Dussiot M, *et al.* Nat Med 2014;20:398-407).
Aims: To determine a safe, tolerable, and effective dose of sotatercept in pts with transfusion-dependent (TD) beta-thalassemia major (TM) and pts with TD or non-transfusion-dependent (NTD) beta-thalassemia intermedia (TI).
Methods: In this ongoing phase 2a, multicenter, open-label, dose-finding study, pts received sotatercept subcutaneously once every 3 weeks; enrollment up to 1.0 mg/kg is complete and analysis ongoing. Efficacy was assessed by hemoglobin (Hb) increase from baseline for NTD pts and RBC transfusion burden reduction for TD pts. Safety was assessed by NCI-CTCAE v4.0. All pts provided informed consent. ClinicalTrials.gov identifier: NCT01571635.
Results: 30 of 46 pts (65%) in the sotatercept 0.1, 0.3, 0.5, 0.75, and 1.0 mg/kg dose groups were NTD (28 TI, 2 Hb-E/beta-thalassemia) and 16 (35%) were TD (12 TM, 4 TI). Of 30 NTD pts, 6, 6, 6, 7, and 5 were included in the different dose groups, respectively; mean baseline Hb for NTD pts was 8.3 g/dL (range 5.9–10.7). Rates of Hb response for NTD pts are shown in the Table. Area under the curve and peak concentration of sotatercept increased with dose (data not shown). Increased exposure was associated with higher mean Hb increases over 9 weeks for NTD pts (r =0.78, P <0.0001) and with reduced transfusion burden over 24 weeks for TD pts (r =0.74, P <0.01). Among TD pts evaluable for efficacy, 8 of 14 pts (57%) showed a ≥20% reduction in transfusion burden on treatment *versus* 6 month transfusion burden at baseline, whereas ≥50% reduction was observed for 1 of 5 pts in the 0.75 mg/kg dose group and 1 of 2 pts in the 1.0 mg/kg dose group; treatment of the 0.3, 0.5, 0.75, and 1.0 mg/kg groups is ongoing. Overall, sotatercept was well tolerated and 25 of 46 pts (54%) remain on treatment; 19 (41%) pts have been on treatment for ≥1 year, 8 (17%) for ≥22 months, and 2 (4%) for ≥2 years. Grade ≥3 treatment-related adverse events leading to discontinuation were seen in 4 pts: worsening grade 3 bone pain in 1 TD pt in the 0.1 mg/kg group; grade 3 ventricular extrasystoles in 1 NTD pt in the 0.5 mg/kg group; and grade 3 hypertension in 2 NTD pts in the 0.75 mg/kg group. Overall, 3 pts discontinued due to lack of efficacy: 1 pt in each of the 0.1, 0.3, and 1.0 mg/kg dose groups. Updated safety and efficacy data will be presented.

Tabella 1. Rates of Hb response for NTD pts.

	Sotatercept dose					
	0.1 mg/kg (n = 6)	0.3 mg/kg (n = 6)	0.5 mg/kg (n = 6)	0.75 mg/kg (n = 7)	1.0 mg/kg (n = 5)	Total (n = 30)
Hb increase ≥ 1.0 g/dL from baseline sustained for ≥ 12 weeks	0	4 (67)	4 (67)	6 (86)	1 (20)	15 (50)
Hb increase ≥ 1.5 g/dL from baseline sustained for ≥ 12 weeks	0	2 (33)	2 (33)	5 (71)	1 (20)	10 (33)

All values are expressed as n (%)

Summary and Conclusions: These preliminary data suggest long-term treatment with sotatercept can increase Hb levels and decrease transfusion burden with a favorable safety profile in pts with beta-thalassemia. These data suggest an exposure-dependent effect for sotatercept. MDC, JP, and OH contributed equally to this abstract.

S138

PHASE 1 MULTIPLE ASCENDING DOSE STUDY OF THE SAFETY, TOLERABILITY, AND PHARMACOKINETICS/PHARMACODYNAMICS OF AG-348, A FIRST-IN-CLASS ALLOSTERIC ACTIVATOR OF PYRUVATE KINASE-R, IN HEALTHY SUBJECTS

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Background: Pyruvate kinase (PK) deficiency is a rare genetic disorder of metabolism resulting in chronic hemolytic anemia, with comorbidities including iron overload and multi-organ dysfunction. Approximately 180 mutations in the *PKLR* gene are recognized to cause functional deficiency of the PK-R isoform. Defective glycolysis in red blood cells leads to increases in the metabolic precursor, 2,3-diphosphoglycerate (2,3-DPG), and deficiency of the product, adenosine triphosphate (ATP). AG-348 is an oral allosteric activator of both wild type and multiple mutant PK-Rs. We report here a multiple ascending dose (MAD) trial of AG-348.
Aims: To assess safety, tolerability, and pharmacokinetics/pharmacodynamics (PK/PD) of AG-348 in healthy volunteers and identify a dosing schedule for future trials in patients with PK deficiency.
Methods: A phase 1, single-center, randomized, double-blind, placebo-controlled MAD study (NCT02149966) was conducted in healthy men and women (18–60 years) in 6 sequential cohorts (each cohort: n=6 AG-348, n=2 placebo [P]). Subjects gave informed consent and received oral AG-348 at 15–700 mg twice daily (q12h) or 120 mg once daily (q24h) for 14 days with follow-up to Day 29. Adverse events (AEs), laboratory parameters, ECGs, and vital signs were monitored. Plasma AG-348 concentrations and whole blood 2,3-DPG and ATP levels were measured in serial blood samples for PK/PD assessment. Hormone levels were monitored due to pre-clinical data suggesting potential modulation.
Results: 48 subjects (42M/6F), mean age 41.5 (range, 25–60) years, enrolled. Final, un-blinded safety data showed ≥1 AE in 16/36 (44%) AG-348-treated and 4/12 (33%) P subjects. Treatment-related AEs (≥1) were noted in 11/36 (31%) AG-348-treated and 3/12 (25%) P subjects. All treatment-related AEs were mild or moderate in severity (only one grade 3 event) and often reversible despite continued dosing. The most frequent AG-348-related AEs were nausea and headache, 5/36 (14%) for each (P: nausea 0/12 [0%]; headache 1/12 [8%]). Gastrointestinal AEs occurred in AG-348-treated subjects only at the highest dose, 700 mg q12h. One grade 3 AE occurred (AG-348 700 mg q12h, elevated liver function tests [LFTs], which resolved after treatment discontinuation). There were 4 AG-348 discontinuations: due to AEs in 2 subjects (grade 2 drug eruption, 60 mg q12h; grade 3 elevated LFTs, 700 mg q12h), and 2 subjects withdrew consent (both had grade 1/2 nausea and grade 1/1 vomiting; both at 700 mg q12h). The highest well-tolerated dose was 360 mg q12h (doses between 360 and 700 mg were not explored). Plasma AG-348 exposure was dose dependent, with low to moderate variability in the PK parameters of AG-348 and its metabolite AGI-8702. There was a dose-dependent decrease in 2,3-DPG and increase in ATP with the effects plateauing at 360 mg q12h. Decrease in 2,3-DPG was robust after Dose 1, while the increase in ATP occurred gradually and was strongly evident at Day 8. Change from baseline in 2,3-DPG and ATP plateaued at ~300 µg/mL (~50% decrease) and ~175 µg/mL (~50% increase), respectively. After final Day 14 dose, 2,3-DPG returned to levels similar to baseline between 72 and 120 hours. ATP levels remained elevated through 120 hours post dose (Figure). Details of all un-blinded data will be presented.

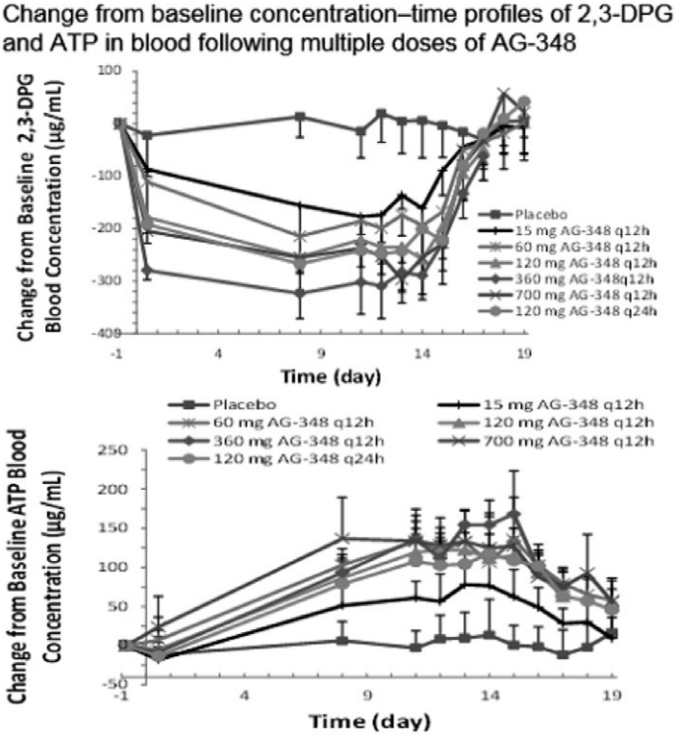

Figure 1.

Summary and Conclusions: AG-348 showed a robust 2,3-DPG/ATP PD glycolytic profile at well-tolerated doses in healthy volunteers. The results support proceeding with a planned phase 2 trial in patients with PK deficiency.

S139

THE SINGLE-UNIT BLOOD TRANSFUSION: EXPERIENCE AND IMPACT IN HAEMATOLOGY PATIENTS

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Background: The Pennine Acute Trust (PAT) is one of the largest non-teaching general hospitals in the UK with an average 30.000 units of blood products are transfused yearly. The Hospital Transfusion Team (HTT) overseeing the transfusion practice, conducted few audits to assess the appropriateness of blood transfusion. These showed that an average of 15-25% of blood transfusion is given inappropriately for various reasons, including the lack of intervention, prevention and diagnosis of anaemia. This has been similarly confirmed by the National Comparative Audits of blood transfusion(1).
Aims: The introduction of the Patient Blood Management (PBM) as a patient-focused model, along with the adoption of a restrictive transfusion triggers, resulted in one of the most effective measures to reduce the inappropriate blood usage, with consequent major benefit for the health care system. The implementation of the single-unit transfusion can considerably reduce the total RBC requirement, as shown in a study of Berger et al (2012), where a change from a double to single-unit transfusion resulted to be safe and achieved a 25% reduction of transfusion requirements(2).
Methods: The HTT introduced the single unit policy for patients without active bleeding in all hospital wards. This involved dispensing one RBC unit at a time. Clinicians were asked to consider each unit transfused as an independent clinical decision and to request the 2nd unit if clinically indicated, after the patient been re-assessed. Recommendations were given to all clinicians to consider alternatives to transfusion, especially, those known with iron deficiency or those with expected transient drop of their haemoglobin level, following treatment, bleeding or sepsis. The main indications suggested for the 2nd unit either the Hb remains<70g/l or patient with on-going chest pain or rise of the haemoglobin less than 8g/l.
Results: The single-unit transfusion policy was introduced in PAT in January 2014. The data analysis within the haematology inpatient ward on the first 12 months of introduction of the policy, and compared with same period of 2013 and matched with the same number of patients, are very encouraging. The analysis revealed an average 31% compliance to single unit policy compared to 10% in 2013. This has resulted in the reduction of the number of RBC units transfused by patient from an average of 3.71 units per patient in 2013 to 3.03 in 2014, with consequent saving of 18% of total units of blood, equivalent to 260 units with a saving of around 40.000 euro/year.
Summary and Conclusions: The introduction of the single unit transfusion policy could result in up to 18% reduction in red blood cell usage in a population with haemato-oncological disorders. These data are very encouraging and demonstrate that the long-standing concept of two RBC units transfusion necessary for an adequate increase of the haemoglobin level must be critically revised. The introduction of this policy will help reducing blood usage, maintain adequate blood supply and optimise healthcare resources. However, further work is needed to improve compliance among clinicians. The introduction of the single unit policy did not have significant impact on the frequency of transfusion. The 72 hours pre-transfusion compatibility specimen expiry allows enough time to consider further transfusion without performing further tests. Although more transfusion events may have occurred in some cases, the overall reduction in transfusion requirement would compensate for the extra work undertaken within the transfusion laboratory.

References

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S140

RAPID REVERSAL OF RED BLOOD CELL SICKLING PROMOTED BY PEGYLATED CARBOXYHEMOGLOBIN BOVINE GAS TRANSFER PROPERTIES

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Background: Sickled Red blood cells (RBCs) have increased rigidity restricting their passage through the microvasculature and cause vasoocclusive crisis (VOC). PEGylated carboxyhemoglobin bovine (Sanguinate™; SG) was designed to release carbon monoxide (CO) to reduce vasoconstriction, counteract inflammatory responses as well as deliver O_2 to hypoxic tissues. Early intervention of VOC with SG treatment could limit the crisis event and reduce pain severity while providing a timely crisis resolution.
Aims: SG treatment effects were evaluated under controlled conditions to determine its capacity for gas exchange with RBCs obtained from healthy and Sickle Cell Disease (SCD) volunteers.
Methods: Carboxyhemoglobin and oxyhemoglobin levels were monitored to determine dose and time effects as well as the repetitive capacity of SG to facilitate gas transfer processes. RBC treated samples were analyzed by light microscopy and image capture flow cytometry to visualize and quantify the effects of SG treatment on reversing sickled SCD RBC. PEG bovine serum albumin (PBSA) product was used as a control.
Results: SG addition to normal oxygenated RBC resulted in CO and O_2 exchange between RBC and SG that followed mass balance and reached equilibrium in closed system. Kinetic analysis revealed SG rapidly transferred its CO component to oxygenated RBC with concomitant O_2 loading of SG. Using experimentally loaded RBC with CO and SG with O_2 produced similar reciprocal gas exchange results. Additionally the primary RBC/ SG reaction products were isolated and cycled demonstrating the ability of SG to continually facilitate gas transfer through multiple exposure events. Similar studies using SG, oxygenated SG (produced by an RBC exchange reaction) or PBSA control were conducted with SCD RBC. Sickling was induced by incubation of RBC in a hypoxic chamber for 3 hours prior to SG or control treatments. After 2 hours of treatment, cells were fixed by addition of glutaraldehyde. Photomicroscopy showed a marked reduction in the sickled RBC population with both SG or oxygenated SG treatments but not with the PBSA control (Figure 1). Results from imaging flow cytometry further supported the microscopy findings and revealed a significant quantitative reduction in the percentage of sickled RBC levels.

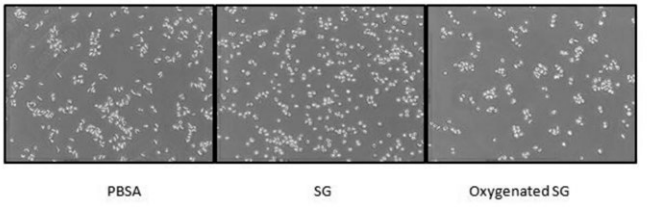

Figure 1. *SG Treatment of Sickled RBC*. Hypoxic SCD RBC treated with SG, oxygenated SG or PBSA for 2 hrs prior to fixation and image capture (40X mag).

Summary and Conclusions: RBCs are negatively impacted by repetitive HbS polymerization/de-polymerization cycling and treatments that reverse RBC sickling during a VOC event could be expected to provide broad clinical benefits. SG was designed to promote CO and O_2 transfer in a concentration dependent manner providing physiological supplementation of O_2 transport/delivery in conditions of hemolytic or ischemic anemia. Additionally since ASH 2015, anti-inflammatory activity on LPS activated samples has been quantified by qPCR and flow cytometry of a selected panel of inflammatory markers. SG pre-treatment of normal and SCD whole blood significantly decreased inflammatory cytokine RNA and protein levels. Studies are ongoing examining the effect of SG on hypoxia induced inflammation. These *ex vivo* data demonstrated for the first time that under controlled conditions, a therapeutic agent serves as an active gas transport agent providing either CO or O_2 to sickled RBCs, prompting rapid unsickling and significant decrease in inflammation markers. Furthermore, image capture flow cytometry provided a quantitative measurement of sickled RBC fraction decrease. This mechanism may provide a useful biomarker test in future clinical studies to monitor SG treatment effects on SCD patients.

Thrombosis and vascular biology

S141

THE THROMBIN INHIBITOR DABIGATRAN IMPAIRS CANCER CELL GROWTH AND PROGRESSION

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Background: Advanced malignancy often correlates with activation of the coagulation system, termed cancer coagulopathy, which is associated with increased mortality rates. Thrombin, a blood-derived serine protease, is the main effector of the coagulation cascade. There is convincing evidence that thrombin, by PAR-1 receptor, regulates numerous critical cellular events, including cell proliferation, cell adhesion, angiogenesis, and invasion. Dabigatran is a selective direct thrombin inhibitor that reversibly binds to thrombin. Dabigatran-bound thrombin is unable to cleave and activate PAR-1.
Aims: The purpose of this study is to explore if dabigatran may affect mechanisms favouring tumor growth by interfering with thrombin-induced PAR-1 activation.
Methods: The U87 glioblastoma cell line, the MCF-7 breast adenocarcinoma cell line and the endothelial cell line HUVEC, all expressing PAR-1, have been used for our experiments. Cell proliferation was measured by a colorimetric assay suitable for determining the number of viable cells. Cell death was quantitated by FACS analysis with PI/annexin. For RNA analysis, Real Time PCR was performed on a ABI PRISM 2600 instrument using SYBR Green. Protein expression was measured by WB analysis. Chemotaxis was studied using transwell plates. Endothelial cord formation was evaluated on Matrigel by enumerating branching points (tube formation) with an inverted microscope.
Results: Exposure of tumor cells to thrombin significantly increased cell proliferation as measured by MTS conversion. Using this approach, dabigatran was effective in antagonizing thrombin-induced proliferation in a dose-dependent manner. In our experimental system, U87 cells underwent cell death when cultured in starving medium. As thrombin has been shown to activate protein kinases interfering with caspases-dependent cell death, we thought to evaluate this property in U87 starved cultures. We found that the fraction of double positive annexin/PI cells was significantly lower in presence of thrombin and this protection was lost when dabigatran was coincubated with thrombin. We then evaluated the regulation of cell cycle proteins by thrombin and dabigatran. In particular, we evaluated the expression of cyclin D1 (promoting cell cycling) and p27 (inhibiting cell cycling) in U87 and MCF-7 starved cultures. Thrombin led to down-regulation of p27 with concomitant induction of cyclin D1. When dabigatran was added to cultures, the pattern of inhibition of growth observed in presence of starving medium was restored (Figure1 A). Treatment of MCF-7 cells with thrombin determined a slight but significant increase of the fraction of cells in S phase. The effect of thombin on cell cycle was completely antagonized by dabigatran. We then evaluated the expression of pro-angiogenetic proteins, Twist and GRO-a, in MCF-7 cells and HUVEC cells following exposure to thrombin and dabigatran. We found that thrombin was significantly effective in inducing up-regulation of Twist and GRO-a mRNA in MCF7 and HUVEC cell lines. Expression of Twist was brought down to control levels when dabigatran was added to colture. WB analysis confirmed RT-PCR results (Figure1 B). We also found that the chemoattractant effect of thrombin on tumor cells was lost in presence of dabigatran, and this effect was directly related to dabigatran concentrations. Finally, vascular tube formation in HUVEC cells was increased in presence of thrombin and the induction of tube formation was progressively lost with dabigatran (Figure1 C).

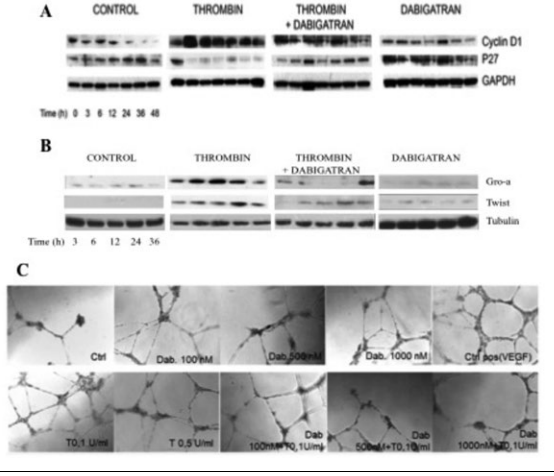

Figure 1.

Summary and Conclusions: Our data support a role of thrombin in inducing the proliferation, migration and pro-angiogenetic effects of tumor cells in-vitro. Dabigatran has shown activity in antagonizing all these effects, thereby impairing tumor growth and progression. In-vivo models may help to understand the relevance of this pathway.

S142

OPTIMAL DURATION OF ANTICOAGULANT THERAPY FOR THE TREATMENT OF CANCER-ASSOCIATED THROMBOSIS

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Background: Cancer-associated thrombosis is associated with a high risk of recurrent thrombosis despite "usual intensity warfarin"; anticoagulant therapy, specifically low-molecular weight heparin, reduces the risk of recurrent thrombosis compared with warfarin. However, the optimal duration of treatment in patients with cancer associated thrombosis remains unclear.
Aims: To evaluate the effects of treatment duration on the outcomes in patients with cancer-associated thrombosis.
Methods: Consecutive patients with symptomatic venous thromboembolism [VTE] (deep vein thrombosis or pulmonary embolism) were prospectively enrolled in the RIETE registry. The patient were categorized into 2 group according to received treatment duration (<6 months *vs* ≥6 months). All the patients were followed up to 5 years from treatment start date. The primary outcome was recurrent VTE and the secondary outcomes were all-cause mortality, VTE-related death and major bleeding.
Results: A total of 4,460 patients with cancer-associated thrombosis were enrolled in the analysis. Of these, 2,937 patients received anticoagulant for less than 6 months, and 1,523 received anticoagulants for more than 6 months. Patients receiving anticoagulant therapy for less than 6 months had an increased risk of recurrent thrombosis (hazard ratio [HR] 2.86, 95% confidence interval [CI] 2.22 to 3.70) when compared to patients received anticoagulant for more than 6 months. The risk of all-cause mortality and VTE-related death were significantly increased higher in patients treated for less than 6 months, HR 5.88 (95%CI 5.00 to 6.67) and HR 6.25 (95% CI 3.03 to 14.29), respectively. We did not observe increased major bleeding in patients who were treated for more than 6 months, HR 0.26 (95% CI 0.19 to 0.38).

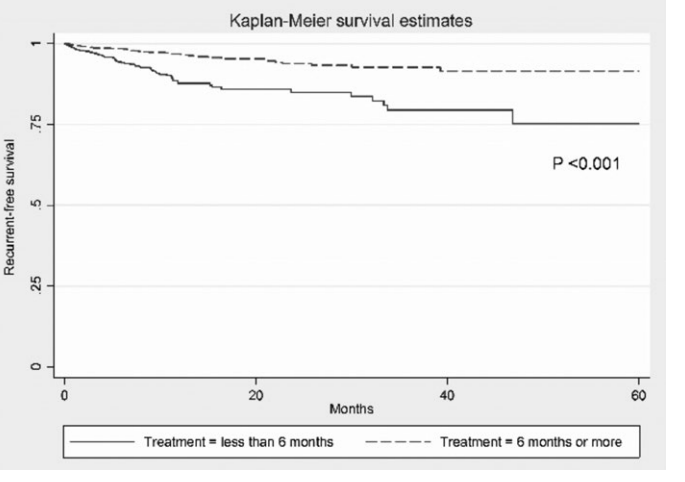

Figure 1.

Summary and Conclusions: We demonstrate that patients with cancer-associated thrombosis patients have a higher risk of recurrent VTE, VTE-related death and all-cause mortality if their anticoagulants are not continued beyond six months from diagnosis.

S143

DEVELOPMENT OF A PREDICTIVE SCORE FOR THE IDENTIFICATION OF LYMPHOMA PATIENTS AT RISK FOR THROMBOEMBOLISM

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Background: There is a paucity of data that pertain to thrombosis in patients with lymphoproliferative diseases. In few published studies the rate of thrombotic complications in lymphoproliferative disease is highly variable ranging from 1.5% up to 59.5% because of the different study types (prospective or retrospective, with hospitalized or ambulatory patients), types of disease (indolent *vs* aggressive) and the disease stage, there are few prediction tools for estimating the risk of thrombosis but they are based on studies performed on hospitalized medical patients without cancer or on hospitalized neutropenic cancer patients without special consideration to hematological malignancies
Aims: Aim of our study was to determine incidence of thromboembolic (TE) events in patients with non Hodgkin lymphoma (NHL), Hodgkin lymphoma (HL) and chronic lymphocytic leukemia (CLL) who were treated in our institution. Also, we assessed predictive model for chemotherapy-associated thrombosis developed by Khorana and create new model for the identification of lymphoma patients at risk for thromboembolism
Methods: We reviewed all medical records of patients with with NHL, HL and CLL diagnosed according to the World Health Organisation classification and treated at our institution between January 2006 and December 2014.
Results: A total of 1054 patients, with malignant lymphoma were eligible for analysis: 576 patients were men (54.6%) and 478 were women (45.4%), and mean age was 54.0 years. A total of 510 patients (48.4%) had high-grade lymphoma, 211 had low-grade lymphoma (20.0%), 153 had Hodgkin lymphoma (14.5%), and 75 (7.1%) had other forms of lymphoma; HLL had 105 (10.0%) patients. In group of our lymphoma patients, 72 (6.8 %) had at least one TE. A total of 40 patients were men and 32 women. Thromboembolic events included deep vein thrombosis (38.9%), jugular vein thrombosis (12.5%), pulmonary embolism (11.1%), CNS thrombosis (6.9%), superficial vein thrombosis (2.8%), acute myocardial infarction (1.4%) and other (26.4%). In total of 49 pts (68%) thrombosis occurred during treatment or up to 3 months after completion of therapy, whereas in 23 pts (32%) thrombosis was diagnosed prior to therapy. Patients with aggressive NHL had significantly higher incidence of TE (8.6%) compared to all other types of lymphoma patients (RR=2.1; 95%CI for RR 1.2-3.6; p=0.009) (incidences of TE in low NHL, HL and other forms of limphoma were 4.3%, 3.3% and 6.7%). Pts with HLL had 8.6% incidence of TE. Patients with high grade lymphomas, overweight patients (BMI>25 kg/m2), patients with reduced mobility (ECOG>2), and patients with recent operative procedure (<4 weeks) had increased risk for thrombosis (p<0.05 for all). No difference regarding age, gender or disease stage was found. Based on previously mentioned risk factors a new TE risk model was developed for patients with lymphoma (excluding HLL). Patients who had at least two out of four risk factors fall into high risk group for TE (RR=13.0; 95%CI for RR 7.4-23.0; p<0.001). Predictive model for chemotherapy-associated TE developed by Khorana was also evaluated on this group of patients (RR=7.0; 95%CI for RR 3.3-14.7; p<0.001). In multivariate analysis our newly developed model for TE events in lymphoma patients were predictor of TE events and independent from Khorana model.
Summary and Conclusions: Prediction tools for estimating the risk of TE events in lymphoma patients have not been established but our newly developed model should be used in addition to chemotherapy-associated model in guiding best practice in the prevention of thromboembolism in patients living with lymphoma.

S144

PRESENCE OF JAK2V617F MUTATION IN ENDOTHELIAL CELLS FROM BUDD-CHIARI SYNDROME PATIENTS IS NOT CORRELATED WITH PH-NEGATIVE MYELOPROLIFERATIVE NEOPLASM

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Background: The role of the endothelial cell in the pathogenesis of Ph-negative MPNs is still not elucidated. Some have reported the presence of the JAK2V617F mutation in endothelial colony forming cells (ECFC) isolated from peripheral blood in patients with Ph-negative MPNs (Teofili L et all, Blood 2011). Others, however, did not find such an association (Piaggio G et al, Blood 2009). In patients with Budd-Chiari Syndrome (BCS), the JAK2V617F mutation has been found in endothelial cell (ECs) isolated by micro dissection from liver biopsies in patients both with and without MPN (Sozer S et al, Blood 2009). Besides the JAK2V617F mutation, other mutations (*e.g.* ASXL1, TET2, DNMT3A, SRSF2) have been described in patients with Ph-negative MPNs, but their presence has not been evaluated in patients with BCS who carried the JAK2V617F mutation.
Aims: 1. To evaluate the presence of the JAK2V617F mutation in CECs from patients with BCS both with and without concomitant Ph-negative MPNs; 2. To determine the mutational landscape of granulocytes in patients with BCS who harbored the JAK2V617F mutation but did not have the clinical diagnosis of a Ph-negative MPN.
Methods: We identified 10 patients from our institution who had a diagnosis of BCS and harbored the $JAK2^{V617F}$mutation in granulocytes. Three patients died from hepatic failure before they could be evaluated by bone marrow biopsy, so 7 patients remain for the analysis. All patients were investigated for the presence of Ph-negative MPNs with bone marrow trephine biopsy. ECs assays were performed according to the method of Hill. Briefly, Ficoll-Paque density gradient–isolated mononuclear cells were plated on fibronectin coated 6-well dishes with EndoCult medium (Stem Cell Technologies) for 48 hours, when non adherent cells were recovered and re-plated in a new dish at 10^6/mL concentration. After an additional 5 days, adherent cells were plucked and analyzed by flow cytometry. The ECs population was sorted using a FACS Aria BD Biosciences sorter according to the following phenotype: CD45-PerCP-negative, CD31-FITC-positve, VEGFR2-PE-positive, CD34-PECy7-positive, CD133-APC-negative. The presence of the $JAK2^{V617F}$mutation was investigated by allele-specific PCR. Paired DNA (sorted CD66b-granulocytes/skin biopsy) from 3 patients with JAK2V617F-positive BCS without a clinical diagnosis of Ph-negative MPN was subjected to whole exome sequencing on a Illumina HiSeq 2000 platform using Agilent SureSelect kit. Tumor coverage was 150x and germline coverage was 60x. Somatic variants calls were generated by combining the output of Somatic Sniper (Washington University), Mutect (Broad Institute) and Pindel (Washington University), followed by in-house filters to reduce false positive calls.
Results: We were able to obtain CECs from all 7 patients. The purity of the CECs populations obtained was over 96% in all cases. Among the 7 patients with BCS, five did not have any clinical feature of a Ph-negative MPN, with a normal bone marrow biopsy. The JAK2V617F mutation was positive in the CECs from 5 cases, including 3 patients who only had BCS. In one patient with BCS solely the reaction did not work, and in another the JAK2 was wild-type in the ECs. The mutation was positive in CECs from both patients with myelofibrosis and BCS. Three patients with BCS solely were evaluated by whole exome sequencing. The only known pathogenic abnormality found was the JAK2V617F mutation, albeit at a low allele fraction (5%, 6%and 12.6%).
Summary and Conclusions: The presence of the JAK2V617F mutation in CECs from patients with BCS who did and did not have a diagnosis of Ph-negative MPN suggest that the mutation plays an important role in the development of vascular complications in these patients. Further studies with a larger number of patients are needed to precisely define the importance of CECs in the pathogenesis of MPNs. The sole presence of the JAK2V617F mutation in circulating granulocytes at a very low allele fraction in patients with BCS without Ph-negative MPNs suggest that these patients have a pre-malignant clone that would probably remain undiagnosed had it been not for the development of hepatic venous thrombosis.

S145

HIGH-DOSE CORTICOSTEROID ASSOCIATED WITH CATHETER-RELATED THROMBOSIS AFTER ALLOGENEIC HAEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Allogeneic haematopoietic stem cell transplantation (Allo-HSCT) recipients are at increased risk of thrombotic complications, most of which are catheter related. Catheter-related thrombosis (CRT) remains challenging in allo-HSCT patients. On one hand, the incidence of CRT varies considerably depending on clinical factors. CRT has major consequences for patients who are already vulnerable, including pulmonary embolism. On the other hand, the benefit of anticoagulation must be weighed against the substantially increased risk of bleeding. However, the underlying pathogenesis of CRT remains unclear. From a clinical perspective, it would be helpful to identify the risk factor of CRT in allo-HSCT patients.
Aims: The aim of this study was to examine the incidence and risk factors of CRT in allo-HSCT recipients.
Methods: We performed a retrospective nested case-control study in patients following allo-HSCT. Patients were reviewed retrospectively from all recipients who underwent allo-HSCT between July 2007 and June 2014 at Peking University People's Hospital, Beijing. Transplantation protocols (donor selection, HLA typing, stem cell harvesting, conditioning therapy, and GVHD prophylaxis) were conducted according to our previously protocols. Thrombotic episodes were diagnosed based on the clinical suspicion of the physician (pain, swelling, etc.) with subsequent central venous catheter (CVC) or peripherally inserted central catheter (PICC) thrombosis confirmed by duplex ultrasound. Cases with CRT and controls were matched for time of HSCT (±5 days), age at HSCT (±5 years) and type of insertion (CVCs or PICC).
Results: During the 6-year period, CVC and PICC were placed in 923 patients undergoing allo-HSCT. A total of 38 patients (4.11%) developed catheter-related thrombosis, among which 12 were associated with CVCs, and 26 were associated with PICCs. The median duration from catheter insertion to thrombosis was 97 days. Among patients with CRT, 20 patients were classified as high risk before transplantation, and 7 experienced relapse. On average, patients received 4.5 rounds of pre-HSCT chemotherapy. Despite reports of an associ-

ation between thrombosis and infection, no patient with CRT experienced a central line-associated bloodstream infection in our study. No significant differences were noted in terms of primary disease, donor type, conditioning regimen and catheter type between cases and controls. On univariate analysis, high-risk classification and relapse were associated with CRT. Grade III-IV GVHD is strongly correlated with CRT. Exposure to high-dose corticosteroids and cyclophosphamide is also related to thrombotic complications. No correlations between CRT and blood counts, coagulation markers, hyperlipidaemia or hypoalbuminaemia were noted in the laboratory values analysed. Multivariate regression analysis identified grade III-IV GVHD and exposure to high-dose corticosteroids as independent risk factors for the development of catheter-related thrombosis after allo-HSCT.

Summary and Conclusions: In conclusion, we demonstrate that CRT occurs among patients following allo-HSCT. The use of high-dose corticosteroids is correlated with the onset of CRT. However, the efficacy and safety of thromboprophylaxis in this population requires further discussion.

Quality of life and health economics

S146

LEVELS OF DISCORDANCE BETWEEN PATIENTS' AND PHYSICIANS' PERCEPTIONS OF PATIENTS' HEALTH-RELATED QUALITY OF LIFE IN RELAPSED/REFRACTORY MULTIPLE MYELOMA-A CROSS-CULTURAL PERSPECTIVE

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Background: Patients' Health-Related Quality of Life (HRQoL) is important, particularly in the relapsed/refractory multiple myeloma (RRMM) setting. Exploring levels of concordance between physicians' and patients' perceptions of HRQoL could improve physicians' understanding of patients' feelings and help in daily patient management. Findings of our study previously published for the overall population showed no major discordance between ratings of HRQoL/functioning domains, but major discordance between the perceptions of physicians and patients for symptom domains.

Aims: To compare between countries the difference in physicians' and patients' perceptions of HRQoL and symptoms.

Methods: An observational study was conducted in Italy, Germany, France, UK/Ireland and Belgium in RRMM patients starting 2nd or 3rd line treatment. Patients and physicians completed three EORTC questionnaires: Quality-of-Life Core Questionnaire (QLQ-C30), with 15 domains (*Global Health Status/QOL, Physical Functioning, Role Functioning, Emotional Functioning, Cognitive Functioning, Social Functioning, Fatigue, Nausea and Vomiting, Pain, Dyspnea, Insomnia, Appetite Loss, Constipation, Diarrhea* and *Financial Difficulties*); QLQ-Multiple Myeloma (QLQ-MY20), with four domains (*Disease Symptoms, Side-Effects of Treatment, Body Image* and *Future Perspective*); QLQ-Chemotherapy-Induced Peripheral Neuropathy (QLQ-CIPN20) with three domains (*Sensory scale, Motor scale* and *Autonomic scale*). Intra Correlation Coefficients (ICC) were calculated to compare patient and physician scores (ICC<0.40 showing major discordance). The difference in patient and physician scores was compared across countries using ANOVA.

Tabella 1. Comparison of EORTC scores reported by physicians and patients at baseline, overall and by country

EORTC	Domain	Total (N=258)		UK/Ireland (N=42)		Germany (N=33)		France (N=43)		Italy (N=83)		Belgium (N=57)		P-value[c]
		Mean diff[a]	ICC[b]	Mean diff[a]	ICC[b]	Mean diff[a]	ICC[b]	Mean diff[a]	ICC[b]	Mean diff[a]	ICC[b]	Mean diff[a]	ICC[b]	
QLQ-C30	Cognitive Functioning	1.0	0.42	-0.4	0.41	-6.5	0.36	-2.0	0.43	-1.0	0.37	11.0	0.46	*
	Emotional Functioning	2.7	0.45	7.7	0.46	-9.1	0.27	-2.0	0.54	0.9	0.51	11.8	0.35	**
	Global Health Status/QoL	-0.1	0.50	5.0	0.65	-10.2	0.44	1.0	0.49	-1.3	0.62	2.7	0.15	*
	Physical Functioning	-0.9	0.76	1.1	0.72	-12.8	0.56	-2.0	0.91	-1.1	0.79	5.6	0.73	***
	Role Functioning	-2.0	0.62	0.0	0.49	-12.0	0.56	-5.8	0.62	-2.1	0.68	5.1	0.63	
	Social Functioning	6.5	0.44	8.1	0.63	-16.1	0.58	5.7	0.40	2.3	0.49	24.7	0.29	***
	Appetite loss	4.7	0.54	0.0	0.20	7.5	0.59	6.7	0.67	1.2	0.55	10.1	0.50	
	Constipation	10.9	0.44	7.7	0.34	11.8	0.40	12.5	0.51	10.6	0.45	11.9	0.30	
	Diarrhea	5.6	0.32	4.5	-0.15	6.5	0.50	8.1	-0.01	3.0	0.44	7.7	0.47	
	Dyspnea	6.9	0.42	2.6	0.56	5.4	0.44	13.8	0.46	1.3	0.55	13.7	0.14	*
	Fatigue	1.4	0.57	2.3	0.30	8.7	0.56	10.4	0.55	-0.2	0.52	-7.7	0.71	**
	Financial Difficulties	4.0	0.29	-8.1	0.36	13.8	0.24	4.9	0.11	7.1	0.47	1.9	0.07	**
	Nausea and Vomiting	2.6	0.36	-0.4	0.43	2.6	-0.17	2.9	0.59	1.7	0.51	6.0	0.02	
	Pain	0.9	0.65	1.7	0.38	17.7	0.60	2.9	0.61	-0.2	0.78	-8.9	0.71	***
	Insomnia	7.1	0.33	1.7	0.00	12.9	0.28	5.7	0.50	8.8	0.44	6.2	0.23	
QLQ-MY20	Body Image	-1.4	0.33	0.9	0.23	-4.4	0.40	-6.8	0.45	-0.4	0.37	1.2	0.00	
	Future Perspective	8.0	0.38	16.8	0.29	-5.7	0.49	1.4	0.40	4.8	0.54	18.9	0.03	***
	Disease Symptoms	3.6	0.58	1.8	0.53	4.3	0.52	-1.7	0.41	5.7	0.63	5.0	0.62	
	Side-Effects of Treatment	7.7	0.37	6.0	0.30	8.4	0.46	10.5	0.22	6.3	0.40	8.7	0.35	
QLQ-CIPN20	Autonomic scale	3.7	0.36	3.9	0.25	6.5	0.21	2.5	-0.08	2.3	0.60	4.9	0.41	
	Motor scale	3.7	0.48	2.2	0.46	1.5	0.53	8.4	0.54	2.4	0.49	4.7	0.42	
	Sensory scale	3.5	0.50	2.1	0.64	-1.6	0.61	8.3	0.32	4.4	0.57	2.7	0.32	

[a] Difference = patient score – physician score

[b] In blue, major discordant scores (ICC <0.40) with better HRQoL/functioning or symptoms score reported by physician than by patient; In yellow, major discordant scores (ICC <0.40) with worse HRQoL/functioning or symptoms score reported by physician than by patient

[c] p-value from ANOVA comparing the mean difference in physician/patient score between countries; * p<0.05; ** p<0.01; *** p<0.001

Results: The population included 33 physicians who enrolled 258 patients (mean age=70; 54% male; mean time since diagnosis=3 years). At baseline, 251 (97%) sets of EORTC questionnaires were completed by patients and 252 (98%) by physicians. Based on ICC (Table 1), most of the domains with major discordance observed between patients and physicians were symptom domains, in all countries but Italy. In almost all cases, physicians underestimated the level of symptoms experienced by their patients. However, major discordance was not observed on the same symptom domains across countries. The number of domains showing major discordance also differed, ranging from none (Italian sites) to nine (UK/Irish sites). Variations across countries were observed on the level of patient-physician discordance, with statistical significance reached for four out of 14 symptom domains. For HRQoL/functioning domains, major patient-physician discordance was also not observed on the same domains across countries. Furthermore, the number of domains showing major discordance differed, ranging from none (French sites) to five

(Belgian sites). Depending on domain and country, physicians either underestimated or overestimated their patients' HRQoL/functioning. Variations across countries were observed on the level of patient-physician discordance, with statistical significance reached for six out of eight domains. The highest variations (p<0.001) were observed for Physical Functioning, Social Functioning, Pain and Future Perspective domains.
Summary and Conclusions: Major discordance was observed across countries on symptom domains, with physicians consistently underestimating their patient's symptoms. However, the specific symptom domains concerned varied between countries. An even more variable picture was observed for HRQoL/functioning domains. This supports the importance to also consider patients' view of their own health.

S147

ACCURACY OF PHYSICIAN ASSESSMENT OF TREATMENT PREFERENCES AND HEALTH STATUS IN PATIENTS WITH HIGHER-RISK MYELODYSPLASTIC SYNDROMES

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Background: Higher-risk myelodysplastic syndromes (MDS) are rarely curable and have a poor prognosis, with short life expectancy. Thus it becomes essential for physicians to accurately evaluate the patients' wishes and preferences in light of the risks, benefits and appropriateness of treatment.
Aims: The primary aim was to investigate the accuracy of physicians' perception of patients' health status and the patients' preferences for involvement in treatment decisions. A secondary objective was to investigate physicians' attitude toward patient involvement in treatment decisions and to examine factors influencing physicians to be more patient inclusive or exclusive in the shared decision-making process.
Methods: We investigated 280 high-risk MDS patients paired with 68 physicians to evaluate physician-patient agreement on preferences for involvement in treatment decisions and self-reported health status. Control Preferences Scale (CPS) and the questionnaire EORTC QLQ-C30 were used to evaluate these parameters, respectively. Simple and weighted κ-coefficient was used to assess the degree of physician-patient agreement. The following variables were ascertained: physician gender and age; the overall number of years in practice; the number of years of experience in treating MDS patients.
Results: The median age of the physicians was 44 years (range 28-64) and their median duration of medical practice was 16 years, with a median of 11 years of expertise in MDS. Analysis was based on 280 patient-physician dyads. Overall concordance was 49% for physician perception of patient preferences for involvement in treatment decisions, with a weighted K-coefficient indicating poor concordance. In 36.4% of comparisons there were minor differences and in 14.6% there were major differences. In 44.7% of the patients preferring a passive role, physicians perceived them as preferring an active or collaborative role. The only factors independently associated with the physicians' attitude toward less involvement of their patients in clinical decisions were absence of the patient's request for prognostic information (P=0.001) and judging the patient as having a poor health status (P=0.036). The degree of agreement between physicians and patients in evaluating overall health status was low. Agreement on health status was found in 27.5% of cases. Physicians most frequently tended to overestimate health status of patients who reported low-level health status (Figure)

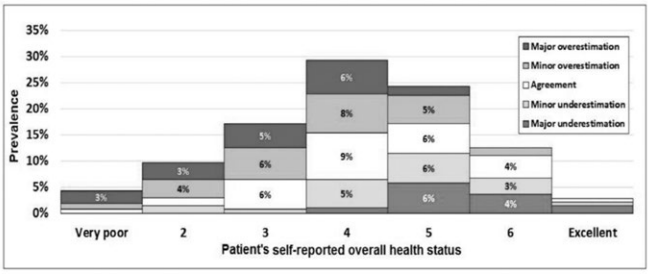

Figure 1.

Summary and Conclusions: Physicians treating MDS patients often have difficulty in understanding what their patients' preferences are and some do not consider the patients' preferences to be important. General concordance on physicians' perception and patients' health status was present in slightly less than one third of comparisons. In particular, physicians tended to overestimate the health status of patients reporting low levels for this parameter. Physicians need to improve their perception of patients' health status in view of the important implications associated with assessment of patient eligibility for inclusion in clinical trials. Moreover, clinicians need to be provided with decision aid tools that can improve their perceptions and communication skills.

S148

AN ONGOING MULTINATIONAL OBSERVATIONAL STUDY IN MULTIPLE MYELOMA (PREAMBLE): A PRELIMINARY REPORT OF DISEASE IMPACT ON QUALITY OF LIFE

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Background: Multiple myeloma (MM) is associated with high morbidity and mortality. In recent years, immunomodulatory drugs (IMiDs) and proteasome inhibitors (PIs) have been approved for MM treatment based on their ability to improve patient (pt) survival. However, a greater understanding of quality of life (QoL) in patients treated with IMiDs and PIs is needed.
Aims: PREAMBLE (Prospective REsearch Assessment in Multiple Myeloma: an oBservationaL Evaluation; NCT01838512) is an ongoing, prospective, multinational, observational cohort study designed to better understand the real-world clinical effectiveness of IMiDs, PIs, and IMiD+PI in pts with relapsed/refractory MM (RRMM). We report QoL data for pts enrolled with ≥6 months' follow-up.
Methods: PREAMBLE includes pts with RRMM aged ≥18 years who have received ≥1 prior therapy and who initiated treatment with an IMiD, PI, or IMiD+PI <90 days prior to or 30 days after enrollment. Administration is according to standard clinical practice. Data are collected at baseline (BL) and every 3 months during Year 1, then every 6 months in Years 2 and 3, or until discontinuation. Data extraction for the present analysis was performed on 12 December 2014. The EuroQol 5 Dimensions (EQ-5D) questionnaire and the European Organisation for Research and Treatment of Cancer (EORTC) QoL Questionnaire-Core 30 (QLQ-C30) were used to assess disease burden. Minimally important differences for EQ-5D and QLQ-C30 have been estimated at 0.06–0.09 (for all cancers)[1] and 6–17 (for MM),[2] respectively.
Results: At the time of data extraction, 273 pts had ≥6 months' follow-up (96% completion rate at BL). Median BL EQ-5D score was 0.69, compared with 0.8 in the general US population[3] and 0.6–0.7 in other MM studies.[4,5] At BL, there was no difference in median EQ-5D score regardless of number of prior lines of therapy. At Month 6, there was a trend towards improvement in median EQ-5D score with increasing numbers of prior lines of therapy (1: 0.69; 2: 0.69; >2: 0.76; 3: 0.76; >3: 0.76). At BL, pts reported some or severe pain and discomfort (63.4% [some] and 14.1% [severe]), anxiety and depression (45.5% [some] and 5.3% [severe]), and inability to conduct usual activities (52.9% [some] and 10.6% [severe]). More pts receiving IMiDs (19.4%) reported severe pain and discomfort at BL than those receiving PIs (8.3%) or IMiD+PI (11.1%). Median QLQ-C30 global health status (GHS) score at BL and Month 12 was 58.3 and 66.7, respectively, compared with 75 (general population), 66.7 (colorectal cancer pts), 66.7 (all cancer pts),[6] and 57–68.7 (previous MM studies).[4,5] Median QLQ-C30 GHS improved over time in pts treated with 3 prior lines of therapy or with IMiDs (Table; higher score=better QoL). At BL, fatigue and pain placed the greatest burden on pts, but improved over time (Table; higher score=worse symptoms).

Tabella 1. Patient-reported QoL outcome measures

EORTC QoL measures	Baseline	6 months	12 months
GHS (EORTC QLQ-C30, median)*			
All patients	58.3	66.7	66.7
By lines of prior therapy			
1	58.3	62.5	50.0
2	50.0	58.3	66.7
>2	58.3	66.7	58.3
3	66.7	83.5	83.3
>3	50.0	66.7	50.0
By class of therapy			
IMiD	50.0	58.3	66.7
PI	66.7	66.7	62.5
IMiD+PI	58.3	66.7	66.7
Individual symptoms (mean)†			
Fatigue			
All patients	46.8	43.0	40.5
By lines of prior therapy			
1	45.1	46.1	41.9
2	47.0	40.5	35.3
>2	50.3	39.9	47.2
3	45.6	33.3	49.2
>3	53.9	45.8	44.4
Pain			
All patients	38.6	34.6	32.8
By lines of prior therapy			
1	36.5	37.3	38.1
2	44.1	35.5	31.2
>2	34.5	27.1	23.6
3	37.7	28.9	19.0
>3	31.9	25.5	30.0

*EORTC QLQ-C30: 0–100 scale; higher score=better health status/QoL
†EORTC QLQ-C30 symptoms: 0–100 scale; higher score=worse symptoms

Summary and Conclusions: Preliminary analysis suggests that MM continues to place a high burden on pts despite new therapies. Although functional scores show a high level of function, pts are particularly troubled by pain, discomfort, and fatigue. As data mature and more pts are enrolled, PREAMBLE will shed light on MM burden and its management.

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S149

HOSPITAL VERSUS HOME CARE FOR PATIENTS WITH HEMATOLOGICAL MALIGNANCIES IN CURATIVE OR TERMINAL PHASE: USE OF THE RESOURCES ANALYSIS, SYMPTOM BURDEN AND COST-EFFECTIVENESS STUDY

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Background: Hospitalization of patients with hematological malignancies often is an inappropriate setting of care since the economic and clinical advantage of the home care is not established.

Aims: To compare the costs, the use of resources, the effectiveness of hospitalization *versus* a program of hematologic home care, funded in partnership by charity and public health service.

Methods: Prospective, observational study. According to disease status two groups of patients were analyzed for 6 weeks: i) in curative phase, for supportive care or for early palliative simultaneous care; ii) in terminal phase, for end of life-care. A mean weekly cost (MWC) for the provider was built of health professional, laboratory, drugs and transfusions costs. Use of resources and costs for families were evaluated, as well as a MWC for the families. To evaluate an economic advantage between settings of care, cost-minimization analysis (CMA) was performed as well as incremental cost-effective analysis (ICER), comparing the relative costs and outcomes (occurring infections). Patient-reported symptoms over time (once per week) were measured with the M.D. Anderson Symptom Inventory (MDASI). This brief self-reported questionnaire consists of 19 items assessing symptom severity (13 items) and symptom interference (6 items).

Results: Out of 119 patients, 60 were cared at hospital, 59 at home, with a prevalence older age, worse anemia, performance status and self-efficiency score in the home care group (<0.001). Infections at first second and third week occurred more frequently in the hospital than in the home-care group (p<0.05). Mean No. of transfusions was similar in both groups. Hospital care was significantly related to a higher MWC (3,830.6 €) and lower cost for families (78.7 €), compared to home care (MWC 931.4 € and 123.2 €, respectively). At home the highest cost driver was for health providers, at hospital for drugs. Compared to hospitalization, CMA showed for home care a weekly-2899.2 € save for the health provider and +44.5 € charge for the family. Patients' families in terminal phase suffered of a mean weekly extra charge of 126.1 €. Cost-effectiveness analysis showed an advantage for home care, with an ICER – 11,315.1 € of prevented days of care for infection for all patients. No statistically significant changes in the two groups (hospital *versus* Home care) over time were found in the mean scores for symptom severity (p=0.139) and symptom interference (p=0.284).

Summary and Conclusions: Although patients at home were older and in worse clinical conditions, in this study the actual determined standard cost of home care resulted one-third of the hospital cost regardless of the phase of disease. In this setting home care resulted also cost-effective by saving money because of a lower number of occurring infections. Also, symptom burden was not different between groups suggesting that symptom management at home is feasible.

S150

HEALTH RESOURCE UTILIZATION WITH CONTINUOUS LENALIDOMIDE TREATMENT (TX) IN ELDERLY PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA (NDMM)

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Background: MM is an incurable condition associated with high Tx costs. NDMM resource consumption is driven by Tx-related hospitalization, which is highest during periods of uncontrolled disease, such as after diagnosis and during relapses (Arikian SR, ASH 2014 [abstract 2656]; Gaultney JG, *J Clin Pharm Ther*, 2013). In the pivotal FIRST trial, continuous Tx with lenalidomide plus low-dose dexamethasone (Rd continuous) was compared with fixed-duration Rd (Rd18) or fixed-duration Tx with melphalan, prednisone, and thalidomide (MPT), each for 18 mos, in NDMM pts aged ≥65 yrs and ineligible for stem cell transplant (SCT).

Aims: This analysis quantified the rates of hospitalization and medical utilization with Rd continuous based on data collected in the FIRST trial.

Methods: The FIRST trial (N =1623) is a pivotal, multinational, randomized, open-label phase 3 study. Resource utilization data was collected until patients discontinued study Tx, presented here with a median follow-up of 37 mos (data cutoff =May 24, 2013). The rates of hospitalization and medical utilization for patients treated with Rd continuous (n =535) were plotted for up to 48 mos to assess whether Rd continuous increased resource utilization over time. Procedures during the Tx period (18 mos) were compared between the 2 fixed-duration Tx arms using negative binomial regression.

Results: Resource utilization among pts treated with Rd continuous declined over time (Figure). The annualized hospitalization rate in the first 3 mos was 3.2 times higher than the average rate for the remaining 45 mos of follow-up (2.02 *vs* 0.62); medical utilization was 4.2 times higher than average (5.66 *vs* 1.34). After 4 yrs of Rd continuous Tx, hospitalization and medical utilization rates were estimated to be 83% and 84% lower, respectively, than those observed in the first 3 mos of Tx, reflecting the long-term disease control observed with Rd continuous. The highest hospitalization rates (per pt-yr) were associated with infections (0.20), musculoskeletal disorders (0.06), cardiovascular disorders (0.06), and respiratory and thoracic disorders (0.05). The mean length of stay per admission was 14.08 days (SD, 21.19). The highest medical utilization rates (interventions per pt-yr) were associated with blood transfusions (0.76), general imaging procedures (0.21), respiratory and thoracic imaging procedures (0.20), and therapeutic interventions (0.09). Hospitalization rates were 0.91 (Rd18) and 0.79 (MPT) per pt-yr of follow-up during the Tx period of 18 mos, adjusted rate ratio (RR) of 1.11 (95% CI, 0.92-1.35; *P* =0.27). The equivalent rates for medical utilization were 3.00 (Rd18) and 2.85 (MPT) inter-

ventions per pt-yr (RR =0.99 [0.81-1.22]; *P* =0.95). A comparison between the 2 fixed-duration arms showed no significant difference in the rates of medical procedures and hospitalizations.

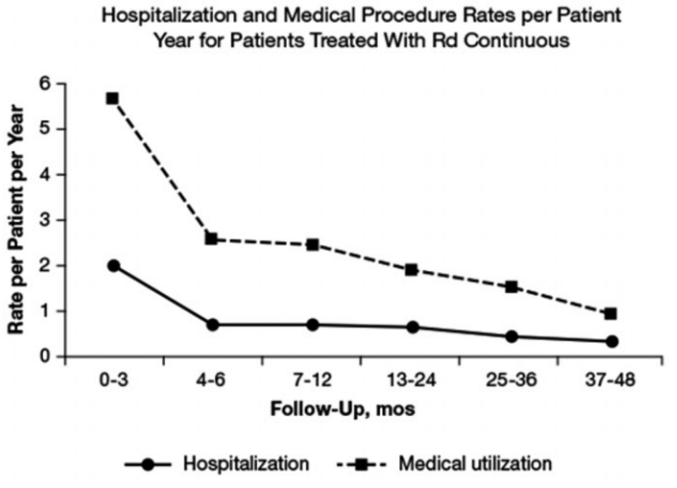

Figure 1.

Summary and Conclusions: The rates of resource utilization among pts treated with Rd continuous dropped substantially after the first 3 mos of Tx and gradually declined thereafter. The findings suggest that Tx with Rd continuous does not require a consistently high use of medical and hospital resources. Note that data on resource utilization were collected only while pts were receiving Tx. Additionally, future analysis should include all costs generated by healthcare resource utilization throughout pt Tx, including costs associated with relapses and Tx-free intervals.

POSTER SESSION

Acute lymphoblastic leukemia - Biology 1

P151

DISTINCTIVE GENOTYPES WITH PRENATAL ORIGINS IN INFANT T-CELL ACUTE LYMPHOBLASTIC LEUKAEMIA

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Background: ALL in infants is often associated with *MLL* rearrangements (*MLL-r*), a high leukocyte count at diagnosis, an immature or pro-B-cell lineage immunophenotype ($CD10^-$) and an *in utero* origin. Infant T-ALL (iT-ALL) is a very rare and poorly defined entity with a poor prognosis.
Aims: The overall aims of this study were to determine the molecular profile of iT-ALL (CNAs, LOH) and to investigate the possible prenatal onset of genetic abnormalities in iT-ALL.
Methods: The availability to us of a unique series of thirteen iT-ALL cases (≤12 months) allowed us to perform matched Genome-Wide SNP6.0 arrays accompanied by NGS (WES). Q-PCR and FISH were used to confirm the SNP-array findings and a 'Backtracking' approach with neonatal blood spots was used to investigate the early onset of these abnormalities.
Results: At diagnosis the median age was nine months, there was no predominance of gender and a high leukocyte count ($\geq 50.10^3$) was observed in 12/13 cases. Immunophenotype analyses revealed that six patients presented a T-IV profile, five T-III, one presented T-I and another a T-II profile. The T-I case (BR4) also expressed classical myeloid markers that, according to previously published criteria, suggest an Early T-cell Progenitor (ETP) leukaemia. All samples were analysed at diagnosis for various classical T-ALL abnormalities. Results showed four cases were mutated for *NOTCH1*, patient BR1 presenting a combined *NOTCH1/FBXW7* mutation, while one case (FR5) harboured a sole *FBXW7* mutation. Three cases presented *PTEN* alterations and all patients were *IL7R* WT. *MLL-r* was confirmed in three cases. Distinct from childhood T-ALL, we observed no infants with either *STIL-TAL1*+ or *TLX3*+. All diagnostic samples were analysed by SNP-array to identify CNAs/LOH. A recurrent somatic chr3 deletion was observed in 3/13 cases. These losses result in the complete deletion of *MLF1* and have not previously been described in ALL. Case FR4 harboured a large 11q14.1-11q23.2 deletion that includes the important 'driver' gene *ATM*, while patient BR5 presented a 13q14.2 deletion that involved *RB1*. Akin to non-infant paediatric T-ALL we observed a *PTEN* deletion in patient BR6 and two further cases with an 11p13 deletion that could lead to *LMO2* activation. However, we observed a lower frequency of *CDKN2A/B* deletions (38%) than found in paediatric T-ALL (70%). WES analyses for BR4 showed mutations in 22 genes previously described to play a relevant role in tumourigenesis and all tested mutations were found in the patients' Guthrie card DNAs, so confirming their presence before birth. For patient BR6 *MLL-r*, *NOTCH1* mutation and *PTEN* deletion (usually considered a postnatal event) were also detected as prenatally acquired alterations.
Summary and Conclusions: In summary, we have genetically analysed a unique series of a rare subtype of paediatric leukaemia; iT-ALLs. We find the genotypes are varied but, overall, different from those of T-ALL in older children and adults. A novel aberration (for acute leukaemia), *MLF1* deletion, was present as a recurrent abnormality in 3/13 cases. Finally, we have provided evidence that some of the genetic abnormalities, including a *PTEN* deletion, were prenatally acquired.

P152

DETAILED GENOME ANALYSES REVEAL EXTENSIVE RAG-MEDIATED REARRANGEMENTS IN ADULT ACUTE LEUKEMIA SUBSETS

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Background: Acute leukemia is characterized by a multi-step accumulation of genetic alterations in hematopoietic stem or progenitor cells. Recent next-generation sequencing (NGS) efforts led to the identification of novel genetic alterations in pediatric acute lymphoblastic leukemia (ALL) subsets, however, their

frequencies, co-occurrence in adult ALL and mutational origin remain unknown. Recent deep-sequencing efforts in pediatric *ETV6-RUNX1*-rearranged ALL cases demonstrated RAG-mediated deletions as the dominant underlying mutational process (Papaemmanuil *et al.*, Nature genetics 2014), however, this analysis was not extended to other ALL subgroups.

Aims: Our goal is to determine novel mutational patterns and to reveal novel mechanisms underlying adult ALL subgroups through integrative analyses of DNA mapping array and NGS data.

Methods: We generated genome-wide copy number profiles of 53 adult B-cell ALL (B-ALL), 20 adult T-cell ALL (T-ALL) cases using Affymetrix 6.0 DNA mapping arrays and 100 adult acute myeloid leukemia (AML) cases using Affymetrix 500K DNA mapping arrays. In addition, 5 B-ALL cases were selected for whole exome sequencing and targeted resequencing of the breakpoint boundaries with the Illumina HiSeq2500.

Results: In comparison to array-based genome characterization studies performed on ALL cohorts, we observed a substantial higher frequency of genetic lesions of well-known lesions in pediatric ALL, *e.g.*, *CDKN2A/B* (44%), *IKZF1* (23%), and *PAX5* (11%). Strikingly, we demonstrated that all T-ALL cases acquired copy number alterations perturbing the *CDKN2A/B* pathway. Interestingly, joint analysis of ALL and AML cases revealed a recurrent deletion affecting both the genes encoding NF1 and SUZ12 in 3 T-ALL and 5 AML cases, an aberration recently shown to be recurrent with concomitant mutations in melanoma and gliobastoma multiforme. We selected 5 representative B-ALL cases with extensive genetic deletions of promoters or gene bodies of genes involved in lymphoid development. Strikingly, all cases belong to the *BCR-ABL1* or *BCR-ABL1*-like ALL subgroup. In total 102 somatic mutations were detected, however, no recurrent somatic mutations were detected. In total, we detected 64 genetic deletions resulting in 128 breakpoints. *De novo* motif detection revealed the RAG-affiliated heptamer sequence CACAGTG (E=5.68x10^{-91}) for 121 out of 128 breakpoints (94.5%). Cryptic recombination signal sequence (cRSS) detection revealed that 58 out of 64 deletions (90.6%) had a 12-bp or 23-bp spacer cRSS on one or both sides of the deletion. We observed that 54 out of the 64 (84.3%) resolved rearrangements demonstrated the incorporation of non-templated sequences at the breakpoint boundaries. Chip-Seq data from the B-lymphoblastic cell line GM12878 revealed that the breakpoint boundaries are enriched for H3K4me3, H3K27ac and RNA polymerase II binding. These epigenetic alterations are typical for V(D)J-foci undergoing RAG-mediated rearrangements. Strikingly, we detected complex insertions or deletions (indels) in cRSS sequences located in promoters of genes associated with leukemogenesis, *e.g. BTLA*, often in addition to large deletions. Further screening for these open-and-shut joints in the *BTLA* promoter revealed 8 additional B-ALL cases in which at least one *BTLA* allele was affected, comprising 6 *BCR-ABL1*/*BCR-ABL1*-like and 2 B-ALL cases with unknown affiliation.

Summary and Conclusions: We demonstrated in a B-ALL subgroup comprising mainly *BCR-ABL1*/*BCR-ABL1*-like ALL cases extensive RAG-mediated rearrangements, as previously shown in *ETV6-RUNX1*-rearranged ALL. Frequently, these cases acquired kinase-activating lesions (Roberts *et al.*, NEJM 2014), providing further grounds for researching associations between these lesions and RAG-mediated rearrangements.

P153

AGE SPECIFIC INCIDENCE OF PARTNER GENE AND SECONDARY ABNORMALITIES IN MLL POSITIVE ACUTE LYMPHOBLASTIC LEUKAEMIA (ALL)

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Background: MLL gene rearrangements (MLL-t) define a unique subgroup in 5-10% patients with ALL. However, there is still heterogeneity in clinical outcomes and genetic composition. The incidence of MLL-t varies by age accounting for 80% infant, 3% children and 10% adults. Patients harbouring MLL-t have a poor outcome relative to their age-matched counterparts and are typically assigned to high risk treatment protocols. The frequency and clinical relevance of the partner gene and cooperating lesions is unclear.

Aims: We investigated the age-specific frequency of partner genes and the spectrum of additional abnormalities in 368 MLL-t cases treated on UK clinical trials over a 20 year period.

Methods: Cytogenetic and FISH analysis was performed on pre-treatment bone marrow samples using the Vysis MLL break apart probe, Kreatech Poseidon MLL fusion probes [t(4;11)(q21;q23), t(11;19)(q23;p13), t(9;11)(p21;q23) and t(6;11)(q27;q23)] and a home-grown AF10 break-apart probe. Copy number alterations (CNA) were assessed by high resolution Affymetrix arrays: SNP6 (n=14) or CytoscanHD (n=12).

Results: Among the 368 patients the most prevalent translocations were t(4;11) n=225 (64%), t(11;19) n=61 (17%), t(9;11) n=28 (8%), t(10;11) n=12 (3%) and t(6;11) n=10 (3%). Seventeen patients harboured other rare partners while the partner gene could not be determined in 15 patients. The presence of t(4;11) was age-dependent: infant (<1 year) 78/137 (57%); young children (1-9 years) 35/77 (48%); older children (10-14 years) 19/33 (58%); young adults (15-24 years) 27/30 (90%); and adults 66/76 (87%). The majority of MLL-t cases had B-cell precursor ALL (93%) but 24 (7%) patients had T-ALL and these were commonly older children and younger adults (11/24, 46%) and included 11 (46%) cases with t(11;19). In order to address the question of outcome heterogeneity by partner gene, we examined the outcome data for 47 patients (1-24 years) treated on UKALL2003 where patients with MLL-t were treated on the high risk arm. There was no evidence that the t(4;11) conveyed an inferior outcome compared with other MLL-t: 5 year event-free and overall survival rates were 74% v 70%, p=0.7 and 74% v 83%, p=0.5, respectively. Among 347 cases with successful cytogenetics, 116 (33%) cases had at least one additional chromosomal abnormality (ACA) whilst in the remaining 225 cases only the 11q23 abnormality was observed. A total of 299 ACA were counted, averaging 0.8 per case but with some variation by age (0.43, 0.70, 0.51, 0.83 and 0.32 in infants, young children, older children, young adults and adults, respectively) as well as partner gene (0.48 in t(4;11) and 1.34 in non-t(4;11)). The most common ACA were gain of chromosomes X, 6, and 8 as well as loss of 17p and i(7q). CNA analysis of 14 children and 12 adults with t(4;11) (n=22) and t(11;19) (n=4) revealed a total of 87 CNA, mostly gains (n=58). SNP array analysis of 24 patients revealed at least one CNA per case and an average of 3.3 per case. This average is fewer than that reported in other cytogenetic subgroups using lower resolution arrays (6-7 per case). Although the number of CNA was low and many were private, the increased resolution offered by the CytoscanHD array identified several regions of the genome which were recurrently affected by CNA and warrant further investigation, including 2q26~31 and 15q26.

Summary and Conclusions: In conclusion, although the number of secondary abnormalities in MLL-t is low there is evidence that it is related to both the partner gene and the age of the patient. In addition, when treated as high risk on a contemporary protocol all children and young adults with a MLL-t can achieve good OS rates.

P154

PROGNOSTIC AND THERAPEUTIC IMPLICATIONS OF RAS/RTK PATHWAY MUTATIONS IN DIFFERENT AGE COHORTS OF B-LINEAGE ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS WITHOUT KNOWN FUSION TRANSCRIPTS

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Background: We previously reported (Messina *et al*, EHA19 ABSSUB-4428) that 27% of B-lineage acute lymphoblastic leukemia without known fusion transcripts (B-NEG ALL) carries RAS/RTK pathway mutations. In particular, *FLT3* mutations were specific of patients older than 15 years, being detected in 9.8% of adolescents/young adults (AYA) and 12.3% of adult cases, while only in 2% of children. On the contrary, *KRAS* and *NRAS* mutations clustered in the pediatric cohort: indeed, they collectively accounted for 30% of pediatric cases and only 14.7% of AYA and 14% of adults. The prognostic role of these mutations has been investigated only in specific ALL subgroups with contrasting results. Moreover, being druggable, these mutations represent an attractive target also from a therapeutic perspective.

Aims: 1) To determine the prognostic impact of RAS/RTK pathway mutations in children, AYA and adult B-NEG ALL. 2) To assess the sensitivity of B-NEG ALL primary cells to gene or pathway specific inhibitors.

Methods: The impact on overall survival (OS) and disease-free survival (DFS) of RAS/RTK pathway mutations was evaluated in 150 B-NEG ALL patients (49 children, 55 AYA, 46 adults) with a median follow-up of 65 months and by grouping AYA and adults, whose mutational profile and therapeutic regimens were comparable. OS and DFS curves were estimated by Kaplan-Meier product-limit method and compared using the log-rank test (SAS software v9.4). Annexin V/7AAD apoptotic test (BD Bioscience), MTT (Sigma Aldrich) and ^{3}H-thymidine (Perkin Elmer) proliferation assays were performed to assess the sensitivity of ALL cells carrying mutations of *FLT3*, *KRAS*, *NRAS* to increasing doses (0.1-10 μM) of selected inhibitors of tyrosine kinases (dasatinib, ponatinib), *FLT3* (quizartinib, crenolanib) and of the PI3K/mTOR/MEK pathway (rapamycin, BEZ235, selumetinib). Drugs were added at time 0 and viability was measured after 72 hours. Cells were plated at 1x10^{6}/ml and each condition was run in triplicate.

Results: Survival analyses showed that AYA and adult B-NEG ALL patients harboring RAS/RTK gain-of-function mutations (*i.e.*ITD and D835 *FLT3* mutations and *KRAS*/*NRAS*G12-13 mutations)displayed a shorter OS at 4 years when compared to WT cases (18% *vs* 49.5%, 95% CI 5.4-59.3 and 38.1-64.4, p=0.074) and also a significantly shorter DFS (p=0.020), being 20% at 4 years

(95% CI 5.8-69.1) compared to 49% (95% CI 35.8-67) for WT cases. At variance, in the pediatric cohort the survival of RAS/RTK mutated cases resembled that observed in WT cases.*in vitro* sensitivity experiments showed that quizartinib and crenolanib (0.1 μM) reduced the proliferation rate of B-NEG ALL primary cells from 3 patients carrying *FLT3* ITD mutations. Indeed, after 72 hours of treatment, quizartinib reduced the percentage of proliferating cells to 31.4±7.9% and crenolanib to 44.1±23.1%. Interestingly, also the 3rd generation multi-target TK inhibitor ponatinib was highly active. Similarly, primary cells from 3 samples harboring *NRAS* or *KRAS* mutations were sensitive to PI3K/mTOR/MEK inhibitors (0.1 μM): in particular, BEZ235 reduced the percentage of proliferating cells to 18.3±12%, rapamycin to 37.3±25.9% and selumetinib to 54.1±12.3%. The same inhibitors also exerted a pro-apoptotic effect in all the samples tested. On the contrary, the non-specific compounds (ruxolitinib and tofacitinib) proved ineffective.

Summary and Conclusions: RAS/RTK pathway mutations are a common event in B-NEG ALL with a different distribution across age cohorts. We conclusively show that these mutations negatively impact on AYA and adult outcome. Our results indicate that the routinary screening of RAS/RTK signaling members in B-NEG ALL may i) improve the risk stratification of AYA and especially adults, whose outcome is still unsatisfactory; ii) open the way to targeted therapeutic strategies, as suggested by *in vitro* experiments.

P155

DUAL SMALL MOLECULE INHIBITORS TARGETING G9A AND DNMTS: A NOVEL STRATEGY FOR TREATMENT OF ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: The indisputable role of epigenetics in hematological malignancies, along with the fact that unfavorable epigenetic alterations might be reversible, has favored the development of novel epigenetic drugs. Although according to the Structural Genomics Consortium there are 377 known epigenetic proteins, currently most epigenetic drugs are inhibitors of DNA methyltransferases (DNMTS) and histone deacetylases (HDACS). Therefore, many epigenetic targets remain to be discovered and exploited.

Aims: The overall goal of our study is the identification of new epigenetic targets and design new small molecules against these targets in Acute Lymphoblastic Leukemia (ALL), with the final aim of improving the treatment and life quality of ALL patients.

Methods: We carried out a screening using a panel of small interfering RNAs (siRNAS): we interfered the expression of 134 genes belonging to the main protein families implicated in the epigenetic mechanism regulation in several cell lines of ALL and determined the effect of the interference in cell proliferation.

Results: In this way, we identified the histone H3 Lysine 9 (K9H3) methyltransferase G9a as a potential target in hematological malignancies. In order to corroborate the results obtained, we nucleofected a greater number of cell lines with two different siRNAs against G9a. As expected, inhibition of G9a expression induced a significant decrease in cell proliferation. Besides, treatment of ALL cell lines with the G9a inhibitors BIX01294 and UNC0638 induced a decreased in cell proliferation. All these data suggest that G9a is an ideal epigenetic target for the treatment of ALL. As DNMT1 and DNMT3B were also overexpressed in ALL cell lines and patient samples and DNTM1 and G9a directly cooperate in coordinated DNA and H3K9 methylation during cell division, we designed and synthesized small molecules with dual activity against G9a and DNMTs. We selected those compounds that presented an optimal affinity against G9a and DNMTs (biochemical assay) and a good *in vitro* G9a-DNMT activity inhibition, decreasing H3K9me2 and 5-methylcytosine (5mC) marks. CM-272 was selected as the lead compound, showing an important *in vitro* anti-tumoral effect with GI_{50} values lying in the nanomolar concentration range. In fact, CM-272 was significantly more active than reference compounds BIX01294 and UNC0638 (G9a inhibitors) or 5-azacytidine and decitabine (DNMTs inhibitors). Moreover, the dual small molecules induced apoptosis in addition to decrease cell proliferation. In order to elucidate the mechanism of action of CM-272 we performed a genome wide RNA-sequencing analysis with and without CM-272 treatment in ALL derived cell lines. In this analysis we observed that CM-272 induces type I interferon response and immunogenic cell death in ALL cell lines. Finally, we performed ADME, toxicity and pharmacokinetic studies in order to carry out the *in vivo* analysis in human CEMO-1 ALL mouse model. Treatment of mice engrafted with CEMO-1 cells with CM-272 induced an *in vivo* decrease of the H3K9me2 and 5mC marks, which was accompanied with a significant prolonged overall survival of treated mice (90.8 days) in comparison with control group (57.3 days). This is the first evidence of *in vivo* efficacy of G9a inhibitors.

Summary and Conclusions: In conclusion, we have identified and validated G9a as an epigenetic target for the treatment of ALL and we have developed dual small inhibitory molecules against G9a-DNMTs. Finally, our results show the therapeutic activity of G9-DNMT small inhibitory molecules in models of ALL and suggest that these compounds may be of clinical value in the treatment of patients with ALL.

P156

CLONAL ARCHITECTURES OF BCR-ABL+ BCP-ALL: IMPLICATIONS FOR ANTI B-CELL DIRECTED THERAPY

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Background: B-cell directed immunotherapies turned out to be effective in the treatment of adult patients with B-cell precursor acute lymphoblastic leukemia (BCP-ALL). However, a lack of target antigen expression in ALL subpopulations may constitute a potential mechanism of relapse and persistence.

Aims: To gain inside in potential mechanisms of resistance to B-cell specific immunotherapies by tracing the distribution of driver mutations within relevant immunophenotypic compartments differing in target antigen expression in a cohort of 27 *BCR-ABL*-fusion positive (*BCR-ABL*+) adult BCP-ALL cases.

Methods: Cryo-conserved samples from initial diagnosis of 27 adult *BCR-ABL*+ BCP-ALL patients treated within the GMALL 07/2003 trial were flow sorted onto slides and screened for *BCR-ABL* and concurrent *CDKN2A/B* and *PAX5* deletions by fluorescence *in situ* hybridization (FISH) using one customized (PAX5) and two commercial probes (Vysis LSI BCR/ABL Dual Color, Dual Fusion Translocation probe, Vysis LSI CDKN2A/CEP 9; both Abbott Molecular, Illinois, USA). The following populations were investigated: CD34+38-19-10-3-, CD34+38+19-3-, CD34+19+20-3-, CD34+19+20+3-, CD34-19+20+3-, CD34-19-20-3+, CD34+19-13/33+10-3/16-, CD34-19-13/33+10-3/16-, CD34-19-13/33+10-3/16+.

Results: Cells with a *BCR-ABL* fusion were detected not only in the B-cell compartment but also in CD19-negative multipotent progenitors (MPP) and in myeloid precursor cells in 8/9 cases expressing the M-*BCR-ABL* transcript, 2/12 patients carrying the m-*BCR-ABL* variant, in 2/3 with both transcripts and in 2/3 cases with unknown *BCR-ABL* transcript. In the remaining patients *BCR-ABL*+ cells were found exclusively within the CD19+ compartment. In 8/10 cases with concurrent *CDKN2A/B* deletions the latter were only found within the CD19+ compartment including one patient with *BCR-ABL*+ MPP. One patient with a unique *BCR-ABL*+ CD16+ compartment showed *CDKN2A/B* deletions also in the MPP and the myeloid compartment. In 4 patients distribution patterns of concurrent *PAX5* deletions resembled the affection patterns of their *CDKN2A/B* deletions. Examination of copy number alterations (CNA) of *ABL*, *BCR* and *BCR-ABL* suggested an evolution of diverse *BCR-ABL*+ subclones in 8/27 patients. The onset of subclonal diversification was observed within the B-determined CD34+19+ compartment in 6/8 cases. However, in two cases diverse *BCR-ABL*+ subclones were detectable within the multipotent CD34+38-19-compartment. Also 6/10 cases with *CDKN2A/B* deletions showed subclonal patterns regarding CNA of 9p21 and the centromeric region of chromosome 9. In these patients we observed the highest burden of *CDKN2A/B* deletions within the CD20+ leukemic compartment.

Summary and Conclusions: Our findings reveal distinct patterns of subclonal evolution in adult *BCR-ABL*+ BCP-ALL. They confirm that the t(9;22) frequently represents an antecedent event in the genesis of BCP-ALL which may occur prior to B-lineage determination. Conversely, leukemic clones were exclusively detected within the CD19+ compartment in other patients. Concurrent deletions of *CDKN2A/B* and *PAX5* are frequently restricted to the CD19+ compartment but may also originate from preleukemic MPP in patients with a *BCR-ABL*+ CD16+ compartment. In the context of B-cell directed treatment, these observations may account for both: resistance and effectiveness. A lack of target antigen expression in leukemic subclones may lead to resistance against CD19 or CD20 directed therapy, however, targeted treatment may eradicate clones with the most complex genetic profile. Our observations may help to define new stratification markers in future chemo-immunotherapeutic regiments for *BCR-ABL*+ BCP-ALL patients.

P157

RESTORING IKAROS TUMOR SUPPRESSION AS A THERAPY FOR HIGH-RISK LEUKEMIA

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Background: Ikaros (*IKZF1*) is a master regulator of hematopoietic differentiation and functions as a tumor suppressor. High-risk acute lymphoblastic leukemia (ALL) characterized by *Ikaros deletion and/or mutation* has a very poor prognosis and high relapse. Treatment is challenging for the high-risk leukemia with Ikaros deregulation because its tumor suppressor mechanisms are not well understood and targeted therapies are not available. Casein Kinase II (CK2) activity is dramatically increased in ALL. CK2 directly phosphorylate Ikaros and suppress Ikaros function. We hypothesize that CK2-mediated down-regulation of Ikaros function also plays an important role in ALL leukemogenesis, and CK2 inhibition will restore Ikaros leukemia suppressor activity.
Aims: To understand the mechanism underlying restoring Ikaros tumor suppressor activity in B-ALL and the therapeutic effect of CK2 inhibition for leukemia therapy.
Methods: Chromatin immunoprecipitation coupled with next generation sequencing (ChIP-SEQ) was used to identify the Ikaros binding targets in B-ALL cells and also the chromatin state of Ikaros targets by characterizing the binding profiling of histone modification markers. Microarray and RNA-seq were used to determine gene expression profiling. The qChIP assay was used for further analysis of protein-DNA association in leukemia cell line and primary leukemia cells. The Ikaros shRNA knockdown and retroviral transduction were used for its function analysis on gene expression and cell proliferation. Human-derived leukemia xenograft mouse model was used to observe the effect of CK2 inhibition on restoring Ikaros tumor suppressor activity and leukemia development with Xenogen IVIS 50 living imager.
Results: The genome-wide Ikaros and histone deacetylase 1 (HDAC1) binding sites were identified in the B-ALL leukemic cells. Ikaros controls cellular proliferation in leukemia by repressing cell cycle regulatory genes and the phosphatidylinositol (PI3K) pathway. Ikaros represses its target genes by forming heterochromatin through two distinct epigenetic mechanisms, one *via* recruitment of HDAC1 histone deacetylase (results in H3K27me3) and the other an HDAC1-independent mechanism (results in H3K9me3). CK2 inhibition result in the increase of more suppressive chromatin in promoter of Ikaros targets for cell cycle progress and PI3K pathway. Moreover, CK2 inhibitor has effect on gene suppression of Ikaros targets and result in cell proliferation arrest of B-ALL cells in an Ikaros-dependent manner. These data indicates CK2 inhibition restore Ikaros function in B-ALL cells. Targeted CK2 inhibition has a strong therapeutic effect on high-risk leukemia cells *in vivo* with both leukemia cell xenograft mouse model and patient-derived leukemia xenograft mouse model through restoring Ikaros leukemia suppressor activity.
Summary and Conclusions: We found the novel, Ikaros-mediated mechanisms of epigenetic regulation that contribute to tumor suppression in leukemia, demonstrated that cellular proliferation is controlled by the CK2-Ikaros axis and identified therapeutic efficacy of a novel therapeutic approach for high-risk leukemia-restoration of Ikaros tumor suppressor activity via inhibition of CK2.

P158

THE STEMNESS INHIBITOR, BBI608, REDUCES THE SELF-RENEWAL OF BCR-ABL1 POSITIVE LEUKEMIA CELLS

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Background: Hematopoietic stem cells and leukemic stem cells share common features, including self-renewal, the capacity to differentiate, resistance to apoptosis, and limitless proliferative potential. Despite these similarities, several stemness factors, such as Hedgehog, Wnt, Notch, and b-catenine show differential activation in normal *versus* leukemia stem cells. BBI608 is an oral first-in-class stemness inhibitor which inhibits the Stat3, b-catenine and Nanog patheways. In a phase I study, BBI608 demonstrated telerability as well as signs of anti-cancer activity in patients with solid tumors.
Aims: We investigated the molecular mechanisms by which BBI608 regulates the self-renewal of primary BCR-ABL1 positive leukemia cells *in vivo*.
Methods: To identify the leukemia-propagating cell fraction of BCR-ABL1-positive leukemia, we serially transplanted human leukemia cells from patients with chronic myeloid leukemia blast crisis (n=1; T315I BCR-ABL1) or ponatinib-resistant Ph-positive acute lymphoblastic leukemia (n=2, Y253H/E255K/T315I BCR-ABL1 and T315I BCR-ABL1) into NOD/SCID/IL-2gc-/-mice. The cell fractions with CD34+CD38-CD19+ and CD34+CD38+CD19+ could self-renew and transfer the leukemia in NOD/SCID mice. To investigate the effects of BBI608 on self-renewal and the relevance as a therapeutic target in ABL-tyrosine kinase-resistant BCR-ABL1 positive leukemia, we examined the activity of BBI608 against CD34+CD38-CD19+, CD34+CD38+CD19+ fractions transferred NOD/SCID mice *in vivo*. NOD/SCID mice were injected intravenously with BCR-ABL1 positive cells then treated with BBI608 (20 mg/kg; p.o.) for 28 days.
Results: All mice demonstrated the engraftment of leukemia by flow cytometry. However, the treatment with BBI608 reduced the population of CD34+CD38-positive cells. We isolated human CD45+ cells from the spleen of mice from each treatment group and injected equivalent numbers of leukemia cells into secondary recipients. Following 30 days, all mice received BCR-ABL1 cells from vehicle treated mice engrafted with leukemia. In contrast, leukemia engraftment was not detected in recipient mice (n=6) from BBI608-treated donors. These results demonstrate the persistent effects of BBI608 on long term self-renewing BCR-ABL1-positive leukemia cells. We further examined the effects of Stat3/Nanog pathway modulation on *in vitro* clonogenic growth. CD34+CD38-CD19+ cells from T315I BCR-ABL1 (n=2) and WT-BCR-ABL1 (n=1) cells were treated with 2 mM of BBI608 for 72 hrs, washed free of drugs, and plated in quadruplicate in methylcellulose. At 14 days, colonies were counted as initial plating. The representative plate was then washed and cells were re-suspended and re-plated. After an additional 14 days, colonies were counted as secondary re-plating. Clonogenic recovery of untreated cells was normalized to 100% and plating results from all treatment groups were expressed as % control. BBI608 had only minimum effects on colony formation after initial plating over control cells. However, upon serial re-plating, secondary colony formations were significantly inhibited by BBI608 ($p<0.001$). To identify the mechanisms that limit the self-renewal of BCR-ABL1-positive cells by BBI608, NOD/SCID mice engrafted with T315I-BCR-ABL1-positive CD34+ CD19+ fractions were treated with BBI608 (20 mg/kg; p.o.) for 14 days. BBI608 induced the expressions of p21Cip1, cleaved PARP and reduced the expression of BMI-1, phospho-Stat3, c-Myc, Sox-2, and Bcl-XL.
Summary and Conclusions: Our preclinical results indicate that BBI608 have potential as an important option for controlling the drug-resistant leukemia initiating cells in BCR-ABL1 positive leukemia. It is expected that the BBI608 may become extremely useful therapeutic interventions in a number of hematological neoplasms, including BCR-ABL1 positive leukemia, where the persistence of cancer stem cells.

P159

TP53 MUTATIONS IN PHILADELPHIA NEGATIVE ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) DEFINE A GROUP OF VERY HIGH RISK PATIENTS: A NEXT GENERATION SEQUENCING (NGS) STUDY

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Background: The Prognosis of adult patients with Acute Lymphoblastic Leukemia (ALL) is estimated at diagnosis on the basis of conventional clinical and biological characteristics and during treatment by the molecular or immunophenotypic evaluation of Minimal Residual Disease (MRD). New biological and molecular features are needed at diagnosis to guide prompt therapeutic strategies. Recently, few studies on heterogeneously treated cohorts of adult ALL patients reported that *TP53* mutations are frequent and correlate with poor response to induction therapy and short survival, but their real impact on ALL outcome is still undefined.
Aims: To investigate the incidence of *TP53* alterations at diagnosis and correlate them with the clinical outcome of 171 adult Philadelphia-negative ALL patients homogeneously treated in the context of a prospective clinical trial (NILG 09/2000, ClinicalTrials.gov identifier:NCT00358072)(Bassan R et al, Blood 2009 and BJC 2014).
Methods: One hundred fourteen B and 57 T-ALL patients were studied (median age 34,6 years, 97 male). Exons 4 to 11 of the *TP53* gene were analyzed on diagnostic DNA and in 3 cases also on relapse DNA by Next Generation Sequencing (NGS, 454 Roche platform). Results were validated, when possible, by Sanger sequencing. Disease-free survival (DFS) and Overall survival (OS) were estimated by the Kaplan Meier method. The log-rank test was used to compare survival probabilities between subgroups of patients.
Results: *TP53* mutations occurred in 8% of patients (14/171) with a higher prevalence in B lineage ALL (9,6 *vs* 5,3% in T-ALL). The allele burden ranged from 5 to 97% pointing out that *TP53* alterations can be present at diagnosis in minor leukemic clones. All alterations were identified in the DNA binding domain region and led to missense or frame-shift mutations introducing a premature stop codon. The found alterations were 12 single nucleotide changes, 2 duplications (4 and 8 nucleotides) and one 11 base pair insertion. The same *TP53* mutations were seen at diagnosis and relapse in three patients for whom DNA was available at both time points, suggesting the presence of a clone bearingmutated*TP53* since disease onset. By univariate analysis, *TP53* mutations were strictly related to older age ($p=0.0003$) but not to other clinical feature. In our cohort, the presence of a *TP53* mutation did not affect CR achievement but relapse rate was significantly higher in mutated cases ($p<0.0001$) (Figure1a). Moreover, patients with mutated *TP53* showed a significantly shorter DFS and OS compared to wild-type patients (DFS $p=0.0007$, OS $p=0.0011$) (Figure 1b-c). NGS analysis also revealed single nucleotides changes that were reported as polymorphism (SNP) in IARC and dbSNP databases. One patient bearing a *TP53* mutation also revealed an unbalanced SNP distribution along the *TP53* sequence confirming the known karyotypic monosomy of chromosome 17. Similarly, other 3 patients showed a *TP53* SNP unbalance without a karyotipic evidence, thus suggesting a cryptic *TP53* gene deletion.

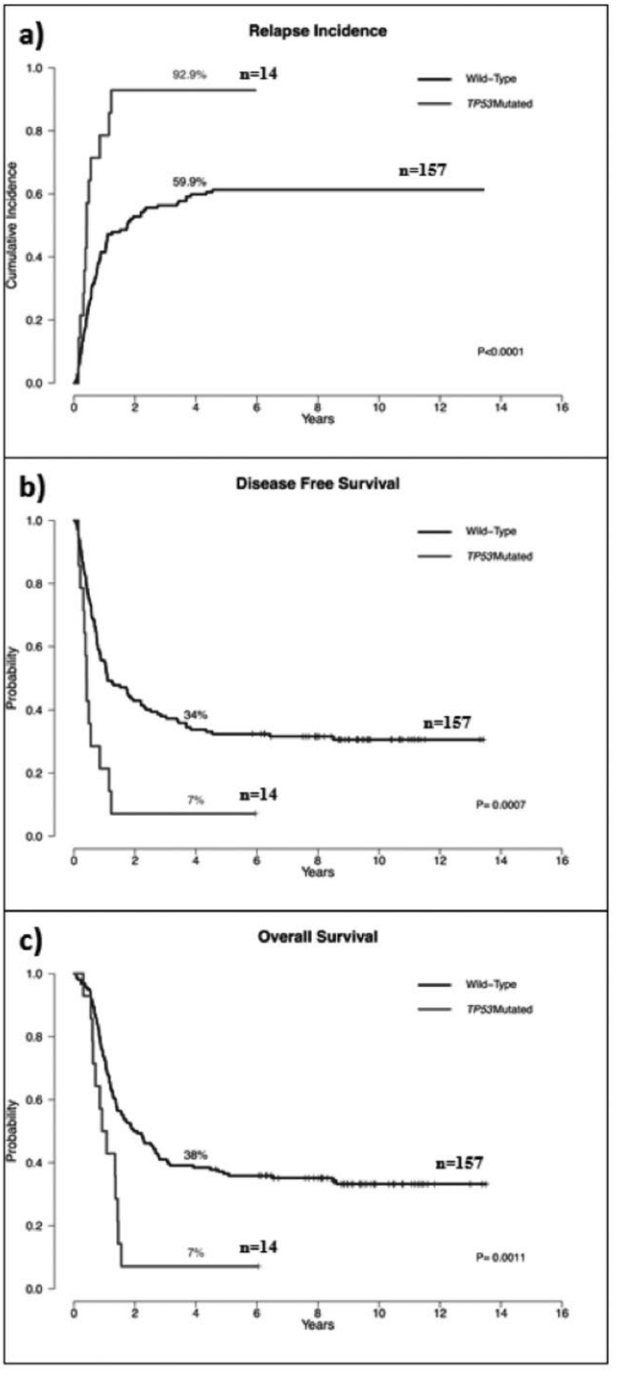

Figure 1. Relapse Incidence (a), Disease Free Survival (b) and Overall Survival (c) in *TP53* mutated vs Wild-type Ph-negative adult ALL. Percentages are indicated at 4 years.

Summary and Conclusions: In our adult ALL cohort, mutated *TP53* does not impair CR achievement, but it identifies a group of patients with higher relapse-rate and dramatically shortened DFS and OS. Therefore, *TP53* aberrations detection must be included in the diagnostic work up of adult ALL to guide treatment strategies. The use of highly sensitive NGS is crucial to identify also minor *TP53* mutated clones that may lead to disease recurrence and to reveal *TP53* gene deletions not detected by conventional karyotype.

P160

TUMOR SUPPRESSOR GENES IKZF1 AND BTG1 COOPERATE IN ACUTE LYMPHOBLASTIC LEUKEMIA DEVELOPMENT AND GLUCOCORTICOID THERAPY RESISTANCE

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Background: Previously, we and others have shown that IKZF1 (IKAROS) gene deletions and mutations independently predict poor prognosis in children with BCP-ALL, and IKZF1 gene alterations have now been incorporated in the risk stratification of Dutch pediatric BCP-ALL patients. However, it remains to be established whether loss of IKZF1 function has a direct impact on chemotherapy responses. Our recent findings show that loss of IKZF1 function promotes resistance towards glucocorticoid-induced apoptosis in normal and leukemic B-cells, which correlates with attenuation of the transcriptional response to glucocorticoids. Furthermore, the effect of other genetic aberrations on therapy outcome of *IKZF1*-deleted patients has not been fully addressed. Our analysis has revealed that the event-free survival of *IKZF1*-deletion positive patients is negatively affected by the co-occurrence of *BTG1* deletions. In addition, *BTG1* deletions appear to occur more frequently in patients with IKZF1 gene alterations (19%) compared to an unselected cohort (9%).
Aims: Westudied the genetic interaction between IKZF1 and BTG1 in leukemia development and glucocorticoid therapy responses using knockout mouse models and human leukemia cell lines.
Methods: Mice haplodeficient for *Ikzf1* were crossed onto a C57BL/6J *Btg1* knockout background and mice were monitored for spontaneous leukemia development over a time period of 18 months. Additionally, glucocorticoid response was determined in lymphoid cells isolated from compound *Ikzf1*$^{+/-}$;*Btg1*$^{-/-}$mice by MTS assay and AnnexinV staining and compared to *Ikzf1*$^{+/-}$, *Btg1*$^{-/-}$and wild-type B cells. Additionally, sensitivity towards glucocorticoid induced apoptosis was determined in human leukemia cell lines displaying loss of IKZF1 and BTG1 function.
Results: We observed a significant acceleration in the onset of T acute lymphoblastic leukemia in mice heterozygous knockout for both *Ikzf1* and *Btg1*, which was even further enhanced in *Ikzf1*$^{+/-}$;*Btg1*$^{-/-}$animals. These leukemias were characterized by clonal TCRb rearrangement and aggressive infiltration into secondary organs. B-cells isolated from 8 to 14 weeks-old *Ikzf1*$^{+/-}$;*Btg1*$^{-/-}$ animals without any signs of leukemia disease were highly resistant against glucocorticoid-induced apoptosis. Similarly, human leukemia cell lines displaying loss of IKZF1 and BTG1 function showed a glucocorticoid-resistant phenotype that was even stronger than by loss of IKZF1 alone.
Summary and Conclusions: Together, our findings establish *BTG1* as a tumor suppressor gene that genetically interacts with *IKZF1* during leukemic transformation and strongly potentiates the IKZF1-mediated glucocorticoid resistance phenotype in normal and leukemic B cells.

Acute lymphoblastic leukemia - Clinical 1

P161

INFLUENCE OF BASELINE FACTORS ON OUTCOMES IN PATIENTS (PTS) WITH RELAPSED/REFRACTORY B-PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA (R/R ALL) TREATED WITH BLINATUMOMAB

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Background: Blinatumomab is a bispecific T-cell engager (BiTE®) antibody construct with dual specificity for CD19 on B-lineage cells and CD3 on T cells. Blinatumomab showed efficacy in Philadelphia-chromosome-negative (Ph–) R/R ALL in a large, multicenter, single-arm, phase 2 study (Topp *et al. Lancet Oncol.* 2015;16:57-66) and is now approved in the United States for this indication.
Aims: To evaluate associations between pt baseline characteristics and outcomes in the phase 2 study.
Methods: Pts (≥18 years) with Ph– B-precursor R/R ALL (primary refractory after induction; relapse <12 months after 1st remission; relapse <12 months after HSCT; relapse or refractory after 1st salvage), ≥10% bone marrow blasts [BMB], and ECOG performance status ≤2 were included in the study. All pts provided informed consent. Blinatumomab was given by continuous IV infusion (4 weeks on, 2 weeks off) for ≤5 cycles (cycle 1: 9µg/d days 1–7, then 28µg/d; subsequent cycles: 28µg/d). The primary endpoint was complete remission (CR, ≤5% BMB, ≥100 000 platelets/µL, absolute neutrophil count [ANC] >1000/µL) or CR with partial hematologic recovery (CRh, ≤5% BMB, >50 000 platelets/µL, ANC >500/µL) within 2 cycles. Associations of baseline factors (platelet count, prior salvage, BMB, peripheral blood blasts, age, geographic region, prior HSCT, number of prior relapses, and lactate dehydrogenase [LDH]) with CR/CRh and overall survival (OS) were assessed using the Cochran-Mantel-Haenszel test and univariate and multivariate logistic regression.
Results: Of the 189 pts treated with blinatumomab, 81 (43%) achieved CR/CRh within 2 cycles. Median (95% CI) relapse free survival was 5.9 (4.8–8.3) months; median (95% CI) OS was 6.1 (4.2–7.5) months. CR/CRh was seen in all groups, including 11 of 25 pts (44%) ≥65 years of age, 29 of 64 pts (45%) with prior HSCT, 52 of 125 pts (42%) without prior HSCT, and 11 of 32 pts (34%) with ≥3 prior salvages. Pts with <50% BMB at baseline were more likely to respond to blinatumomab treatment; 43 of 59 pts (73%) with <50% BMB achieved CR/CRh, while 38 of 130 pts (29%) with ≥50% BMB achieved CR/CRh. In the final multivariate model, lower BMB and higher platelet count were associated with CR/CRh; higher platelet count and lower LDH were associated with longer OS (Table).

Tabella 1.

Response (CR/CRh)			
	OR	95% CI for OR	*P*[a]
Platelet Count (10^9/L)			0.0341
< 50 vs ≥ 100	0.3	(0.1–0.8)	
50 to < 100 vs ≥ 100	0.3	(0.1–1.0)	
BMB			< 0.0001
< 50% vs ≥ 50%	5.1	(2.5–10.5)	
Survival (OS[b])			
	HR	95% CI for HR	*P*[c]
Platelet Count (10^9/L)			< 0.0001
< 50 vs ≥ 100	4.2	(2.3–7.8)	
50 to < 100 vs ≥ 100	2.6	(1.3–5.4)	
LDH			0.0082
≥ 230 vs < 230	1.9	(1.2–3.1)	

[a]*P* value based on logistic regression model (Wald test). [b]OS model controlled for age.
[c]*P* value based on Cox proportional hazard model. OR: odds ratio. HR: hazard ratio.

Summary and Conclusions: In this analysis, low baseline BMB count was associated with response to blinatumomab. This supports administration of blinatumomab when disease burden is low. For pts with higher tumor burden, cytoreduction/higher blinatumomab doses may be considered. Responses to blinatumomab have been observed in pts in remission with minimal residual disease (Gökbuget *et al. Blood.* 2014;124:379), suggesting that pts have low biologic resistance to blinatumomab even after intensive chemotherapy.

P162

CHARACTERIZATION OF LEF1 HIGH EXPRESSION AND NOVEL MUTATIONS IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Aberrant activation of the Wnt pathway plays pathogenetic roles in tumors and has been associated with adverse outcome in acute lymphoblastic leukemia (ALL). Lymphoid enhancer binding factor 1 (*LEF1*), a key mediator of Wnt signaling, has been linked to leukemic transformation, *and LEF1* mutations have been identified in T-ALL.
Aims: To characterize the *LEF1* high expression and novel mutations in Chinese adult ALL.
Methods: Bone marrow (BM) samples from 131 newly diagnosed patients (82 male and 49 female) with ALL (87 B-ALL, 43 T-ALL and 1 T-/B-ALL) were collected between June 2008 and July 2013 at the First Affiliated Hospital of Nanjing Medical University. All the patients provided their written informed consent in accordance with the Declaration of Helsinki before enrollment in the study. The study was approved by the Institutional Review Board of the Nanjing Medical University. We screened mutations of*LEF1*exons 2 and 3, the hotspot regions in T-and B-ALL with the pretreatment BM samples of the cohort. The *LEF1* mutations were created by site-direct mutagenesis and confirmed by sequencing.The transient luciferase assay was performed in HEK293T cells using the Promega' luciferase assay reagents and measured with luminometer following the manufacture's instruction. The colorimetric cell proliferation assay (WST-1 reagent from Roche Applied Science) was performed in Nalm6 and Molt4 leukemia cells stably expressed LEF1-WT or its mutants. The Whitney U –test for quantitative parameters, a $\chi 2$ test for qualitative parameters, and Kaplan –Meier survival analysis were used in the cohort study. All statistical analyses were performed using the SPSS 17.0 and $p<0.05$ was considered statistically significant.
Results: Patients were divided into high and low*LEF1*expression groups (Q4 *vs* Q1-Q3). We found *LEF1* was highly expressed in 25.0% adult ALL patients and *LEF1* high expression was associated with high-risk leukemia factors (high WBC, Philadelphia chromosomepositive and complex karyotype), shorter event-free survival (EFS), and high relapse in patients with B-ALL. In addition, *LEF1* high expression was observed to associate with high mutation rate of *Notch1* and *JAK1* in T-ALL. Moreover, we identified 2 novel *LEF1* mutations (K86E and P106L) in 4 of 131 patients with ALL, and those patients with high-risk ALL (high WBC, complex karyotype). These results suggest a role for *LEF1* mutations in leukemogenesis. We further explored the effect of the mutations on cell proliferation and found both mutations significantly promoted the proliferation of ALL cells. We also observed the effect of *LEF1* and its mutations on the transcription of its targets, *c-MYC* and *Cyclin D1*. We found *LEF1* increased the promoter activity of its targets *c-MYC* and *Cyclin D1*, and *LEF1* K86E and P106L mutants further significantly enhanced this effect. We also observed that the *c-MYC* and *Cyclin D1* mRNA levels were significantly increased in patients with *LEF1* high expression compared with those with low expression.
Summary and Conclusions: Our findings indicate high *LEF1* expression and mutations are associated with high-risk leukemia. Our data also revealed that *LEF1* high expression and/or gain-of-function mutations are involved in leukemogenesis of ALL.

P163

HIGH RATE OF COMPLETE HEMATOLOGICAL RESPONSE IN ELDERLY PH+ ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) PATIENTS BY INNOVATIVE SEQUENTIAL USE OF NILOTINIB AND IMATINIB: A GIMEMA CLINICAL TRIAL LAL 1408

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Background: Tyrosine Kinase Inhibitors (TKI) have been shown to be very effective for the treatment of Acute Lymphoblastic Leukemia (ALL), with a Complete Hematologic Response (CHR) rate close to 100%, and a high rate of Complete Cytogenetic and Molecular responses (CCgR and CMR). However, when they are used alone, as single agents, most patients relapse, so that they are currently used in combination with chemotherapy and as a preparation to allogeneic stem cell transplantation (SCT). Since Ph+ ALL is more frequent in the elderly, many patients cannot tolerate intensive chemotherapy and are not eligible for SCT. We have explored if the administration of two TKIs, Nilotinib (NIL) and Imatinib (IM) can improve the results without increasing the toxicity.
Aims: To evaluate the response and the outcome of Ph+ ALL patients treated with the sequential administration of NIL and IM, and to investigate the type and number of BCR-ABL kinase domain mutations developing during and after the study.
Methods: We have designed a study (ClinicalTrials.gov. NCT01025505) in which patients more than 60 years old or unfit for intensive chemotherapy and SCT where treated with two TKIs, NIL 400 mg twice daily, and IM 300 mg twice daily, alternating for 6 weeks for a minimum of 24 weeks (study core) and indefinitely in case of response. The 6-weeks rotation schedule was respected, irrespectively of temporary discontinuations. The primary end-point was the rate of Disease Free Survival (DFS) at 24 weeks (4 courses of treatment); the secondary end points included the evaluation of CHR, CCgR and CMR rates. Mutation analysis was performed by nested RT-PCR amplification of the *ABL* kinase domain of the *BCR-ABL* transcript (codons 206 through 421). Amplified products were screened by denaturing-high performance liquid chromatography (D-HPLC). Samples scored positive for the presence of sequence variations were then subjected to direct automatic sequencing to characterize the mutation.
Results: 39 patients have been enrolled in 15 Italian hematologic Centers (median age 66 years, range 28-84, M/F=19/20). Among these, 8 patients were unfit for standard chemotherapy or SCT (median age 50 years, range 28-59). 27 patients were p190, 5 were p210 and 7 were p190/p210. After 6 weeks of treatment, 37 patients obtained a CHR (95%) and 2 a PHR (5%). All the patients were evaluable for response after 12 weeks of treatment: 35 patients were still in CHR (90%), 2 relapsed (5%) and one patient died. 25/26 patients who have completed the study core (24 weeks), were in CHR, and 22 continued therapy in the protocol extension phase.Thus, the OS at 1 year is 82%, and 64% at 2 years (median follow up 34.9 months, range 3.5-42.4 months). Overall, 25 patients relapsed, with a median time to relapse of 9.2 months (range 1.2-28.4 months), for a DFS of 48.7% at 12 months. Most of mutations detected at relapse were T315I, E255K, E255V and Y253H. Further details about Cytogenetic and Molecular responses, and about Adverse Events will be provided on site.
Summary and Conclusions: In this small cohort of Ph+ ALL elderly/unfit patients, the rates of relapse and progression were not likely to be different from the rates observed with Imatinib alone. It's important to notice that the mutations that occurred at the time of relapse were sensitive to other TKIs (Dasatinib and Ponatinib).
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P164

INTERNATIONAL MULTI-LABORATORY NEXT-GENERATION SEQUENCING FOR MRD ANALYSIS IN ALL. A PILOT STUDY BY THE EUROCLONALITY-NGS CONSORTIUM

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Background: Amplicon-based next generation sequencing (NGS) of immunoglobuline (IG) and T-cell receptor (TR) gene rearrangements to quantify minimal residual disease (MRD) in lymphoid neoplasms is already the focus of intense research, development, and application. This is mainly due to its potential to overcome limitations of the current molecular gold standard, real-time quantitative (RQ)-PCR, as it avoids laborious design and testing of patient specific assays and allows quantification of MRD with a more specific readout than RQ-PCR. However, standardization, quality control and validation of the technology in a multicentre, scientifically controlled and independent setting is highly warranted, but currently lacking.
Aims: To investigate an optimized IG/TR based NGS approach (including quality controls and calibrators) for sensitive and quantitative MRD assessment in lymphoid neoplasms within the EuroClonality NGS Consortium.
Methods: Within a European Multicentre trial a newly designed quality controlled one-step IGH NGS approach was tested in seven institutes (Kiel, Salamanca, Milano, Bristol, London, Prague, and Torino). Serial dilutions of diagnostic DNA and follow-up samples of thirty B-cell precursor ALLs with known complete IGH rearrangements as well as a set of positive and negative controls were analysed. All samples were spiked with pre-defined copy numbers of five reference IGH sequences. One-step PCR was used for library preparation with sets of FR2 primers harbouring sample specific barcodes and 500ng DNA per sample (75.000 copies), subsequently being sequenced with the Illumina V2 kit on the Illumina MiSeq NGS platform. Purpose-built bioinformatics methods were used to analyse data. MRD results of ALL follow-ups were compared to results of EuroMRD based RQ-PCR.
Results: A total of 312 samples were sequenced within 24 deep sequencing runs producing 168 million reads. Regression analysis of reference IGH allele reads was used to calculate the read coverage per copy, and correct for different B-cell content per sample. Ratios of reads between pairs of reference IGH alleles were highly consistent in intra-and inter-centre analyses, independent of the total number of reads in the sample. This high consistency in the usage of the reference IGH alleles was confirmed through redundancy analysis based on normalized reads (adjusted p-value=0.24). When outliers were identified, indicating poor assay performance, those were excluded from further analysis. The IGH gene rearrangements of all 27 pooled positive B-cell line controls were identified in all centres with a high intra-and inter-centre consistency. NGS based MRD analysis in 116 ALL follow-up samples revealed MRD positivity in 69/116 samples, and RQ-PCR in 66/116 samples, with a good overall concordance between both approaches (R^2=0.81). Discrepancies mainly affected samples with low MRD levels being consistent with Poisson sampling.
Summary and Conclusions: The approach newly developed by the EuroClonality NGS Consortium shows great promise to allow for quality controlled NGS based MRD analysis in a multicentre setting with a high intra-and inter-laboratory reproducibility. The critical next step, as part of the Consortium´s strategy, involves validation of this technology, prior to its implementation into routine practice, within multicentre clinical trials, in a scientifically controlled, independent, and multidisciplinary setting.

P165

RETREATMENT WITH BLINATUMOMAB AFTER CD19-POSITIVE RELAPSE: EXPERIENCE FROM 3 TRIALS IN PATIENTS WITH RELAPSED/REFRACTORY B-PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA (R/R ALL)

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Background: Blinatumomab is a bispecific T-cell engager (BiTE®) antibody construct designed to link CD19-positive B cells to cytotoxic T cells and induce tumor cell lysis. Blinatumomab recently showed efficacy in Philadelphia-chromosome-negative (Ph–) R/R ALL in a large, multicenter, single-arm, phase 2 study (Topp *et al. Lancet Oncol.* 2015;16:57-66).
Aims: To determine the potential benefit of retreatment with blinatumomab among patients with R/R ALL who had previously responded to blinatumomab.
Methods: In this combined analysis, we evaluated outcomes among patients with R/R ALL enrolled in 3 open-label, phase 2 studies (NCT01471782: pediatric and adolescent R/R ALL, study 205; NCT01209286: adult R/R ALL, study 206; and NCT01466179: adult R/R ALL, study 211) who had a CD19-positive hematological relapse after initial response to blinatumomab and were retreated with blinatumomab. Patients were <18 (study 205) or ≥18 (studies 206 and 211) years of age with Ph– R/R B-precursor ALL. All patients or their legal representatives provided informed consent. Blinatumomab was given by continuous IV infusion for up to 5 cycles. A cycle was 4 weeks on, 2 weeks off. The primary endpoint of the 3 studies was complete response or complete response with partial recovery of peripheral blood counts within the first 2 cycles. Patients who relapsed following a response that lasted ≥3 months could receive up to 3 cycles of retreatment; patients with GVHD or CNS involvement were ineligible.
Results: A total of 11 patients received retreatment with blinatumomab (study 205, n=2; study 206, n=5; study 211, n=4). Seven patients (64%) were male; mean (range) age was 31 (4–77) years. At the time of initial treatment, 9 patients (82%) had ≥1 line of prior salvage chemotherapy; 7 (64%) had prior HSCT. Among the 10 patients who achieved hematological remission during the 2 cycles of initial treatment, the median (range) duration of response prior to

relapse was 9 (3–11) months. Five patients (45%) had HSCT between the first blinatumomab response and relapse. Outcomes and adverse events (AEs) during retreatment are shown in the Table. Four of 11 patients (36%) with R/R ALL who relapsed following an initial response to blinatumomab responded to retreatment with blinatumomab. One patient was retreated a second time and achieved a complete response.

Tabella 1. a≤5% marrow blasts and full, partial, or incomplete recovery of peripheral blood counts. bThere were no grade 4 or grade 5 neurologic AEs.

	Retreated Patients (n=11)
Median (range) duration of retreatment, days	28 (4–85)
Hematological remission (responders), n (%)	4 (36%)a
Minimal residual disease (MRD) responses among responders, n (%)	
MRD<10-4	4 (100%)
Complete MRD responses	1 (25%)
Patients with any AEs, n (%)	10 (91%)
Grade≥3 AEs	8 (73%)
Grade≥3 neurologic AEs	3 (27%)b
Cytokine release syndrome	0 (0%)

Summary and Conclusions: Our results suggest that patients with R/R ALL who have responded to treatment with blinatumomab and have then relapsed may respond to retreatment. Overall, rates of AEs during retreatment were in line with recent clinical experience with blinatumomab.

P166

CENTRAL NERVOUS SYSTEM AS AN IMMUNOLOGICAL SANCTUARY FOR ACUTE LYMPHOBLASTIC LEUKEMIA: ROLE OF NATURAL KILLER CELLS

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Background: Central nervous system acute lymphoblastic leukemia (CNS-ALL) is a major clinical problem. Prophylactic therapy is neurotoxic and a third of the relapses involve the CNS. We have previously reported that increased expression of interleukin 15 (IL-15) in leukemic blasts is associated with increased risk for CNS-ALL. As IL-15 is a strong activator of natural killer (NK) cells we hypothesized that NK cells may be involved in regulating CNS leukemia.
Aims: To characterize the role of IL-15 and NK cells in CNS-ALL.
Methods: We have created two in-vivo mouse models: Syngeneic S49 T lymphoma leukemia in BALB/c and human ALL cell lines and primary human ALL xenografts in NOD/SCID and NOD/SCID/IL2Rγ null (NSG) mice. NK cells were manipulated in-vivo by antibody mediated depletion and by human peripheral blood NK (PBNK) cell therapy of xenografted mice. In-vivo studies were complemented by in-vitro killing assays and NK ligand and receptor characterization. Results from mouse models were verified by a case-control study involving RNA and flow cytometry analyses of diagnostic bone marrow leukemia samples from prospective BFM trials of childhood ALL. Ethical approvals were obtained for all mouse and human experiments.
Results: Transplantation of IL-15 expressing S49 leukemia cells in neonatal mice caused marked isolated CNS and ophthalmic leukemia and activation of NK cells. A human B-cell precursor ALL cell line, 018Z, expressing endogenous IL-15 caused isolated CNS leukemia in NOD/SCID, an immunodeficient mouse strain with proficient NK cells. Yet it caused systemic CNS/peripheral leukemia in NSG mice that lack NK cells. Antibody mediated depletion of NK cells in NOD/SCID mice resulted in aggressive combined systemic and CNS leukemia, suggesting that induction of NK cells prevented peripheral but failed to control CNS leukemia. Therapeutic experiment with human PBNK confirmed that NK cells do not enter the brain even in the presence of leukemic infiltration. The killing of human leukemia lymphoblasts by NK cells depended on the expression of the NKG2D receptor. These findings were confirmed in primary human ALLs. The degree of peripheral/CNS leukemia of seven independent primary human ALL xenografts in NOD/SCID mice significantly correlated with IL-15 expression and in-vivo activation of NK cells (P<0.04). Expression of NKG2D receptor evaluated by qRT-PCR and by flow cytometry of NK cells infiltrating diagnostic bone marrows of children treated by BFM protocols for ALL correlated with subsequent CNS involvement (P=0.001). Conversely, the expression of secreted inhibitory "decoy" NK ligands ULBP1 and ULBP3 inversely correlated with CNS positivity (P<0.0001)
Summary and Conclusions: The CNS may be an immunological sanctuary for ALL cells protected from NK cell activity. Markers of activation of NK cells (higher IL15, NKG2D and lower (ULBP1+ULBP3)/ULBP2 ratio) at the time of diagnosis of ALL may be biomarkers for increased risk of CNS relapse. Addition of CNS prophylactic therapy should be considered in NK based therapeutic protocols for hematopoietic malignancies.

P167

RITUXIMAB PLUS MULTIAGENT CHEMOTHERAPY FOR NEWLY DIAGNOSED CD20-POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA: A PROSPECTIVE MULTICENTER PHASE II STUDY (RADICAL; KALLA0804) BY KOREAN ADULT ALL WORKING PARTY

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Background: Clinical significance of CD20 expression in adult acute lymphoblastic leukemia (ALL) has been evaluated previously, showing that the expression of CD20 was correlated with a bad prognosis in ALL. The addition of rituximab to the conventional treatment showed a promising result in a prospective observational study in a center, although no well-designed prospective interventional study has been performed until now.
Aims: This prospective study has been performed to find out the efficacy and safety of rituximab plus multiagent chemotherapy for patients with CD20-positive acute lymphoblastic leukemia.
Methods: Subjects with newly diagnosed ALL who aged ≥15 years old and were in their good condition were eligible when their leukemic blast cells of bone marrow aspirate express ≥20% of CD20 antigens at time of diagnosis. All patients received induction treatment composed of vincristine/prednisolone/daunorubicin/L-asparaginase (for Ph-neg) or imatinib (for Ph-pos) plus rituximab (375mg/m^2) at day 8. After achieving hematologic complete remission (HCR), subjects received five cycles of consolidation therapy with concomitant rituximab followed by 2 years of maintenance (non-alloHCT). Allogeneic hematopoietic cell transplantation (alloHCT) could be performed whenever after achieving HCR. All subjects received 8 times of rituximab during the whole treatment, irrespective of the adoption of alloHCT-if he/she continued to alloHCT during the consolidation, rituximab was administered monthly from day 90 of alloHCT. Data from 103 patients who were treated with the same cytotoxic drug combination without rituximab in Asan Medical Center from 1997 until 2010 were collected as a historical control group. Toxicity was graded according to National Cancer Institute Common Toxicity Criteria (version 4.0). Data were frozen up in May 2014 for this interim analysis.
Results: Thirty six subjects were eligible for interim analysis-3 subjects were excluded for various causes. The median age of subjects was 42.0 (range 18-71), 31% of them were Ph-positive and 75% were high risk. The median value of CD20 expression was 52.5%. Hematologic CR (HCR) rate was 97% and only one subject failed to achieve HCR owing to death in aplasia during induction. Fourteen subjects received allogeneic hematopoietic cell transplantation (alloHCT) after achieving HCR. Median number of consolidation therapy was 3, and rituximab was introduced 5 times (median) to each subject-rituximab was skipped only 3 times during the cytotoxic chemotherapy schedules of all subjects. After 11.5 months of median follow-up for surviving subjects, the 1-year and 21 –month relapse-free survival (RFS) were 71.5% and 49.1%, respectively, which were significantly higher than those of historical control (55.5% and 38.9%, *p*=0.041). The overall outcomes were not significantly dif-

ferent by the risk group, Ph-status, and the performance of alloHCT. Whereas, the 1-year RFS of subjects with high CD20 expression level (the same or more than the median value) was superior to those with low level (91.7% *vs* 46.4%, *p*=0.033), and this difference of outcome was not observed among the historical control group. Hematologic/non-hematologic toxicity profiles were all tolerable. Only 10 times of infusion-related adverse events associated with rituximab happened, except 1 episode of grade-3 anaphylaxis. Especially, the incidence of acute graft-*versus*-host disease (GVHD) of ≥grade 2 was 21%, and that of chronic GVHD was 21%, which were all tolerable.
Summary and Conclusions: In this interim analysis of the prospective study, combination of rituximab with multiagent chemotherapy was feasible; all adverse events were tolerable, and all the outcomes were not inferior to the results of our historical control. Based on these results, we will continue to recruit subjects up to a total of 77 ones. (Clinicaltrials.gov NCT01429610).

P168

ADAM28 OVEREXPRESSION REGULATED THROUGH PI3K/AKT PATHWAY IS ASSOCIATED WITH POOR PROGNOSIS IN DE NOVO ADULT B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: B-cell acute lymphoblastic leukaemia (B-ALL) in adults is a very challenging disease. Most patients will go into complete remission, but relapse often occurs. Relapse following remission induction chemotherapy remains the major barrier to patient survival, and relapse is an important part of overall mortality in B-ALL patients after haematopoietic stem cell transplantation. Many relapse patients are currently stratified as standard risk; therefore, additional more accurate biomarkers for relapse and prognosis are needed to improve patient stratification. ADAM28 is an ADAM (a disintegrin and metalloproteinases) metalloproteinase that can cleave myelin basic protein. Studies have demonstrated that ADAM28 is overexpressed in several human tumours and is related to cell proliferation and lymph node metastasis (Takashi Ohtsuka, *et al*. Int. J. Cancer 2005). To date, no information is available on the prognostic role of ADAM28 in B-ALL.
Aims: The aim of this study was to assess ADAM28 expression levels with regard to the potential role of ADAM28 as a prognostic biomarker in B-ALL.
Methods: In total, 50 consecutive patients with de novo B-ALL and 23 healthy donors were enrolled in this study. After obtaining informed consent, ADAM28 expression levels were analysed by flow cytometric assessment and real-time quantitative polymerase chain reaction. Relapse-free survival (RFS), event-free survival (EFS) and overall survival (OS) data after a 2-year follow-up were prospectively collected for prognostic evaluation. *in vitro*, B-ALL cells were isolated from the bone marrow of patients to assess the effect of arsenic trioxide (ATO) on ADAM28 expression. Using selective pharmacologic inhibitors, the PI3K/Akt pathway was demonstrated to be involved in the regulation of ADAM28 expression.
Results: Our data are the first to demonstrate that ADAM28 expression in leukaemic cells from B-ALL patients was significantly increased compared with donor bone marrow cells (P=0.0003). A significant inverse correlation was observed between ADAM28 levels in bone marrow cells and serum expression levels (r^2=0.7512; P=0.0025). Despite considerable variability in expression levels in B-ALL cells, ADAM28 levels were not associated with established risk factors, such as white blood cell count at diagnosis, immunophenotype and karyotype. However, patients experiencing disease relapse or death exhibited significantly increased ADAM28 expression compared with those with a favourable outcome (p=0.0043). Notably, ADAM28 overexpression was associated with a lower probability of relapse-free survival and event-free survival (P<0.001) and was a significant prognostic factor (P=0.010, 0.003, respectively) in a multivariate analysis. *in vitro*, the expression of ADAM28 in leukaemic cells from B-ALL patients was inhibited by ATO in a time-and dose-dependent manner. A significance increase in migratory activity was noted in B-ALL cells overexpressing ADAM28 using the Boyden chamber method. The PI3K/Akt pathway inhibitor down-regulated ADAM28 expression and decreased cell migratory activity.
Summary and Conclusions: Our results are the first to indicate that ADAM28 overexpression in B-ALL patients is correlated with poor outcome. ADAM28 overexpression in B-ALL identifies patients with a high risk of early relapse and reduced RFS, EFS and OS. ADAM28 overexpression enhances cell migration, which is potentially regulated by the PI3K/Akt pathway. These data suggest that ADAM28 might serve as a novel biomarker to evaluate the prognosis of B-ALL and a potential therapeutic target in B-ALL patients.

P169

ALLOGENEIC STEM CELL TRANSPLANTATION FOR PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA WHO HAD CENTRAL NERVOUS SYSTEM INVOLVEMENT: A STUDY FROM THE ADULT ALL WORKING GROUP OF THE JSHCT

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Background: The prognosis for adult acute lymphoblastic leukemia (ALL) patients with central nervous system (CNS) involvement (CNS+ patients) who received allogeneic hematopoietic stem cell transplantation (allo-SCT) remains unclear.
Aims: We compared the clinical outcomes of allo-SCT in CNS+ patients with those in CNS-patients, and also evaluated prognostic factors for CNS+ patients.
Methods: Clinical data for patients were collected from the Japan Society for Hematopoietic Cell Transplantation database (TRUMP). The eligibility criteria for this study were as follows: diagnosis of ALL or acute biphenotypic leukemia, aged more than 16 years, allo-SCT between 2005 and 2012, and first SCT. Burkitt leukemia or lymphoblastic lymphoma and secondary leukemia were excluded from this study.
Results: Data for 2582 patients including 136 CNS+ patients and 2446 CNS-patients were used for analysis. As compared with CNS-patients, CNS+ patients were younger (median age: CNS+ 36.5 years *vs* CNS-40 years, *P*=0.02), higher frequency of T-cell phenotype (*P*=0.03), had worse disease status at SCT (CR at SCT: CNS+ 55.9% *vs* CNS-80.5%, *P*<0.01) and had worse performance status (PS) at SCT. A myeloablative dose of total body irradiation was more frequently used for CNS+ patients. Other characteristics were not different between the two groups. Incidence of neutrophil engraftment was marginally worse in CNS+ patients (Gray, *P*=0.07), and mortality rate before engraftment was significantly higher in CNS+ patients (Gray, *P*=0.04). Incidences and severity of acute graft-*versus*-host disease (GVHD) and chronic GVHD were not different between CNS+ patients and CNS-patients. Incidence of relapse was higher in CNS+ patients (Gray, *P*=0.02), and the rate of CNS relapse was also higher (9.7% in CNS+ patients *vs* 2.3% in CNS-patients, *P*<0.01). CNS+ patients developed CNS complications more frequently than CNS-patients (*P*=0.02). Incidences of other transplant-related complications and transplant-related mortality were not different between the two groups. At the median follow-up day of 1000 after SCT (range: 15-3137 days), 3-year overall survival (OS) rates were 41.2% in CNS+ patients and 55.8% in CNS-patients, and the difference was statistically significant by univariate analysis (log-rank, *P*<0.01). However, in patients who received SCT in CR, there was no difference in OS rates between CNS+ and CNS-patients (log-rank, *P*=0.38). We performed multivariate analysis (MVA) using Cox's proportional-hazards regression model to adjust the differences of variables. In the MVA, CNS involvement at diagnosis had not an unfavorable effect on OS (hazard ratio: 1.08, 95% confidence interval: 0.85-1.38, *P*=0.53), on the other hand, age, disease status and PS at SCT remained significant prognostic factors for OS. In the MVA focusing in patients who had CNS involvement at diagnosis, disease status and PS at SCT were significant risk factors for OS, and age of the patient was marginally significant.
Summary and Conclusions: Patients who had CNS involvement showed unfavorable OS due to poor disease status and poor PS at SCT. Patients who achieved CR and/or good PS before SCT showed preferable OS even if they had CNS involvement.

P170

REDEFINE CLINICAL RISK CLASSIFICATION IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA USING INITIAL ABSOLUTE LYMPHOCYTE COUNT

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Background: Excellent outcome in childhood acute lymphoblastic leukemia (ALL) treatment has been achieved by using risk-stratified therapy. Children with ALL are currently stratified based on various clinical and molecular characteristics to a specific risk group in order to receive an appropriate therapeutic

regimen. Post induction Minimal Residual Disease (MRD) has been used routinely to identify higher risk patients that previously resided in standard risk group by clinical classification. However, the use of MRD is still limited in most developing countries due to the high complexity and high cost of MRD testing. Therefore, a new basic clinical marker is needed to facilitate a better risk classification for children with ALL in resource-limited countries. Absolute lymphocyte count (ALC) during and post induction therapy which is a routine clinical testing has recently been found to associate with treatment outcome in children with ALL.

Aims: To define the level of ALC during induction chemotherapy that has a meaningful correlation to ALL treatment outcomes in Thai children.

Methods: A retrospective cohort of 200 newly diagnosed pediatric ALL patients treated at King Chulalongkorn Memorial Hospital during January 1996-August 2012 were reviewed.The study was approved by Chulalongkorn University Institutional Review Board. ALC level during and post induction period was documented together with other established risk factors. Lowess smoothing Martingale residuals plot was used to determine the optimal cut-point of ALC level. Univariate analysis was performed using Cox proportional hazards model. Multivariate models were developed including covariates with P<0.1 in univariate models and hazard ratios (HR) were estimated with 95% confidence interval. Overall survival and event free-survival were analyzed using the method described by Kaplan and Meier**.**

Results: High ALC at induction Day 1 (ALC-1) and/or Day-28 (ALC-28) were associated with favorable outcome in ALL treatment. Children with ALC-1≥800 cells/μL or ALC-28≥1,860 cells/ μL had a significantly better 10-year overall survival (10Y-OS) [85% (ALC-1≥800 cells/μL) *versus* 60% (ALC-1<800 cells/μL), P<0.001 and 90% (ALC-28≥1,860 cells/ μL) *versus* 75% (ALC-28<1,860 cells/ μL), P=0.02] and 10-year event free survival (10Y-EFS) [80% (ALC-1≥800 cells/μL) *versus* 55% (ALC-1<800 cells/μL), P=0.005 and 80% (ALC-28≥1,860 cells/ μL) *versus* 76% (ALC-28<1,860 cells/ μL), P =0.63]. We can define prognostic subgroups in more details when combining ALC-1 and ALC-28. Children with ALC-1<800 cells/ μL and ALC-28<1860 cells/μL displayed the worse treatment outcome compared to other groups (10Y-OS=52%, HR=6.9, P=0.001 and 10Y-EFS=48%, HR=4.3, P=0.002). Furthermore, ALC can sub-classify the treatment outcome of patients in each established clinical risk group. Standard risk patients with high ALC-1 had a significantly better 10-Y OS [90% (ALC-1≥800 cells/μL) *versus* 60% (ALC-1<800 cells/μL), P=0.01] (Figure1A) and 10-Y EFS [80% (ALC-1≥800 cells/μL) *versus* 45% (ALC-1<800 cells/μL), P=0.002] (Figure1B). Similarly, High ALC-1 in high risk patients also predicted better 10Y-OS [77% (ALC-1≥800 cells/μL) *versus* 50% (ALC-1<800 cells/μL), P=0.05] and 10-Y EFS [80% (ALC-1≥800 cells/μL) *versus* 62% (ALC-1<800 cells/μL), P =0.14].

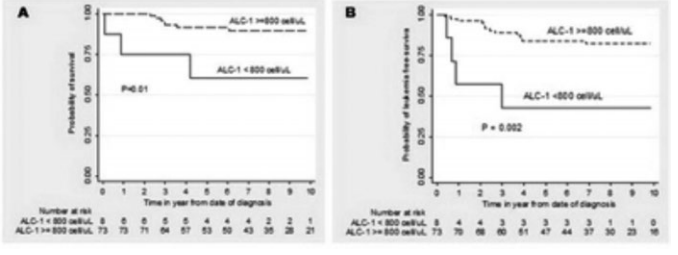

Figure 1. Kaplan-Meier estimate of overall survival (A) and event free survival (B) among standard risk group with high and low ALC-1.

Summary and Conclusions: Unlike most studies which focused mainly on the end of induction ALC, our finding indicated that ALC-1 and/or ALC-28 are new powerful independent prognostic factors in pediatric ALL**.** ALC; which is a simple and readily available clinical tool, can potentially be used as an additional clinical factor to improve risk classification for children with ALL in resource-limited countries.

Acute myeloid leukemia - Biology 1

P171

THE CXCR4 ANTAGONIST BL-8040 DIRECTLY AFFECTS AML BLASTS BY INDUCING THEIR TERMINAL DIFFERENTIATION AND BLOCKING SURVIVAL SIGNALS

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Background: Acute Myeloid Leukemia (AML) is a heterogeneous group of diseases characterized by uncontrolled proliferation and survival of hematopoietic stem and progenitor cells. An important characteristic of AML blasts is their ability to avoid terminal differentiation into mature cells. The chemokine CXCL12 and its receptor CXCR4 are key players in the retention of AML blasts in the protective bone marrow (BM) microenvironment. The CXCL12/CXCR4 axis is also critical for the survival and maintenance of AML blasts in their stemness state. In addition, CXCR4 overexpression is associated with poor prognosis in AML patients. We have studied the effect of BL-8040 (BKT140) on the survival, proliferation and differentiation of human AML blasts, using in-vitro systems, AML mice model and correlative studies in human samples obtained from the ongoing relapsed/refractory AML clinical trial (NCT01838395).

Aims: Tostudythe effect of BL-8040 on the survival, proliferation and differentiation of human AML blasts.

Methods: The ability of BL-8040 to induce differentiation of AML blasts was tested in-vitro using MV4-11, HL-60, THP-1, NB4 and U937 cells. The in-vivo effect of BL-8040 on differentiation, survival and apoptosis of human AML MV4-11 cells was tested using AML model of NOD scid gamma (NSG) mice. In humans, as part of an ongoing Phase IIa clinical trial (NCT01838395) samples from subjects who signed informed consent, were evaluated for differentiation and survival of AML blasts following two days of BL-8040 monotherapy.

Results: We have studied the signaling pathways involved in the survival of AML blasts. Our results revealed that these cells are dependent on both, increased survival factors mediated by the PI3K/mTOR/AKT axis and reduced apoptotic potential attributed to increased BCL2 expression. Treatment of AML cells with BL-8040 directly inhibited cell growthand increased cell death. This BL-8040 effect was mediated by inhibition of the mTOR and AKT signaling and by down regulation of BCL2 expression *in vitro* and *in vivo*. Moreover, the treatment with BL-8040 distinctively induced myelomonocytic and granulocytic differentiation *in vitro* and *in vivo*. In the clinical setting, two days of BL-8040 monotherapy triggered a dramatic decrease in the amount of leukemic blasts in the BM in the majority of patients (9/10 patients tested). This decrease was accompanied by an increase in the number of blasts positively stained for apoptosis using cleaved caspase 3 antibody (6/8 patients tested). Furthermore, in the majority of treated patients (7/10 patients tested) the reduction in the number of leukemic blasts in the BM was associated with induction of monocytic and granulocytic terminal differentiation.

Summary and Conclusions: Treatment with BL-8040 as a single agent rapidly and efficientlyinduces cell death of AML cells both in-vitro andin-vivo. This effect is mediated by reduction of the expression of BCL2 and by blocking the survival signals through the PI3K/mTOR/AKT pathway. Furthermore, the present study demonstrates for the first time that CXCR4 inhibition is associated with terminal differentiation of AML blasts in patients. By relieving the differentiation block that characterizes AML blasts along with blockade of survival signals, BL-8040 affects AML blasts survival. Moreover, the current study highlights an important role for BL-8040 as a differentiation therapy which has the potential to translate into clinical benefit.

P172

TARGETING ABERRANT NCAM (NEURAL CELL ADHESION MOLECULE; CD56) EXPRESSION IN ACUTE MYELOID LEUKEMIA

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Background: Acute myeloid leukemia (AML) is a heterogenous disease of the hematopoietic progenitor cell driven by the subsequent acquisition of genetic alterations. Although new technologies (*e.g.* NGS) have substantially contributed to our understanding of the genetic complexity of the disease, cellular consequences often remain elusive. Approximately 20% of AML patients show strong expression of CD56 (neural cell adhesion molecule; NCAM), a cell surface marker typically found in natural killer cells. Expression of NCAM is asso-

ciated with poor overall survival, however, the functional role of aberrant NCAM expression has not been investigated to date.
Aims: The goal of this study is to examine the biological role of NCAM in AML and to explore whether NCAM represents a potential therapeutic target.
Methods: The function of NCAM was investigated in several human AML cell lines. Cells were infected with lentivirus expressing a Doxycyclin-inducible shRNA directed against NCAM or scrambled shRNA. Genetically modified cells were analyzed for proliferation, cell death and downstream signaling pathways. In addition, we performed xenotransplantation experiments to explore the role of NCAM as a therapeutic target. Upstream regulatory mechanisms were investigated by DNaseI-hypersensitive assays and modified qRT-PCR mapping. To analyze NCAM effects at the stem cell level we made use of a well-established murine MLL-AF9 leukemia model using C57BL/6 wild-type or NCAM-/-mice and performed serial replating assays and bone marrow transplantation experiments.
Results: NCAM was expressed in several human cell lines (NOMO1, MOLM-14, THP1, SKM1 and others) at high levels and correlated with MLL-rearranged leukemias. Knockdown of NCAM using three different shRNA clones caused diminished proliferation, G1-arrest and finally apoptotic cell death. Suppression of NCAM sensitized leukemic blasts to genotoxic stress *in vitro* and prolonged survival of mice in xenotransplantation experiments. Applying DNaseI-hypersensitivity assays we demonstrate accessible binding sites for the transcription factors MEIS1, MEF2c and STAT1. shRNA-mediated knockdown of MEIS1, MEF2c and MLL-AF9 resulted in downregulation of NCAM cell surface expression, suggesting an upstream regulatory role for MLL-AF9. Analysis of downstream signaling pathways upon knockdown of NCAM demonstrated activation of SMAD2/3, p38 and upregulation of CDKN1a. To analyze NCAM expression in leukemia-initiating cells (LICs), we infected wild-type and NCAM-/-derived bone marrow cells with MLL-AF9 expressing viral particles and transplanted syngeneic recipient mice. Analysis of wild-type LICs ($Lin^-c\text{-}Kit^+CD34^+FcgR^+$) demonstrated strong surface expression of NCAM, whereas normal HSCs ($Lin^-c\text{-}Kit^+Sca1^+$) were NCAM-negative. Recipients of $NCAM^{-/-}$-MLL-AF9_cells developed acute leukemia with prolonged disease latency, and in colony assays replating activity was diminished. $NCAM^{-/-}$MLL-AF9 cells had lower CD117 and Gr-1 expression, but higher expression of Mac-1 and, in some samples, aberrant B220 co-expression. To analyze whether aberrant NCAM-expression is essential to maintain self-renewal activity we currently perform serial bone marrow transplantation experiments.
Summary and Conclusions: Targeting aberrant expression of NCAM by shRNA-mediated gene knockdown demonstrated strong antileukemic activity *in vitro* and sensitized leukemic blasts to genotoxic stress. *in vivo*, depletion of NCAM resulted in prolonged disease survival in syngeneic and xenotransplantation experiments and diminished self-renewal capacities. Our data suggest that NCAM represents a promising therapeutic strategy and likely targets AML cells at the LIC level.

P173

GENETIC AND EPIGENETIC EVOLUTION DURING THE PROGRESSION OF CYTOGENETICALLY NORMAL ACUTE MYELOID LEUKEMIA (CN-AML)

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Background: Clonal evolution in acute myeloid leukemia (AML) has been previously described either in studies of large patient cohorts with focus on only a restricted number of AML-associated genes or in smaller series of relapsed patients studied by genome-wide techniques.
Aims: To better understand the genetic and epigenetic mechanisms of relapse and therapy-resistance of AML, we performed exome sequencing and DNA-methylation profiling of matched bone marrow or peripheral blood samples taken at diagnosis, remission and relapse from 47 patients with cytogenetically normal AML (CN-AML) with a median age at diagnosis of 65 (range 21-89).
Methods: Paired-end sequencing (Illumina) with a mean target coverage of >100x resulted in an average of 95% of the exome covered at least 10-fold. Variants at diagnosis or relapse were called with a minimum variant allele frequency (VAF) of 20%. Mutations with a VAF≤5% at remission were defined as somatic, whereas mutations in known AML-associated genes (n=40, *e.g. DNMT3A*, *IDH2*, *SRSF2*) with VAF>5% at remission were considered as persistent during remission. After exclusion of common germline polymorphisms (dbSNP 138; MAF≥1%) and known error-prone genes (*e.g. MUC4*), we filtered for mutations with translational consequences and validated them by custom genepanel sequencing (HaloPlex, Agilent) with a mean target coverage of >300x. On average, 94% of the target region was covered at least 20-fold. DNA-methylation was analyzed using Illumina Infinium HumanMethylation450 BeadChip technology.
Results: Overall, 104 genes were recurrently mutated, while 7 genes were recurrently altered only at diagnosis (*e.g. CBL*) and 16 genes were recurrently altered only at relapse (*e.g. KDM6A*, *SRSF2*, *SF3B1*). The median number of somatic mutations per patient was 7 at diagnosis and 8 at relapse, whereas a gain or loss of mutations could be detected in 41 patients (87%). A median of 1 mutation is lost in relapse (*e.g. FLT3* point mutations, range 0-5), a median of 2 mutations were acquired during AML progression (*e.g. WT1*, range 0-5) while mutations in several AML-associated genes (*e.g. DNMT3A*) remained stable during the course of disease. In 60% of patients, the major clone at diagnosis remained predominant at relapse whereas expansion of a subclone during disease progression was observed in 40% of patients. A total of 5 patients acquired chromosomal alterations at relapse, with trisomy 8 as the only recurrent chromosomal abnormality present in 3 patients. A total of 22 patients (47%) had persistent mutations at remission with 40/54 (74%) mutations showing a dynamic pattern in their VAFs (relative change ≥20%). *DNMT3A* was the most frequently affected gene (Figure 1A), and patients with persistent *DNMT3A* mutation had a significantly shorter relapse-free survival compared to patients with not-persisting *DNMT3A* mutation (Figure 1B, n=13, median 270 days, range 81-586 *vs* n=7; median 508 days; range 235-1697; p=0.012). *DNMT3A* R882 mutations correlated with a specific DNA-methylation profile, characterized by hypomethylation of HOX genes at diagnosis. Of note, even at remission we were able to measure the *DNMT3A* R882 mutation-specific DNA-methylation pattern in patients with persistent mutations at VAF≥25%. These results suggest altered DNA-methylation at the pre-leukemic stage.

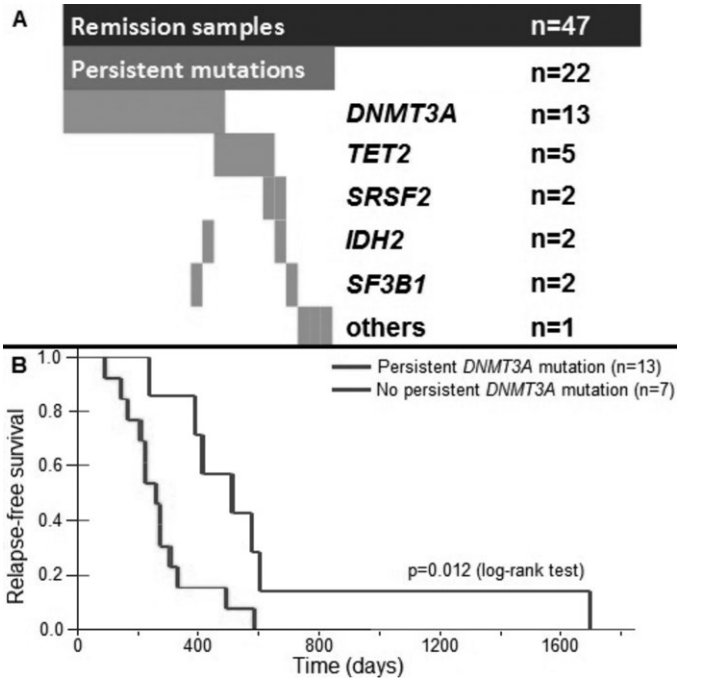

Figure 1.

Summary and Conclusions: In summary, our findings provide insights into the genetic and epigenetic evolution during the course of disease in a large cohort of relapsed CN-AML. Information about the dynamics of genetic lesions (*e.g.* persistent or relapse-specific mutations) may have prognostic applications and provide the basis for tailored approaches to treat or to prevent relapse of AML.

P174

GENOTOXIC STRESS MODULATES EVI1 INTERACTION WITH CTBP1 IN ACUTE MYELOID LEUKAEMIA CELLS

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Background: EVI1 proto-oncogene overexpression is associated with adverse prognosis in acute myeloid leukaemia (AML). EVI1 is a DNA binding transcriptional regulator. It forms complexes with multiple protein partners including chromatin re-modellers and transcriptional repressors, such as C-terminal binding protein (CtBP). Understanding EVI1 protein interaction dynamics will be important for novel therapeutic approaches.

Aims: The identification and characterisation of EVI1 protein interactions modulated by EVI1 phosphorylation.
Methods: EVI1 phosphorylation was detected by MIDAS mass spectrometry in EVI1 overexpressing AML SB1690CB cells. Flag-tagged wild type EVI1 and phosphorylation site mutated constructs were generated. Mouse bone marrow or HEK293T cells overexpressing the constructs were used for re-plating experiments, co-immunoprecipitation and immunofluorescence. Genotoxic stress was induced by 2Gy radiation and hydrogen peroxide(H_2O_2) treatment.
Results: We detected EVI1 phosphorylation at the C-terminal SQSP motif (serine858 and860). EVI1 constructs with alanine-mutated phospho-sites (A/A-EVI1) maintained the EVI1 transforming capabilities in transduced mouse bone marrow serial replating experiments. Proteomic interactome studies comparing WT-and A/A-EVI1 in an overexpression system confirmed interaction with co-repressor CtBP1. Co-immunoprecipitation showed significantly reduced CtBP1 binding with A/A-EVI1 compared to WT-EVI1 after induction of DNA damage. Higher levels of co-immunoprecipitated CtBP1 bound to EVI1 in damaged *versus* undamaged cells were confirmed in SB1690CB cells. Immunofluorescence analysis showed significantly higher co-localisation of WT-EVI1 with CtBP1 and reduced co-localisation of A/A-EVI1 with CtBP1 after DNA damage compared to undamaged cells.
Summary and Conclusions: Genotoxic stress induces EVI1 C-terminal phosphorylation and modulates EVI1 interactions with the co-repressor CtBP1. This links the DNA damage response to transcriptional repression in EVI1 overexpressing AML.

P175

COMBINING IMMUNE CHECKPOINT INHIBITION WITH AMG 330 TO ENHANCE T CELL MEDIATED LYSIS OF PRIMARY AML CELLS

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Background: Bispecific T-cell engaging (BiTE®) antibody constructs are very efficient in recruiting and activating T cells. Through the strong T-cell activation, proinflammatory conditions are generated, favouring the upregulation of immune checkpoint molecules on cancer cells. Blockade of immune-suppressive receptor-ligand interactions could potentially enhance BiTE® mediated cytotoxicity.
Aims: In this study we tested the relevance of immune checkpoint molecules on CD33/CD3 BiTE® antibody construct (AMG 330) mediated lysis of primary AML cells in *ex vivo* cytotoxicity assays.
Methods: The expression profiles of PD-L1 (n=123), CD277 (n=70) and ILT3 (n=55) were assessed by flow cytometry (Specific fluorescence intensity, SFI) in newly diagnosed AML patient samples. A long-term culture system supporting the growth of primary AML cells for up to 36 days *ex vivo* was used to determine the functional relevance of immune checkpoint molecules on AMG 330 mediated lysis. The long-term culture system enabled us to analyze the dynamic process of inhibitory molecule expression and receptor-ligand interaction over time.
Results: No constitutive expression could be detected for PD-L1 at primary diagnosis in 83.7% of the cases. In contrast, constitutive expression of CD277 and ILT3 was detected in 77% and 100% of patient samples respectively. As has been reported previously, the expression pattern of immune checkpoint molecules can be modulated through proinflammatory conditions. Therefore, the expression profile of the inhibitory molecules on different AML cell lines and primary AML cells after addition of IFN-γ and TNF-α was analyzed. PD-L1 was strongly induced on AML cell lines and primary AML cells (mean fold change SFI: AML cell lines 3.5, n=6; primary AML cells 9.2, n=6). In contrast, no further increase in ILT3 expression levels was detected (mean fold change SFI: AML cell lines 0.7, n=5; primary AML cells 1.2, n=3). The cytokine-mediated upregulation of other immune checkpoint molecules including CD277 on AML cells is currently being evaluated. In several studies it was shown, that BiTE® antibody constructs induce strong T-cell activation, which is accompanied by secretion of IFN-γ and TNF-α. Using our long-term culture system we observed a considerable upregulation of PD-L1 on primary AML cells after 1-4 days of *ex vivo* cytotoxicity assay (SFI control BiTE® (cBiTE®) *vs* AMG 330 1.8: 9.4, *p<0.0001;* n=27). Blocking of the PD-1/PD-L1 interaction significantly enhanced AMG 330 mediated lysis efficacy (% lysis: AMG 330 58% *vs* AMG 330+α-PD-1/PD-L1 blocking antibody 75%, n=9, *p=0.03)* which was accompanied by a significant increase in T-cell proliferation (fold change $CD2^+$ cells: cBiTE® 0.8 *vs* cBiTE®+α-PD-1/PD-L1 blocking antibody 1.0, *p=0.06*; AMG 330 8.0 *vs* AMG 330+α-PD-1/PD-L1 blocking antibody 11.1, n=9, *p=0.008*). In addition, IFN-γ secretion was significantly enhanced (IFN-γ secretion: cBiTE® 7.8 pg/ml *vs* cBiTE®+α-PD-1/PD-L1 blocking antibody 16.5 pg/ml, *p=0.03;* AMG 330: 1573 pg/ml *vs* AMG 330+α-PD-1/PD-L1 blocking antibody: 2060 pg/ml, n=8, *p=0.008)*. The functional relevance of other immune checkpoint molecules in AMG 330 mediated lysis efficacy is currently being evaluated in analogous experiments.
Summary and Conclusions: AMG 330-mediated cytotoxicity is modulated by blockade of inhibitory molecules on AML cells. Our results support the use of combinatorial approaches of BiTE® antibody constructs with PD-1/PD-L1 immune checkpoint blockade.

P176

DIFFERENTIAL RESPONSIVENESS OF PML-RARA CARRYING VARIOUS POINT MUTATIONS TO ARSENIC TRIOXIDE

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Background: Expression of the fusion gene, promyelocytic leukemia (*PML*)–retinoic acid receptor-α (*RARA*), initiates acute promyelocytic leukemia (APL) and plays a crucial role in APL pathogenesis. Arsenic trioxide cures APL through targeting PML-RARA oncoprotein for degradation and further eradicating the malignant cells. A growing amount of evidence supports arsenic trioxide as a front-line drug in APL treatment and the overall survival of APL patients has been improved dramatically. Unfortunately, there are not a few patients developed arsenic resistance during therapy and their outcomes were extremely dismal. Previous study has reported that A216V and L218P mutation in the PML moiety of PML-RARA were detectable in two APL patients at relapse. More recently, we identified a mutational hot spot (including previously reported A216V, newly identified S214L, A216T, L217F and S220G) within PML-RARA transcripts from 9 of 13 patients with relapsed APL. Whether all these acquired mutations play a role underlying the mechanism of clinical arsenic resistance are still unknown.
Aims: To explore if each of the identified mutations virtually plays a role and functions similarly underlying the mechanism of arsenic-resistance.
Methods: The recombinant plasmids of wild type and mutant PML-RARA were transfected into cultured Hela cells followed by the treatment with or without As_2O_3. The protein levels and cellular localization were detected by immunoblotting and immunofluorescence analysis. A retrospective analysis was performed to validate whether various point mutations of PML-RARA functioned distinctly in APL patients. The morphological characteristics and the expression levels of PML-RARA transcripts in bone marrow of included patients were monitored before and after reinduction treatment with arsenic.
Results: Immunoblotting analysis showed that total protein levels of PML-RARA mutants with L217F or S220G were significantly decreased upon As_2O_3 treatment (regular dose), similar with the phenotype of wild type PML-RARA. This finding was in parallel with the enhanced nuclear matrix association and SUMOylation of L217F and S220G mutant proteins triggered by As_2O_3. Whereas mutants with A216V, S214L or A216T did not respond to As_2O_3 treatment in the same experimental conditions. Importantly, the varying responsiveness of different point mutations to arsenic observed *in vitro* was conserved in the clinical setting. Relapsed APL patients respectively with A216V, S214L or A216T mutation had strong resistance to the reinduction therapy with arsenic. Temporarily moderate drug resistance was found in a patient with S220G mutation. In contrast,one patient with L217F mutation achieved a complete remission after reinduction treatment. Furthermore, we showed that high dose of As_2O_3 was capable to reverse the primary arsenic resistance driven by indicated mutations in the PML B2 domain of PML-RARA in cultured cells.
Summary and Conclusions: Our results reinforce the correlation of genetic mutations found in the PML part of PML-RARA transcript with arsenic resistance during APL therapy. We also propose a novel insight regarding that the acquired mutations exhibited varying responsiveness to arsenic treatment both *in vitro* and *in vivo*. Our findings may help in predicting the prognosis and selecting more effective strategies for the management of relapsed APL.

P177

SURVIVAL PREDICTION AND IDENTIFICATION OF KEY PROGNOSTIC PROTEINS IN ACUTE MYELOID LEUKEMIA USING REVERSE PHASE PROTEIN ARRAY

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Background: Novel proteomic technologies, allow for accurate and rapid profiling of the cellular protein ecosystem in Acute Myeloid Leukemia (AML). The high throughput data they generate could be used to build better prognostic models, to identify new targets for therapy, and to individualize treatment.
Aims: To use high-dimensional survival models to identify key prognostic proteins in AML and to compare the performance of proteomics based survival models to that of commonly used prognostic factors.

Methods: We used reverse-phase protein array (RPPA) to measure the level of total and phosphorylated expression of 230 proteins in leukemia-enriched cell samples from 384 newly diagnosed AML patients. We used Cox regressions with the Least Absolute Shrinkage and Selection Operator regularization to construct multivariate prediction models for overall and relapse-free survival (OS&RFS) and to identify the key prognostic proteins. To calculate coefficients and evaluate model performance we employed a 100 iteration bootstrapping procedure. We repeated the analyses for the subset of patients with normal karyotype AML and younger patients (≤60).
Results: Multivariate prognostic models based on proteomics performed at least as well as models based on commonly used prognostic covariates including age, sex, performance status, prior malignancy or hematological disease, cytogenetics and FLT-ITD mutation analysis (C-Index 0.71 *vs* 0.69 respectively for RFS). Multivariate survival models highlighted multiple proteins associated with survival. Of these, the most pronounced were ARC, IGFBP2, INPP5D, TP53 and CCND3 which appeared in univariate and multivariate analyses. STAT3, XIAP, and CDK2 were identified only by multivariate models, suggesting they are of relevance only in the context of the expression of other proteins. In younger patients we further identified SMAD5, phospho-ERBB2, phospho-MTOR, and CCNE2. Genetic alterations associated with many of these proteins have been previously implicated in the pathogenesis of AML and provide an external validation to our results. For most however this work is the first evaluation of their prognostic significance. Finally, using proteomics based risk scores we were able to discern subgroups of patients with differing survival. This risk stratification outperformed stratification based on cytogenetics. Further, we identified distinct subgroups among patients with a normal karyotype (RFS not-reached, 150 and 39 weeks, p<0.0001). Our results bare a considerable similarity to risk stratification based on genetic alterations presented in other studies, though the validity of such a comparison is limited due to variability in patient populations and a lack of standardized reporting.

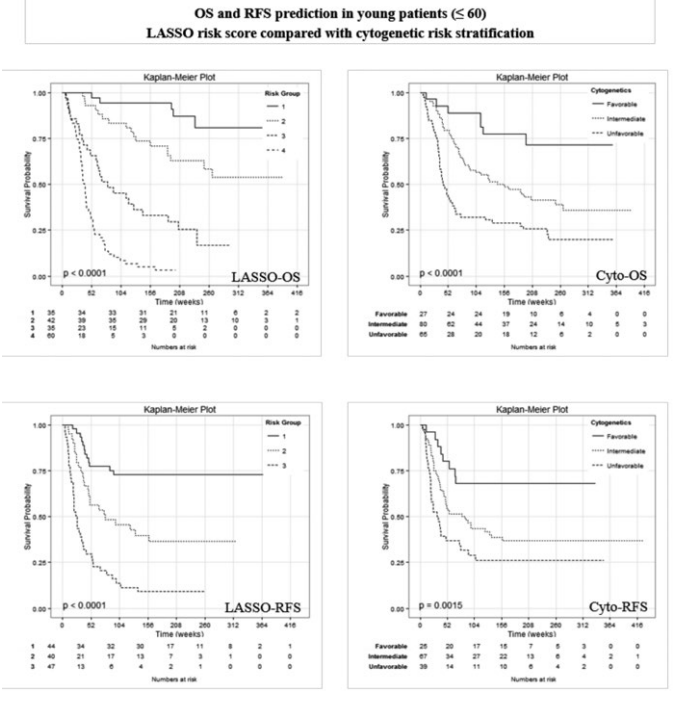

Figure 1.

Summary and Conclusions: RPPA is an efficient tool for rapid and high throughput proteomic profiling. Using the proteomic data with advanced survival modeling can reliably identify multiple proteins with a significant prognostic value as targets for future research and novel therapeutics.

P178

MICRORNA-182, C/EBPA AND E2F GENERATE AN AUTOREGULATORY FEEDBACK NETWORK WHICH IS DYSREGULATED IN ACUTE MYELOID LEUKEMIA

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Background: The transcription factor CCAAT enhancer binding protein alpha (C/EBPα) is a master regulator of granulopoiesis and is inactivated in approximately 50% of all acute myeloid leukemia (AML) cases. MicroRNAs, a class of small non-coding RNAs, were identified as important regulators of normal hematopoiesis and leukemia development. We have already shown that microRNAs, such as miR-223, miR-34a and miR-30c, are essential elements in C/EBPα triggered granulocytic differentiation.But to our knowledge nothing is known about inactivation of C/EBPα by microRNAs in acute myeloid leukemia.
Aims: Functional characterization of miR-182 in AML.
Methods: We used Next Generation Sequencing to detect novel C/EBPα related microRNAs. To investigate the function of miR-182, we used several *in vitro* and *in vivo* systems, including cell lines, primary AML cells and a transplantation based mouse model.
Results: In this study, we identified a novel network between C/EBPα, miR-182 and E2F. In a next generation sequencing approach based on inducible K562-C/EBPα-ER cell line, we found miR-182 strongly downregulated by wild-type C/EBPα. We could further demonstrate an inverse correlation between C/EBPα protein amount and miR-182 expression level in several biological systems, including leukemic cell lines, G-CSF treated primary human CD34$^+$ progenitor cells *in vitro* and sorted murine bone marrow subpopulations *in vivo*. Additionally, we found elevated miR-182 expression levels in lineage-negative, c-kit-positive myeloid progenitors of *CEBPA* Knock-Out mice. To discover the mechanism how miR-182 is blocked by C/EBPα, we analyzed the minimal promoter region of miR-182 and performed chromatin immunoprecipitation (ChIP). Here, we could demonstrate a strong binding of C/EBPα to the miR-182 promoter, particularly to a conserved E2F binding site. Because E2F is a well known inhibitor of C/EBPα function, we tested whether E2F also effects miR-182 expression. An overexpression of E2F1 in U937 cells leads to an elevated miR-182 expression level. In addition, we measured the expression of miR-182 in bone marrow from AML patients regarding to their CEBPA mutation status. We could show that only patients with mutations in the C-terminal region of C/EBPα showed increased miR-182 expression levels, while patients with N-terminal CEBPA mutations revealed no abnormal miR-182 expression compared to healthy donors or AML patients with no CEBPA mutation. The C-terminal domain of C/EBPα is necessary for E2F inhibition. These findings illustrate the importance of C/EBPα-E2F interaction during miR-182 regulation. Next, we found a highly conserved binding site of miR-182 in the 3'UTR of CEBPA itself, suggesting a possible negative feedback loop. To test this, we performed overexpression of miR-182 in U937 cells, umbilical cord blood mononuclear cells (UCB-MNCs) and primary blasts from AML patients. Here, we observed a strong reduction of C/EBPα protein after miR-182 overexpression in all cell types. Furthermore, we could demonstrate a direct binding of miR-182 to the 3'UTR of *CEBPA* via luciferase activity assay. Finally, we were interested in the functional impact of miR-182 in myeloid differentiation and leukemia development. We showed that stable overexpression of miR-182 blocks granulocytic differentiation in G-CSF treated 32D cells and enhances replating capacity of primary mouse bone marrow mononuclear cells. Moreover, we could demonstrate that enforced expression of miR-182 in mouse Lin-Sca-1+c-Kit+ early hematopoietic progenitors by lentiviral infection leads to a strong reduction of mature Mac1+Gr1+ granulocytes in the peripheral blood *in vivo*.
Summary and Conclusions: Taken together, we identified miR-182 as novel oncogenic microRNA that directly blocks C/EBPα during myeloid differentiation and leukemia development. Thus, our data display a potential new strategy for therapeutics in C/EBPα dysregulated AML.

P179

THE LEUKEMIA-ASSOCIATED RUNX1/ETO ONCOPROTEIN CONFERS A MUTATOR PHENOTYPE

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Background: One of the key processes in oncogenesis is the transition from a premalignant to a fully malignant state. The chromosomal translocation (8;21) is an initiating event in acute myeloid leukaemia (AML) and generates the *RUNX1/ETO* fusion gene.*RUNX1/ETO* drives self-renewal, but is insufficient for leukaemogenesis, requiring further mutations for transformation. We postulated that this leukaemic fusion protein may affect the acquisition of mutations.
Aims: In this project, our aims were to investigate whether and how RUNX1/ETO impacts on the acquisition of secondary mutational hits, thereby promoting the generation of fully developed leukaemia.
Methods: We investigated the effect of*RUNX1/ETO*expression on susceptibility to spontaneous and exposure driven mutation in TK6 cells. After lentiviral transduction with *RUNX1/ETO*, we isolated TK6 clones withvarying levels of *RUNX1/ETO*expression. We used the *PIGA* assay as an initial measure of spontaneous and exposure-induced mutation. Loss of this glycosyl-phosphatidylinositol(GPI) anchor protein results in an absence of GPI-linked proteins from the cell membrane, including CD55 and CD59.Cells that are simultaneously negative for two GPI-linked proteins are considered to be*PIGA*mutants

and are detected using fluorochrome-conjugated antibodies and analysed by flow cytometry. We also used the*thymidine kinase(TK)* gene as a second mutational target for exposure-induced mutation.
Results: We determined the *PIGA* mutation frequency (M*f*) in several independent *RUNX1/ETO*-expressing and vector control clones 8-10 weeks after initial cloning. The mean *PIGA* M*f* in *RUNX1/ETO* clones (7.5×10^{-4}) was 5 times higher than in vector control clones (1.5×10^{-4}) (p=0.032). We next investigated whether *RUNX1/ETO* cells were sensitive to mutagenesis following exposure to genotoxic agents. Fusion protein positive and vector control clones were exposed to sub-cytotoxic doses of doxorubicin (100nM) or ionising radiation (3Gy), and M*f* was measured 2 weeks after treatment. The mean treatment-induced *PIGA* M*f* was strongly increased in *RUNX1/ETO* clones compared to vector control clones following doxorubicin (Mean M*f* =6.5×10^{-4} *vs* 2.1×10^{-4}, p=0.09) or radiation treatment (Mean M*f* =5.9×10^{-4} *vs* 2.1×10^{-4}, p=0.008). This was confirmed by assaying at the *TK* locus, where mean treatment-induced M*f* was also significantly higher in RUNX1/ETO cell clones compared to vector control clones following either doxorubicin (Mean M*f* =3.5×10^{-6} *vs* 4.3×10^{-7}, p=0.014) or radiation treatment (Mean M*f* =6.4×10^{-6} *vs* 1.5×10^{-6}, p=0.002). For both *PIGA* and *TK*, the M*f* in vector control clones following doxorubicin or radiation treatment was not significantly different from mock-treated cells. Additionally, using ChIP, we showed that RUNX1/ETO directly binds to and represses the OGG1 base-excision repair DNA glycosylase and that RNAi mediated depletion of *RUNX1/ETO* in t(8;21) cell lines significantly increases OGG1 transcript and protein levels, providing a plausible reason for the increased M*f* shown in RUNX1/ETO cells.
Summary and Conclusions: We have shown that RUNX1/ETO predisposes to the acquisition of mutations, both spontaneously and after treatment with genotoxic agents. RUNX1/ETO actively promotes the acquisition of secondary mutations thereby expanding genetic heterogeneity in the population from which malignant clones can emerge. We also suggest that late-relapse in t(8;21) AML may be due to the presence of RUNX1/ETO positive, genetically unstable pre-leukaemic clones in the bone marrow, which are prone to mutation acquisition, leading to leukaemia relapse.

P180

EVALUATION OF MINIMAL RESIDUAL DISEASE IN CHILDREN WITH CORE BINDING FACTOR ACUTE MYELOID LEUKEMIA-RESULTS OF THE FRENCH MULTICENTER ELAM02 TRIAL

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Background: Acute myeloid leukemia (AML) with t(8;21)(q22;q22) or inv(16)(p13q22) chromosomal rearrangement corresponding to the RUNX1-RUNX1T1 or CBFb-MYH11 fusion transcripts respectively, belongs to the core binding factor (CBF) AML, a favorable-risk subgroup of AML. Nonetheless, 30% of patients with CBF-AML relapse. Prognostic factors have been identified in adult studies to detect patients at higher risk of relapse, such as older age, high white blood cell count, additional cytogenetic abnormalities or gene mutations, and minimal residual disease response to therapy. Especially in adult CBF-AML patients, it has been shown that a log reduction under 3-log in transcript level after 2 courses of chemotherapy, was associated with a significant increased risk of relapse. In children CBF-AML accounts about 20% of de novo AML with an unexpected relatively high incidence of relapse in the RUNX1-RUNX1T1 subgroup. There is need to identify factors able to predict a high risk of relapse. Few studies have reported the prognostic relevance of minimal residual disease in pediatric CBF-AML.
Aims: The main aim of the study was to quantify and follow the minimal residual disease of CBF fusion transcripts during therapy in order to determine its prognostic relevance and to identify patients who might benefit of allogeneic hematopoietic stem cell transplantation in first complete remission in the future protocol.
Methods: 438 children were treated between 2005 and 2011 in the French pediatric ELAM02 trial. We focused on 97 patients with CBF-AML including 61 AML with t(8;21)(q22;q22) and 36 AML with inv(16)(p16q22). RUNX1-RUNX1T1 and CBFb-MYH11 transcripts were quantified by real-time PCR at diagnosis, and after each course of chemotherapy (MRD1, MRD2, MRD3, MRD4) according to the trial design.
Results: Patients with inv(16)(p16q22) AML had higher WBC and more frequent CNS involvement than those with t(8;21)(q22;q22). N/K-RAS mutations were more frequent in the inv(16)(p16q22) subset, ASXL2 mutations more frequent in t(8;21)(q22;q22) subset, whereas KIT gene mutations were equally frequent in both subsets. At diagnosis, the median fusion transcript ratio was higher in RUNX1-RUNX1T1 patients [258.7, range 20-1685] than in CBFb-MYH11 patients [94.4, range 31.5-447]. Incidence of relapse was 39.3% and 16.7% in t(8;21) and inv(16) respectively. In the RUNX1-RUNX1T1 subset, MRD log reduction evaluation shows that 19 (41%) of 46 patients at MRD2 and 8 (21%) of 38 patients at MRD3 did not reach 3-log reduction in fusion transcript ratio level. In the CBFb-MYH11 subset, MRD log reduction evaluation shows that 20 (67%) of 30 patients at MRD2 and 18 (67%) of 27 at MRD3 did not reach 3-log reduction in fusion transcript ratio level. The 5-year DFS was not significantly different at any point between MRD positive and MRD negative in both subgroups: 48±11% *versus* 49±12% at MRD2 and 48±10% *versus* 37±17% at MRD3 in RUNX1-RUNX1T1 subset and 100% *versus* 79±9 at MRD2 and 89±10% *versus* 75±11% at MRD3 in CBFb-MYH11 subset. The 5-year disease free survival (DFS) was 53±2% in all-ELAM02, 53±7% in t(8;21) and 80±7% in inv(16). Similarly the 5-year overall survival (OS) did not shown any significant difference and was 71±2% in all-ELAM02, 87±4% in t(8;21) and 100% in inv(16) subgroups.
Summary and Conclusions: In the ELAM02 trial we confirmed how frequent CBF-AML patients are slow-responders to chemotherapy but we were unable to confirm the prognostic impact of 3-log MRD reduction to predict relapse as in adult. Of note, children did not received anti-CD33 for chemotherapy as it was in published adult trials. Furthermore comparison between flow-cytometry and fusion transcript MRD could be helpful in CBF-AML.

Acute myeloid leukemia - Clinical 1

P181

HIGH PROGNOSTIC VALUE OF PRE TRANSPLANT MINIMAL RESIDUAL DISEASE ASSESSMENT BY COMBINED WT1 EXPRESSION AND FLOW CYTOMETRY IN ACUTE MYELOID LEUKEMIA PATIENTS

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Background: Allogeneic bone marrow transplantation (BMT) offers the greatest chance of cure for most patients affected by acute myeloid leukemia (AML). Persistence of disease or high levels of pre BMT minimal residual disease (MRD) have been reported to predict relapse risk after BMT. WT1 expression levels and multicolor flow cytometry (MFC) are the most widely used tools to evaluate MRD.
Aims: We recently reported that combined MRD evaluation by WT1 and MFC after induction therapy strongly impacts on relapse risk in AML patients.The aim of the present study was to perform the same MRD assessment in the pre BMT setting in order to evaluate its reliability in predicting relapse after transplant.
Methods: We retrospectively analyzed outcome of 257 AML patients with both WT1-based and MFC-based MRD evaluation on bone marrow samples before transplant. Median age at transplant was 44 years. One hundred thirty-three patients were transplanted in first, 54 in second and 72 in third or subsequent complete remission. Induction regimens included fludarabine-containing regimens or standard "3+7" induction. Median follow-up was 48 months (95% CI 37.5-50.5 months). Relapse-free survival (RFS) was calculated from the time of transplantation until last follow-up or documented leukemic relapse. Overall Survival (OS) was calculated from the time of transplantation until death by any cause or last follow-up. A positive MFC MRD was defined by the presence of no less than 25 clustered leukemic cells/10^5total events (threshold of $2.5x10^{-4}$residual leukemic cells) at four-color flow-cytometry. Real-time PCR for WT1 was performed on DNA Engine 2 (Opticon®, MJ Research®). WT1 copy number/Abl copy number $500x10^4$ was used as cut-off value for abnormal WT1 expression.
Results: Eighty-nine relapses (35%) were reported. Median RFS was 84 months. The probability of disease relapse was significantly affected only by disease status (first or subsequent CR, p<0.001) and MRD status before transplantation, measured with any method (p <0.001). Specifically, MFC-MRD was the strongest predictor of longer relapse free survival (p <0.001) since only two relapses occurred in the eleven MFC-MRD negative patients. Among MFC-MRD positive patients a further stratification of risk is obtained by the evaluation of WT1 MRD status that was able to identify patients with significantly worse RFS (p <0.01, Figure1). Multivariate RFS analysis revealed that the combined MRD evaluation was the only independent predictor of RFS (p <0.001). The predictive value of MRD resulted independent from induction schedules, donor type, disease status at BMT and risk group. Similarly, univariate OS analysis showed that BMT year, disease status at BMT and MRD evaluation with any methodic significantly influenced OS duration (p <0.001, <0.001 and <0.001, respectively). Multivariate OS analysis confirmed that combined MRD evaluation was an independent strong predictor of long survival, alongside with BMT year and disease status at BMT (p <0.003, <0.001 and <0.002, respectively)

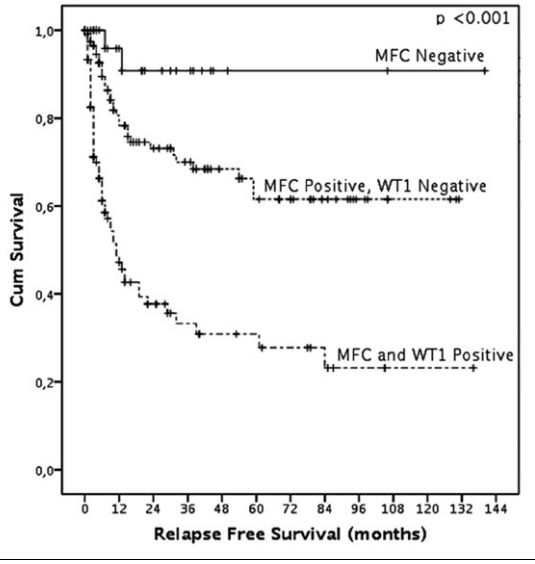

Figure 1. RFS according to risk group

Summary and Conclusions: Pre transplant MRD evaluation by WT1 and MFC on bone marrow samples is a reliable predictor of relapse risk. Patients with both negative pre-BMT MRD markers have a significantly longer DFS, while patients with both positive MRD markers display an higher risk of relapse. Identifying patients who are at higher relapse risk may allow to modulate post BMT follow up, with the aim of detecting disease recurrence earlier and/ or applying pre-emptive therapeutic strategies in order to delay or prevent AML relapse.

P182

A COMPARISON OF DAUNORUBICIN/ CLOFARABINE AND FLAG-IDA IN HIGH RISK ACUTE MYELOID LEUKAEMIA: RESULTS FROM THE UK NCRI AML17 TRIAL

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Background: We have previously shown that consolidation of high risk patients with high dose ara-c was less effective than combination chemotherapy (including etoposide amsacrine and mitoxantrone) (Burnett AK *et al.* J.Clin.Oncol. *31*,3360-3368, 2013). In the NCRI AML17 Trial (ISRCTN55675535) 3215 untreated non-APL adults, generally <60 years, were given an initial induction course of Daunorubicin/Ara-C with or without Etoposide and/or Gemtuzumab Ozogamicin (GO). On recovery patients were assigned a risk group. To be high risk patients required to fulfil one of the following criteria: 1) Group A: in CR but with adverse features assimilated into a validated risk score (based upon cytogenetics, age, presenting WBC, secondary disease and response to course 1 of therapy) predicting an expected survival of around 24% (Burnett AK et al *Blood* 108 (11):10, 2006) ; 2) Group B: not in CR after a second induction course; or 3) Group C: relapsed disease.
Aims: We compared Daunorubicin/C.lofarabine with FLAG-Ida in a randomised fashion in patients who were high risk.
Methods: 393 patients were identified (311 group A; 5 group B; and 77 group C) and randomised 2:1 between Daunorubicin ($50mg/m^2$ days 1,3 and 5) + Clofarabine ($20mg/m^2$ days 1-5) or FLAG-Ida (Fludarabine $30mg/m^2$ d2-6, Ara-C $2g/m^2$/day d2-6, G-CSF days 1-7 and Idarubicin 8 mg/m^2 on days 4,5,6). All high risk patients were candidates for allo-BMT. For the purpose of this analysis groups B & C are combined.
Results: Overall 393 patients entered the comparisons of DClo *vs* FLAG-Ida: 311 in group A and 82 in groups B+C. The characteristics of group A and of B+C were: median age 55 (18-69) and 47 (19-63); de novo 221 and 78; secondary disease or high risk MDS 90 and 4; performance score >1 13 and 3; cytogenetics favourable 0 and 14; cytogenetics intermediate 151 and 58; cytogenetics adverse 160 and 3. The median follow up is 25.8 months for group A and 12.7 months for group B+C. In group A, the median number of courses received was 1 for FLAG-Ida and 2 for DClo. The overall survival at 4 years for group A was 35%. RFS was 46% in the FLAG-Ida patients *vs* 34% in those on DClo (HR 1.25 (0.89-1.75) p=0.2). Survival was non-significantly superior in the FLAG-Ida patients (48% *vs* 30%, HR 1.30 (0.95-1.79) p=0.10). The objective of the chemotherapy was to deliver patients to allograft. 53% of FLAG-Ida and 56% of DClo received a transplant. When the RFS and OS are censored at time of transplant the RFS was FLAG-Ida 22% *vs* DClo 18%, and OS was 22% *vs* 22%. The overall survival at 3 years for group B+C was 20%. CR was achieved in 2/2 refractory and 18/25 relapsed patients allocated FLAG-Ida *vs* 1/3 and 41/52% for DClo. Survival was non-significantly better in the FLAG-Ida patients (35% *vs* 11%, HR 1.26 (0.73-2.17) p=0.4). 56% of FLAG-Ida and 65% of DClo received a transplant. When the OS are censored at time of transplant the OS at 18 months was FLAG-Ida 38% *vs* DClo 30%. If groups A, B+C are analysed together using standard meta-analytic approaches, the hazard ratio for survival favours FLAG-Ida (HR 1.29 (0.98-1.70) p=0.07). Although FLAG-Ida resulted in slower count recovery and required significantly more supportive care (RBC and platelets/ antibiotics/ hospital days), it resulted in survival which was not inferior to DClo.

Tabella 1.

AML17: Overall Survival
High risk randomisation

	Deaths/Patients DClo	Deaths/Patients FLAG–Ida	Statistics (O–E)	Statistics Var.	O.R. & 95% CI (DClo : FLAG–Ida)
Type of patient:					
Post Course 1	122/207	49/104	10·3	38·6	1.30 (0.95, 1.79)
Relapsed/Refractory	40/55	17/27	3·0	12·9	1.26 (0.73, 2.17)
■ **Total:**	**162/262**	**66/131**	**13·2**	**51·5**	**1.29 (0.98, 1.70)**

0·0 0·5 1·0 1·5 2·0
DClo better | FLAG–Ida better

Test for heterogeneity (2 groups): χ^2_1 = 0·0; P = 0·9; NS

Effect 2P = 0·07

Summary and Conclusions: FLAG-Ida resulted in at least as good survival when compared with DClo for high risk patients in remission or relapsed or refractory patients.

Acknowledgements: This study received research support from Cancer Research UK. Genzyme/Sanofi provided Clofarabine for the trial.

P183

FLUDARABINE-CONTAINING INDUCTION INCREASES MINIMAL RESIDUAL DISEASE CLEARENCE AND IMPROVES SURVIVAL IN AML PATIENTS WITH CONCOMITANT NPM1 AND FLT3-ITD MUTATIONS

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Background: The negative prognostic impact of FLT3-ITD mutations in acute myeloid leukemia (AML) patients is well known. This unfavorable effect is mostly due to high relapse rate and short disease free survival (DFS). On the contrary, NPM1 gene mutation has been shown to confer a good prognosis. The co-existence of both mutations is common with most groups reporting a predominant negative prognostic impact of FLT3.
Aims: We performed a retrospective analysis of the outcome of 124 de novo AML patients carrying FLT3 and/or NPM mutation receiving a fludarabine containing regimen or conventional daunorubicin and cytarabine (3+7) schedule as front line treatment, with the aim of evaluating whether the combination of fludarabine to Ara-C and anthracycline may increase DFS in FLT3 or NPM mutated patients, especially in double-mutated patients.
Methods: One-hundred twenty four consecutive FLT3-ITD and/or NPM1 positive non M3 AML patients (age 17-78 years) treated in two Hematology centers of Northern Italy were retrospectively included in this study. In center 1, all patients received FLAI-5 regimen (fludarabine 30 mg/sqm and ARA-C 2g/sqm on days 1 to 5 plus idarubicin 10 mg/sqm on days 1-3-5), whereas in center 2 patients were given either FLAI or 3+7 induction. Overall 90 patients received FLAI, and 34 patients received 3+7 regimen. In both centers, patients with FLT3-ITD mutation underwent allogeneic bone marrow transplantation (BMT) in first complete remission if feasible. Minimal residual disease was evaluated on marrow samples in most patients using multicolor flow-cytometry (MFC) and NPM expression levels. Demographical, cytogenetic and molecular characteristics were comparable in the series of patients treated in the two centers and in patients receiving the two induction regimens.
Results: Two patients (5.9%) died during first induction in the 3+7 arm,whereas two patients (2.2%) died in the FLAI arm, mostly due to infections. One further patient in the FLAI arm died during second cycle. Overall 60-days mortality was therefore 4%. After two induction cycles, 105 patients achieved CR (88%) and 14 did not respond (16%). Forty-two/105 (40%) CR patients underwent BMT in first CR. CR rate after cycle 1 was significantly higher in FLAI-5 cohort (86% *vs* 69%, p<0.05). Patients treated with FLAI-5 had significant higher probability of achieving MRD clearance, both on NPM and MFC-based evaluation (p<0.001 and p<0.05, respectively, Figure 1A and D). After a median follow up of 51 months, 2-year DFS was 53.4% (median 101 months). DFS duration was significantly influenced by induction type (p <0.001, Figure1 B), risk group, status for FLT3 and NPM1, NPM-MRD and MFC-MRD status (p <0.03, p<0.05, p<0.05, p<0.001, p<0.01, respectively). Survival did not significantly differ between patients achieving MFC-MRD negativity with FLAI 5 or "3+7". Among patients with FLT3-ITD and concomitant NPM mutation, the difference between FLAI and 3+7 cohorts in term of DFS was higher (p <0.0001, Figure 1 C). No significant DFS difference was observed in FLT3-ITD/NPM wild type patients. Overall Survival analysis led to similar results.

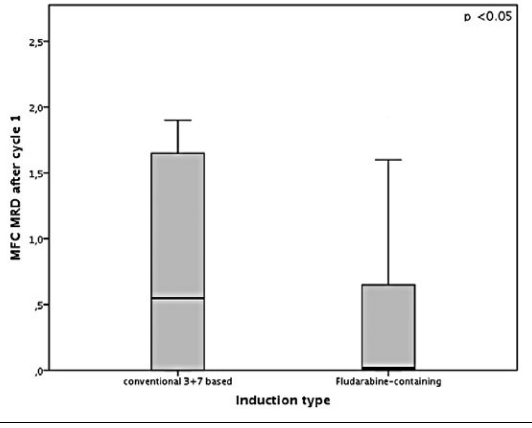

Figure 1. A: Multicolor Flow Cytometry MRD levels after cycle 1

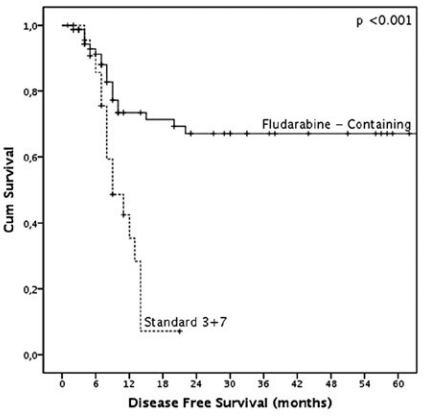

Figure 1. B: DFS in All Patients according to induction therapy.

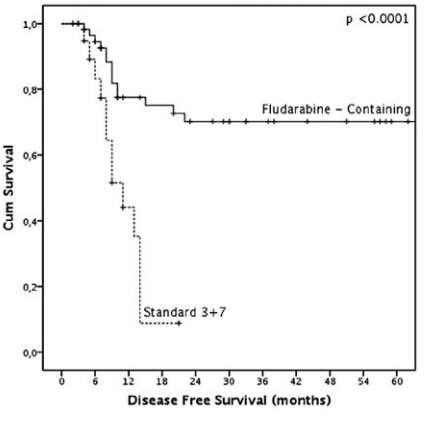

Figure 1. C: DFS in NPM1 Patients according to induction therapy.

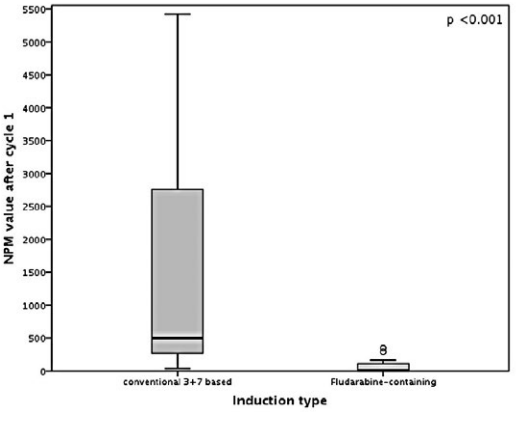

Figure 1. D: NPM1 MRD levels after cycle 1.

Summary and Conclusions: Despite the small size of patients cohorts and the potential bias due to the retrospective nature of the analysis, our study seems to indicate that fludarabine containing induction improves quality of response in patients carrying NPM1 mutation compared to 3+7 regimen. It is possible that the favorable impact of adding Fludarabine to Ara-C and anthracycline in double mutated AML patients may overcome the negative prognostic impact of FLT3-ITD.

P184

THE EFFECT OF AZACITIDINE ON HEALTH-RELATED QUALITY OF LIFE (HRQL) IN OLDER PATIENTS WITH NEWLY DIAGNOSED ACUTE MYELOID LEUKEMIA (AML): RESULTS FROM THE AZA-AML-001 TRIAL

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Background: Older patients (pts) with AML generally have a poor prognosis. While treatment (Tx) may extend overall survival (OS) for pts with AML, it may also cause significant toxicity and impairment of HRQL (Cheng, *Leukemia*, 2014). In the large, international, phase 3 AZA-AML-001 study, median OS for older pts with AML treated with azacitidine (AZA) was 10.4 months *vs* 6.5 months for pts who received conventional care regimens (CCR; HR=0.85; p=0.1009) (Dombret, EHA, 2014). HRQL was a prespecified secondary endpoint of the study.
Aims: To evaluate changes in HRQL during Tx among pts in AZA-AML-001.
Methods: Pts were aged ≥65 years with newly diagnosed *de novo* or secondary AML (>30% bone marrow blasts). Before randomization, pts were preselected to receive 1 of 3 CCR per investigator choice: induction chemotherapy, low-dose cytarabine (LDC), or best supportive care only. Pts were then randomized to AZA or CCR, in which case, they received their preselected Tx. Most pts (n=312, 64%) were preselected to receive LDC. HRQL was assessed by EORTC QLQ-C30 questionnaire at baseline, day 1 of every other Tx cycle, and at the end-of-study visit, which occurred at different time points for individual pts. Analyses included only pts who completed the baseline and at least 1 post-baseline HRQL assessment. An HRQL-specific statistical analysis plan (SAP) was finalized before database lock. HRQL changes were evaluated prospectively for the AZA and CCR cohorts, and *post hoc* for the pt subgroup preselected to LDC who received AZA or LDC. Four of the 15 QLQ-30 domains were prespecified in the SAP as most relevant: Fatigue (primary), Global Health Status/QoL, Physical Functioning, and Dyspnea (secondary). HRQL was evaluated through cycle 9 (~32 to 34 weeks) due to subsequent small cohort sizes. A prespecified 10-point minimally important difference (MID) threshold represents meaningful change.
Results: Rates of HRQL assessment compliance were fairly high overall (>77% both Tx groups) except at the end-of-study visit (AZA=42%, CCR=37%). Overall, 157 AZA pts and 134 CCR pts were evaluable for HRQL (the AZA-AML-001 ITT population included 488 pts, AZA N=241, CCR N=247). The rate of attrition of evaluable pts during early Tx was higher in the CCR arm than in the AZA arm. During Tx, mean change from baseline scores with AZA or CCR showed general improvement in the 4 relevant domains (Figure). Few changes were statistically significant (p<0.05) and fewer met the MID threshold. Pts receiving CCR achieved meaningful improvement in Fatigue (cycles 7, 9) and Global Health Status/QoL (cycle 9). No HRQL detriment was seen with AZA or CCR during Tx. Notably, scores varied substantially among individual pts in both Tx groups. Within the LDC preselection group, HRQL outcomes with AZA and LDC were largely consistent with the primary HRQL analysis (Figure). Pts receiving AZA achieved meaningful improvement in Fatigue (cycle 9).

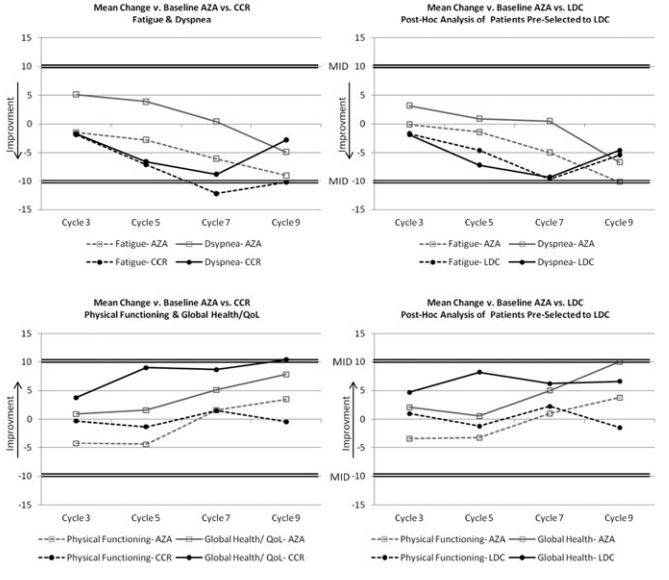

Figure 1. Mean Changes from Baseline EORTC QLQ-30 Domain Scores.

Summary and Conclusions: During Tx, AZA and CCR were associated with general improvement in HRQL in the 4 relevant domains, but improvements were not consistently meaningful. Importantly, for these 4 domains, there was no meaningful HRQL deterioration at the group level during Tx. Results were largely similar for AZA *vs* LDC.

P185

DEFINITION OF CURE IN ADULT PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background: Advances in diagnosis, treatment, and supportive care have improved rates of complete remission (CR) in adult patients (pts) with newly diagnosed acute myeloid leukemia (AML). Unfortunately, disease relapse is common, accounting for the majority treatment failure, and explaining the marginal improvements in long-term survival. However, there are subsets of pts who achieve CR and continue to maintain very long term remissions, without relapse-the so-called 'cure-fraction'. Although long-term follow-up of AML pts is standard, the time point after which a patient is considered 'cured' of AML is a critical piece of information for both pts and physicians.
Aims: The aim of the current study is to clarify the definition of cure in adult pts with AML in 1stCR.
Methods: For the purposes of the study, cure was defined as the point after which the probability of relapse (POR) was ≤5%. In subgroups where this probability was not achievable in the first 5 yrs, a threshold of ≤10% was applied. Pts who had received an allogeneic stem cell transplant were excluded. Pts with acute promyelocytic leukemia (APL) were analyzed separately and not included in the larger AML cohort. We evaluated the cumulative risk of relapse, time to relapse, relapse-free survival, and overall survival in a cohort of pts treated at our institution from 2000-2012, with a median follow up of 26.2 months.
Results: A total of 1096 pts were analyzed (median age 57, range [18-88]), of whom 159 (14.5%) had APL. Of the remaining 937 pts, 493 (53%) were age ≥60 yrs, and 444 (47%) were <60 yrs. In pts with APL, the POR after 1 and 2 yrs was ≤5% and ≤1%, respectively. In the entire (non-APL) cohort, the POR level of ≤5% was achieved after 4 yrs in CR. In pts <60 yrs, the POR of ≤5% was achieved after 3 yrs in CR. In pts ≥60 yrs, the POR of ≤5% was not achieved in the time of follow-up. In these pts, we observed a POR of ≤10% after 4 yrs in CR. In pts with core-binding factor (CBF) AML, the POR was ≤5% at 2 yrs and ≤1% at 3 yrs after CR. In pts with diploid karyotype a POR of ≤5% was achieved after 3 yrs in CR and in the non-diploid pts after 5 yrs in CR.(Figure 1).

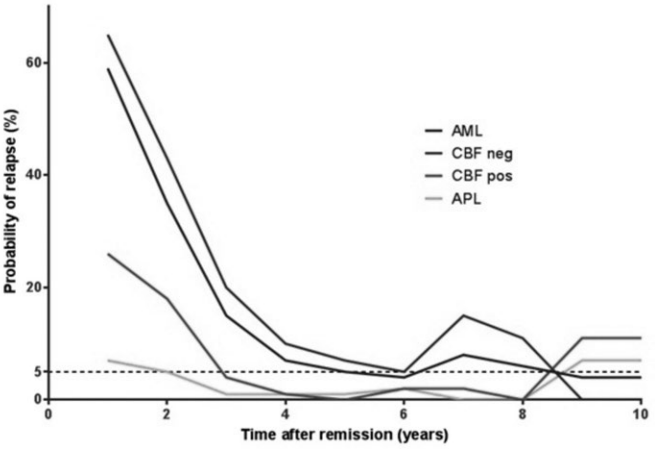

Figure 1. The illustration shows the correlation between the probability of relapse and the time passed from achieving the 1st complete remission for the each subgroup of patients. Abbreviations: AML (acute myeloid leukemia), CBF neg (core-binding factor negative AML), CBF pos (core binding factor positive AML), APL (acute promyelocytic leukemia).

Summary and Conclusions: Although late relapses were observed, using a threshold of ≤5% for POR, pts with APL and CBF AML may be considered 'cured' 1 and 2 yrs after achieving a CR, respectively. In younger pts with AML, cure may be considered after 3 yrs in CR, while older pts did not achieve a POR ≤5% during our follow-up. Using a POR of ≤10%, a 'cure' in older pts may be considered after 5 yrs in CR.
Conflict of interest: This work was funded by a Conquer Cancer Foundation of ASCO Long-term International Fellowship. Any opinions, findings, and conclusions expressed in this material are those of the authors and do not necessarily reflect those of the American Society of Clinic Oncology or the Conquer Cancer Foundation.

P186

RESIDUAL HEMATOPOIETIC STEM CELLS REFLECT MINIMAL RESIDUAL DISEASE AND PREDICT OUTCOME IN ACUTE MYELOID LEUKEMIA

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Background: It has been demonstrated that functionally normal hematopoietic stem cells (HSC) can be separated from leukemic cells in a subgroup of patients with acute myeloid leukemia (AML) using the $CD34^+CD38^-ALDH^+$ phenotype. Our recent studies further showed that patients suitable for this separation can

be identified prospectively by their low percentages of cells with high ALDH activity (ALDH$^+$ cells) (<1.9%; ALDH-rare AML) and that these patients represent a cohort with favorable outcome. However, frequencies of residual HSC varies within this subgroup and individuals with very low numbers appear to have extremely poor survival.
Aims: In this study we sought to investigate the relationships of residual HSC to survival and disease status in diagnostic AML and follow-up samples. We further aimed to test if HSC numbers correlate to leukemia initiation potential in xenotransplantation assays.
Methods: Between 2012 and 2013 bone marrow (BM) aspirates of 73 ALDH-rare AML patients were collected after written informed consent and patients stratified according to HSC frequencies determined by 34$^+$38$^-$ALDH$^+$ cells in HSC$^-$AML (□0.01% of total MNC) and HSC$^+$ AML (≥0.01% of total MNC). HSC numbers were determined by FACS-analysis at diagnosis and various follow-up time-points. Engraftment potential was evaluated by transplantation of 2x10e6 bulk AML cells in immune deficient NOD/SCID-IL2R^{null} (NSG) mice.
Results: Survival analysis showed that HSC$^-$AML represents a subgroup of patients with significantly worse overall survival (OS) (mean: 455 days) and disease-free survival (DFS) (mean: 209 days), compared to HSC$^+$ AML (mean OS: 878 days; mean DFS: 896 days). Survival analysis of patients with cytogenetic intermediate-risk also showed significantly worse OS and DFS for HSC$^-$AML with mean OS of 439 days, compared to 872 days for HSC$^+$ AML and mean DFS of 218 days, compared to 848 days. We also analyzed matched sample series from diagnosis, various remission time points and relapse (if available), and found that HSC recovered after chemotherapy in cases that achieved complete remissions. However, in cases of persistent disease HSC remained rare. Correlation of disease status to HSC numbers during therapy revealed that residual HSC are a reliable qualitative marker of minimal residual disease with a negative correlation to frank or molecular relapse. To test if HSC numbers correlate to leukemia initiation potential we transplanted 44 AML cases in NOD/SCID-IL2R^{null} (NSG) mice and observed only abnormal engraftment (47% AML-and 53% no engraftment) upon transplantation of HSC$^-$cases. In contrast HSC$^+$ cases mostly lead to normal, multi-lineage engraftment (79%).
Summary and Conclusions: Frequency of residual HSC reliably predicts outcome in AML patients and the quality of NSG mouse engraftment. Our AML stratification strategy is especially helpful in identifying cases with poor prognosis in the intermediate-risk group with HSC$^-$AML representing patients with poor overall and disease free survival. In addition HSC frequency can also be used as a qualitative marker of MRD status tabling it as indicator of BM-niche occupation of persistent leukemic cells.

P187

ALLOGENEIC TRANSPLANT IN PATIENTS ≥60 YEARS OF AGE WITH FIRST RELAPSED OR REFRACTORY ACUTE MYELOID LEUKEMIA AFTER TREATMENT WITH VOSAROXIN OR PLACEBO PLUS CYTARABINE: RESULTS FROM VALOR

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Background: Increasing numbers of older patients with acute myeloid leukemia (AML) receive allogeneic hematopoietic cell transplant (HCT) due to wider donor availability and improvements in supportive care that reduce transplant-related morbidity/mortality. HCT in patients ≥60 y/o with first relapsed or refractory (R/R) AML were examined from VALOR, a large phase 3 adaptive design, randomized, double-blind, placebo-controlled trial evaluating vosaroxin plus cytarabine (vos/cyt) *vs* placebo plus cytarabine (pla/cyt) [NCT01191801].
Aims: The aim of this posthoc subgroup analysis was to assess outcomes of HCT in patients ≥60 y/o with R/R AML.
Methods: Patients were randomized 1:1 to receive cyt (1 g/m^2 IV over 2 hr, d 1-5) plus either vos (90 mg/m^2 IV over 10 min, d 1 and 4; 70 mg/m^2 in subsequent cycles) or placebo. We assessed complete remission (CR) rates prior to HCT, posttreatment HCT rates, HCT outcomes, and overall survival (OS) by treatment arm in R/R AML patients ≥60 y/o.
Results: Overall, 451 patients ≥60 y/o received vos/cyt (n=226) or pla/cyt (n=225). Posttreatment HCTs were performed in 47 (20.8%) patients on vos/cyt and 44 (19.6%) patients on pla/cyt. Of the 91 HCT patients, 27 had achieved CR after vos/cyt *vs* 16 after pla/cyt. An additional 7 patients (vos/cyt) and 6 patients (pla/cyt) received subsequent therapy and went on to achieve CR, resulting in totals of 34 vos/cyt *vs* 22 pla/cyt patients who achieved CR prior to transplant. Median OS for patients who underwent posttreatment HCT on the vos/cyt arm was 20.2 mo *vs* 12.2 mo on the pla/cyt arm (HR 0.699; one sided P=0.088). There were no clinically meaningful differences between treatment arms with respect to transplantation type, complications associated with HCT (including graft-*vs*-host and veno-occlusive disease), 100-day mortality, or achievement of engraftment.
Summary and Conclusions: While HCT rates were comparable between treatment arms in this older R/R AML population, higher pretransplant CR rates in the vos/cyt arm enabled more patients ≥60 y/o to undergo transplant while in CR as compared to the pla/cyt arm. With median OS lengthened by 8 months, a trend toward an OS benefit was observed for vos/cyt-treated patients. Additional follow-up is being conducted to further assess the impact of vos/cyt treatment on posttreatment HCT. Funded by Sunesis Pharmaceuticals, Inc., South San Francisco, CA.

P188

RELAPSE CHARACTERISTICS AND RISK FACTORS FOR CENTRAL NERVOUS SYSTEM INVOLVEMENT IN ACUTE PROMYELOCYTIC LEUKAEMIA IN THE ORAL ARSENIC TRIOXIDE ERA-A 13-YEAR FOLLOW-UP STUDY

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Background: Arsenic trioxide (As_2O_3)-based regimensareeffective in inducing a remission in more than 90 percent of relapsed APL. Treatment failure following As_2O_3 and central nervous system (CNS) involvement is associated with a poor prognosis. The optimal post-remission therapy in relapsed APL is undetermined. Identifying the clinicopathologic characteristics and risk factors for CNS relapse will be a useful guide to stratify patients to the most optimal post-remission therapy.
Aims: The aim of this study was to prospectively evaluate the clinicopathologic characteristics and risk factors for CNS involvement in patients with relapsed APL treated with oral arsenic trioxide-based therapy.
Methods: A total of 188 patients with APL were prospectively followed for 13 years, of whom 71 patients had relapsed APL. The clinicopathologic characteristics at relapse were determined. Prognostic factors for CNS involvement at relapse were determined.
Results: There were 41 men and 30 women, at a median age of 43 (21–78) years. The leukemia was *de novo* in 68 patients (five of which were of microgranular variant morphology), and therapy-related in 3 patients. Additional karyotypic abnormalities were seen in 11 patients (15.5%). Internal tandem duplication of the fms-like tyrosine kinase 3 gene (*FLT3*-ITD) was detected in 13 patients. During prior first complete remission (CR1), only 15 patients (21.1%) had received oral-As_2O_3 based maintenance. A second complete remission (CR2) was achieved with an oral-As_2O_3-based re-induction in all patients in first relapse, who then received oral As_2O_3-based consolidation and maintenance. At relapse, the median leucocyte count and peak leucocyte count was 2.7 (0.4–94.7) x 10^9/L and 9.2 (0.6–130.4) x 10^9/L, and the median platelet count was 59 (7–281) x 10^9/L. APL differentiation syndrome (DS) occurred in 11 patients (15.5%) during relapse. At a median follow up of 93 (21–392) months, 28 patients (39.4%) had two or more relapses. In the whole cohort of patients, the patterns of relapses were: isolated bone marrow (BM), N=54 (76.1%); concurrent BM and CNS, N=9 (12.7%); and isolated CNS, N=8 (11.3%). In total, 22 patients relapsed during oral As_2O_3-based maintenance at various disease stages. On univariate analysis, factors significantly associated with CNS relapse included peak leucocyte count at diagnosis >20 x 10^9/L (P=0.03), oral As_2O_3-based maintenance at CR1 (P=0.004), leucocyte count at R1 >10 x 10^9/L (P=0.04), 2 or more relapses (P<0.001), and relapse during oral As_2O_3-based maintenance (P<0.001). On multivariate analysis, peak leucocyte count at diagnosis >20 x 10^9/L (P=0.04) and relapse during oral As_2O_3-based maintenance (P=0.01) were significantly associated with CNS involvement at relapse. CNS involvement at relapse predicted worse overall survival (HR=4.19; P=0.002; 95% CI:1.72-10.19).
Summary and Conclusions: CNS involvement was associated with a poor prognosis. High peak leucocyte count at diagnosis and relapse during oral As_2O_3-based therapy were the main risk factors.

P189

PROSPECTIVE STUDY ON 220 NEUTROPENIC EPISODES IN 109 AML PATIENTS. ROLE OF BED-SIDE ULTRASOUND (BUS) IN NEUTROPENIC ENTEROCLITIS (NEC): INCIDENCE AND SURVIVAL WITH DIFFERENT CHEMOTHERAPY REGIMEN

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Background: Neutropenic enterocolitis (NEC) is a life threatening complication of leukemic and solid tumors patients (pts) treated with chemotherapy (CHT) with mortality rate up to 50-100%. It's a clinical syndrome in neutropenic patients (pts) characterized by abdominal pain (AP), fever (F) and diarrhoea (D). Ultrasound (US) was used to evaluate bowel-wall thickening (BWT), and >4 mm is considered diagnostic of NEC. Perforation occurs in 5%>10% of cases. Early diagnosis is crucial to start conservative medical management (CMM) which appears the optimal strategy for most cases.
Aims: To evaluate prospectively if Bed-side-US(BUS) can detect early signs of NEC and guide a prompt treatment (CMM or surgical) in order to reduce mortality and to evaluate the impact of different CHT regimens on mucosal damage and NEC occurrence
Methods: in the last 7 years all AML pts admitted in Our Hematology Unit wards at University of Pisa (Italy), undergoing chemotherapy (CHT) were enrolled (n=109). Abdominal US was performed, baseline before treatment, and as only one symptom (or a combination) appeared within 12h from onset: F and/or D and/or AP in CHT-related neutropenic pts.
Results: N=220 chemotherapy-related neutropenic episodes (NE) occurred in 109 pts. N=17 episodes of NEC were diagnosed (7.7 %incidence rate). N=2 pts had 2 separate episodes of NEC and both survived. CHT regimens received (total number of cycles/NEC episodes) was: 3+7 (Idarubicin+ARAC) N=62/10, Idarubicin (AML-M3) N=9/1, 3+3+5 (Idarubicin,VP-16,ARAC) N=48/3; 2+5 (Idarubicin+ARAC) N=24/1; Clofarabine (40mg) N=5/0; Clofarabine (20mg) 14/0; Clofarabine+ARA-C (Cofa 20mg) N=18/1; FLANG N=13/1; HD-ARA-C (3gr/mq for 3 consecutive days) N=26/0. Overall 3 pts died out of 17 NEC episodes (mortality rate 17.6%). All pts were promptly treated as soon as BUS was diagnostic of NEC. Overall 3 pts died /17 NEC episodes (17.6% mortality rate).Median time to response from beginning of CMM was 24h and the first sign of was a decrease in AP followed by F, D and BWT. Amelioration of symptoms occurred with pts still neutropenic. Symptoms at Dx were:F+AP+D N=9, F+D N=1, F+AP N=1, Dia+AP N=4,D N=0, F N=0, AP N=2. Fever at Dx was absent in N=6 episodes (35%). Statistically (St) CHT regimens mostly associated with NEC were: 3+7 odds ratio (OD) 6.00 (P<0.0001), 3+3+5 OD=1.75 (P<0.0001). There was not a St significant association of the following CHT regimens with NEC occurrence: Clofarabine (20 or 40mg) N=0 NEC, 2+5 (P=0.125), Idarubicin (AML-M3) (P=0.111), Clofa 20mg+ARAC (P=0.167), HD-ARAC N=0 NEC, FLANG (P=0.143). The association of ARAC to Clofarabine *vs* Clofarabine (20 or 40mg) alone was not St significant in NEC occurrence (P=0.9). The likelihood of NEC Dx in a discriminant model (Bayes theoreme) for pts with BWT and AP=100%, AP+D=100%, AP+D+F=100%,AP+F=100%.
Summary and Conclusions: BUS allowed to detect early signs of NEC and to start prompt treatment in this life threatening complication, with a 76% survival rate. With BUS pts do not live the isolation room. Early diagnosis and intervention allowed to reduce mortality. Images of BUS and CT were superimposable. Fever is not a condition sine qua non for NEC diagnosis. Different chemotherapy regimens do have a different impact on mucosal damage. A prompt BUS in neutropenic patients as just one symptom presents allows to make early diagnosis of this life threatening complication and guide prompt treatment (conservative or surgical) eventually reducing mortality.

Acute myeloid leukemia - Clinical 2

P190

MGA GUIDED INTENSIVE INDUCTION WITH HIGH DOSE CYTARABINE PLUS IDARUBICIN IN 149 AML ELDERLY PATIENTS

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Background: The poor outcome of elderly AML patients is mainly due to the biological properties of the disease and their frailty. To improve both the efficacy and the tolerance of chemotherapy, different approaches have been tried and tested in this setting.
Aims: We explored the feasibility and efficacy of an intensive induction regimen in elderly AML patients evaluated by a simplified Multidimensional Geriatric Assessment (MGA).
Methods: 149 consecutive AML patients older than 59 years, have been prospectively evaluated by MGA: 91 patients (61%) who resulted fully or partially fit, were eligible forintensive induction chemotherapy; the remaining 58 frail patients received best supportive care (BSC). Frail patients turned out to be older, have a poorer PS score and significantly more secondary and poor prognostic karyotype AML than the fit subgroup of patients. In this latter group only 6.2% of patients had favourable-risk cytogenetics while 40.7% had unfavourable-risk cytogenetics. Thirty-six patients had a secondary disease and 3 patients had a 3 WHO performance status (PS) score. The induction chemotherapy consisted in the association of High-Dose Aracytin, combined with a single high-dose of Idarubicin, combined with Amifostine, in order to reduce the toxicity.
Results: Among the 91 patients who received intensive treatment the overall chemotherapy-related mortality rate (TRD during induction and consolidation) was 14.2% while the induction TRD rate was 5.5%. The main toxicity of the intensive induction chemotherapy consisted in myelosuppression with neutrophils (>1,500/µl) and platelets (>20,000/µl) recovery was achieved on days +15 and +16 respectively, with a median duration of hospitalization of 30 days (range: 15-69); we did not observe severe extrahematological toxicities. Sixty-seven (73%) patients achieved the Complete Remission (CR) and 61 received intensive consolidation, followed by ASCT, SCT or Gemtuzumab Ozogamicin, depending on mobilization outcome and donor availability.On intention to treat basis the 8-year OS of the 91 patients receiving intensive induction, was 20%, with a median duration of 11.4 months, significantly higher than that observed in the 58 BSC patientssince all died within 18 months with a median OS of 1.5 months (p<0.001). Patients with poor cytogenetic, no hyperleukocytosis and those with hyperleukocytosis ≥50,000/µl had respectively a 1.8and 3 relative risk of dying when compared with other patients (p=0.005) (Figure 1).

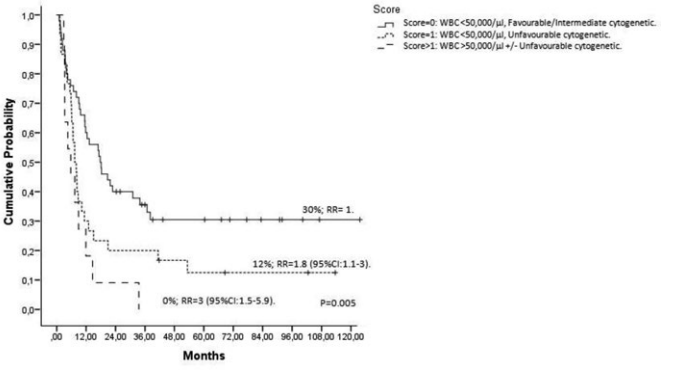

Figure 1. OS in 91 AML elderly patients by prognostic score.

Summary and Conclusions: Our simplified MGA selected 60% of elderly AML patients eligible for an intensive, feasible and effective protocol, making tailored post-consolidation possible in 53.8% of patients with long term survival double that reported in the literature.

P191

CD200 EXPRESSION IS ASSOCIATED WITH INFERIOR RESPONSE AND POOR SURVIVAL IN PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background: The evasion of immune system by neoplastic cells is emerging

as a mechanism of survival after conventional chemotherapy both in cells of hematologic and solid tumors. Many molecules with "immune attenuator" activity have been identified over the last years; among them, CD200 has a central role by inhibiting different effectors in immune response and has been associated with poor prognosis in several cancers, including acute myeloid leukemia (AML).

Aims: In the present work we investigated the aberrant expression of CD200 in 244 adult AML (173 "de novo" and 71 secondary) to evaluate its impact on response to therapy and on survival. Secondary aim was to evaluate the possible association of CD200 expression with particular subsets of disease.

Methods: Two hundred forty-four patients with non-promyelocytic AML treated at our two Institutions were included in this analysis. Blast cells immunophenotype and CD200 expression were evaluated by multiparametric flow cytometry.

Results: CD200 aberrant expression was found in 137/244 cases (56%), with significantly higher frequency in secondary compared to "de novo" AML (52/71, 73% *vs* 85/173, 49%; p=0.0006). No association was found according to age and FAB classification, but CD200 positivity was higher in CD34+ cases (99/129, 77% *vs* 36/113, 32%; p<0.0001). CD200 expression was associated with FLT3 unmutated status (105/170, 62% *vs* 17/46, 37%; p=0.004) and with NPM wild-type status (99/145, 68% *vs* 19/65, 29%; p=0.001). Considering cytogenetic profile, CD200 expression was more frequent in the unfavorable group (44/67, 66%) than in favorable/intermediate ones (74/153, 48%; p=0.01). Complete remission (CR) was obtained in 73% of CD200 negative and in 58% of CD200 positive patients (p=0.01). The negative impact of CD200 expression on CR was confirmed also in multivariable analysis (HR: 0.49, 95%CI: 0.20-0.95; p=0.04). With a median follow-up of 56 months, 107 patients (44% of the total) were alive. Overall survival (OS) was significantly lower in CD200 positive patients, with 3-year OS of 45%, compared to 31% in the CD200-negative group (p=0.01) [Fig 1]. Moreover, a strong association was observed between intensity of CD200 and survival, as patients with high CD200MFI (>11) had a 3-year OS of 10% compared to 40% in CD200 negative or low-expressing cases (p=0.03). In multivariable analysis CD200 expression intensity retained its negative impact on OS (HR: 0.59, 95%CI: 0.32-98; p=0.04). Finally we evaluated the effect of CD 200 expression in patients with unfavorable karyotype: 3-year OS was 39% in CD200 negative and 12% in CD200+ patients (p=0.045).

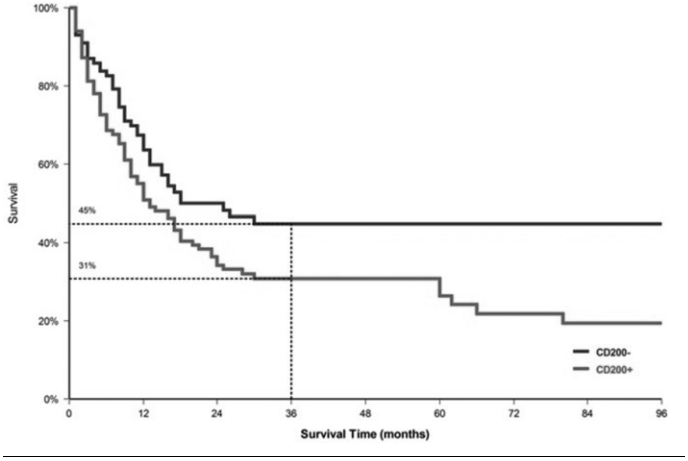

Figure 1.

Summary and Conclusions: Aberrant CD200 expression is associated with low remission rate and poor survival in AML. The negative effect is particularly evident in the unfavorable cytogenetic group. New therapeutic strategies directly targeting CD200 or modulating the tolerant microenvironment induced by CD200 should be explored to improve the still largely unsatisfactory results of conventional chemotherapy.

P192

NOVEL FUSIONS AND POINT MUTATIONS DETECTED IN NEWLY DIAGNOSED AML PATIENTS BY RNA-SEQ IDENTIFY POTENTIAL NEW TREATMENT OPTIONS

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Background: Specific genetic alterations in AML represent a critical information for both diagnosis and treatment choice.

Aims: 20 AML patients at diagnosis were subjected to RNA-Seq to identify gene fusions; mutations and indels in a predefined set of 45 genes were also evaluated.

Methods: RNA sequencing data were generated using an Illumina Genome Analyser IIx following standard library-prep protocols. Alignment to the reference GRCh38/hg38 genome was performed using STAR. Alignment data were processed using Samtools. Single nucleotide and small indels detection was performed using in-house software and applied to the following list of genes involved in AML pathogenesis (CEBPA, NPM1, FLT3, RUNX1, MLL, WT1, EZH2, NF1, MECOM(EVI1), KIT, H-RAS, K-RAS, TET2, IDH1, IDH2, DNMT3A, BCOR, BCORL1, NUP98, ASXL1, ABCB5, BAALC, CEP72, DIP2C, ROBO1, KLC1, TP53, IGFBP7, SETBP1, JAK2, NRAS, NOTCH1, CDKN2A, MPL, SF3B1, BRAF, PTPN11, SRSF2, IKZF1, GATA1, MYD88, ATM, CBL, PHF6, BCL2). The presence of fusions was assessed using FusionAnalyser.

Results: We identified a total of 10 fusions and 29 single nucleotide variants with a median number of 1 single nucleotide variant per patient (range 0-4). 5 patients had fusions that were previously detected with standard techniques (1AML-ETO, 3 PML-RARA and 1 CBFB-MYH11); for all these patients known fusions were confirmed by RNA-sequencing. In 5 patients previously unreported fusions were detected. Two of them were already known from the literature (ZMYM2-FGFR1 and MLL-MLLT10) and 3 were new (ETS2-ERG, WDFY3-WAS and KDM2B-ETV6). Thus the number of fusions present in this cohort of patients was doubled when compared to fusions identified by standard techniques. The ZMYM2-FGFR1 is particularly interesting, because it is potentially targetable by using drugs such as ponatinib, dovitinb or nintedanib; indeed, *in vitro* treatment of patient cells with nanomolar concentration of ponatinib caused a potent and specific growth inhibition (see figure). Among the patients in whom point mutations were identified, new treatment options could have been selected in 14 out of 20 cases, harboring mutations in genes such as NOTCH1, IDH1/2, RAS, KIT, TET, EZH2 and KMT2A.

A

Patient	Known Fusions	Fusion #1	Fusion #2	Mutations
AML001	RUNX1-RUNXT1	RUNX1-RUNX1T1 (AML-ETO)		KIT N822K
AML002	PML-RARA	PML-RARA	ETS2-ERG	
AML003	PML-RARA	PML-RARA		
AML004	NO			TET2 V1718L, NRAS G61L
AML005	NO			EZH2 C606S, CBL C384Y
AML006	NO	ZMYM2-FGFR1		RUNX1 T322*
AML007	PML-RARA	PML-RARA		NRAS G12V, ATM K482Q, PTPN11 T239I
AML008	NO			
AML009	Inv(16)	CBFB-MYH11		NRAS G13A
AML010	NO			IDH1 R132H, NPM1 L287SV
AML011	NO	MLL-MLL10 (MLLT10-KMT2A)		KMT2A I2714V
AML012	NO			NPM1 L287SV
AML013	NO			IDH2 A18P
AML014	NO			IDH1 A132C
AML015	NO			RUNX1 G49A
AML016	NO			NRAS G12A, BCOR L459*
AML017	NO			IKZF1 A138V
AML018	NO			NOTCH1 V2443L, NOTCH1 L1797P, RUNX1-IT1 T75A
AML019	NO			IDH2 A172L, NOTCH1 A1946C, RUNX1 M347T, PHF6 C243S
AML020	NO	KDM2B-ETV6		IDH2 A132C, JAK2 A923H

B

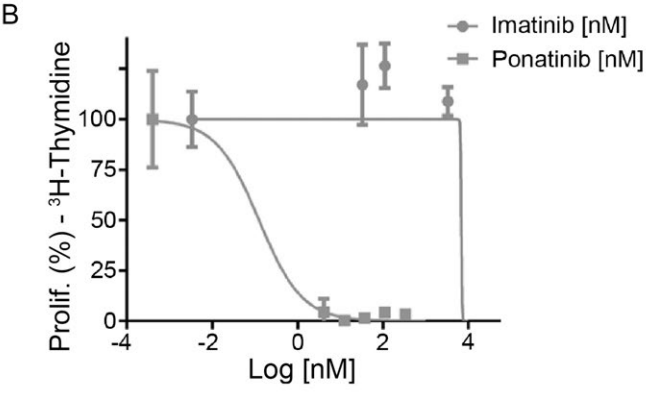

Figure 1.

Summary and Conclusions: NGS technologies, such as RNA-Seq, can result useful to identify genetic lesions and to tailor treatment in AML patients.

P193

SALVAGE REGIMENT WITH FRACTIONATED GEMTUZUMAB OZOGAMICIN, INTERMEDIATE DOSE CYTARABINE AND MITOXANTRONE (MYLODAM) FOR REFRACTORY AND RELAPSED AML: A SINGLE CENTER EXPERIENCE

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Background: Gemtuzumab ozogamicine (GO, mylotarg ®) has been used for

refractory or relapsing AML with a response rate of 30% when used alone and 60% when used in combination with other chemotherapy. In the largest published study reporting the combination of a single dose of mylotarg® 9mg/m^2 with mitoxantrone and intermediate dose of cytarabine in relapsed AML, the 2 years OS and EFS were 41% and 33% respectively. Concerns about hepatic toxicity have limited mylotarg® use, particularly for patients who had previously received SCT. Several studies have shown good results and reduced toxicity when using mylotarg® in a fractionated dose schedule.

Aims: In this study, we report a one center experience of a new association with fractionated mylotarg® 3mg/m^{2}on day 1, 4, 7, mitoxantrone 12mg/m^{2}on day 1 to 3 and cytarabine 1g/m^2/12h on day 1 to 5 (MYLODAM), for refractory or relapsed CD33+ AML.

Methods: Twenty-four patients were treated with MYLODAM between January 2012 and December 2014. Median age at time of treatment was 55 years old (range 19-67). Seven patients received the MYLODAM salvage course for primary refractory CD33+ AML, 7 for relapsed AML after chemotherapy and 10 for relapsed AML after stem cell transplant (2 auto-SCT, 8 allo-SCT, in a median time between transplant and MYLODAM of 300 days). Cytogenetic risk groups were distributed as follow: 7 adverse karyotype, 9 intermediate risk and 7 favorable risk. Among the 12 patients with normal karyotype, 6 had NPM1 mutation and 5 had FLT3 ITD mutation. After reaching CR or CRp, 8 patients received at least one consolidation containing GO and 19 underwent allogeneic transplant (3 second allo-SCT, 16 first allo-SCT).

Results: Overall response rate was 19/24 (79%), including 6 CR and 13 CRp (with incomplete platelet recovery). One year estimated OS and EFS were 62 % and 60% respectively. Median OS and EFS were not reached, with a median follow-up of 12 months (figures 1A and 1B). We found no significant difference in terms of EFS and OS whether patients had refractory disease, post transplant or post chemotherapy relapse (p=0.41). Minimal residual disease was under the threshold of detection after salvage therapy for 11/ 15 responding patients with molecular evaluation (5/6 with NPM1 mutation, 5/6 with WT1 over expression and 1/3 for AML1-ETO transcript). The median duration of thrombopenia (<50G/L) and neutropenia (<500/mm^3) were 49 and 42 days respectively, for patients reaching CR or CRp. Early death (<30 days) occurred for one patient. Liver toxicity was frequent (elevation of transaminase levels: grade 0-1: 38%, grade 2-3: 54%, grade 4: 8% and hyperbilirubinemia ≥grade 2 in 2 cases). We report 2 cases of sinusoidal obstruction syndrome, none of them having received previous SCT; one patient died 18 days after the beginning of chemotherapy, the other patient was treated with defibrotide and is still alive.

Figure 1A:

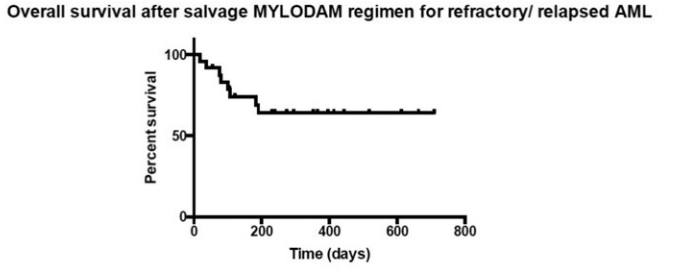

Figure 1B:

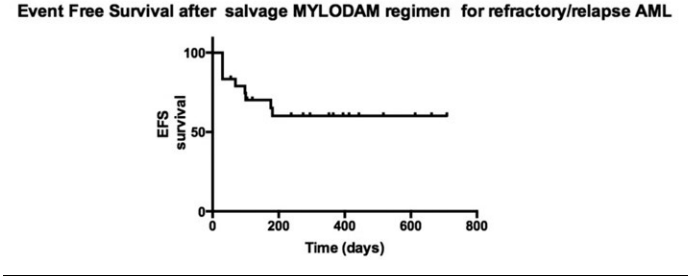

Figure 1.

Summary and Conclusions: MYLODAM is an efficient salvage regimen for refractory and relapse CD33+ AML. This combination based on fractionated mylotarg® has an acceptable safety profile, even for the 10 patients who were treated after SC transplant.

P194

EARLY PERIPHERAL BLAST CELL CLEARANCE PREDICTS MINIMAL RESIDUAL DISEASE STATUS AFTER INDUCTION IN ACUTE MYELOID LEUKEMIA

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Background: Identification of patients (pts) with acute myeloid leukemia (AML) having high likelihood to respond to standard therapy and those with low probability to do well and being candidates for more aggressive treatment is of major clinical importance. Most prognostic factors are surrogate for disease's chemosensitivity; among them, genetics is the most relevant, representing the framework of European Leukemia Net (ELN) risk stratification. It defines subgroups with relatively good survival (ELN-favorable) and at the opposite a category with poor prognosis (ELN-adverse). However, in the absence of genetic determinants, ELN system merges pts with heterogeneous diseases. At any rate, the response to induction therapy remains a powerful prognostic parameter, expressing actual chemosensitivity of AML cells. In responsive pts, minimal residual disease (MRD), usually evaluated after induction or consolidation cycles, is an accurate tool to refine risk category. We have previously demonstrated that an earlier assessment, based on the speed of peripheral blast clearance (PBC) as assessed by flow cytometry during induction therapy, strictly correlated with AML outcome.

Aims: To correlate PBC after the first 3 days of induction therapy with MRD assessment after induction at hematopoietic recovery.

Methods: Eligible pts had untreated non-M3 AML according to the World Health Organization criteria and were treated according to a standard induction therapy. At diagnosis, bone marrow (BM) aberrant leukemia-associated immunophenotypes (LAIP) were identified by flow cytometry. We quantified LAIP+ cells by single platform before treatment on day 1 and then following the first 3 days of therapy (day 4). PBC of each individual pt was expressed as the ratio between absolute LAIP cell count on days 1 and 4 converted to a logarithmic scale. By ROC curves we identified 1.5 log as the most accurate cut-off separating pts with high and low PBC index, respectively (PBC-hi, PBC-lo). In pts achieving CR after a single induction cycle, we searched for LAIP+ cells in BM expressing MRD as percentage on overall BM cellularity; pts were considered MRD+ when LAIP+ cells were detected as at least 0.01% of total BM cells.

Results: Between 2007-2013, 104 pts achieving CR had available LAIP and were studied both by PBC and MRD after induction. As concerns PBC, 34 (32.7%) pts were PBC-lo and 70 (67.3%) were PBC-hi. PBC status significantly correlated with ELN prognostic stratification of patients (p=0.0007). Regarding MRD, 50 (48.1%) pts were classified as MRD+ and 54 (51.9%) as MRD-. MRD status correlated with ELN categories as well (p=0.0067). Both PBC and MRD influenced disease-free and overall survival in univariate analysis. Most important, PBC was able to predict MRD status after induction; specifically, 45/70 (64.3%) PBC-hi and 9/34 (26.4%) PBC-lo pts were MRD-negative, respectively (Fisher's test p=0.0004). The combination of both parameters separated two categories of patients with favorable (PBC-hi/MRD-) and very poor (PBC-lo/MRD+) outcome, as depicted in Figure 1. Discordant cases showed intermediate prognosis.

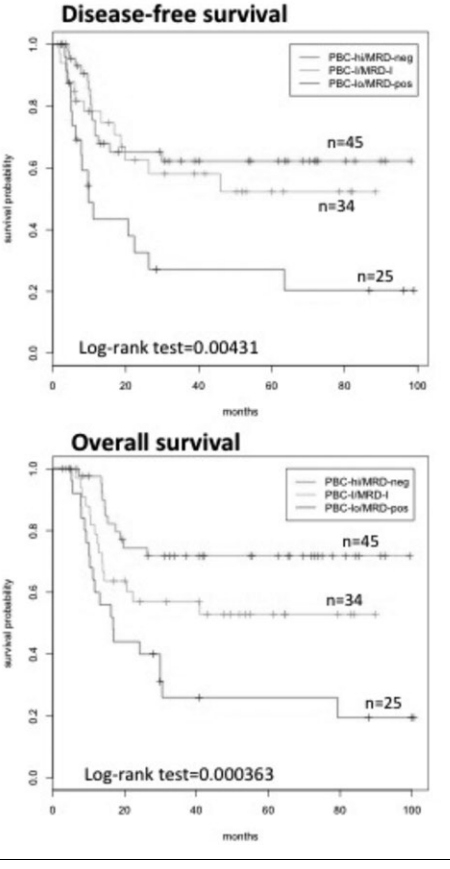

Figure 1.

Summary and Conclusions: PBC is very early and powerful outcome predictor in AML, providing a real time quantification of AML burden reduction in the first days of chemotherapy. PBC well correlated with MRD status after induction and the combination of both parameters identified pts with strikingly different outcome.

P195

MICRODUPLICATION 17Q21.31 AND 17P11.2 ASSOCIATE WITH AML DIAGNOSIS OR RESPONSE TO THE THERAPY

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Background: Genetic work on leukemia documented the predictive power of chromosomal abnormalities as to the response to treatment. GTG and FISH techniques are of clinical value. More recently, it became apparent that heritable or acquired characteristics of non transcribing portion of the genome may influence the risk of cancer and the response to the treatment.
Aims: In the present study we looked at the copy number variations (CNV) to document the possible relations between the pattern of CNV in some regions of the genome and the risk of AML course in patients with normal karyotype (NK). This approach is supported by the observation that CNV pattern influences the expression of genes being in their vicinity; therefore, their examination offers better inside into genetic potential of leukemic cells. In an advent of the wide use of specific kinase inhibitors in the treatment of AML patients with Flt3 mutation we investigated the relation between the presence of this mutation and the CNV abnormalities to find out whether Flt3 internal tandem duplication (ITD) mutation associates with a more complex genetic background in NK patients.
Methods: 82 patients with AML (median age: 57,5 years old (range 21-81 years), 79 primary and 3 secondary AML) were investigated. GTG karyotyping was technically valid in 70 patients and in 25 FISH analysis was performed (XY, inv(3),-5/5q-,-7/7q-,+8, MLL, RUNX1, PML/RARA or RARA, inv(16) and MLL). Patients without abnormalities (46 patients) were classified as those with NK. Twenty six patients were Flt3 ITD positive. All fit patients received chemotherapy with intention to treat. Unfit patients were on the low dose AraC. All patients who proceeded to the consolidation therapy phase and those unfit having a stable number of blasts at three month after the treatment started were classified as good responders all others as poor. DNA was extracted from mononuclear cells (Ficoll separation) of the marrow aspirated at the diagnosis. One microgram portions of DNA were subtracted to the analysis employing WG Catalog NimbleGene 12x270K (Roche) and Catalog Agilent Cancer CGH+SNP 180K microarrays according to the manufacturer's instruction then were scanned in the Roche NimbleGene MS 200 instrument. Data was analyzed with the use of Nexus 7.5 (BioDiscovery) program.
Results: The difference in CNV was compared between Flt3 ITD positive and negative NK AML patients. It was found that the difference in the number of CNV was statistically significant only in the analysis of chromosome region chr17q21.31: 41576590-41682478. Flt-3 negative but not Flt3 ITD positive patients had CNV gains in that region (25% *vs* 0%, p=0.03). The presence of these microduplications barely affected their response to the treatment (37% *vs* 16%, n.s.). Working further on the relationship between the response to the treatment and CNV gains in chromosome 17. We found that NK patients with CNV gains in 17p11.2: 19065233-19083457 responded poorly to the induction therapy (66% *vs* 13%, P=0.013) and suffered from poor survival (p=0.037). Notably, none of the 7 patients having gains in the 17p11.2 region had TP53 mutation evaluated by the FISH technique.
Summary and Conclusions: From the present study we cannot conclude whether CNV abnormalities found in two chromosome 17 regions are inherited or acquired; however, these genetic traits may affect either the risk of leukemia (NK patients) or response to the treatment and survival in NK AML patients.
Supported by INNOMED/I/1/NCBR/2014 CellsTherapy grant

P196

THE SIGNIFICANCE OF RELATIVE MUTANT LEVEL FOR C-KIT MUTATION IN CORE-BINDING FACTOR ACUTE MYELOID LEUKEMIA BY ALLELE-SPECIFIC REAL-TIME PCR

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Background: The chromosomal aberrations such as t(8;21)(q22;q22), which result in *RUNX1-RUNX1T1* translocation, and inv(16)(p13q22)/t(16;16)(p13;q22), which result in *CBFβ-MYH11* translocation are collectively referred to as core binding factor acute myeloid leukemia (CBF AML). CBF AML is associated with a relatively good prognosis although some CBF AML cases are difficult to manage due to relapse. Recently, *c-KIT* mutations have been identified as a poor prognostic factor. However, the prognostic impact of *c-KIT* mutation still remains controversial. In this study, we investigated the relationship between *c-KIT* mutations and prognosis in CBF AML patients using highly sensitive detection method, quantitative allele-specific real-time PCR.
Aims: We performed this study to evaluate the prognostic impact of qualitative and quantitative determination of *c-KIT* mutations in CBF AML using highly sensitive method, although there have been few studies on detection of *c-KIT* mutations using allele-specific real-time PCR.
Methods: We analyzed the *c-KIT* mutations in 111 newly diagnosed adult CBF AML patients (74 with t(8;21) and 37 with inv(16)) in St. Mary's Hospital from April 2009 to July 2013. DNA isolated from paired bone marrow samples at the time of disease relapse and complete remission was also investigated. We used allele-specific real-time PCR to detect D816 and N822 mutations in *c-KIT* exon 17 and determined exon 8 mutations using direct sequencing analysis.
Results: Of the total 111 patients, 62% (69/111) and 8% (9/111) had c-KIT mutations in exon 17 and 8, respectively. CBF AML patients with a *c-KIT* exon 17 mutation showed significantly higher percentage of blasts in bone marrow aspirates at diagnosis (77.0% *vs* 73.0%, *P* =0.031), and significantly lower *WT1* expression (0.5 *vs* 0.7, *P* =0.048) than those without a mutation. CBF AML patients with *c-KIT* exon 17 mutations had worse event free survival (EFS) than patients without mutations. The estimated 5-year EFS rates were 86.1% for patients without mutations and 61.5% for patients with mutations (*P* =0.022). The estimated 3-year EFS was the lowest in patients with high mutant allele level (37.5%, *P* =0.003). In multivariate analyses, the high level of *c-KIT* exon 17 mutant alleles was associated with a statistically significant adverse impact on both OS (HR, 7.4, *P* =0.001) and EFS (HR, 4.6; *P* =0.004). Of the 17 relapsed patients, 15 had *c-KIT* mutations at diagnosis. *C-KIT* mutations were lost at relapse in 5 patients, and 2 patients who harbored double *c-KIT* mutations at diagnosis lost the major mutations while retaining the minor at relapse. *C-KIT* mutations showed the mutation disappeared at complete remission. There was a similar trend between the level of *c-KIT* mutant and the fusion transcripts in serial analyses.
Summary and Conclusions: The small populations of leukemic cells with *c-KIT* mutations could be detected by high sensitive quantitative method and high mutant level was associated with poor outcome. The quantitative measurement of the *c-KIT* exon 17 mutant allele burden could be a valuable tool in evaluation of prognosis at diagnosis and for subsequent patient monitoring.

P197

IMPROVED SURVIVAL IN PATIENTS ≥60 WITH FIRST RELAPSED/ REFRACTORY ACUTE MYELOID LEUKEMIA TREATED WITH VOSAROXIN PLUS CYTARABINE VS PLACEBO PLUS CYTARABINE: RESULTS FROM THE PHASE 3 VALOR STUDY

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Background: Prognosis for older patients with relapsed or refractory (R/R) acute myeloid leukemia (AML) is poor, with lower response rates and shorter survival compared with younger patients. Safe and effective salvage regimens are urgently needed. Vosaroxin is a first-in-class anticancer quinolone derivative that is active in AML, is minimally metabolized, evades P glycoprotein receptor–mediated efflux, and has activity independent of p53 status. VALOR, a phase 3, randomized, double-blind, placebo-controlled trial, evaluated vosaroxin plus cytarabine (vos/cyt) *vs* placebo plus cytarabine (pla/cyt) in patients with R/R AML (NCT01191801).
Aims: To evaluate the efficacy and safety of vos/cyt *vs* pla/cyt in patients ≥60 yrs of age enrolled in the VALOR trial.
Methods: Patients were randomized 1:1 to receive cytarabine (1 g/m^2 IV over 2 hr, d 1-5) plus either vosaroxin (90 mg/m^2 IV over 10 min d 1 and 4; 70 mg/m^2 in subsequent cycles) or placebo. Up to 2 induction and 2 consolidation cycles were administered. Eligible patients had refractory disease (persistent disease after induction, or first complete remission [CR1] duration <90 d) or were in first relapse (early relapse: CR1 duration of 90 d to 12 mo; late relapse: CR1 duration of 12 mo to 24 mo). Patients had 1-2 cycles of prior induction

chemotherapy including at least 1 cycle of anthracycline/anthracenedione and cytarabine. Randomization was stratified by age (<60, ≥60 yrs), disease status (refractory, early relapse, late relapse), and geographic location (US, non-US). Primary endpoints were overall survival (OS) and 30-and 60-day mortality. Here we report results of predefined subgroup analyses in patients ≥60 yrs.
Results: Between Dec 2010 and Sept 2013, 711 patients were randomized at 101 sites; 63% (n=451) were ≥60 yrs (n=226 randomized to vos/cyt and n=225 to pla/cyt). At final analysis, OS was improved with vos/cyt in patients ≥60 yrs (7.1 mo *vs* 5.0 mo with pla/cyt; HR=0.75; *P*=0.003) (**Figure**). Event-free survival was also improved (2.1 mo *vs* 1.3 mo with pla/cyt; HR=0.61; *P*<0.0001). CR was achieved in 31.9% of patients ≥60 yrs treated with vos/cyt *vs* 13.8% treated with pla/cyt (*P*<0.0001). Responses were durable; median leukemia-free survival among patients ≥60 yrs who achieved CR was 10.3 mo with vos/cyt *vs* 6.5 mo with pla/cyt (*P*=0.20). The rate of subsequent allogeneic transplant in patients ≥60 yrs was 20.2%. In a predefined sensitivity analysis in which patients were censored at allogeneic transplant, the survival benefit in patients ≥60 yrs was maintained (6.7 mo with vos/cyt *vs* 5.0 mo with pla/cyt; HR=0.75; *P*=0.009). Thirty-day and 60-day all-cause mortality in patients ≥60 yrs was similar between treatment arms (30-day: 10.2% *vs* 9.0%; 60-day: 20.4% *vs* 22.6% with vos/cyt *vs* pla/cyt, respectively). Serious AEs in the ≥60 population were more common with vos/cyt treatment (57% *vs* 33% with pla/cyt); most common serious AEs were febrile neutropenia (9.3% with vos/cyt *vs* 5.9% with pla/cyt), sepsis (9.3% *vs* 5.0%), pneumonia (7.5% *vs* 4.5%), bacteremia (7.5% *vs* 2.3%), and stomatitis (4.4% *vs* 1.8%). Serious and non-serious cardiac, renal, neurologic, and hepatic AEs were comparable between treatment groups.

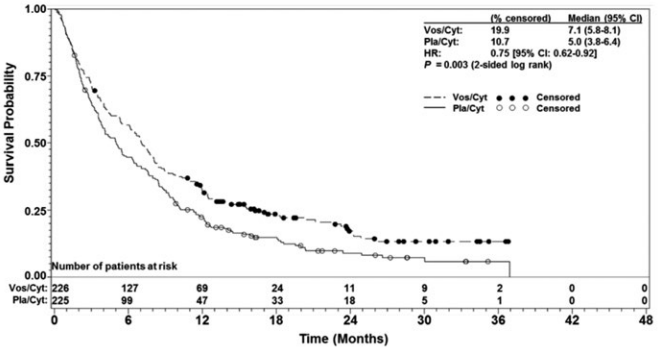

Figure 1. OS in patients age ≥60 years, by treatment arm (n=451).

Summary and Conclusions: The addition of vosaroxin to cytarabine improved OS and increased CR rates without increasing early mortality in older patients with R/R AML, a population with few treatment options. The additional toxicity observed in the vosaroxin arm was acceptable in light of the benefit received. VALOR results support the use of vos/cyt as a standard of care in patients ≥60 yrs of age with R/R AML.

CLL - Biology: Microenvironmental interactions

P198

IMPORTANCE OF THE B-CELL RECEPTOR ISOTYPE FOR SIGNALING AND FUNCTIONAL RESPONSES IN B CELLS FROM PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: B-cell receptor (BCR) signaling is a central pathogenetic mechanism in chronic lymphocytic leukemia (CLL). CLL cells express immunoglobulins of both IgM and IgD isotypes, but the relevance of these isotypes for signaling and functional responses remains incompletely understood.
Aims: The aim of the present study is to characterize functional differences between IgM and IgD signaling.
Methods: Purified CLL cells from 73 CLL patients (40 M-CLL and 33 U-CLL) were stimulated with anti-IgM and anti-IgD; phosphorylation levels of the BCR-related molecule HS1 and of the downstream kinase ERK was analyzed in serial samples after 2, 5, 15, 30, and 60 minutes of stimulation by Western Blot and flow cytometry; BCL6 expression were analyzed after 3, 6, 9, 12, 24 hours of stimulation by Western Blot. 48-hour cell viability was analyzed by flow cytometry; CCL3 and CCL4 chemokine production was measured by ELISA after 3, 6, 9, 12, and 24 hours of stimulation.
Results: sIgM and sIgD levels were significantly higher in U-CLL. Relative surface expression of IgM was 34.81±5.346 in M-CLL, as compared to 85.71±10.51 in U-CLL (p<0.0001). Relative surface expression of IgD was 32.30±4.463 in M-CLL, as compared to 59.74±8.054 in U-CLL (p=0.0048). Anti-IgM stimulation protected CLL cells from *in vitro* apoptosis to a greater extent than anti-IgD and this difference was more evident when considering the U-CLL subset of patients, which showed a mean relative viability after anti-IgM stimulation of 178±21.79% as compared to anti-IgD stimulation, which only reached 114.6±7.171% (p=0.0023). IgM stimulation induced protracted HS1 and ERK phosphorylation, up to 30 minutes following receptor engagement. In contrast, IgD stimulation induced short-lived phosphorylation of both proteins. CCL3 and CCL4 chemokines were secreted only after IgM stimulation, and surprisingly low, if any, induction was noted after IgD stimulation. When U-CLL cells were stimulated with 10 mg/mL anti-IgM, CCL3 levels in the surnatants were 2155±719.1 pg/mL, as compared to 96.70±23.38 pg/mL after 10 mg/mL anti-IgD. CCL3 and CCL4 chemokine secretion peaked after 9-12 hours of IgM stimulation, and was concomitant to BCL6 down-regulation, in particular in U-CLL cases. Interestingly, when CLL cells were co-cultured for 14 days with NLCs, relative surface IgM levels were down-modulated from 47.36±18.95 to 41.39±16.08 in M-CLL (p>0.05), and from 80.01±18.19 to 43.45±7.824 in U-CLL (p=0.0195). Consistent to this finding, CCL3 and CCL4 chemokines were secreted in CLL-NLC cocultures, in particular in U-CLL cases. The mean CCL3 levels in U-CLL surnatants were 923.9±250.8 pg/mL, as compared to 256.7±117.3 pg/mL for M-CLL (p=0.0062); CCL4 levels were also higher in U-CLL, but did not reach statistical significance.
Summary and Conclusions: IgM stimulation induces more durable signaling responses, increased CLL-cell survival, CCL3 and CCL4 secretion, and concomitant BCL6 down-regulation, in particular in U-CLL. In contrast, IgD responses are more transient and have no effect on CLL-cell survival or chemokine secretion. BCL6 is a known transcriptional repressor of the CCL3 gene in normal B cells, and our findings suggest that this mechanism is also recapitulated in CLL cells. In addition, stimulation provided by NLCs is associated with IgM receptor downmodulation and CCL3 and CCL4 chemokine secretion in U-CLL. Taken together, these evidences support the prevalence for IgM signaling in CLL pathogenesis.

P199

HIGHER-ORDER IMMUNOGLOBULIN SEQUENCE RELATIONS FOR MAJOR SUBSETS OF CHRONIC LYMPHOCYTIC LEUKEMIA: UNIQUENESS *VERSUS* EQUIVALENCE

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Background: Studies of the B cell receptor immunoglobulin (BcR IG) in chronic lymphocytic leukemia (CLL) revealed that ~30% of cases can be assigned to stereotyped subsets. In our previous publication of 7,596 CLL BcR IG gene sequences, we focused on 19 major subsets, collectively accounting for 12% of the cohort. This process was based on the implementation of stringent criteria focusing on high sequence relatedness. However, we have been aware of BcR IG sequences similar to those in or even between major subsets, yet set apart due to not fulfilling the adopted criteria.
Aims: We systematically searched for previously obscured sequence relatives of major BcR IG stereotypes with the aim of obtaining a wider, better connected, and thus more insightful view into IG repertoire restriction in CLL.
Methods: Sequence relatives of major subsets were sought for through applying a series of criteria in reference to each respective subset: (i) usage of the same IGH genes, (ii) VH CDR3 length difference ranging from-2 to +2 amino acids (aa); and, (iii) expression of the same VH CDR3 sequence motif. A total of 20331 productive IGHV-IGHD-IGHJ gene rearrangements from a multi-institutional series of CLL patients were evaluated, with assignment to major subsets performed with purpose-built bioinformatics methods.
Results: Overall, 2450/20331 (12.1%) CLL sequences formed the 19 major subsets, each containing 44 to 548 sequences. The relative frequency of each major subset was highly similar between the present and the previous analysis, justifying their consideration as major. The quest for sequence relatives to major subsets was performed individually for each subset. For major subsets with mutated IGHV genes (M-CLL), generally, very few non-subset sequences showed adequate VH CDR3 similarity, and even these utilized different IGHV genes, thus not satisfying our search criteria. Amongst subsets with unmutated IGHV genes (U-CLL), a striking case of relatedness was observed between major subsets #1 (501 cases) and #99 (70 cases), characterized by the usage of the same IGH genes and the presence of a shared QWL motif at the same positions within VH CDR3. Their single important difference concerned VH CDR3 length (subset #1: 13 aa, subset #99: 14 aa). For the remaining major U-CLL subsets, certain subsets remained "unique", contrasting others for which we identified closely related sequences we now deem as "satellite" subsets. In particular, in the case of subset #8 (IGHV4-39) we identified two "satellite" subsets that consisted of 35 and 10 cases and displayed relative sizes of 37.6% and 10.8% (compared to subset #8), respectively. Even more pronounced was the case of subset #31 (IGHV3-48), with its "satellite" comprising of 51 sequences and a relative size of 86.4% compared to subset #31, thus almost equal to its established relative.
Summary and Conclusions: Major M-CLL stereotyped subsets remained isolated, with unique sequence characteristics. In contrast, a clear trend was evident for many major U-CLL subsets in having "satellites", potentially functionally equivalent, and often large in size. These findings could be attributed to the intrinsic differences between M-CLL *versus* U-CLL subsets in terms of IG gene repertoire and VH CDR3 features. Considering that major stereotyped subsets may indeed represent distinct disease subgroups, our highlighting of higher-order sequence relations has implications for clinicobiological research aimed at dissecting the heterogeneity of CLL towards identifying distinct profiles that would assist in clinical decision making.

P200

SPECIFIC T-CELL IMMUNE RESPONSES AGAINST AUTOANTIGENS RECOGNIZED BY CHRONIC LYMPHOCYTIC LEUKEMIA CELLS

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Background: The etiology of chronic lymphocytic leukemia (CLL) remains elusive, but there is growing evidence indicating a role of autoimmunity as well as relevance of antigen stimulation in pathogenesis of CLL. Appearance two groups of patients with mutated and unmutated status of the rearranged immunoglobulin heavy variable *(IGHV*) genes and more frequent occurrence of B-cell receptor (*BCR*) gene sequences, that are virtually-identical in unmutated CLL suggest that an unknown antigen could be involved in development of CLL. Recent studies proved occurrence of monoclonal antibodies directed against cytoskeletal proteins including non-muscle myosin heavy chain IIA (MYHIIA), vimentin, and cofilin-1, that are exposed on the surface during apoptosis. These molecules could represent autoantigens recognized by the specific BCR of the CLL cells.
Aims: Current study aimed to characterize response of T cells specific to epitopes derived from three autoantigens including MYHIIA, VIM and CFL1.
Methods: *MYH9, VIM,* and *CFL1* genes expression was analyzed using qRT-PCR in 212 CLL patients. HLA-A2-restricted epitopes derived from MYHIIA, cofilin-1 and vimentin were selected *in silico* using 2 independent prediction algorithms. The affinity of MHC class I-restricted peptides was determined by cellular-peptide binding affinity assay utilizing T2 cell line. Mixed lymphocyte peptide cultures (MLPC) were performed to define immunogenicity of synthesized peptides *in vitro.* To detect specific immune response ELISpot assays for specific IFN-γ release were evaluated. Finally, the frequency of the specific T cells directed against selected peptide with the highest along with detailed phenotypical characteristics was assessed using dextrameric peptide-specific complexes in 7-colour flow cytometry panel *ex vivo*.
Results: In CLL patients analyzed with qRT-PCR *CFL1* overexpression in comparison with healthy volunteers was found, while there were no differences in *MYH9* and *VIM* expressions between CLL and HV. The *in silico* analyses followed by *in vitro* HLA-A2 cellular-peptide binding assay enabled to define 9 immunogenic peptides derived from MYHIIA, vimentin and cofilin-1. MLPC with ELISpot assays revealed higher specific cytotoxic immune response for peptides derived from MYHIIA in 4/6 (66.7%), vimentin 7/8 (87.5%) and cofilin-1 5/8 (62.5%) in CLL patients in comparison with healthy volunteers 3/8 (37.5%).Multicolor flow cytometry panel demonstrated low frequencies of autoreactive peptide-specific T cells against MYHIIA, vimentin and cofilin-1 in CLL patients *ex vivo*; most of detected cells presented effector-memory phenotype. Results obtained from functional assays were compared with mutational status of *IGHV* genes in CLL patients including subset analysis. Interestingly, we found 6 CLL patients with stereotypic BCR represented subset clones subset no.2 [*IGHV3-21*M], subset no.4 [*IGHV4-34M*], subset no.5 [*IGHV1-69*UM], subset no.34 [*IGHV1-69*UM], subset no. 59 [*IGHV1-58*UM] and subset no. 64B [*IGHV3-48*UM]. All of these patients demonstrated reactivity against peptides derived from MYHIIA, vimentin or cofilin-1. Interestingly, we found presence of autoreactive cofilin-1-derived peptide specific cytotoxic T lymphocytes in patient characterized by subset no. 5 [*IGHV1-69UM*] *ex vivo*.
Summary and Conclusions: The presence of autoreactive T lymphocytes that might be involved in pathomechanism of CLL during stimulation of an autoreactive B cells in proliferative compartment, indicate on important role of autoimmunity in the pathogenesis of CLL. Autoantigenic stimulation through apoptotic cells could lead to the proliferation of leukemic cells, possibly maintained or at least accompanied by autoreactive T cells.

P201

STEM CELL FACTOR KLF4 IS EPIGENETICALLY INACTIVATED AND REPRESSED BY NOTCH SIGNALING IN CLL

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Background: Whole genome sequencing revealed CLL as a disease of the genome and epigenome characterized by somatic mutations and aberrant DNA-methylation. A number of genes have already been identified that show aberrant DNA methylation in CLL cells. Examples are *e.g.* ZAP70 that is involved in B cell and T cell signaling and whose promoter DNA-methylation is correlated with its decreased expression. Of note, DNA-methylation of a single CpG dinucleotide is important for ZAP70 expression and predictive of prognosis. Similarly, the

MYB proto-oncogene is directly methylated in CLL, WNT signaling activation has been related to hypermethylation of WNT inhibitor genes and also miRNA transcriptional deregulation in CLL has been linked to aberrant DNA-methylation. DNA-methylation has been reported to be stable over time in leukemic cells of CLL patients and to be similar in resting and proliferative compartments. Of interest, in addition to comparison with healthy tissue, recent publications have shown intratumor heterogeneity of DNA methylation. We therefore focused on identifying functionally relevant aberrant DNA-methylation of promoters in CLL that correlates with transcriptional activity and is therefore most likely to impact on gene regulation. With this approach we could identify the stem cell factor KLF4 to be epigenetically deregulated in CLL, and could further show that this defect is possibly caused by aberrant activation of NOTCH1.
Aims: To identify genes that: display an aberrant DNA-methylation in their promoter in CLL cells; whose promoter DNA methylation correlates with their transcriptional activity; to understand their functional role in the cellular pathomechanism of CLL.
Methods: In order to uncover the impact of aberrant DNA-methylation on the transcriptional profile of CLL cells, gene expression and methylation array profiling was performed in CLL-and B-cells using standard and custom microarrays. Methylated DNA was precipitated using a methyl-cytosin binding fusion protein (MCIp) and detected with arrayed 60bp oligonucleotides that span all RefSeq human gene promotors-3.8 to +1.8kbp from the transcriptional start sites. Functional analyses of candidate epigenetically deregulated genes were performed using transient transfections in primary CLL cells and subsequent phenotypic analysis of target gene expression and apoptosis of transfected cells. In addition, NOTCH1 signaling was inhibited in primary CLL cells using g-secretase inhibitors to assess the impact on KLF4 levels.
Results: 1866 genes were differentially expressed in CLL ($log_2FC \leq |1|$, $p \leq 0.05$). DNA-methylation covered a greater heterogeneity in CLL cells compared to the healthy counterpart. In total, 2191 genes displayed aberrant methylation within their promoter ($log_2FC \geq |0.5|$, $p \leq 0.05$). However, only 33 of these genes also showed a significant negative correlation between expression and methylation, including CBX7, ADRB2, CXCR3, LILRA4 and KLF4. We focused on the transcriptional regulator KLF4 that was recently shown to play an important role in B cell development and maturation and to act as tumor suppressor in NHL and cHL. KLF4 downregulation was correlated in CLL with hypermethylation in a region downstream of its TSS. Expression was also correlated with levels of BCL2 family members BAK, BAX, BCL2 and with the cell cycle regulator CCND1. Inhibition of NOTCH1 signaling, which is constitutively activated and frequently mutated in CLL patients, led to re-expression of KLF4 in 8 patient samples and 6 leukemia-and lymphoblastoid cell lines, indicating regulation by NOTCH1 in B cells. Intriguingly, ectopic overexpression of KLF4 in CLL cell lines resulted in the deregulation of genes involved in signaling pathways including BCR signaling.
Summary and Conclusions: The detected epigenetic deregulation of the stem cell factor KLF4 is possibly caused in CLL by aberrant activation of NOTCH1. It underlines a defect in the developmental program of CLL and suggests aberrant arrest of CLL cells in a terminally differentiated state.

P202

NOTCH2 AND NOTCH3 HAVE OPPOSITE ROLES IN THE REGULATION OF APOPTOSIS IN CLL CELLS

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Background: Chronic lymphocytic leukemia (CLL) cells express constitutively activated NOTCH2. We have recently shown that the NOTCH2 transactivation inhibitor gliotoxin induces apoptosis in CLL cells by a mechanism involving the induction of *NOTCH3*. However, the regulation and possible functions of NOTCH3 in resting and activated CLL cells remain to be clarified.
Aims: To elucidate the different roles of NOTCH2 and NOTCH3 in CLL cells we compared the effects of gliotoxin and the clinical relevant γ-secretase inhibitor (GSI) RO4929097 on CLL cells *in vitro*.
Methods: The dose and time dependent effects of these compounds on CLL cell viability and on the induction of apoptosis, respectively, was determined by MTT-assays and by FACS analysis. The influence of gliotoxin and RO4929097on the expression of *NOTCH* and apoptosis related genes was analysed by RT-PCR.
Results: Gliotoxin rapidly induced apoptosis in all CLL cases whereas RO4929097 affected CLL cell viability only in a subgroup of cases after a prolonged incubation time. All *NOTCH1* mutated CLL samples clustered in the RO4929097 sensitive group. Chronic activation of CLL cells with low doses of PMA (1ng/ml) enhanced NOTCH2 signaling, inhibited spontaneous apoptosis, and strongly induced the proliferation marker *MYC* mimicking the proliferative compartment in the lymphoid microenvironment. Inhibition of NOTCH2 by gliotoxin led to the concomitant induction of homotypic NOTCH3 signalling as indicated by the induced mRNA expression of *NOTCH3* and its ligands *JAG2* (in unstimulated CLL cells) or *DLL1* (in PMA activated CLL cells) together with an upregulation of the activation induced cell death (AICD) mediator *NR4A1*. In contrast, RO4929097 inhibited NOTCH3 signalling, *NR4A1* transcription, and counteracted gliotoxin mediated apoptosis in GSI resistant CLL cases indicating a tumour suppressive role for NOTCH3 in the leukemic cells. In line with this hypothesis, the downregulation of NOTCH2 activity in unstimulated CLL cells *in vitro* was associated with upregulation of *NOTCH3* mRNA expression and increased spontaneous apoptosis especially in CLL samples derived from Rai/Binet I/A patients. In this context, RO4929097 inhibited *NOTCH3* and *NR4A1* expression an inhibited spontaneous apoptosis depending on the GSI sensitivity of NOTCH2.
Summary and Conclusions: In summary, these data indicate that NOTCH2 and NOTCH3 have counteracting roles in the regulation of CLL apoptosis which should be considered in therapeutic approaches aimed to target NOTCH signaling in CLL. Constitutive active NOTCH2 might inhibit tumour suppressive NOTCH3 signaling in CLL cells thereby enabling the activation dependent progredient expansion of the malignant clone. Thus, gliotoxin selectively targets oncogenic NOTCH2 signalling in a wide spectrum of CLL cases while GSIs may be effective onlyin a subgroup of CLL patients including *NOTCH1* mutated cases.

P203

EXPRESSION AND FUNCTIONAL ROLE OF CORTACTIN IN AGRESSIVENESS AND DIFFUSION OF CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Cortactin is an actin-binding protein involved in several cell functions, *i.e.* the assembly and the organization of cytoskeleton. Its overexpression was observed in several human cancers and experimental data support the role of cortactin in metastatic capability through the regulation of cell motility and the release of matrix metalloproteinase-9 (MMP-9). The activity of this protein is regulated by its phosphorylation in Tyr residues by Src kinase family. We previously demonstrated that in leukemic cells from CLL patients the Src kinase Lyn is overexpressed, activated and involved in the resistance to apoptosis. Recently, we found that cortactin is overexpressed in patients with CLL.
Aims: Here we investigated the involvement of cortactin in the release of MMP-9 and, therefore, in the progression of CLL.
Methods: Blood samples were collected from 7 controls and 17 CLL patients. Informed consent was obtained according to the Declaration of Helsinki. Untouched peripheral blood B cells were purified using the *RosetteSep* for human B cells isolation kit. The samples that were used had at least 95% of normal CD19+ or neoplastic CD5+/CD19+ cells, as assessed by flow-cytometry (FC). Purified B cells (2×10^6cells/ml) were cultured in RPMI medium with or without CXCL12 (100ng/ml) for the evaluation of MMP-9 production. MMP-9 release by neoplastic B cells was also investigated after cortactin silencing in 4 patients expressing high level of cortactin. The protein was silenced by *SMART*pool siRNA collection (Dharmacon, Thermo Scientific), according to the manufacturer's instructions. Immunohistochemical (IHC) staining was performed on formalin-fixed, paraffin-embedded tissue sections using a fully automated platform.
Results: By IHC and FC we confirmed the increased expression of cortactin in CLL patients with respect to controls. Moreover, we identified to groups of patients: one characterized by high expression the other by low expression of protein. By gelatin zymography we found that the release of MMP-9 by neoplastic B cells correlated to the expression of cortactin after 5 and 24-hrs culture. To investigate whether cortactin was involved in MMP-9 secretion in CLL, a cortactin-targeted siRNA silencing system was used to knockdown this protein in 4 patients with high cortactin expression. We found that following cortactin knockdown, leukemic cells showed a defect in MMP-9 secretion, as assessed by ELISA test. This protease also showed a decreased gelatinolytic activity in culture medium, confirming the hypothesis that cortactin is involved in the regulation of MMP-9 secretion in CLL malignant cells. Finally, we found that the incubation of leukemic cells with PP2 or other kinase inhibitor, decreased Tyr phosphorylation level of cortactin and shut down the release of MMP-9 in culture medium, also following CXCL12 triggering.
Summary and Conclusions: The overexpression of cortactin in neoplastic B cells and the correlation between cortactin levels, activity and MMP-9 release suggest a role of this protein in metastatic invasion and in the CLL aggressiveness. In addition, cortactin might represent a biomarker for diagnosis and prognosis as well as target for new therapeutic strategies.

P204

ROLE OF HAUSP/PTEN NETWORK IN CHRONIC LYMPHOID LEUKEMIA PATHOGENESIS AND THERAPY

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Background: PTEN tumor suppressor was shown to play a role in Chronic Lymphocytic Leukemia (CLL). In particular, it was demonstrated that PTEN is phosphorylated in the tail by Casein Kinase II, favoring its inactivation. Further-

more, it was also proposed that PTEN levels are significantly reduced in some CLL patients. PTEN tumor suppressive functions are also regulated by proper cellular compartmentalization through mono-ubiquitination. In particular, PTEN mono-ubiquitination promotes PTEN nuclear localization, while PTEN de-ubiquitination by HAUSP promotes PTEN nuclear exclusion with dramatic consequences on the tumor suppressive function.
Aims: Assessment of the biological role of CKII/HAUSP/PTEN network in CLL in order to evaluate HAUSP as a rheostat in the regulation of PTEN. Evaluation of the therapeutic role of HAUSP inhibitors in CLL.
Methods: Primary CLL cells were collected from untreated and informed patients accordingly to ethical committee approved protocol, during routinely diagnostic procedures. Cells were enriched in CD19 fraction using the Miltenyi anti CD19 kit. CLL CD19 cells were used for different analyses. Immunofluorescence was performed to investigate PTEN cellular compartmentalization. Protein samples were used for HAUSP and PTEN protein levels quantification and evaluation of those proteins regulated by HAUSP (p53, p21, MDM2). The same material was used for PTEN ubiquitination, PTEN phosphatase assay and Casein Kinase II kinase assay. Transfected CLL cell lines with GFP-PTEN and mutant constructs were used to evaluate the contribution of PTEN in promoting growth arrest and/or apoptosis induction. HAUSP inhibitors were used in CLL cell lines and proliferation and apoptosis were evaluated with MTT technology and Annexin V-FITC/Propidium PE detection by flow cytometry.
Results: By immunofluorescence we observed that a portion of CLL patients is characterized by PTEN nuclear exclusion. PTEN protein levels did not correlate with cellular compartmentalization, suggesting that the two phenomena are regulated by different mechanisms. Forcing PTEN expression into the nucleus of a CLL cell line, with a PTEN-NLS expressing vector, was associated with strong apoptosis induction and growth arrest. These observations suggest that PTEN is functionally inactivated in CLL. Next, we assessed PTEN ubiquitination in CLL primary cells. We observed that PTEN is de-ubiquitinated, clearly demonstrating a direct role of HAUSP in PTEN nuclear exclusion of CLL cells. Our data suggested a dual regulatory mechanisms that activate HAUSP towards PTEN. HAUSP could be either over-expressed and/or regulated by Casein Kinase II, which phosphorylates HAUSP on serine residues. Interestingly, treatment of CLL cells with HAUSP small molecule inhibitors is associated with re-localization of PTEN into the nucleus, which in turn promotes cell growth arrest and apoptosis induction.
Summary and Conclusions: Our data demonstrate that (1) PTEN is functionally inactivated by nuclear exclusion in CLL; (2) PTEN can be reactivated in CLL through HAUSP and Casein Kinase II inhibitors, with significant therapeutic implications.

P205

UPREGULATED COBLL1 IN CHRONIC LYMPHOCYTIC LEUKAEMIA (CLL) PATIENTS WITH UNMUTATED IGHV IDENTIFIES A COHORT WITH INFERIOR PROGNOSIS

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Background: Chronic lymphocytic leukaemia (CLL) is a heterogeneous disease; patients are stratified into two prognostic groups with either mutated (M-CLL) or unmutated (U-CLL) *IGHV*. CLL cells are also characteristic by upregulated expression of a transmembrane tyrosine-protein kinase (ROR1), a member of Wnt/planar cell polarity (PCP) pathway, which influences migration of CLL cells and chemotaxis. We have identified recently Cordon-bleu protein-like 1 (COBLL1) as a ROR1 binding partner. The significance of COBLL1 for CLL patient stratification remains unknown.
Aims: Our aim was (i) to examine expression of *COBLL1* in CLL cells and (ii) to analyse *COBLL1* expression in relation to the patients' prognosis.
Methods: We analysed *COBLL1* expression in 174 previously untreated patients (82 U-CLL, 92 M-CLL) by qRT-PCR and correlated the results with prognostic markers (*TP53* defect, *IGHV* status, del 11q, del 13q, trisomy 12, *ATM, BIRC3*, *MYD88* and *NOTCH1* mutation). We also examined how chemotaxis of CLL cells towards chemokines CCL19 and CXCL12 differs depending on *COBLL1* expression (11 M-CLL, 10 U-CLL *COBLL1*-low, 6 U-CLL *COBLL1*-high).
Results: *COBLL1* expression in CLL cells is heterogeneous. *COBLL1* is upregulated in all M-CLL (M-CLL *vs* U-CLL, p<0.0001, Mann Whitney test), whereas its expression in U-CLL shows a bimodal pattern and distinguishes two prognostically different subgroups. Intriguingly, the U-CLL *COBLL1*-high patients suffer from more aggressive disease than the U-CLL *COBLL1*-low patients, which results in their shorter time to first treatment (TTFT), time to second treatment (TTST) and overall survival (OS) (TTFT: medians 20 *vs* 33 months, p=0.0226; TTST: 36 *vs* 57, p=0.0055; OS: 75 *vs* 207, p=0.0037, Mantle Cox test). Analysis of biological markers revealed lower somatic hypermutation load in U-CLL *COBLL1*-high cells (p=0.0062, Mann Whitney test). No other genomic differences which might result in survival differences within U-CLL patients have been found. Frequency of *TP53* defect was not statistically significant (29% U-CLL *COBLL1*-high *vs* 22% U-CLL *COBLL1*-low). Therefore, we analysed the cell migration abilities of U-CLL *COBLL1*-high towards chemokines CCL19 and CXCL12 and found impaired migration compared to M-CLL and U-CLL *COBLL1*-low cells (CCL19: M-CLL *vs* U-CLL *COBLL1*-high, p=0.0182, Mann Whitney test). In basal migration, the trend was reversed, *i.e.* basal migration of U-CLL *COBLL1*-high cells was increased compared to the M-CLL and U-CLL *COBLL1*-low cells.
Summary and Conclusions: *COBLL1* expression in CLL cells differs dramatically and identifies a novel subgroup within U-CLL patients with inferior prognosis. Their shorter survival cannot be explained by any other prognostic marker tested. Aggressive disease course of U-CLL *COBLL1*-high patients can be a result of a deregulated response to microenvironment stimuli caused by deregulated Wnt/PCP pathway. Supported by MUNI/A/1180/2014, IGA NT13493-4/2012, GACR P301/11/0747, 15-29793A, CZ.1.07/2.3.00/30.0009.

P206

EXTRACELLULAR VESICLES FROM MESENCHYMAL STROMAL CELL MICROENVIRONMENT PROTECT CHRONIC LYMPHOCYTIC LEUKEMIA B-CELLS FROM APOPTOSIS

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Background: The interactions between chronic lymphocytic leukemia (CLL) cells and the microenvironment (primarily composed by mesenchymal stromal cells-MSC) play an important role in promoting the increased survival of leukemic B cells. Extracellular vesicles (E*Vs*) produced by leukemic cells and the microenvironment may be implicated in this cross-talk. E*Vs*, including microparticles and exosomes, are small plasma membrane fragments with sizes ranging from 0.01 to 1µm, and contain products specific to the original cell, such as microRNA, mRNA and proteins.
Aims: Our objective is to assess the role of E*Vs* in the cross-talk between malignant cells and their microenvironment.
Methods: Ultracentrifugation at 150000 x g during 1h was applied to isolate E*Vs* from supernatant of MSC culture. Protein concentration was measured by BCA kit and Nanodrop. Different concentrations of E*Vs* were added to CLL-B-cells to evaluate their impact on cell proliferation and survival. PKH67 labeling and qRTPCR were performed to prove the inclusion of E*Vs* in CLL B-cells (18 samples were analyzed).
Results: We first demonstrated that E*Vs* from MSCs are able to enter in CLL B-cells. By flow cytometry with PKH67-labelled E*Vs*, we observed that 44.2, 93.8 and 100% of CLL B-cells had integrated fluorescent E*Vs* after 1, 3 and 24h respectively. Two highly expressed mRNA (collagen and fibronectin) in MSC, also detected in MSC-derived E*Vs* by qRTPCR, were increased in CLL-B cells after 24h of incubation with E*Vs* confirming EV-mediated mRNA transfer to target cells. Further analysis of apoptosis in CLL cells were assessed by flow cytometry using an Annexin/7AAD staining: addition of increasing concentrations of E*Vs* showed a protective effect on CLL B-cells from cell death (mean increase of 11% of live cells, n=19/p-value=0.007).
Summary and Conclusions: We demonstrated, by two methods, that MSC-derived-E*Vs* enter into CLL B-cells. These vesicles protect CLL cells from spontaneous apoptosis and affect mRNA expression involved in CLL cell functions. This study provides evidence of the critical role played by E*Vs* in the interactions between leukemic cells and their microenvironment.

P207

FUNCTIONAL INVOLVEMENT OF THE SRC KINASE LYN IN THE SURVIVAL OF CHRONIC LYMPHOCYTIC LEUKEMIA CELLS VIA MAINTENANCE OF ANTI-APOPTOTIC B CELL RECEPTOR SIGNALING

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Background: The Src family tyrosine kinase Lyn shows high protein levels and activity in chronic lymphocytic leukemia (CLL) cells and pharmacological inhibitors of Lyn cause apoptosis.
Aims: In a chemical genetics approach using inhibitor-resistant mutant kinases, the functional roles in CLL pathogenesis of the Src kinases Lyn and Lck and of Abl were validated with the aid of Src/Abl inhibitors in current clinical use or development. To dissect the effects of these inhibitors with relatively broad selectivity and to unequivocally assign partial contributions to blocked Lyn activity, we monitored the protection from inhibitor effects by mutant Lyn with a modified binding pocket.
Methods: For this purpose the CLL-derived, EBV-transformed lymphoblastoid

cell line JVM-3 was retrovirally transduced to express Lyn-T319I or wild type (WT) in addition to endogenous Lyn. Cellular survival of JVM-3 cells and CLL samples after inhibitor treatment was determined by annexin V/7AAD staining. Signaling analysis was performed using Western Blotting and electrophoretic mobility shift assays. Transient down-regulation of Lyn expression was achieved after nucleofection with specific siRNA. In addition, the impact of Src/Abl inhibitors on purified Lyn and Abl was determined in a FRET-based kinase activity assay.

Results: The activity of purified Lyn and Abl was inhibited to similar degrees by Src/Abl inhibitors at >5000-fold lower concentration as required for cytotoxicity for malignant B cells and more strongly by dasatinib than bosutinib. JVM-3 cells showed similar sensitivity for Src-Abl inhibitors as CLL cells isolated from patients' blood, *e.g.* approximately 2.5 times higher cytotoxicity of bosutinib than dasatinib. The activating autophosphorylation of the activation loop tyrosine of Src kinases was diminished by Src-Abl inhibitors in parental JVM-3 cells and in transductants expressing Lyn-WT, but maintained with Lyn-T319I expression. In the same manner survival of JVM-3 cells was reduced in the presence of Src-Abl inhibitors and rescued by expression of Lyn-T319I, but not by Abl-T315I. Src-Abl inhibitors as well as siRNA knock-down of Lyn led to phosphatidylserine exposure, increased poly(ADP)ribosyl polymerase cleavage and reduced Mcl-1 levels. Stronger cell killing by siRNA knock-down of Lyn than of Lck corresponded to a higher degree of protection from inhibitor-induced apoptosis by Lyn-T319I than by Lck-T316I. In the presence of Src/Abl inhibitors, Lyn-T319I restored the activating phosphorylation of the direct Lyn substrates Syk and phospholipase C-γ and of Akt at serine 473, as well as the DNA binding activity of the transcription factors NFκB and STAT3. Lyn determined the maintenance of high levels of the anti-apoptotic protein Mcl-1 mainly via transcriptional mechanisms.

Summary and Conclusions: The described chemical genetic approach permitted to confirm a functional role of Lyn at nodes of anti-apoptotic signalling networks and to quantitatively compare the contributions of Lyn and other kinases.

P208

THE COMBINATION OF ARSENIC TRIOXIDE AND IBRUTINIB INDUCES SYNERGISTIC APOPTOSIS IN CHRONIC LYMPHOCYTIC LEUKAEMIA THAT IS POTENTLY ASSOCIATED WITH PI3K-AKT DOWN-REGULATION

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Background: Chronic lymphocytic leukaemia (CLL) is generally treated by chemoimmunotherapy, and there is an unmet medical need for novel targeted therapies or combination therapies. Ibrutinib is an efficient therapeutic agent for CLL. However, some patients do not respond to ibrutinib, and the long-term safety of ibrutinib has not been established. (Chung *et al.*, Pharmacotherapy2014) By combining ibrutinib with other agents, higher responses are expected in CLL patients. Our previous study also suggested that arsenic trioxide (As_2O_3) induces CLL cell apoptosis and serves as an efficient therapeutic agent for CLL alone or in combination with other agents.

Aims: The aim of the study is to investigate whether As_2O_3 combined with ibrutinib inhibits CLL cell proliferation synergistically *in vitro* and *in vivo*.

Methods: The CLL cell line MEC1 and primary B lymphocytes (PBL) from CLL patients were used to examine the effects of As_2O_3 and ibrutinib alone or in combination on CLL proliferation and apoptosis. The combinatorial effects of As_2O_3 and ibrutinib on the growth and apoptosis of MEC1 cells and PBLs were evaluated. Chou–Talalay analyses were preformed to evaluate the combinatorial effects of As_2O_3 and ibrutinib on CLL cells *in vitro*. To identify the possible signal pathway involved in the synergistic effect, microarray analysis was conducted on PBLs from 5 independent patients with CLL before and after treatment with As_2O_3 and ibrutinib alone or in combination. *in vivo* studies were conducted using MEC1 cells xenografted in BALB/c nude mice. Human MEC1 cells were inoculated subcutaneously into nude mice to establish a human leukaemia xenograft model.

Results: The effect of the As_2O_3 and ibrutinib combination is synergistic in CLL cells. The combination of As_2O_3 and ibrutinib has a minor effect on the viability of normal peripheral blood lymphocytes. The results from the gene expression profiling analysis indicate that As_2O_3 causes the down-regulation of PI3K target genes. Ibrutinib exerted only modest effects on PI3K-AKT signatures, whereas the combination had an even more profound effect compared with As_2O_3 alone. Signatures associated with NF-κB were strikingly down regulated by ibrutinib and to a lesser extent by As_2O_3. Finally, STAT3 target genes were reduced by treatment with ibrutinib; As_2O_3 had a lesser effect. The cytotoxicity of combination therapy was associated with increased Caspase-3,-8,-9 and Bax expression and a more effective reduction of PI3K-AKT. In an adoptive transfer MEC1 mouse model of CLL, As_2O_3in combination with ibrutinib affected disease progression. In this model, the$As_2O_3$and ibrutinib combination resulted in tumour regression and inhibited CLL progression more dramatically than either drug alone. The combination of As_2O_3 with ibrutinib induces significantly more tumour cell apoptosis. The expression of p-AKT proteins was significantly reduced, whereas the expression of Caspase-3 and Bax increased in the combination therapy group.

Summary and Conclusions: The combination of As_2O_3 and ibrutinib inhibits tumour growth and induces apoptosis synergistically in CLL cells *in vitro* and *in vivo*. The combination induces a minor effect on the viability of normal peripheral blood lymphocytes. The combinatorial effect against CLL cells is mediated by Caspase pathway activation and the inhibition of PI3K-AKT. The combination of As_2O_3 and ibrutinib may have clinical potential for CLL treatment.

Chronic lymphocytic leukemia - Clinical 1

P209

REFINING PROGNOSIS OF CHRONIC LYMPHOCYTIC LEUKEMIA WITH SOMATICALLY HYPERMUTATED B-CELL RECEPTORS: A NOVEL PROGNOSTIC INDEX ON BEHALF OF THE EUROPEAN RESEARCH INITIATIVE ON CLL (ERIC)

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Background: Chronic lymphocytic leukemia (CLL) patients with somatically hypermutated IGHV genes (M-CLL) within their B-cell receptor immunoglobulin (BcR IG) generally display favorable biological profiles with an indolent disease course. However, neither the biological background nor the clinical presentation and outcome of M-CLL is uniform, thus justifying the quest for parameters that would assist in refining prognosis.
Aims: Development of a novel prognostic index tailored to M-CLL.
Methods: Cox-regression models were used to assess the impact of demographic, clinical, genomic and immunogenetic features on time-to-first-treatment (TTFT) in 1359 M-CLL cases.
Results: Main features of the studied cohort were as follows: median age: 64 years (22-92); males: 810/1359 (60%), Binet A/B/C: 1169/79/53 (90%/6%/4%); CD38 expression (CD38+): 161/1225 (13%). Except for del(13q) (494/836, 59%) and trisomy 12 (+12, 142/1072, 13%), other genomic aberrations were relatively rare [del(11q): 49/1076 (4.5%), *TP53* abnormality (*TP53*abn) *i.e.* del(17p) and/or *TP53* mutations: 52/1144 (4.5%), *NOTCH1* mutations (*NOTCH1*m): 23/1333 (2%), *SF3B1*m: 33/897 (4%), *MYD88*m: 23/556 (4%)]. Borderline mutated IGHV genes (germline identity, GI: 97-97.99%) were expressed by 101 cases (7%). Thirty-three (2.5%) and 26 (2%) patients were assigned to stereotyped subsets #4 (IGHV4-34/IGKV2-30) and #2 (IGHV3-21/IGLV3-21), respectively. All the aforementioned parameters were evaluated for their impact on TTFT, using univariate regression analysis. As expected, Binet stage C at diagnosis emerged as the most powerful marker (HR: 9.44, 95% CI, 7.45-11.97, p<0.0001). Among the remaining cases (Binet A/B), representing 96% of the cohort, the following parameters were significantly associated with shorter TTFT: Binet B (HR: 7.35, 5.51-9.81, p<0.0001), subset #2 membership (HR: 4.24, 2.32-7.76, p=0.0001), CD38+ (HR: 2.62, 1.98-3.50, p<0.0001), *TP53*abn (HR: 2.56, 1.64-4, p=0.0003), +12 (HR: 1.75, 1.27-2.41, p=0.002), male gender (HR: 1.52, 1.2-1.9, p=0.004). In contrast, a trend for favorable outcome was observed for subset #4 membership (HR: 0.43, 0.16-1.18, p=0.06). Taking into consideration the above HRs as well as median TTFT, we defined 4 groups among Binet A/B patients (n=934) with different TTFT (Figure 1): (i) high risk (n=88, 9%): Binet B or subset #2 membership (median TTFT: 2.1 years, 65% and 85% probability of treatment initiation (TI) at 5 and 10 years from diagnosis, respectively); (ii) intermediate risk (n=199, 22%): Binet A and occurence of one or more of: CD38 expression/*TP53*abn/+12 (median TTFT: 12 years, 33% and 45% TI at 5 and 10 years, respectively); (iii) low risk (n=619, 66%): Binet A with no CD38+/*TP53*abn/+12 (median TTFT: not yet reached, 15% and 25% TI at 5 and 10 years, respectively); and (iv) very low risk (n=28, 3%): subset #4 membership (median TTFT not yet reached, 0% and 10% TI at 5 and 10 years, respectively).

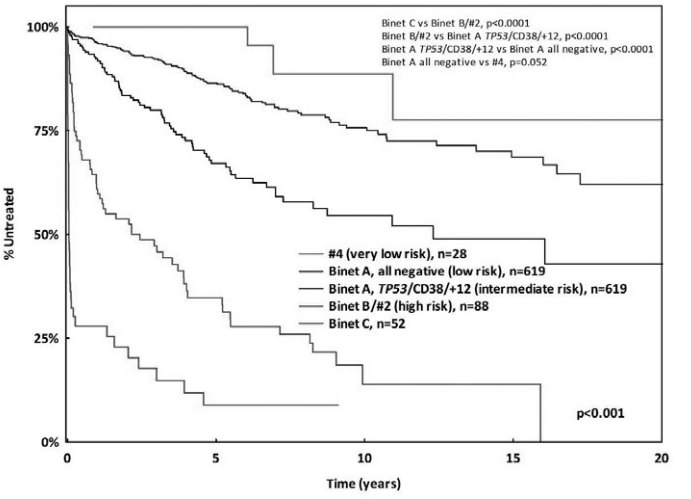

Figure 1. Kaplan-Meier curves for time-to-first-treatment (TTFT) in M-CLL.

Summary and Conclusions: We report a simple prognostic index for M-CLL capable of identifying patients who will experience short TTFT as well as those who will probably never require treatment, applicable even within early clinical stage CLL. At the ends of the clinical spectrum described by this novel index lie stereotyped subsets #2 (aggressive) and #4 (indolent). This further highlights the importance of distinct BcR IG configuration, likely linked to distinct immune signaling, for CLL ontogeny and evolution and indicates that subclassifying M-CLL based on stereotypy is relevant in the context of everyday routine practice.

P210

LONG-TERM FOLLOW-UP OF A PHASE IB TRIAL OF IDELALISIB IN COMBINATION WITH CHEMOIMMUNOTHERAPY (CIT) IN PATIENTS WITH RELAPSED/REFRACTORY (R/R) CLL INCLUDING PATIENTS WITH DEL17P/TP53 MUTATION

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Background: Idelalisib (IDELA) (Zydelig™) is a first-in-class PI3Kδ inhibitor approved in combination with rituximab for patients with relapsed CLL.
Aims: To determine the safety and clinical activity of idelalisib in combination with 6 commonly used chemo-immunotherapeutic agents for the treatment of R/R CLL.
Methods: Patients with R/R CLL were treated continuously with 150 mg BID oral IDELA and a limited number of cycles (C) of CIT to evaluate safety and efficacy of combination regimens. Patients could enroll in extension study after 48 weeks. Responses were evaluated by published criteria (Hallek 2008; Cheson 2012).
Results: 114 patients (37F/77M) median age 65 (range 41-87) years enrolled with: extensive prior therapies (median: 3, range 1-9), refractory disease (51%), high-risk Rai (60%), del17p/*TP53* mutation (29%), del11q (13%), unmutated *IGHV* (79%). Median exposure was 14.6 (range 0-49) months. 61 patients (54%) enrolled in extension study. 21 (34%) were continuing on study. Most common and select AEs independent of causality (any Grade/Gr ≥3): diarrhea/colitis (52%/19%), pyrexia (45%/4%), cough (37%/1%), nausea (29%/1%), fatigue (32%/4%), pneumonia (23%/15%), dyspnea (22%/3%), rash (21%/4%), pneumonitis (4%/4%). AST/ALT elevation Gr ≥3 was seen in 12%. Most common reasons for discontinuation were AEs (25%) or PD (25%). 2 patients discontinued due to AST/ALT elevation, 1 due to Richter's transformation. 20 (18%) deaths were reported on study; 6 patients experienced PD before death. ORR was 82.5% in all patients, 70% in patients with del17p/*TP53* mut, and 87% among patients without. SD/PD was reported in 10%/3%. Median overall PFS was 26.1 months, 20.3 months for patients with del17p/*TP53* mut, and 36.8 months for patients without. Median OS for all patients or patients with del17p/*TP53* mut was not reached. Estimated OS at 36 months was 73.1% for all patients, 57.3% for patients with del17p/*TP53*, and 78.3% for patients without.

Tabella 1.

Idelalisib +						
Rituximab (R) N = 19	Ofatumumab (O) N = 21	Bendamustine (B) N = 18	BR N = 15	Fludarabine (F) (oral) N = 12	Chlorambucil (Chl) N = 15	ChlR N = 14
8 wks	up to 6 C				up to 12 C	

Summary and Conclusions: IDELA in combination with CIT shows a manageable safety profile without increased toxicities and has substantial clinical activity in heavily pretreated, refractory, and high-risk CLL including presence of del17p/*TP53* mutation. Phase 3 trials of IDELA with O or BR in patients with R/R CLL are ongoing (NCT01659021, NCT01732926).

P211

A RANDOMISED DE-ESCALATION STUDY OF ORAL FLUDARABINE, CYCLOPHOSPHAMIDE AND RITUXIMAB IN FIT ELDERLY PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKAEMIA: WELL TOLERATED AND SUPERIOR OUTCOMES WITH FCR

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Background: Fludarabine (F), cyclophosphamide (C) and rituximab (R) gave superior progression free (PFS) and overall survival (OS) *versus* (*vs*) FC in the CLL8 Study.
Aims: We aimed to assess the safety, tolerability and efficacy of FCR based therapy in elderly patients (pts).
Methods: Previously untreated pts with CLL aged≥65 were randomised to one of 3 therapy arms: (i)*FR5*: F 24mg/m^{2}po D1-5 + R (375mg/m^{2}cycle 1, 500mg/m^{2}cycles 2-6) iv D1, (ii)*FCR3*: F 24mg/m^{2}po and C 150mg/m^{2}po D1-3 + R iv D1 or (iii)*FCR5*: F 24mg/m^{2}po+ C 150mg/m^{2}po D1-5 + R iv D1 all at 4 weekly intervals for an intended 6 cycles. Cycles could be delayed up to 2 weeks for grade 3+ toxicity, and if unresolved by 2 weeks were off study.
Results: The eligible cohort was 116. Median age was 71 (range 65-82) years; 78 males (67%) and 39 females (33%). Binet stage was progressive A-19 (16.2%), B-55 (47.0%), C-43 (36.8%). Response and grade 3+ toxicity data are shown in the table. All 6 protocol cycles were completed in 69% but less on FCR5 44% *vs* FR5 89% and FCR3 76% (p<0.001). FCR3 *vs* FR5 was not statistically significant (NSS). Reasons for non-completion were death, intercurrent illness, withdrawn consent, stable or progressive disease, unacceptable toxicity and doctor decision.

Tabella 1.

Response 2 months Post-Rx or at Rx end	Treatment arm			Total (n=116)
	FR5 (*N*=37)	FCR3 (*N*=41)	FCR5 (*N*=38)	
Complete remission (CR) BM Confirmed	10 (27%)	18 (44%)	17 (45%)	45 (39%)
CR (Bone Marrow confirmed)	9 (24%)	13 (32%)	8 (21%)	30 (26%)
CR-i (BM confirmed)	1 (3%)	5 (12%)	9 (24%)	15 (13%)
Total MRD Negative in PB	14 (38%)	21 (51%)	30 (79%)	65 (56%)
Nodular Partial Remission (nPR)	11 (30%)	13 (32%)	3 (8%)	27 (23%)
Partial Remission (PR)	10 (27%)	5 (12%)	4 (11%)	19 (16%)
Stable Disease (SD) / PD / early death	2 (6%)	2 (4%)	1 (3%)	5 (5%)
Overall Response Rate (ORR)	35 (95%)	39 (95%)	37 (97%)	111 (96%)
At least 1 grade 3+ AE	21 (57%)	34 (83%)	35 (92%)	90 (78%)
Early cessation due to toxicity	2 (5.6%)	1 (2.4%)	13 (34%)	16 (14%)

Toxicity was lower with FR5 compared to FCR3 and FCR5 (p=0.004) (FCR3 *vs* FCR5 NSS). Dose delay occurred in 43 pts (37%): FR5 32%, FCR3 34%, FCR5 44% (p=0.653), but early cessation due to toxicity was more common with FCR5 (p<0.001). 11/13 pts stopping early due to toxicity received ≥3 cycles of therapy (mean 3.5). ITT full dose FCR5 CR rates were significantly higher (79%), but also higher rates of incomplete marrow recovery, haematological toxicity and earlier cessation of therapy.
Summary and Conclusions: Final analysis shows oral FCR therapy is generally safe and well tolerated in CLL pts aged ≥65 years requiring first-line treatment, *when early stopping is utilised if prolonged toxicity occurs.* Toxicity was mostly hematological and manageable. Response rates were very high with ORR of 95% and 97% and CR rate of 44% and 45% with reduced dose and full dose FCR. Full dose FCR is highly effective and while cessation due to toxicity appears important, a dose intensity effect may exist. Dose reduced FCR provides a balance of effectiveness, safety and tolerability as first-line therapy for fit elderly pts.

P212

CLINICAL ASSESSMENT OF CHRONIC LYMPHOCYTIC LEUKEMIA SAMPLES FOR SOMATIC HYPERMUTATION STATUS BY NEXT-GENERATION SEQUENCING AND SANGER SEQUENCING

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Background: Somatic hypermutation (SHM), is an important process to increase the affinity of immunoglobulin molecules. The presence of ≥2% SHM is an important prognostic factor for CLL patients. The current method used to determine SHM status requires two steps: a PCR/capillary electrophoresis (CE) to detect clonality, followed by Sanger sequencing. This multistep approach is labor intensive and time consuming. We developed the next generation sequencing (NGS) LymphoTrack® assays to address these limitations. Here we report the results of a pilot study of SHM detection using both the NGS and CE/Sanger approaches from 50 anonymized, blinded CLL samples.
Aims: to assess the clinical performance of NGS SHM assay
Methods: LymphoTrack® SHM assays have been developed for both the MiSeq and PGM NGS platforms. Both assays and NGS platforms were used in this study. The MiSeq SHM Assay employs two master mixes. One amplifies genomic DNA between the upstream leader (VHL) region and the downstream joining (J) region of the IGH gene. The other amplifies from the framework1 (FR1) to J region. Amplicon products from VHL/J primers span the entire variable (V) region. Amplicon products from FR1/J primers encompass portions of the FR1 region to the downstream J region. The PGM Assay only employs FR1/J primers. The proprietary V and J consensus primers were designed and adapted to enable the PCR products to be sequenced on either the MiSeq or PGM platform. Multiplexed PCR was followed by amplicon purification using the AMPureXP PCR system. Purified equimolar amounts of amplicons were pooled to form a library which was loaded onto the MiSeq or PGM. The sequencing data was analyzed using Invivoscribe LymphoTrack® bioinformatics software, generating frequency distributions, DNA sequences, V-J assignment and usage, and SHM status. DNA from clinical samples was obtained from Memorial Sloan-Kettering Cancer Center.
Results: The analytical performance of the SHM assays was evaluated using contrived samples, with known V-J rearrangement and SHM status. For limit of detection (LOD), limit of blank (LOB), linearity, and precision and reproducibility (P/R) the assay demonstrated excellent linearity (R^2>0.99), sensitivity of 2.5% for clonality, and reproducibility (<20% CV). In addition, good concordance (R^2>0.99) was demonstrated between the NGS assay and the traditional method of using gel extraction and Sanger sequencing in evaluating the SHM status of cell line DNAs. The clinical performance of the LymphoTrack® SHM assays was evaluated on 50 CLL samples that have also been tested using the traditional CE/Sanger method. NGS assays were able to detect all 50 samples while 2 samples were non-evaluable by CE/Sanger method and were excluded from comparison. Between the LymphoTrack® FR1 MiSeq and CE/Sanger, 100% concordance has achieved for SHM status with linear regression of R^2=0.91 for SHM rates. The LymphoTrack SHM FR1 MiSeq assay is in 100% agreement with the LymphoTrack SHM FR1 PGM assay for both SHM status and rates. Only one discordant sample between the FR1 MiSeq and Leader MiSeq for SHM status, resulting a 98% concordance for SHM status and linear regression of R^2=0.98 for SHM rates.
Summary and Conclusions: A comprehensive NGS assay has been developed for both MiSeq and PGM platforms that identifies clonal IGH V-J rearrangements, associated specific V-J region DNA sequences and determines the SHM status in CLL specimens. This NGS assay has demonstrated excellent clinical concordance for determining SHM status with the traditional method.

P213

HEALTH RELATED QUALITY OF LIFE AND PATIENT REPORTED OUTCOMES IN PATIENTS RECEIVING OFATUMUMAB MAINTENANCE VERSUS OBSERVATION IN THE PROLONG TRIAL

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Background: The randomized phase III PROLONG study in 474 relapsed CLL patients in remission after 2nd or 3rd line induction treatment has demonstrated a statistically significant improvement in PFS by ofatumumab maintenance treatment as compared to observation: 29.4 months *versus* 15.2 months respectively (HR=0.50, p<0.0001; van Oers, 2014).
Aims: However, given that a watch and wait strategy is the current standard of care and patients were treated for up to 24 months with ofatumumab maintenance, it is important to assess the impact of maintenance therapy on health related quality of life (HRQoL).
Methods: During the PROLONG trial, the QLQ-C30 and the QLQ-CLL16 patient questionnaires were administered in all pre-progression patients during both treatment and during the follow up stage. Primary endpoints in patient reported outcomes were health related quality of life as reported by the QLQ-C30 questionnaire and a B-symptom index including patient reported symptoms of fatigue, night sweats, temperature changes and weight loss as reported by the QLQ-C30 and QLQ-CLL16 questionnaires.
Results: In HRQoL, there was no statistical difference (p=0.14) or clinically relevant difference between the arms at any time point measured during treatment with the ofatumumab arm demonstrating a mean 0.2 point decline in HRQoL on a scale of 0-100 and the observational arm demonstrating a mean 1.9 point decline in HRQoL. The B symptom index demonstrated a minor worsening across all B symptoms in patients in the observation arm (p=0.002): during treatment with ofatumumab no change occurred in B symptoms (0.0 on a 0-100 scale) whereas in the observation arm there was a 2.8 point worsening. There was a trend to a difference between the arms as to the question about worry for future health, where patients on ofatumumab reported to be less likely to worry (4 point difference on 0-100 scale, p=0.06).
Summary and Conclusions: These results clearly demonstrate that ofatumumab maintenance therapy does not negatively affect HRQoL. At present it is not known whether the observed differences in the B symptom index and future health score are based on the psychological effects of being treated instead of watchful waiting, or reflect improved quality of remission upon ofatumumab maintenance.
Reference: Van Oers, M, *et al.* (2014) Ofatumumab (OFA) Maintenance prolongs PFS in Relapsed CLL: PROLONG Study Interim Analysis Results. *Blood (ASH Annual Meeting Abstracts),*124. Abstract 21

P214

QUALITY OF LIFE BENEFITS OF IDELALISIB WITH RITUXIMAB FOR PATIENTS WITH PREVIOUSLY TREATED CHRONIC LYMPHOCYTIC LEUKAEMIA

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Background: Patients with chronic lymphocytic leukaemia (CLL) experience disease-related symptoms including fatigue, dyspnea, abdominal pain, weight loss, lymphadenopathy and sleep disturbances. Robust data on health-related quality of life (HRQL) in the form of utility scores for CLL patients are, however, scarce. Longitudinal EQ-5D data were collected in a phase III randomized controlled trial of idelalisib with rituximab *versus* rituximab monotherapy for patients with previously treated CLL unsuited to cytotoxic therapy (Study 116, ClinicalTrials.gov Identifier: NCT01539512). These data can provide further insight into the health-related quality of life (HRQL) of CLL patients.
Aims: This study aims to analyse longitudinal EQ-5D data from Study 116 to better understand the HRQL of previously treated patients receiving further treatment for CLL, and more specifically to gain insight on the HRQL profile associated with idelalisib with rituximab for previously treated CLL.
Methods: EQ-5D questionnaires were administered in Study 116 at baseline and at regular intervals: Weeks 2, 4, 6, 8, 12, 16, 20, 24, 30, 36, 42, 48, every 12 weeks thereafter prior to disease progression, and at the end of treatment. A generalised estimation equation (GEE) regression was carried out to determine absolute HRQL scores across treatment arms and whether there was a difference in quality of life between idelalisib with rituximab and rituximab monotherapy while patients were receiving treatment. GEE regression was used as this method accounts for potential autocorrelation of patient quality of life scores.
Results: Compliance rates for EQ-5D completion were good (>70%) across all time points and in total there were 1,667 observations over both treatments arms. Table 1 shows estimated mean HRQL estimates for patients receiving treatment in Study 116, across treatment arms. Estimated utility was high across treatment arms, and a significant treatment effect was found (p=0.031), with patients receiving idelalisib with rituximab having a better quality of life than those receiving rituximab monotherapy (absolute difference of 0.0652).

Tabella 1. EQ-5D estimates for patients receving treatment in Study 116

Treatment arm	Estimate	Standard Error
R utility	0.7475	0.0159
IR treatment effect vs. R	0.0652	0.0216
IR utility	0.8127	

Key: IR, idelalisib with rituximab; R, rituximab

Summary and Conclusions: The tolerability and efficacy profile of idelalisib with rituximab may provide previously treated CLL patients with high quality of life, both in absolute terms and in relation to treatment with rituximab monotherapy.

P215

CHRONIC LYMPHOCYTIC LEUKEMIA MD ANDERSON CANCER CENTER PROGNOSTIC INDEX IS SUCCESSFUL BUT STILL CAN BE IMPROVED BY INCORPORATING SERUM LDH LEVEL

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Background: MD Anderson Cancer Center (MDACC) nomogram and prognostic index is an easy prognostication system in chronic lymphocytic leukemia (CLL) because it depends on routinely available parameters (Blood 2007; 109: 4679). External validation of this prognostication system has been done recently (Br J Haematol 2014; 167: 224). Lactate dehydrogenase (LDH) level has prognostic value in many lymphoid neoplastic disorders. However to the best of our knowledge no routinely used prognostication system in CLL uses the LDH level.
Aims: In this study we aimed to test the prognostic value of MDACC scoring system in a multicenter CLL cohort and to investigate if incorporation of LDH could improve the MDACC index.
Methods: All previously untreated CLL patients who were admitted between 1997 and 2014 with available lab and clinical data for MDACC prognostic scoring and survival analysis and a baseline serum LDH level were included in the study. Patients with comorbidities affecting survival expectation and/or serum LDH level were eliminated. Survival analyses were done according to the Kaplan Meier method. Cox regression analysis was done to determine if MDACC index and LDH were independently associated with survival. If both parameters were independently associated with survival, deriving an integrated scoring method depending on Cox regression analysis odds ratios of the MDACC index and LDH was intended. All statistical tests were done using SPSS v17.

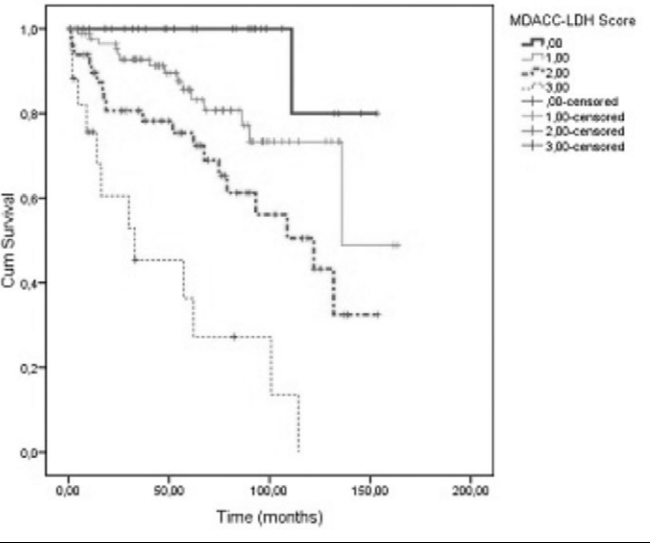

Figure 1. MDACC-LDH scores corresponded to survival durations.

Results: The cohort was composed of 326 patients (207 male, 119 female; median age [range] =63 [31-93], median f/u duration=53.5 months). The

patients in low, intermediate and high MDACC index categories at diagnosis composed 16%, 68%, and 16% of the cohort, respectively. 112 (36.6%) patients had an elevated LDH level at diagnosis. Both MDACC index (not reached, 131.8 [14.9, 102.5-161], and 62.1 [3.8, 54.5-69.6] months median [standard error, 95% confidence interval] survival durations in low, intermediate and high risk groups respectively, p=.000) and LDH level (not reached *versus* 81.1 [12.7, 56-106.1] months, p=.000) were very closely and independently (Exp(B)=2.59, p=.000 for MDACC score and Exp(B)=2.63, p=.000 for LDH) associated with overall survival. All 3 MDACC risk groups could be effectively subdivided into 2 different prognostic subgroups depending on associated normal or increased LDH level. Depending on these results 0, 1 and 2 points were assigned to the MDACC low, intermediate and high risk groups, respectively. Normal and high LDH levels corresponded to 0 and 1 points. The cumulative MDACC-plus-LDH (MDACC-LDH) score divided the patients into 4 distinct prognostic groups. MDACC-LDH scores 0 (17% of the patients), 1 (48%), 2 (26%), and 3 (9%) corresponded to median (standard error, 95% confidence interval) survival durations of not reached, 135.8 (incomputable), 122 (24.1, 74.7-169.3), and 33 (22.6, 0-77.4) months, respectively (p=.000) (Figure 1). MDACC-LDH scores 1 and 2 could be merged together for practical reasons leading to low (0 points), intermediate (1 or 2 points) and high (3 points) MDACC-LDH score levels.

Summary and Conclusions: In conclusion, MDACC index is a successful prognostic index in treatment-naïve CLL. Serum LDH level can divide each MDACC prognostic group into two different subgroups. MDACC index and LDH can be successfully incorporated into a new score.

P216

GENETIC BASIS OF HAIRY CELL LEUKEMIA (BRAF) REVEALS HIGH FREQUENCIES OF MUTATIONS IN BRAF V600E, U2AF1 AND MAP2K1 GENES

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Background: Hairy cell leukemia is a distinct B cell neoplasm with characteristic morphological and immunophenotypic features. The somatically acquired V600E mutation of the *BRAF* gene has been recently described as a molecular marker of hairy cell leukemia. In those cases which are *BRAF* negative mutations in *MAP2K1* and RNA spliceosome encoding gene *U2AF1* have recently been described.
Aims: Identification of baseline frequencies of BRAFV600E, MAP2K1 and RNA spliceosome encoding gene U2AF1 in a large series of HCL in our centre.
Methods: Genomic DNA was extracted from stained bone marrow aspirate smears of hairy cell leukemia patients. DNA was subjected to a modified ARMS PCR technique followed by capillary electrophoresis on an ABI3500 genetic analyzer. The PCR was designed to amplify a 200bp control product, a 142bp *BRAF* V600E specific amplicon and a 92bp wild type specific amplicon. Exons2 & 3 of *MAP2K1* and exon2 of *U2AF1* genes were sequenced in *BRAF* negative cases by Sanger sequencing.
Results: Majority of patients 89.1% (41/46) with a morphological and immunophenotypic diagnosis of HCL were positive for *BRAF* V600E mutation. Of the five patients that were *BRAF* negative a single patient harboured P124L mutation in exon2 of MAP2K1 gene. This mutation has been described in papillary carcinoma of the thyroid but not in patients of HCL.
Summary and Conclusions: Conclusions: Our findings point that analysis of *BRAF* mutations is a potential diagnostic tool to distinguish HCL from other B-cell lymphomas with similar features such as the HCL variant and splenic marginal-zone lymphoma. This distinction is clinically relevant, since HCL, but not HCL-like disorders, responds to therapy with interferon or purine analogues.
Summary: *BRAF*V600E mutation present in 89.1% patients of HCL in our cohort represents a reliable molecular marker for the accurate diagnosis of hairy cell leukemia.

P217

LLC-LENAR-08 A PHASE I/II STUDY LENALIDOMIDE-RITUXIMAB IN RELAPSE/REFRACTORY CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Phase II studies showed lenalidomide monotherapy is active in relapsed and refractory CLL. A synergistic activity between rituximab and lenalidomide against CLL has been suggested and a previous study has evaluated the combination of lenalidomide and rituximab in this population with encouraging results (J Clin Oncol, 2012,31: 584), but the optimal dose and schedule for this combination remains unknown.
Aims: The endpoint in phase I of this study is to define the optimum dose of lenalidomide in combination with rituximab in R/R CLL patients. The endpoint in phase II is to evaluate effectiveness and toxic profile of the resulted schedule.
Methods: We carried out a multicenter phase I/II study on R/R CLL patients, in the dose finding phase we started with a continuous lenalidomide daily dose of 2,5mg and rituximab (375mg/m2 cycle 1 and 500mg/m2 cycles 2-6/28 days) with cohorts of 3 patients (if no DLT move to a higher dose; if one DLT occurred in the cohort expand 3 more patients). In the second phase we evaluated efficacy (*NCI WGC criteria*), toxic profile (*CTCAE v3.0 scale)* OS and TTP with *Kaplan Meier.* Study registered in ClinicalTrials.gov:NCT01185262.
Results: We reported our completed Phase I/II study, 29pt from 7 Spanish sites were enrolled, 4 of them were screening failures because criteria not fulfilled, and 5 withdrew consent to participate before starting treatment. Median age 75 yr (45-86), male/female 11/9, ECOG 0/1/2: 7/12/1, median time from diagnosis 7.8 yr, median number of previous lines 2 (1-4) all but 2 pt had received fludarabine. In phase I the MTD of lenalidomide was 15 mg. Effectiveness: In phase II ORR 7 pt (53%), all PR, estimated TTP 15.4 months, estimated OS 21.9 months. Toxicity: 34 SAE were reported, in 6 cases treatment was discontinued definitively (infection 4 pt, progressive multifocal leukoencephalopathy 1pt, amyotrophic lateral sclerosis 1pt). Grade 4 neutropenia was the main adverse event associated with the regimen.
Summary and Conclusions: In our experience 15 mg is the MTD of daily lenalidomide in combination with rituximab every 28 days in R/R CLL patients. The combination was effective and well tolerated with a 53% ORR and 21.9 months estimated OS.

LB218

IBRUTINIB COMBINED WITH BENDAMUSTINE/RITUXIMAB IN PREVIOUSLY TREATED CHRONIC LYMPHOCYTIC LEUKEMIA/SMALL LYMPHOCYTIC LYMPHOMA (CLL/SLL): FIRST RESULTS FROM A RANDOMIZED, DOUBLE-BLIND, PHASE 3 STUDY

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Background: Despite considerable therapeutic advances, virtually all patients with CLL/SLL will relapse and bendamustine/rituximab (BR) is often used in this case. Ibrutinib is a first-in-class, once-daily, oral covalent inhibitor of Bruton's tyrosine kinase, an essential enzyme in the B-cell receptor signaling pathway and a key mechanism promoting disease progression in B-cell malignancies. Clinical trials of ibrutinib have demonstrated a favourable benefit/risk profile in several B-cell malignancies, including previously-treated CLL, leading to the approval of ibrutinib single agent therapy in multiple countries.
Aims: The international, multicentre, double-blind, placebo-controlled phase 3 HELIOS study evaluated the efficacy and safety of ibrutinib in combination with BR (ibrutinib+BR) compared with placebo plus BR (placebo+BR) in patients with previously treated CLL/SLL. The preplanned interim analysis reported here showed that the primary end point was met, upon which the Independent Data Monitoring Committee recommended unblinding the study.
Methods: Patients were randomized 1:1 to receive BR (≤6 cycles) with either ibrutinib (420 mg daily) or placebo. Stratification factors included whether the patient was refractory to purine analogs and the number of prior therapies. Patients with del17p (>20% of cells) were excluded. The primary end point was independent review committee (IRC)-assessed progression-free survival (PFS). Secondary end points included IRC-assessed overall response rate (ORR) and overall survival (OS).
Results: In total, 578 patients were randomized (289 per arm); median age was 64 yrs; 38% had Rai Stage III/IV disease; median of 2 prior therapies. Six cycles of BR were completed in 81.9% and 77.4% of patients in the ibrutinib

and placebo arms, respectively. At a median follow-up of 17.2 months, IRC-assessed PFS was significantly longer with ibrutinib+BR *vs* placebo+BR (median not reached *vs* 13.3 months; HR: 0.203, 95% CI: 0.150-0.276, *P*<0.0001); PFS results were consistent across all subgroups. IRC-assessed ORR was 82.7% *vs* 67.8% (*P*<0.0001) and the rate of complete response/complete response with incomplete marrow recovery (CR/CRi) was 10.4% *vs* 2.8% with ibrutinib+BR *vs* placebo+BR, respectively. Investigators reported ORRs of 86.2% *vs* 68.9%, P<0.0001, and rates of CR/CRi of 21.4% *vs* 5.9%, respectively. Median OS was not reached (HR 0.628: 95% CI 0.385, 1.024; *P*=0.06). Ninety patients (31%) in the placebo+BR arm with confirmed progressive disease crossed over to receive ibrutinib, as permitted per the protocol. The incidence of most adverse events (AEs) was similar between arms. The most common all-grade AEs with ibrutinib+BR and placebo+BR respectively were neutropenia (58.2% *vs* 54.7%) and nausea (36.9% *vs* 35.2%); the most common grade 3/4 AEs were neutropenia (53.7% *vs* 50.5%) and thrombocytopenia (15.0% each arm). Rates of grade 3/4 atrial fibrillation were 2.8% and 0.7%, and major hemorrhage were 2.1% and 1.7%.

Summary and Conclusions: The addition of ibrutinib to BR significantly reduced the risk of progression or death by 80% compared with placebo+BR. The ORR was also significantly improved with ibrutinib+BR *vs* placebo+BR. Safety of ibrutinib+BR was consistent with the known profiles for ibrutinib and for BR. The data further support ibrutinib as an important treatment option for patients with previously treated CLL/SLL.

LB219

OFATUMUMAB (O) IN COMBINATION WITH FLUDARABINE (F) AND CYCLOPHOSPHAMIDE (C) (OFC) VS. FC IN PATIENTS WITH RELAPSED CHRONIC LYMPHOCYTIC LEUKAEMIA (CLL): RESULTS OF THE PHASE III STUDY COMPLEMENT 2

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Background: The addition of anti-CD20 monoclonal antibody (mAb) rituximab to the FC chemotherapy combination has shown improved response rates, progression free survival, and overall survival compared to FC alone in previously untreated and relapsed CLL. Ofatumumab is a human anti-CD20 mAb that is more effective than rituximab when used as monotherapy in patients with CLL that is refractory to fludarabine and alemtuzumab, and has shown activity in patients with rituximab-refractory CLL. Ofatumumab has also shown activity in combination with chlorambucil and bendamustine in patients with previously untreated CLL, as well as maintenance monotherapy in patients with relapsed CLL.

Aims: This report describes the clinical effect of ofatumumab in combination with fludarabine and cyclophosphamide (OFC) *vs* fludarabine and cyclophosphamide (FC) alone in a randomised phase III study in patients with relapsed CLL (Study COMPLEMENT 2; OMB110913).

Methods: Patients with relapsed CLL (progressive disease after ≥6 month response to at least one prior therapy) who required therapy per the 2008 NCI-WG guidelines were randomised (1:1) to receive either OFC or FC. F and C were administered as intravenous (IV) infusions (F: 25mg/m^2, Days 1-3 every 28 days for 6 cycles; C: 250mg/m^2, Days 1-3 every 28 days for 6 cycles). O was also administered as IV infusions (Cycle 1: 300mg day 1 and 1000mg day 8, subsequent cycles: 1000mg at day 1). O premedication included acetaminophen, antihistamine and glucocorticoid. The primary endpoint was progression-free survival (PFS) assessed by an Independent Review Committee (IRC) and secondary endpoints included overall response rate (ORR), time to next CLL therapy (TNT), overall survival (OS), and safety.

Results: 365 patients from 18 countries were randomized, 359 received treatment. Baseline demographics and disease characteristics were balanced between the two arms. Median age was 61 years. All Binet stages were represented (A 16%, B 55%, C 28%), and 6% of patients had 17p deletion. Median duration of treatment for both arms was 6 cycles. PFS assessed by the IRC was prolonged in the OFC arm (28.9 months) compared to the FC arm (18.8 months, HR=0.67 [95%CI=0.51, 0.89]; p=0.0036). The ORR (95% CI) by IRC assessment was 84% (77%, 89%) for OFC and 68% (60%, 74%) for FC (p=0.0004). Median OS was 56.4 months in the OFC arm and 45.8 months in the FC arm (HR=0.78; [95%CI=0.56, 1.09]; p=0.1404) with a median follow-up of 34 months. Grade ≥3 AEs from start of treatment to 60 days post last dose occurred in 74% of patients given OFC and in 69% of patients given FC. Grade ≥3 infusion reactions were reported in 9% of patients given OFC and in 3% given FC. Grade ≥3 cytopenias in both arms included neutropenia (OFC: 53%; FC: 39%), thrombocytopenia (OFC: 14%; FC: 25%), and anaemia (OFC: 8%; FC: 12%). Grade ≥3 infections were reported in 19% of patients given OFC and in 14% given FC.

Tabella 1. Baseline Characteristics, Time to event Endpoints and Response.

	OFC (N=183)	FC (N=182)
Characteristic	**Median (range)**	
Age, years	62 (38-83)	61 (32-90)
	% of patients	
Male	57	64
Rai Stage III–IV at screening	32	35
Binet Stage C at screening	28	29
Del 17p	4	8
Del 11q (no 17p)	24	20
Unmutated IGHV	68	69
Time-to-event endpoints	**Median (95% CI), months**	
Progression-free survival (IRC)	28.9 (22.8,35.9)	18.8 (14.4,25.8)
	HR: 0.67 (0.51,0.89); p=0.0036	
Time to next therapy	48.1 (40.5,60.4)	40.1 (32.1,48.4)
	0.72 (0.50,1.03); p=0.0613	
Overall survival	56.4 (44.2,nr)	45.8 (37.3,nr)
	HR: 0.78 (0.56, 1.09); p=0.1404	
Response	**% of patients**	
Best OR (95% CI) (IRC)	84 (77%,89%)	68 (60%,74%)
	P=0.0004	
Complete response (IRC)	28	8
Partial response (IRC)	55	59
Stable disease (IRC)	11	28
Progressive disease (IRC)	0	0
Not evaluable (IRC)	4	2
Missing (IRC)	1	2

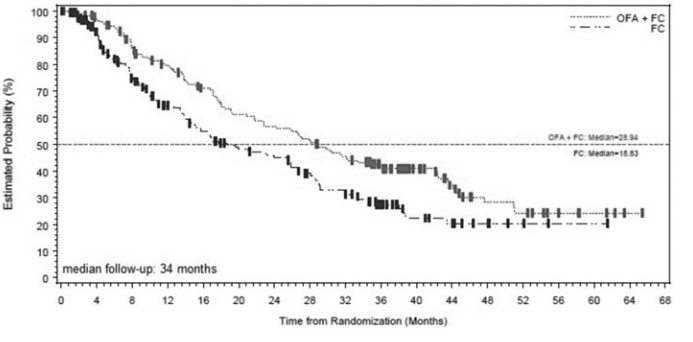

Figure 1. Kaplan-Meier Graph of IRC-assessed Progression Free Survival.

Summary and Conclusions: The results of this randomised study of ofatumumab added to FC demonstrate clinically important improvements in efficacy compared with FC alone, and the combination of OFC showed a manageable side effect profile in patients with previously treated CLL.

Chronic myeloid leukemia - Biology

P218

NON GENOMIC LOSS OF FUNCTION OF TUMOR SUPPRESSORS IN CML: BCR-ABL PROMOTES P53 NUCLEAR EXCLUSION THROUGH THE INTERACTION WITH IKB-ALPHA

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Background: The function of tumor suppressors (TS) can be tightly controlled by various non-genomic mechanisms, such as epigenetic silencing, regulation by non-coding RNAs and post translational modifications. The identification of mechanisms that cause non genomic loss of function of TS could have tremendous consequences from the therapeutic standpoint. Targeting pathways that inactivates TS could indeed promote the re-activation of a specific tumor suppressor with strong biological consequences. IκBα is commonly known as the inhibitor of the transcription factor (TF) NFκB, which in turn regulates the transcription of many genes involved in immune and inflammatory responses, as well as genes regulating cell proliferation and survival, including IκBα itself. IκBα is also able to physically interact with the tumor suppressor protein p53. In the 20% of blast crisis Chronic Myeloid Leukemia (CML) patients, p53 is mutated, while it was never found mutated/deleted during the chronic phase of the disease.
Aims: The aim of this study was to demonstrate that BCR-ABL promotes the formation of a ternary complex with IκBα and p53 in the cytoplasm causing loss of p53 tumor suppressive nuclear pool.
Methods: HEK293T and HeLa cells transfected with IκBα expression vector alone or in combination with Bcr-Abl expression vector and primary cells collected from CML patients at the diagnosis were analysed by immunofluorescence, immunoprecipitation and western blot in order to evaluate IκBα and p53 protein levels, interactions and cellular compartmentalization. Furthermore, kinase assays have been performed with purified proteins to examine the phosphorylation status of IκBα. CML/healthy subjects and transfected cell line mRNA were analysed for NFKBIA (IκBα) and p21 expression by Real-TimePCR
Results: While assessing the cellular compartmentalization of IκBα in BCR-ABL transfected HeLa cells, we observed that IκBα is expressed mostly in the cytosol, while in parental HeLa cells IκBα showed a cyto/nuclear localization. Similarly, primary CML cells are characterized by IκBα expression exclusively in the cytosol. Using BCR-ABL transfected cells, we demonstrate that BCR-ABL physically interacts with IκBα through the BCR portion of the chimeric protein BCR-ABL. *in vitro*, BCR-ABL does not appear to directly phosphorylate IκBα on tyrosine residues. Next, we investigated whether BCR-ABL regulates IκBα expression and stability. RealTimePCR analysis does not show IκBα mRNA expression differences between normal and CML BM, and between empty and BCR-ABL transfected cell. Incubation of BCR-ABL positive cells with proteasome inhibitor MG-132 shows that BCR-ABL regulates IκBα protein stability at a post-translational level. IκBα is known to negatively regulate the NFkB pathway through the interaction with p65 subunit. Importantly, it was also reported that IκBα can interact with the p53, with consequent inhibition of some of its functions. Interestingly, here we observed that in CML primary cells, p53 is also delocalized into the cytosol and that IκBα is physically bound to both p53 and BCR-ABL in the cytosol of CML cells. The delocalization of p53 is associated with impairment of its function. The p21 mRNA levels, a downstream gene of p53, are indeed markedly reduced in CML primary cells, compared to normal bone marrow.
Summary and Conclusions: In this work, we demonstrate that BCR-ABL promotes p53 nuclear exclusion through the interaction with IκBα. Our data suggest that expression of BCR-ABL could promote non genomic loss of function of the tumor suppressor p53.

P219

TRANSCRIPTION FACTOR MEF2C IS UPREGULATED DURING CML DISEASE PROGRESSION AND INHIBITS C/EBPA EXPRESSION

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Background: Although tyrosine kinase inhibitors (TKI's) have revolutionized CML therapy, the disease progresses to advanced blast crisis which is characterized by differentiation arrested blasts. The progression primarily happens due to the presence of secondary mutations and/or C/EBPα down regulation. MEF2C belongs to the MEF2 (Myocyte enhancer factor) family of proteins which comes under MADS–box group of transcription factors. MEF2C expression is abundant in CMP (Common Myeloid Progenitors) and gradually decreases with granulocytic commitment and differentiation. Earlier it was shown that C/EBPα regulates the myeloid specific miRNA-223 and we have recently shown that miRNA-223 negatively regulates *MEF2C* in CML.
Aims: Given that MEF2C antagonizes myeloid differentiation it may repress the C/EBPα transcription and play a significant role in CML disease progression.
Methods: Affymetrix Genechip® Human exon 1.0 ST arrays (Affymetrix, Inc, USA) were used for the hybridization. Blood samples were collected from CML-BC patients with their consent and the study was approved by Institutional human ethicalcommittee. RQ-PCR was performed in Light cycler 480 II with LC 480 SYBR Green master mix reagent. ANOVA and correlation statistics was performed by using Graphpad Prism software 5.0.
Results: *MEF2C* is a confirmed target of miR-223 and its expression was negatively correlated with miR-223 expression in CML samples. To identify the genes targeted by MEF2C, microarray experiment was performed in K562 cells treated with scrambled and MEF2C siRNA. Among the differentially expressed genes, a total of 21 genes which are known to have a role in myeloid differentiation were detected using GO analysis. Four transcription factors including the C/EBPα were among the 21 genes. C/EBPα up regulation was validated by RQ-PCR in the cells. To understand the relationship between *MEF2C* and *C/EBPα* in CML, we deduced their expression pattern from publicly available Radich CML dataset GSE4170. We observed the existence of a negative correlation between *MEF2C* and *C/EBPα* and found that MEF2C expression was significantly up regulated whereas *C/EBPα* expression was significantly down regulated with CML disease progression. *C/EBPα* mRNA expression was also up regulated in imatinib treated 32D BCR-ABL cells. This shows that C/EBPα can also be regulated at mRNA level in CML progression. MEF2C expression also showed a positive correlation with the blast count, the clinical parameter which is used to determine the stage of the disease. We have also isolated peripheral blood mononuclear cells (PBMC) from a CML-BC patient and the blast cells were treated *ex-vivo* with 2μM imatinib. Imatinib treatment was able to reduce MEF2C protein and up regulate *C/EBPα* and *CSF3R* mRNA expression. We found the existence of a MEF-2 binding site in the C/EBPα promoter. The MEF-2 binding site was found to be highly conserved between human and mouse. Since role of mouse MEF2C α1 isoform is established in antagonizing the myelopoiesis we cloned the mouse C/EBPα promoter and performed a luciferase assay with MEF2C α1 isoform and found that MEF2C significantly represses the *C/EBPα* promoter.
Summary and Conclusions: The study shows that MEF2C expression increases with CML disease progression and it is BCR-ABL dependent since addition of imatinib was able to reduce MEF2C protein. MEF2C also negatively regulates the C/EBPα promoter as it affects the luciferase activity. Thus MEF2C up regulation was associated with decreased *C/EBPα* mRNA expression which can favour disease progression.

P220

MESENCHYMAL STEM CELLS FROM CML PATIENTS REGULATE MYELOID DERIVED SUPPRESSOR CELLS ACTIVATION

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Background: The complex interplay between cancer cells and immune system allows neoplastic cells to evade immune surveillance and expand. Recently, it has been demonstrated that a subpopulation of myeloid cells, defined as "myeloid-derived suppressor cells" (MDSCs), plays an important role of immune escape in chronic myeloid leukemia (CML) patients inducing T cell tolerance. Mesenchymal stem cells (MSCs) are a heterogeneous population of stromal adult stem cells with immunomodulatory properties that contribute to form a cancer stem niche where tumor cells are protected and sustained in their growth.
Aims: The aim of this study was to evaluate the influence of MSCs on expansion and activation of MDSCs in CML patients.
Methods: In a first instance, using real time PCR, we evaluated the expression of immune modulatory factors (arginase 1, NOS2, COX2, TNFα, TGFβ, IL6, IL10, IL1β) by CML MSCs (n=8) compared with healthy donors (HD) ones (n=6). Human peripheral blood mononucleated cells (PBMCs) isolated from healthy volunteer donors were cultured alone and with CML or HD MSCs (1:100 ratio). After one week, PBMCs were collected and MDSCs were then isolated using anti-CD66b magnetic microbeads. The phenotype of MDSCs (identified as CD11b+CD33+CD14-HLADR-cells) was confirmed by cytofluorimetric analysis.
Results: CML MSCs showed higher COX2,TGFβ and IL6 expression ($p<0.05$). Subsequently, we investigated the capacity of MSCs from CML patients and HD to generate MDSCs. The immunosuppressive capacity of the generated MDSCs was analyzed. Myeloid cells were co-cultured with autologous CFSE-labeled T cells stimulated by phytohaemagglutinin (PHA). Only MDSCs generated by co-culture with CML MSCs were suppressive, decreasing T cell proliferation of 31±12% ($p<0.01$) while myeloid cells generated by co-culture with HD MSCs and control (isolated from PBMCs cultured in medium alone) did not show any suppressive effect on T cell proliferation. Analyzing the expression of immune modulatory factors by MSCs after 48 h of co-culture with PBMCs, we observed higher expression of IL10 and TGFβ ($p<0.05$) andIL6 ($p<0.01$) by CML MSCs than HD ones. These data suggest that multiple mechanisms are involved in MDSCs induction by CML MSCs.
Summary and Conclusions: From our experiments we can conclude that MSCs from CML patients are able to generate and activate MDSCs and might favor cancer immune evasion in CML patients.

P221

DO ENDOTHELIAL CELLS BELONG TO THE PRIMITIVE LEUKEMIC CLONE IN CML? ROLE OF EXTRACELLULAR VESICLES

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Background: The expression of BCR/ABL in hematopoietic stem cells is a well defined primary event in chronic myeloid leukemia (CML). Some reports have described the presence of BCR-ABL on endothelial cells from CML patients, hypothesizing that the disease may arise in a primitive hemangioblastic cell. On the other hand, it has been described that extracellular vesicles (E*Vs*) released by CML leukemic cells are involved in angiogenesis modulation in the disease and that endothelial cells can incorporate them.
Aims: To assess if E*Vs* released from BCR-ABL$^+$ cells carry inside the oncogene and if their incorporation into endothelial cells leads to the expression of both BCR-ABL mRNA and the oncoprotein in the recipient endothelial cells.
Methods: For the study K562 cells and primary samples of newly diagnosed CML patients were used (after signed informed consent was obtained) as well as the endothelial EA.hy926 cell line. For E*Vs* isolation and characterization, E*Vs* obtained from K562 cell culture medium and plasma from CML patients were isolated by differential centrifugation and characterized by transmission electron microscopy and Western blot. For incorporation assays, E*Vs* from K562 cells and from plasma of CML patients were incubated with the EA.hy926 cell line for 4, 24, 48 and 72h. The incorporation was analyzed by flow cytometry and fluorescence microscopy. Total RNA from E*Vs* or cells was extracted using TRIzol Reagent and the expression was quantified by using commercial TaqMan Universal Mastermix and the Step One Plus Real-Time PCR System. The polyclonal rabbit anti-human c-ABL (BCR-ABL) antibody was used and the proteins were visualized using the enhanced chemiluminescence system.
Results: E*Vs* from K562 cells and plasma from newly diagnosed CML patients were isolated and characterized and they showed the typical cup-shaped morphology and were positive for the exosome marker CD63. By RT-PCR, the presence of BCR-ABL RNA in the E*vs* from both K562 cells and plasmatic E*Vs* of CML patients was demonstrated. Next it was verified by flow cytometry and fluorescence microscopy that after 24h of culture K562-derived E*Vs* were incorporated into EA.hy926 cells The presence of mRNA BCR-ABL on endothelial EA.hy926 cells that incorporated the E*Vs*-K562 for 4h, 48h and 72h was also detected by RT-PCR. In addition, the presence of BCR-ABL mRNA in EA.hy926 cell line after incubation with plasma-derived E*Vs* from CML patients was detected. The presence of BCR-ABL on endothelial cells incubated with Philadelphia+ E*Vs* was also confirmed by Western blot.
Summary and Conclusions: Endothelial cells acquire BCR-ABL RNA and the oncoprotein after incubation with E*Vs* released from Ph+ positive cells (either from K562 cells or from plasma of newly diagnosed CML patients). This results challenge the hypothesis that endothelial cells may be part of the Philadelphia+ clone in CML.

P222

PPAR-GAMMA LIGANDS INCREASE ANTILEUKEMIC ACTIVITY OF 2ND AND 3RD GENERATION TYROSINE KINASE INHIBITORS IN CHRONIC MYELOID LEUKEMIA CELLS

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Background: Introduction of imatinib into clinical practice revolutionized the treatment of BCR-ABL1-positive leukemias and allowed for successful development of new tyrosine kinase inhibitors (TKIs). So far several 2nd and 3rd generation TKIs has been approved for the treatment of chronic myeloid leukemia (CML). Unfortunately, a significant number of patients do not benefit from the therapy because of TKIs resistance or severe drug toxicity. Recently, the success of 2nd and 3rd generation TKIs in CML was shadowed by the reports on serious side effects of these drugs including increased risk of cardiovascular events in patients treated with nilotinib and ponatinib. Pioglitazone, peroxisome proliferator-activated receptor gamma (PPAR-gamma) ligand used in the treatment of type 2 diabetes, reduces cardiovascular risk in various clinical settings and is currently tested for secondary prevention after ischemic stroke and transient ischemic attack in patients with diabetes. In addition, although it is not classic anticancer drug, pioglitazone exerts antitumor activity and is used to potentiate various anticancer therapies in pre-clinical and clinical trials. Therefore we decided to study the influence of PPAR-gamma ligands, including pioglitazone, on 2nd and 3rd generation TKIs efficacy.
Aims: Comprehensive analysis of the influence of PPAR-gamma ligands on antileukemic properties of 2nd and 3rd generation TKIs in chronic myeloid leukemia cell line K562.
Methods: To study the cytotoxicity of PPAR-gamma ligands (pioglitazone, prostaglandin J2, rosiglitazone) and TKIs (dasatinib, nilotinib, bosutinib and ponatinib) XTT and trypan blue exclusion assays were used. The influence of the studied drugs on cell proliferation and clonogenic potential was studied using APO-BRDU and clonogenic assays. Western blotting analysis was used to study proteins involved in the regulation of cell cycle (p21, p27, cyclin D1) and apoptosis (cleaved caspase 3 & 9). In addition, phosphorylation of CrkL was measured as a marker of BCR-ABL1 kinase activity inhibition. Activation of caspase 7 was measured using fluorescent microscopy. Cell cycle was analysed using propidium iodide DNA staining (flow cytometry).
Results: PPAR-gamma ligands synergistically increased cytotoxicity of dasatinib, nilotinib, bosutinib and ponatinib (Figure1A). In addition, pioglitazone significantly enhanced the antiproliferative effect of the studied TKIs. Moreover, the addition of pioglitazone induced cell cycle arrest in G0/G1 and sensitised CML cells to TKIs as observed by increased number of cells in subG1 phase in TKI + pioglitazone group (Figure1B). Cell cycle arrest was confirmed by Western blotting analysis of p21, p27 and cyclin D1. In result of cell cycle arrest, pioglitazone significantly increased proapoptotic activity of TKIs as observed in Western blotting (cleavage of caspase 3 & 9) and microscopy (caspase 7 activity).

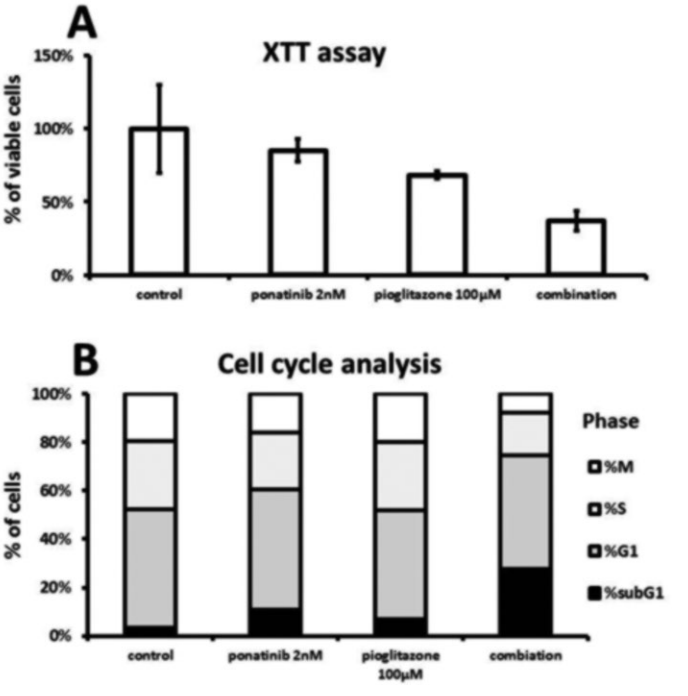

Figure 1.

Summary and Conclusions: Reduction of cardiovasular risk after administration of pioglitazone observed in patients with type 2 diabetes suggests that this drug can be useful in prevention of severe complications related to administration of 2nd and 3rd generation TKIs. In addition to its influence on cardiovascular risk, pioglitazone may enhance therapeutic efficacy of 2nd and 3rd generation TKIs through induction of cell cycle arrest and sensitization of CML cells to TKI-induced apoptosis. Therefore, the addition of pioglitazone to standard CML therapy may become potent treatment modality for the patients treated with 2nd and 3rd generation TKIs and increased cardiovascular risk.

P223

IDENTIFICATION OF BRD4 AS A NOVEL MOLECULAR TARGET IN PH+ CML

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Background: Chronic myeloid leukemia (CML) is a hematopoietic stem cell disease defined by leukemic expansion of myeloid progenitor cells and the BCR/ABL1 oncoprotein that promotes growth and survival of leukemic cells. In most patients growth of clonal cells can be kept under control by BCR/ABL1 tyrosine kinase inhibitors (TKI), including imatinib. For patients who develop resistance or intolerance against imatinib, second-line and third-line TKI (nilotinib, dasatinib, bosutinib, ponatinib) are available. However, in advanced CML, patients may develop resistance against most or all TKI. Therefore, current

research is attempting to identify novel drug targets in CML, in order to overcome TKI resistance. One class of promising targets may be epigenetic regulators of cell growth. In acute myeloid leukemia (AML), the epigenetic reader BRD4 has recently been introduced as a novel drug target; and the BRD4-targeting drug JQ1 was found to induce growth inhibition in AML cells. However, so far, little is known about the expression and function of BRD4 in CML cells.
Aims: In this study we examined the expression of BRD4 in CML cells and asked whether BRD4 serves as a target of therapy in this leukemia.
Methods: BRD4 expression was determined by qPCR and immunocytochemistry and the effects of JQ1 on proliferation and survival of CML cells were evaluated by ^{3}H-thymidine uptake and Annexin V staining.
Results: Both CML cell lines investigated, namely KU812 and K562, express BRD4 mRNA and the BRD4 protein. The BRD4-targeting drug JQ1 was found to inhibit proliferation in KU812 cells in a dose-dependent manner (IC_{50}: 0.25-0.75 μM). Interestingly, however, no substantial growth-inhibitory effect of JQ1 was seen in K562 cells (IC_{50}: >5 μM). Moreover, we were able to show that JQ1 inhibits growth of primary CML cells with IC50 values ranged between 0.1 to 5 μM. Corresponding results were obtained when analyzing cell survival. In particular, JQ1 was found to induce apoptosis in KU812 cells but did not induce apoptosis in K562 cells. In addition, we were able to show that KU812 cells and K562 cells display MYC mRNA. Exposure to JQ1 was followed by a decrease in expression of MYC mRNA levels in both cell lines. Similar results were obtained by Western blotting where JQ1 was found to decrease the expression of the MYC protein in KU812 and K562 cells. Moreover, we were able to show that MYC expression in CML cells is BCR/ABL1-dependent. Imatinib, nilotinib, ponatinib and dasatinib were found to decrease expression of MYC mRNA as well as MYC protein expression in K562 cells and KU812 cells. Finally, we were able to show that JQ1 cooperates with imatinib, nilotinib, ponatinib and dasatinib in producing growth inhibition in KU812 cells and K562 cells.
Summary and Conclusions: Together, our data show that BRD4 serves as a potential target in CML cells, and that the BRD4-blocker JQ1 synergizes with BCR/ABL TKI to induce growth-inhibition. Whether BRD4 inhibition is a pharmacologically meaningful approach in patients with TKI-resistant CML remains to be determined.

P224

QUANTITATIVE EVALUATION OF BOTH POINT MUTATED AND ALTERNATIVELY SPLICED BCR-ABL IN CML-CP PATIENT WITH SUBOPTIMAL MOLECULAR RESPONSE TO IMATINIB: RESULT OF HIGHLY-SENSITIVE, DEEP SEQUENCING STUDY

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Background: Tyrosine kinase inhibitors (TKIs) have dramatically improved outcomes of chronic myeloid leukemia (CML) patients. However, a fraction of patients fail in TKI treatment. One of the major mechanisms of resistance to TKIs is point mutations in the BCR-ABL kinase domain (KD). Alternatively-spliced (AS) BCR-ABL transcript variants, such as retention of 35bp intronic nucleotides at Exon 8/9 splice junction (Ins35) are important obstacles to achieve optimal response to TKI (Gaillard JB, *et al.* Mol Cancer Ther 2010; T O'Hare, *et al.* Blood 2011). This BCR-ABL variant possesses a stop codon in KD residues, resulting in generation of "function dead" BCR-ABL. However, this variant showed structural changes, resulting in a failure to TKI bindings. We hypothesized that the emergence of these BCR-ABL variants may render such CML clones insensitive to TKIs, contributing to the persistence of CML clones.
Aims: We evaluated both point mutated and AS BCR-ABL variants in pts with suboptimal response (SoR) to Imatinib (IM) by highly-sensitive assay system.
Methods: In the Study to Evaluate Nilotinib (NIL) in CML Patients with Suboptimal Response (SENSOR, NCT0104387), all pts with SoR—complete cytogenetic response but no major molecular response (MMR) after ≥18 months (mo) of frontline IM, were switched to NIL 400mg BID. cDNA was synthesized from extracted RNA samples, and BCR-ABL was amplified by long-range nested PCR method. These amplicons were deeply sequenced to detect BCR-ABL variants by using HiSeq 1500 (illumina) (Figure 1). The cumulative amounts of AS BCR-ABL are defined as the area under the curve from baseline to 24 mo after switching to NIL. These cumulative amounts are thought to be a potential persistence of AS BCR-ABL.
Results: Of 45 pts enrolled in the main study, 41 pts were evaluated for BCR-ABL variants. At 24 mo, 10 pts could not achieve MMR, whereas 27 pts could achieve MMR, including 5 pts with MR4.0. The remaining 4 pts could not be evaluated serially. The remaining 4 pts could not be evaluated serially. We analyzed 422 samples from 41 pts who have at least one sample: 412 samples with an average sequence depth on ABL-1 exon 8 achieved over 1,000 were considered as reliable for further statistical analysis. Two types of splicing variants became evident: the Ins35 and the Intron 8-retained BCR-ABL that possesses inappropriately spliced Intron 8 of ABL-1. AS BCR-ABL transcript variants were detected in all 41 pts and serially detected during NIL treatment. A representative cumulative curve in a patient who could not achieved MMR is shown in Figure 2. After switching NIL, AS BCR-ABL was serially detected at any points fo treatment. In addition, various point mutations were detected all along during NIL treatment at any depth of molecular response (Table 1). We analyzed the relationship between the cumulative amounts of Ins35 for 0-24 mo and molecular response rate: These cumulative amounts had an impact on the achievement rate of MMR (Table 2).

Figure 1: Quantitative deep sequencing of BCR-ABL KD by using NGS

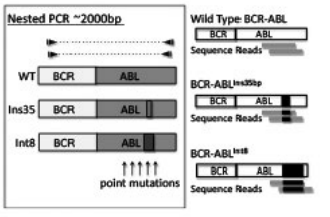

Figure 2: Molecular dynamics of BCR-ABL transcript variants after switching to nilotinib from imatinib

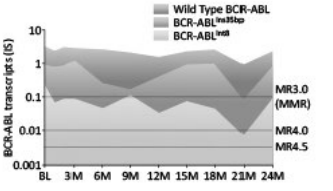

Table 1: Detected mutation type by molecular response at 24 mo

	MR4.0 (N=5)		MMR including MR4.0 (N=27)		No MMR (N=10)	
	Baseline	Post-Baseline	Baseline	Post-Baseline	Baseline	Post-Baseline
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
No mutation	0 (0.0%)	2 (40.0%)	0 (0.0%)	12 (44.4%)	0 (0.0%)	9 (90.0%)
AS BCR-ABL (Ins35 and Int8)	5 (100.0%)	0 (0.0%)	27 (100.0%)	0 (0.0%)	10 (100.0%)	0 (0.0%)
Point mutation (Insensitive) E255K	0 (0.0%)	0 (0.0%)	1 (3.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Point mutation (Other*)	2 (40.0%)	3 (60.0%)	4 (14.8%)	15 (55.6%)	0 (0.0%)	1 (10.0%)

*L201F, K219N, P230L, Y232A, M244V, G249C, G250R, G251V, G259S, G259V, E308D, V335L, N368K, H375R, K378N, F401L, T406A, E409G, K415R, L429P, S438F, S446F, L452I, E459K, stopgain, synonymous

Table 2: Cumulative amounts of BCR-ABL^{Ins35bp} for 0-24M and molecular response rate at 24 mo after switching to nilotinib

	MR4.0 (n=5)	MMR including MR4.0 (n=27)	No MMR (n=10)
Median (% month) [range]	0.14407 [0.0259-0.2909]	0.20851 [0.0247-2.9180]	1.28703 [0.3154-12.7419]

Figure 1.

Summary and Conclusions: Our highly-sensitive quantification system revealed that cumulative amounts of Ins35 for 0-24 mo might correlate with failure to achieve MMR after switching to NIL. Point mutations were detected in only 1 patient (K219N) out of 10 pts who could not achieve MMR at 24 mo. In contrast, AS BCR-ABL was detected in all 10 pts, suggesting that AS BCR-ABL might be related to SoR to TKIs rather than point mutation. These data suggest that CML clones with AS BCR-ABL may not be addicted to BCR-ABL kinase activity and were difficult to eradicate by TKI alone. Thus, quantification of AS BCR-ABL variants might be a beneficial method to predict clinical outcomes and to find out the mechanism of TKI resistance in CML pts.

P225

AN ITALIAN MULTICENTRIC EVALUATION OF THE Q-LAMP TECHNOLOGY APPLIED TO THE MOLECULAR DETECTION OF BCR-ABL TRANSCRIPTS IN ARCHIVED RNA FROM PHILADELPHIA POSITIVE ONSET SAMPLES

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Background: The identification of the BCR-ABL transcripts for the purpose of molecular diagnosis is actually based on conventional RT-PCR. The novel RT-Q-LAMP molecular method represents a semi-automated alternative, which allows rapid differential detection of BCR-ABL p190 and p210 fusion transcripts in a close, single step format.
Aims: Evaluation of the Q-LAMP assay performances on archived RNA samples previously tested by conventional qualitative RT-PCR .
Methods: BCR-ABL RT-Q-LAMP is a fluorescent isothermal method for retro-

transcription, amplification and differential detection of the Minor (p190) and Major (p210) t(9;22) transcripts and the endogenous Gusb RNA, that acts as internal control. The test result is obtained starting directly from patient RNA in a single, sixty minute step. RT-Q-LAMP is carried out on the Liaison IAM instrument that incubates at constant temperature, displays fluorescent signals in real time and return final elaborated data. Overall 165 samples (111 BCR-ABL positive, 54 negative) consisting of archived RNA (500ng per reaction), have been tested and the results compared with one previously obtained by RT-PCR. 11 samples presented a chemical contamination, showing A260/A230 ration below 1 (0,24-1). Further 8 rare isoform samples (e19a2 n=3; e6a2 n=3; e8(-44nt)/+(intr abl1 30nt)/a2 n=1; p210 presenting a deletion in the fusion region) have been also analysed.
Results: The RT-Q-LAMP and RT-PCR data resulted fully concordant (100%). 3 samples initially discordant (negative by RT-PCR, weakly positive by Q-LAMP) have been further investigated by Quantitative PCR confirming the positive status. The positive samples have been identified by Q-LAMP in, as average, 22,7 minutes and 18,3 minutes for the p190 and p210 respectively. The 11 samples presenting poor A260/A230 ratio have been detected in 20,8 min as average. The rare isoforms e19a2 and e6a2 have been amplified, as well as the p210 positive sample presenting a deletion in the fusion region, which prevents quantitative PCR from amplification. The rare isoform e8(-44nt)/+(intr abl1 30nt)/a2, detectable by nested RT-PCR did not produce amplification in Q-LAMP.The negative samples presented amplification of the housekeeping gene, validating the result. Q-LAMP also demonstrated robustness to contamination by extraction chemical reagents. The close tube format and the ready-to-use lyophilized reagents provided an easier the set-up that may decrease the classical risks of multistep procedures. The software elaborates and stores the data ensuring traceability. The limitations identified are the inability to distinguish the p210 b2a2 from the b3a2 isoform and to detect the rare isoforms in which the exon 2 of ABL is deleted.
Summary and Conclusions: The Q-LAMP technique produced results concordant with RT-PCR, in a much lower time-to-results (about 20 minutes). The Q-LAMP method represents a valid option for reliable and rapid detection of BCR-ABL and the one step, close tube format could easily be adopted in not highly specialized centers for an effective diagnosis of Philadelphia Positive Leukemias.
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P226

DYNAMIC EXPANSION AND FUNCTIONAL TUNING OF NATURAL KILLER CELLS IN CHRONIC MYELOID LEUKEMIA

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Background: Previous studies indicate that Natural Killer (NK) cells are deficient in Chronic Myeloid Leukemia (CML) patients, although the mechanisms behind the dysfunction are not completely understood. Current therapeutic strategies influence these innate lymphoid cells and successful results may be partially explained by the advantageous effects on their cytotoxicity against cancer cells. Due to recent advances in the knowledge of NK cell´s biology, there is an increasing interest in mapping NK-cell responses in cancer.
Aims: The aim of the present study was to analyze NK cells in CML patients and the effect of therapy and dose-dependent mechanisms on essential features of NK cells.
Methods: In this study, we analyzed blood samples from 67 CML patients treated with IFN-α and/or different generations of tyrosine kinase inhibitors (TKI). Extended analysis of NK-cell receptor repertoire and functional properties was performed by multiparametric flow cytometry, cell sorting, Luminex xMAP technology and real-time quantitative PCR.
Results: Relative frequency of NK cells was found reduced at CML diagnosis and recovered after treatment. CML therapy induces an increase of $CD62L^{+}CD56^{bright}$ NK cells, associated to the capacity of migration to secondary lymphoid organs. Activation of NK cells and the increased expression of CD137 and CD137L were interpreted as a significant effect of therapy response. Activatory (KIR2DS1) and inhibitory (KIR2DL1, KIR2DL2) receptors were found altered in CML. The expression of KIR2DS1 by $CD56^{dim}CD16^{+}$ NK cells was highest in CML patients undergoing Dasatinib therapy. Treatment also increased the NKG2C/NKG2A (activatory/inhibitory) ratio. Lower expression of NKp30 and NKp44 was compensated by the increase of $NKp46^{+}$ NK cells. Production of IFN-γ and suppression of TGF-β+ and IL-10+ NK cells was also a beneficial effect of treatment protocol. IFN-γ production decreased with an increased TKI dose. Dasatinib induced the expression of KIR2DS1 (activating receptor) on NK cells, improving NK cell ability to kill cancer cells.
Summary and Conclusions: NK cells are affected during CML and current therapeutic protocols ameliorate NK-cell performance. In the future, combination of NK cell-based immunotherapy with pharmacological interventions should be investigated in order to eradicate cancer cells and discontinuation of therapy.
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P227

IS THE HIGH-THROUGHPUT DROPLET DIGITAL PCR A NEXT-GENERATION TECHNOLOGY FOR ACCURATE AND SENSITIVE BCR-ABL1 QUANTIFICATION FOR DEEP MOLECULAR RESPONSE MONITORING IN CML?

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Background: A sensitive and standardized monitoring of deep molecular response (MR) based on BCR-ABL1 transcript level quantification is an essential part of CML tyrosine kinase inhibitor (TKI) stopping trials. Detailed laboratory recommendations, developed as a part of the European Treatment and Outcome Study (EUTOS) for CML, to enable testing laboratories to score MR in a reproducible manner based on reverse transcription quantitative PCR (RT-qPCR) has been recently published (Cross *et al.* Leukemia 2015). A new quantification technology droplet digital PCR (ddPCR) is tested in more and more laboratories with promising high quantification accuracy and sensitivity.
Aims: To assess differences in accuracy and sensitivity of BCR-ABL1 transcript levels quantification using ddPCR and standardized RT-qPCR. In addition to that to compare the levels of BCR-ABL1 at mRNA and DNA levels measured by ddPCR.
Methods: The RT-qPCR used in our EUTOS $MR^{4.5}$ laboratory is standardized having regularly validated conversion factors for data reporting in the international scale (IS) using ABL1 or GUSB control genes. The system QX-200 (Biorad) was used for ddPCR. The same RT-qPCR primers and probes were applied for ddPCR. Unlike to singleplex RT-qPCR, TaqMan probes were differently labelled for multiplex ddPCR. Using each method all samples were analyzed in doublets.
Results: The WHO certified ERM-AD623 standards (n=6) were used to compare the quantification accuracy of both methods. Obtained gene copies ratios (BCR-ABL1/ABL1 and BCR-ABL1/GUSB) in the standards by singleplex RT-qPCR were in range 0.63-1.11 (Me=0.85). Singleplex ddPCR determined ratios in range 0.92-1.29 (Me=1.02). Multiplex ddPCR improved quantification accuracy with ratios 0.93-1.0 (Me=0.99).
Samples of 31 CML patients treated with TKIs with BCR-ABL1 mRNA levels ranging 0.01-10% IS were used for data comparison. The Bland-Altman bias plot was used to determine bias between RT-qPCR and ddPCR showing antilog bias 0.54-fold in 31 paired results.
To compare BCR-ABL1 detection sensitivity of both methods we tested 66 samples from the time of $MR^{4.0}$ confirmation and after TKI discontinuation of 7 patients enrolled in the EURO-SKI study (Europe Stops TKI in CML). The total number of paired results from RT-qPCR *vs* ddPCR of samples with BCR-ABL1 mRNA levels below 0.01% IS was 37 with determined antilog bias 0.23-fold. The number of BCR-ABL1 negative samples by RT-qPCR *vs* ddPCR was 26/66 in $MR^{4.5}$-$MR^{5.0}$ sensitivity *vs* 6/66, respectively. Moreover, 26 samples of 4/7 EURO-SKI patients were assessed by ddPCR for the levels of genomic BCR-ABL1 fusions using patient-specific assays. We found significant correlation between DNA and mRNA BCR-ABL1 levels ($r=0.9038$, $P<0.0001$).
Summary and Conclusions: We found a higher quantification accuracy of ddPCR in contrast to RT-qPCR for which standard calibration curves need to be performed to mathematically calculate cDNA copy numbers in unknown samples. This probably represents an important variable. We found comparable results of both methods for BCR-ABL1 mRNA quantification within the range 0.01-10% IS. Approximately a log higher BCR-ABL1 mRNA levels in EURO-SKI $MR^{4.0}$-$MR^{5.0}$ samples were detected by ddPCR in comparison to RT-qPCR, assuming that higher sensitivity of ddPCR may be important for TKI stopping treatment management. Interestingly, BCR-ABL1 DNA and mRNA levels measured by ddPCR correlated significantly. A larger cohort of samples needs to be analyzed to elucidate the clinical importance of DNA measurement. Supported by MZCR project no. 00023736 and EURO-SKI research consortium.

Chronic myeloid leukemia - Clinical 1

P228

EFFICACY AND SAFETY OF NILOTINIB VS IMATINIB IN PATIENTS WITH NEWLY DIAGNOSED CHRONIC MYELOID LEUKEMIA IN CHRONIC PHASE: 6-YEAR FOLLOW-UP OF ENESTND

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Background: In the Evaluating Nilotinib Efficacy and Safety in Clinical Trials–Newly Diagnosed Patients study (ENESTnd), nilotinib (NIL) demonstrated improved efficacy *vs* imatinib (IM) throughout 5 y of follow-up (f/u), with higher rates of major molecular response (MMR; *BCR-ABL*on the International Scale[BCR-ABL^{IS}] ≤0.1%) and MR$^{4.5}$(BCR-ABL^{IS} ≤0.0032%). The protocol was amended in January 2012 to extend the f/u period to 10 y, from the initial 5 y, to collect further data on efficacy parameters, including deep molecular response and survival, and on safety. Here, we report 6-y f/u data.
Aims: The efficacy and safety of NIL *vs* IM were evaluated in adult patients (pts) with newly diagnosed Philadelphia chromosome–positive (Ph+) chronic myeloid leukemia (CML) in chronic phase (CP).
Methods: Pts were randomized to NIL 300 mg twice daily (n=282), NIL 400 mg twice daily (n=281), or IM 400 mg once daily (n=283). "On core treatment" analyses included all events that occurred during core treatment with the assigned study drug. "On study" analyses included all events that occurred during core treatment, extension treatment, or posttreatment f/u. Progression and survival data were collected prospectively every 3 mo for 5 y, then every 6 mo, including after discontinuation of study treatment.
Results: After a minimum f/u of 6 y, more pts remained on core treatment in the NIL 300 mg and 400 mg arms than in the IM arm (53.5%, 55.2%, and 44.9%, respectively). In both NIL arms, cumulative rates of MMR and MR$^{4.5}$ by 6 y were higher than in the IM arm (Table). In all 3 arms (NIL 300 mg, NIL 400 mg, and IM), MR$^{4.5}$rates by 6 y were highest in pts with BCR-ABL^{IS} ≤1% at 3 mo (73.6% [106/144], 75.0% [102/136], and 72.1% [31/43], respectively) and lowest in pts with BCR-ABL^{IS} >10% at 3 mo (8.3% [2/24], 21.4% [6/28], and 15.9% [14/88], respectively); more pts on NIL *vs* IM achieved BCR-ABL^{IS} ≤1% and fewer pts on NIL *vs* IM had BCR-ABL^{IS} >10% at 3 mo. By 6 y, fewer pts on NIL *vs* IM had progressed to accelerated phase/blast crisis (AP/BC) on study and on core treatment (Table). Since the 5-y data cutoff, 1 new progression to AP/BC on study (NIL 300 mg arm, ≈5 y after discontinuation of core treatment) was reported; no new progressions on core treatment were reported. Fewer deaths due to advanced CML occurred in the NIL arms than in the IM arm. Since the 5-y data cutoff, 5 deaths were reported, including 3 on core treatment and 2 on study after discontinuation of core treatment (Table); no new deaths due to advanced CML occurred in any arm. The safety profiles of NIL and IM were consistent with previous reports. Very few events of pleural effusions, pericardial effusion, or pulmonary edema occurred in any arm (Table). More pts in the NIL arms than in the IM arm had cardiovascular events (CVEs); CVEs were reported at a consistent frequency within each arm throughout the study (Table). A recent protocol amendment allowed for a dose reduction to NIL 300 mg twice daily in pts in the NIL 400 mg twice daily arm, and a subsequent dose increase to the originally assigned 400 mg twice daily dose, based on a pt's individual benefit/risk assessment.

Tabella 1.

ENESTnd 6-y Data	Nilotinib 300 mg Twice Daily (n = 282)	Nilotinib 400 mg Twice Daily (n = 281)	Imatinib 400 mg Once Daily (n = 283)
Pts still on study,[a] n (%)	231 (81.9)	238 (84.7)	224 (79.2)
Pts still on core treatment, n (%)	151 (53.5)	155 (55.2)	127 (44.9)
MMR, % (*P* value vs IM[b])	77.3 (< .0001)	79.0 (< .0001)	61.1
MR$^{4.5}$, % (*P* value vs IM[b])	55.7 (< .0001)	54.8 (< .0001)	32.9
Progressions to AP/BC[c] on study, n (*P* value vs IM[b])	11 (.0661)	6 (.0030)	21
Progressions to AP/BC[c] on core treatment	2 (.0059)	3 (.0185)	12
Estimated rates of 6-y OS, % (*P* value vs IM[b])	91.6 (.7085)	95.8 (.0314)	91.4
Deaths due to any cause, n	21	11	23
Deaths due to advanced CML,[d] n	6	4	16
Deaths after 5-y data cut-off,[e] n	3	1	1
AEs of interest (all causes, all grades), n (%)	**n = 279**	**n = 277**	**n = 280**
Peripheral edema	28 (10.0)	40 (14.4)	56 (20.0)
Fluid retention	0	2 (0.7)	7 (2.5)
Pleural effusion	5 (1.8)	3 (1.1)	3 (1.1)
Pericardial effusion	2 (0.7)	2 (0.7)	3 (1.1)
Pulmonary edema	1 (0.4)	0	0
Pulmonary hypertension	0	2 (0.7)	1 (0.4)
Retinal vein occlusion	1 (0.4)	0	0
Thrombophlebitis	1 (0.4)	3 (1.1)	0
Superficial thrombophlebitis	0	1 (0.4)	0
Deep venous thrombosis	1 (0.4)	1 (0.4)	1 (0.4)
CVE[f]	28 (10.0)	44 (15.9)	7 (2.5)
IHD[f]	14 (5.0)	28 (10.1)	6 (2.1)
ICVE[f]	4 (1.4)	9 (3.2)	1 (0.4)
PAD[f]	12 (4.3)	9 (3.2)	0
Other[f,g]	4 (1.4)	3 (1.1)	0
CVEs by y of treatment,[f,h] n (%)			
< 2 y	8 (2.9)	16 (5.8)	2 (0.7)
≥ 2 y to < 4 y	11 (3.9)	10 (3.6)	2 (0.7)
≥ 4 y to < 6 y	6 (2.2)	15 (5.4)	2 (0.7)
≥ 6 y to < 8 y	3 (1.1)	3 (1.1)	1 (0.4)

AE, adverse event; AP/BC, accelerated phase/blast crisis; CVE, cardiovascular event; ICVE, ischemic cerebrovascular event; IHD, ischemic heart disease; MMR, major molecular response (BCR-ABL^{IS} ≤ 0.1%); MR$^{4.5}$, molecular response 4.5 (BCR-ABL^{IS} ≤ 0.0032%); OS, overall survival; PAD, peripheral artery disease.
[a] On core or extension treatment or in posttreatment f/u.
[b] *P* values are nominal, provided for descriptive purposes only, and were not adjusted for multiple comparisons.
[c] Includes progressions to AP/BC or deaths due to advanced CML.

Summary and Conclusions: NIL continued to demonstrate improved efficacy over IM, with higher rates of molecular response, fewer progressions, and fewer deaths due to advanced CML. No new safety signals were detected. Results from ENESTnd continue to support use of NIL 300 mg twice daily as a standard-of-care frontline therapy option for pts with newly diagnosed Ph+ CML-CP.

P229

ENESTCMR 4-Y RESULTS: PATIENTS (PTS) WITH CHRONIC MYELOID LEUKEMIA IN CHRONIC PHASE (CML-CP) AND RESIDUAL DISEASE MORE LIKELY TO ACHIEVE DEEP MOLECULAR RESPONSE FOLLOWING SWITCH TO NILOTINIB (NIL)

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Background: ENESTcmr has demonstrated that switching to NIL results in higher rates of deep molecular response *vs* continued imatinib (IM) in pts with CML-CP with residual disease after long-term IM treatment; among pts without major molecular response (MMR; *BCR-ABL1* ≤0.1% on the International Scale [IS]) after ≥2 y of IM, substantially higher rates of MMR were also observed with switching to NIL *vs* continuing IM.
Aims: Here we present results with 4 y of follow-up.
Methods: The phase 3 ENESTcmr study evaluated pts with Philadelphia chromosome–positive (Ph+) CML-CP with complete cytogenetic response but with detectable *BCR-ABL1* by real-time quantitative polymerase chain reaction (RQ-PCR; sensitivity of ≥4.5 logs) on the IS after ≥2 y of treatment with IM. Pts were randomized to receive NIL 400 mg twice daily (BID; n =104) or continue IM (400 or 600 mg once daily [QD]; n =103). Crossover to the NIL arm was allowed

for pts in the IM arm with detectable *BCR-ABL1* transcripts after 2 y on study or with treatment failure/confirmed loss of response (≥2 consecutive assessments) at any time.

Results: Of 103 pts randomized to the IM arm, 46 crossed over to NIL, most commonly due to detectable *BCR-ABL1* after 2 y (n =41/46; 89%). The 4-y cumulative incidence of $MR^{4.5}$ (*BCR-ABL1*^{IS} ≤0.0032%) among pts without $MR^{4.5}$at baseline (intention-to-treat analysis; NIL, n =98; IM, n =96) was 52% (51/98 pts) in the NIL arm and 42% (40/96 pts) in the IM arm (nominal *P* =.1318; Table). Among the 46 pts who crossed over to NIL, 20 (43%) achieved $MR^{4.5}$by the end of the study (13 achieved $MR^{4.5}$for the first time [5 of these pts did not have an RQ-PCR evaluation at the crossover visit], 4 maintained $MR^{4.5}$, and 3 regained $MR^{4.5}$ that was lost prior to crossover). When excluding responses achieved after crossover to NIL, 52% of pts in the NIL arm *vs* 28% of pts in the IM arm (nominal *P* =.0004) achieved $MR^{4.5}$ by 4 y. Median time to $MR^{4.5}$ was 24 months in the NIL arm and was not reached in the IM arm by 48 mo. At 2 y, 78 pts were eligible for crossover to NIL due to molecular detectable disease; of these, 13/41 pts who switched to NIL *vs* 5/37 pts who remained on IM achieved undetectable disease by 4 y. The safety profiles of NIL and IM were consistent with previous reports. By 4 y, cardiovascular events were reported in 13 pts in the NIL arm (ischemic heart disease [IHD], 4; cerebrovascular event [CVE], 4; peripheral arterial disease [PAD], 7), 3 pts who crossed over from IM to NIL (IHD, 1; CVE, 1; PAD, 1), and 2 pts in the IM arm (excluding events after crossover; IHD, 1; CVE, 1). The median time on treatment was 3.9 y in the NIL arm and 4.0 y for pts in the IM arm who did not cross over; for pts who crossed over from IM to NIL, median time on treatment was 2.1 and 1.8 y before and after crossover, respectively.

Tabella 1.

	NIL 400 mg BID (n = 98)	IM 400 or 600 mg QD (n = 96)	*P* Value[a]
$MR^{4.5}$ in pts without $MR^{4.5}$ at baseline (intention-to-treat analysis)	n (%)	n (%)	
By 1 y	32 (33)	13 (14)	.0020
By 2 y	42 (43)	20 (21)	.0006
By 3 y	46 (47)	35 (37)	.1146
By 4 y	51 (52)	40 (42)	.1318
$MR^{4.5}$ in pts without $MR^{4.5}$ at baseline (excluding responses achieved after crossover[b] to NIL)			
By 3 y	46 (47)	25 (26)	.0015
By 4 y	51 (52)	27 (28)	.0004

[a] *P* values are nominal and are provided for descriptive purposes only.
[b] On study, crossover to the NIL arm was allowed for detectable *BCR-ABL1* transcripts after 2 y or treatment failure/confirmed loss of response at any time on IM.

Summary and Conclusions: Results from ENESTcmr showed that for pts with Ph+ CML-CP and residual disease after ≥2 y of IM treatment, more pts achieved $MR^{4.5}$with switching to NIL (52%) *vs* continuing IM (42%; when accounting for confounding effects of crossover, 28%) by 4 y. In addition, more pts with detectable disease at 2 y who switched to NIL than those who remained on IM were able to achieve undetectable disease by 4 y. Overall, results from ENESTcmr suggest that an earlier switch to NIL (at randomization) allows earlier achievement of $MR^{4.5}$ *vs* remaining on IM or delaying switch to NIL (at crossover) and enables a larger proportion of pts to achieve deeper molecular responses and become eligible for treatment-free remission trials sooner.

P230

DEEP MOLECULAR RESPONSE TO NILOTINIB AS FIRST-LINE TREATMENT OF BCR-ABL+ CML

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Background: In the ENESTnd trial, nilotinib (NIL) showedsuperior efficacy compared to imatinib (IM); based on these results, NIL has been approved for frontline treatment of chronic myeloid leukemia (CML). The treatment-free remission (TFR) is an emerging treatment goal in CML and a sustained deep molecular response (DMR, MR4 or better) is a pre-requisite to achieve TFR. The 5-year update from the ENESTnd trial showed a superiority of NIL over IM in terms of achievement of DMR, but differences concerning the stability of DMR have not been reported yet. Independent studies are extremely relevant to confirm and to extend the results of company-sponsored trials.

Aims: To assess the efficacy of NIL as first-line treatment in terms of achievement of DMR and stability of DMR

Methods: A phase 3b study was conducted by the GIMEMA CML WP (CML0811; NCT01535391). The primary endpoint was the rate of MR4 at 24 months. Key secondary objectives: evaluation of the kinetics of molecular response and stability of DMR, assessment of the safety profile, evaluation of the outcome. The starting NIL dose was 300 mg BID, with dose escalation to 400 mg BID (in absence of safety issues)in case of suboptimal response or failure (ELN 2009 criteria), with the exception of progression to ABP or BCR-ABL mutations insensitive to NIL. The molecular response was assessed in GIMEMA standardized molecular laboratories (Labnet network). The MR4 was defined as either detectable disease ≤0.01% BCR-ABL or undetectable disease with ≥10.000 ABL copies; the MR4.5 was defined as either detectable disease ≤0.0032% BCR-ABL or undetectable disease with ≥32.000 ABL copies. Sustained MR4 or MR4.5: MR4 or MR4.5 for at least 1 year a, with at least 3 evaluable analysis. A prospective evaluation of glucose metabolism and serum lipids was performed. All the analysis were performed according to the ITT principle.

Results: 130 CML patients in early chronic phase have been enrolled in 32 italian hematologic centers; median age, 50 years (range 18-85); high risk patients, 22%, 6% and 8% according to Sokal, Euro and EUTOS scores, respectively; clonal chromosomal abnormalities in Ph+ cells at baseline, 5%; e13a2 BCR-ABL transcript, 34%. The median follow-up is 29 months (24-37 months). At the last contact, the patients still on treatment with NIL were 100/130, 77% (86 with 600 mg, 9 with 300 mg or less, 5 with 800 mg daily), while 30/130 patients, 23%, permanently interrupted the study drug for the following reasons: 3% progression to ABP, 5% failure or suboptimal response (dose escalation not feasible or not effective), 8% adverse events, 1% TFR, 5% other reasons (including consent withdrawal and pregnancy). At 3 months, 80% of patients had BCR-ABL transcript levels <10%; at 6 months, 78% of patients had BCR-ABL transcript levels <1%. The major molecular response rates at 12 and 24 monthswere 57% and 65%, respectively. The rates of MR4 at 6, 12, 18 and 24 months were 12%, 28, 31% and 46%, respectively. Seventy-six patients (58%) achieved a MR4 at least once; the patients with a sustained MR4 were 39/76 (51%, or 30% of the total). The rates of MR4.5 at 6, 12, 18 and 24 months were 2%, 7%, 11% and 17%, respectively.Eleven patients achieved a sustained MR4.5. A significant increase of glycosylated hemoglobin was not observed. The total cholesterol, and both LDL and HDL cholesterol fractions significantly increased during treatment. Triglicerid concentrations had not significant variations. Six patients (5%) had a cardiovascular event, including myocardial infarction and arterial thrombosis. All the patients are still alive.

Summary and Conclusions: The molecular response rates seem to be superior to the historical data of IM. NIL 300 mg BID as frontline treatment of BCR-ABL+ CML, with dose optimization in case of non optimal response, may improve the proportion of patients able to discontinue TKI treatment. Due to the metabolic effects, a baseline selection is important to maximize the therapeutic benefit and to minimize the cardiovascular risks.

P231

SMOOTHENED (SMO) INHIBITOR LDE225 COMBINED WITH NILOTINIB IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA (CML) RESISTANT/INTOLERANT (R/I) TO AT LEAST 1 PRIOR TYROSINE KINASE INHIBITOR: A PHASE 1B STUDY

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Background: Currently available therapies may have limited potential to eliminate leukemic stem cells (LSCs). LDE225 is an antagonist of the transmembrane protein SMO, which plays a role in the Hedgehog (Hh) pathway. Inhibition of the Hh pathway diminished survival signals in LSCs and increased the sensitivity of LSCs to tyrosine kinase inhibitors (TKIs).[1]Preclinical studies[2] have shown synergistic effects between SMO inhibitors and BCR-ABL TKIs. This combination is being explored in early phase clinical trials.

Aims: We evaluated the feasibility of the combination of nilotinib (NIL), a potent BCR-ABL TKI and LDE225 in patients with Philadelphia chromosome-positive CML in chronic phase R/I to at least 1 prior TKI.

Methods: This phase 1b, single-arm, multicenter study had the primary objective of determining the maximum tolerated dose (MTD) and/or the recommended phase 2 dose (RP2D) of LDE225 (planned doses of 400/600/800 mg once daily) in combination with NIL 400 mg twice daily. Dose-limiting toxicities (DLT) were identified during the first 8 weeks of dosing. An adaptive Bayesian logistic regression model guided by the escalation with overdose control principle was used. Secondary objectives included safety, pharmacokinetics, and efficacy (as measured by the kinetics of molecular and cytogenetic responses).
Results: Eleven patients (cohort 1 [LDE225 400 mg + NIL]: n=4; median age 58 years; cohort 2 [LDE225 600 mg + NIL]: n=7, median age, 46 years) were enrolled. The median duration of exposure to study treatment in cohort 1 and cohort 2 was 11.27 and 8.25 months, respectively. Two patients in cohort 2 experienced 1 DLT (elevated creatine phosphokinase [CPK]: n=1, grade [G] 3; n=1, G4). One patient in cohort 1 experienced 1 serious adverse event (SAE; G4 elevated CPK), and 2 patients in cohort 2 experienced 2 SAEs each (G4 elevated CPK + G1 elevated troponin T and G3 appendicitis + G2 sepsis). The most frequently occurring (>30%) adverse events (AEs; all grades) in the overall patient population included alopecia (73%), elevated CPK (64%), dysgeusia (64%), muscle spasms (64%), folliculitis (46%), and weight loss (37%). AEs leading to study discontinuation included 1 patient in cohort 1 (G1 elevated CPK) and 4 patients in cohort 2 (G1 folliculitis, G3 folliculitis, G2 dysgeusia, and G2 alopecia). No deaths occurred during the study. No other clinically significant laboratory abnormalities were observed during the study. One patient in cohort 1, who had a major molecular response (MMR; ≤0.1% BCR-ABL^{IS}) at baseline, achieved deep molecular response (MR$^{4.5}$; ≤0.0032% BCR-ABL^{IS}) temporarily at 2 months and 1 patient who had no MMR at baseline achieved MMR post baseline (from 1 month until 9 months). In cohort 2, 1 patient who had no MMR at baseline achieved MMR at 12 months. The study was terminated early, as the benefit of addition of LDE225 to NIL did not appear to outweigh additional risks in patients with CML.

Tabella 1.

Treatment-Related AEs: All Grades, n (%)[a] (> 30% Incidence) (Safety Set)	LDE225 200 mg qd[b] + Nilotinib 400 mg bid n = 1	LDE225 400 mg qd + Nilotinib 400 mg bid n = 3	LDE225 600 mg qd + Nilotinib 400 mg bid n = 7	All Patients N = 11
Any AE	1 (100)	3 (100)	7 (100)	11 (100)
Alopecia	1 (100)	3 (100)	4 (57)	8 (73)
Blood CPK increase	1 (100)	3 (100)	3 (43)	7 (64)
Dysgeusia	1 (100)	0	6 (86)	7 (64)
Muscle spasms	0	2 (67)	5 (72)	7 (64)
Folliculitis	1 (100)	0	4 (57)	5 (46)
Responses, n (%)[a]	**LDE225 400 mg qd + Nilotinib 400 mg bid n = 4**		**LDE225 600 mg qd + Nilotinib 400 mg bid n = 7**	
	Baseline	**12 mo[c]**	**Baseline**	**12 mo[c]**
MR$^{4.5}$	0	0	1 (14)	0
MMR	2 (50)	2 (50)	2 (29)	2 (29)
CCyR	3 (75)	3 (75)	7 (100)	5 (71)
MCyR	3 (75)	3 (75)	7 (100)	5 (71)

AE, adverse event; bid, twice daily; CCyR, complete cytogenetic response; CPK, creatine phosphokinase; MCyR, major cytogenetic response; MMR, major molecular response (≤ 0.1% BCR-ABL^{IS}); MR$^{4.5}$, ≤ 0.0032% BCR-ABL^{IS}; qd, once daily.

[a] All percentages have been rounded to the nearest hundredth.
[b] One patient in the assigned LDE225 400 mg + nilotinib 400 mg group received LDE225 200 mg qd during the e study due to a dosing error. For safety-related analyses, this patient was analyzed according to this treatment gr
[c] MR$^{4.5}$ and MMR rates at 12 mo; CCyR and MCyR rates by 12 mo.

Summary and Conclusions: The MTD was not determined; no RP2D for the combination of NIL and LDE225 was found with respect to an acceptable risk/benefit ratio for patients with CML. No evidence of clinical benefit or impact of the combination was observed. The SAEs of elevated blood CPK were consistent with the safety profile of LDE225. No significant AEs were seen due to NIL. Study outcomes were similar to another phase 1 study, which showed lack of efficacy and tolerability when a SMO inhibitor was used in combination with dasatinib.[3]

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P232

FINAL LASOR RESULTS: SWITCH TO NILOTINIB (NIL) IN PATIENTS (PTS) WITH CHRONIC MYELOID LEUKEMIA IN CHRONIC PHASE (CML-CP) AND SUBOPTIMAL CYTOGENETIC RESPONSE (CYR) TO FRONTLINE IMATINIB (IM)

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Background: LASOR is the only randomized study evaluating IM dose escalation *vs* switch to NIL in pts with Philadelphia chromosome–positive (Ph+) CML-CP and suboptimal response to frontline IM. When accounting for crossover, results from the 1-y analysis demonstrated an overall benefit of switching to NIL *vs* IM dose escalation.
Aims: Here we present final results from LASOR with 2 y of follow-up.
Methods: Adult pts (N =191) with CML-CP and a complete hematologic response but suboptimal CyR, defined per 2009 European LeukemiaNet (ELN) criteria (no CyR ≥3 to <6 mo [Ph+ >95%], no partial CyR ≥6 to <12 mo [Ph+ 36%–95%], or no complete CyR [CCyR] ≥12 to <18 mo [Ph+ 1%–35%] on IM 400 mg once daily [QD]), were randomized to receive NIL 400 mg twice daily (BID; n =96) or IM 600 mg QD (n =95). The primary endpoint was CCyR at 6 mo after randomization. Consistent with standard of care at the time of the study, pts were allowed to cross over to the alternate treatment arm for failure to achieve CCyR by 6 mo or development of intolerance/loss of response at any time. As a result, additional analyses were performed to evaluate the effects of crossover.
Results: Crossover was more frequent in the IM arm (56 pts [60%] crossed over to NIL) than in the NIL arm (13 pts [14%] crossed over to IM). Among pts who crossed over, median time to crossover (from 1 day after randomization to the last known date a pt took randomized study drug) was 6 mo in each arm; the most common reasons for crossover were lack of CCyR after 6 mo (IM, 31%; NIL, 6%), intolerance (IM, 17%; NIL, 5%), and loss of best CyR (IM, 8%; NIL, 1%). The median time on randomized treatment (including time to crossover for crossover pts and time on treatment for pts who did not cross over) was 22 mo with NIL and 8 mo with IM. Median time on alternative treatment after crossover was higher for pts who switched to NIL (16 mo) *vs* IM (6 mo). Accounting for crossover by considering pts who crossed over without achieving response by the time point as nonresponders, 66% of pts on NIL *vs* 40% on IM achieved CCyR by 1 y (nominal *P* =.0005; Table). Overall, the CCyR rate by 1 y was 67% in the NIL arm *vs* 63% in the IM arm (nominal *P* =.6509; intention-to-treat [ITT] analysis). When considering pts who crossed over without achieving response by the time point as nonresponders, 42% of pts on NIL *vs* 19% of pts on IM achieved a major molecular response (MMR; *BCR-ABL1* ≤0.1% on the International Scale) by 1 y (nominal *P* =.0009; by 2 y, 51% and 26%, respectively [nominal *P* =.0006]). MMR was achieved by 42% of pts randomized to the NIL arm and 28% of pts randomized to the IM arm by 1 y (nominal *P* =.0688; ITT analysis) and by 51% and 48%, respectively, by 2 y (nominal *P* =.7729). Estimated rates of 2-y event-free survival (EFS) are reported in the table. The safety profile of both drugs was similar to that reported at 1 y. Cardiovascular events occurred in 5 pts on NIL (ischemic heart disease, n =4; peripheral arterial occlusive disease and arteriosclerosis, n =1) and 0 pts on IM.

Tabella 1.

	NIL 400 mg BID (n = 96)	IM 600 mg QD (n = 95)		NIL 400 mg BID (n = 96)	IM 600 mg QD (n = 95)	
	ITT Analysis			Analyses Accounting For Crossover[a]		
	Pts, %	Pts, %	*P* Value[b]	Pts, %	Pts, %	*P* Value[b]
CCyR[c]						
By 1 y	67	63	.6509	66	40	.0005
By 2 y	72	75	.7440	70	41	.0001
MMR[d]						
By 1 y	42	28	.0688	42	19	.0009
By 2 y	51	48	.7729	51	26	.0006

	NIL 400 mg BID (n = 96)	IM 600 mg QD (n = 95)	Crossover to NIL (n = 56)	Crossover to IM (n = 13)
EFS on treatment[e]				
KM-estimated 2-y EFS (95% CI), %	67 (54-77)	52 (20-76)	-	-
Hazard ratio vs NIL (95% CI)	.851 (0.486-1.492)		-	-
P value[b] vs NIL	.5739		-	-
KM-estimated 2-y EFS before crossover[f] (95% CI), %	71 (59-81)	58 (34-76)	-	-
Hazard ratio vs NIL (95% CI)	1.048 (0.554-1.983)		-	-
P value[b] vs NIL	.8848		-	-
KM-estimated 2-y EFS after crossover (95% CI), %	-	-	66 (35-85)	39 (1-82)
Hazard ratio vs NIL (95% CI)	-	-	.382 (.103-1.419)	
P value[b] vs NIL	-	-	.1363	

KM, Kaplan-Meier.
[a] For analyses by indicated time points, pts who achieved the response prior to crossover and by the indicated time point were considered as responders.
[b] *P* values are nominal and are provided for descriptive purposes only.
[c] Pts with missing cytogenetic evaluations by the indicated time point were considered as nonresponders.
[d] Pts with missing polymerase chain reaction evaluations by the indicated time point or with atypical transcripts at baseline were considered as nonresponders.
[e] Date of event is the earliest date of any of the following events during treatment on study: death due to any cause, progression to accelerated phase or blast crisis, loss of partial CyR, loss of CCyR, loss of complete hematologic response. Time is censored at the date of the last on-treatment assessment for patients without an event.
[f] For patients who crossed over to the alternate treatment arm without an event, time is censored at the date of crossover.

Summary and Conclusions: With 2 y of follow-up and accounting for crossover, final results from LASOR demonstrated clinically meaningful improvements in CCyR and MMR in pts who switched to NIL following suboptimal CyR (2009 ELN criteria) with frontline IM. Although the primary endpoint (ITT analysis) was not met, data accounting for crossover suggest such pts (classified as warning/failure according to current ELN recommendations) may be more likely to achieve recommended ELN treatment milestones with switch to NIL *vs* IM dose escalation.

P233

HEALTH-RELATED QUALITY OF LIFE IN CML PATIENTS UNDER NILOTINIB FIRST-LINE TREATMENT: RESULTS OF A GERMAN SUB-STUDY WITHIN THE ENEST1ST TRIAL

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Background: For chronic myeloid leukemia (CML), many treatment options have been established. So far, none of them provided a statistically significant and clinically relevant survival advantage as compared with the others. Thus, endpoints like health-related quality of life (HRQoL) move into focus. HRQoL might become essential for deciding on treatment strategies. Data on HRQoL in CML are rare (Efficace *et al.*, 2011), especially for patients receiving second generation tyrosine kinase inhibitor treatment.
Aims: We evaluated HRQoL in German patients of the ENEST1st study (Evaluating Nilotinib Efficacy and Safety in clinical Trials as First-Line Treatment, NCT01061177). Main interest was the comparison of HRQoL between diagnosis and during treatment and the presence of adverse events and symptoms.
Methods: The EORTC QLQ-C30 questionnaire (including global health status and five functioning scales) and an ad-hoc 15-item symptom scale were sent out to German participants of the study after informed consent was given at diagnosis (visit 1, V1), month 3 (V2), month 6 (V3), month 12 (V4), month 18 (V5), and at the end of the study (month 24, V6). Patients with completed questionnaires at visits 1 and 6 were included in this analysis; patients ending the study prior to 24 months were counted as visit 6 at their last QoL assessment. Incomplete questionnaires were not considered. Functional scales and global health status of the EORTC questionnaire were calculated in accordance with Aaronson (1993) and Fayers (2001). With functional scales and global health status ranging from 0 to 100, 8 points are regarded as a minimally important difference suggesting benefit or harm (Efficace *et al.*, 2013). Results at V1 and V6 were compared with the Wilcoxon signed rank test handling zeros and ties as suggested by Pratt (1959). Level of significance was 0.05.
Results: A total of 133 patients consented to the sub-study, 53 patients were included in this analysis. The median age was 50 years (range 19-83), 40% were female, and 7.6% had a high Euro score (Hasford *et al.*, 1998). This was comparable with the total study cohort (n=1164) with a median age of 53 (range 18-91) and 9.1% Euro-high risk. The results of all patients of the EORTC-QLQ-C30 items are summarized in Table 1. For most items, the score at diagnosis did not substantially decrease or increase over time. However, there was an increase of social and role functioning for all patients. Despite minimally important differences in social and role functioning, results of global health status and the functional scales at V1 and V6 were not statistically significantly different. Regarding symptoms there was a substantial change in skin problems. At diagnosis patients scored skin problems not at all (n=32), a little (n=10), quite a bit/very much (n=6), whereas at last visit the numbers were 12, 17 and 19 respectively. Other symptoms like edema, joint pain, weight loss, sexual function and headache were scored equally. Skin adverse events were documented according WHO as grade 1 in 22, grade 1 or 2 in 7 and grade 3 in 2 patients.

Tabella 1.

	Visit 1			Visit 6			p-value
Variable	N	Mean	Standardd eviation	N	Mean	Standard deviation	
Global health status / QoL	50	63	19	49	71	22	ns
Role functioning	50	70	28	49	78	29	ns
Social functioning	50	72	28	49	81	24	ns
Emotional functioning	50	65	24	49	70	25	ns
Physical functioning	50	85	14	49	84	20	ns
Cognitive functioning	50	84	21	49	80	23	ns

ns=not significant

Summary and Conclusions: The HRQoL of CML patients increased over time under first-line treatment with nilotinib with the exception of cognitive functioning. However, the differences were not statistically significant which might be due to low patient numbers. Regarding the ad hoc symptom scale, skin problems showed the most notable changes.

P234

PONATINIB EFFICACY AND SAFETY IN HEAVILY PRETREATED LEUKEMIA PATIENTS: 3-YEAR RESULTS OF THE PACE TRIAL

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Background: Ponatinib is an approved oral tyrosine kinase inhibitor (TKI) with potent activity against native BCR-ABL and resistant mutants, including the T315I mutant.
Aims: The phase 2 PACE trial evaluated the efficacy and safety of ponatinib (starting dose 45 mg once daily).
Methods: Patients with chronic myeloid leukemia (CML) or Philadelphia chromosome–positive acute lymphoblastic leukemia (Ph+ ALL) resistant/intolerant to dasatinib/nilotinib or with T315I were enrolled in PACE (NCT01207440) and gave informed consent. Of 449 patients, 58% received ≥3 prior TKIs. Arterial occlusive events (AOEs) led to recommended dose reduction in October 2013. Exposure-adjusted incidence rates of new AOEs were calculated as (number of first events in interval)/(total exposure for interval in patient-years) x 100. Rates for later intervals exclude patients with prior events.
Results: As of October 6, 2014: 33% of all patients and 45% of chronic-phase (CP)-CML patients remained on study. Median follow-up was 34.2 (0.1–48.6) months overall and 38.4 (0.1–48.6) months for CP-CML patients. Main reasons for discontinuation were progression (21% overall, 9% CP-CML) and adverse events (AEs) (15% overall, 17% CP-CML). Among CP-CML patients, 59% achieved major cytogenetic response (MCyR), 39% achieved major molecular response (MMR) or better, and 22% achieved MR4.5. Responses were durable, with an estimated 83% of responders maintaining MCyR at 3 years; estimated rates of progression-free survival and overall survival at 3 years were 61% and 82%, respectively. Among accelerated-phase CML, blast-phase CML, and Ph+ ALL patients, estimated rates of overall survival at 3 years were 59%, 9%, and 16%, respectively. Treatment-emergent AEs in ≥25% of patients were thrombocytopenia (43%), abdominal pain (42%), rash (41%), constipation (37%), headache (37%), dry skin (36%), fatigue (30%), pyrexia (29%), arthralgia (29%), hypertension (28%), nausea (28%), and neutropenia (25%). Rates of AOEs (any/serious) were 22%/17%, including cardiovascular (12%/8%), cerebrovascular (8%/6%), and peripheral vascular (8%/6%) events. Rates of venous thromboembolic events (VTEs; any/serious) were 5%/4%. Of the 99 patients with AOEs, 42 remained on study and 33 were still in MCyR. Exposure-adjusted incidence rates of new AOEs (events per 100 patient-years) were 14.5 in Year 1, 14.1 in Year 2, and 10.8 in Year 3. Exposure-adjusted incidence rates of new VTEs (events per 100 patient-years) were 3.5 in Year 1, 1.8 in Year 2, and 1.8 in Year 3. One year after recommended dose reduction, 61 (95%) of 64 CP-CML patients maintained MCyR, 44 (94%) of 47 CP-CML patients maintained MMR, and 5 (7%) of 70 ongoing patients without a prior AOE had an AOE.
Summary and Conclusions: Ponatinib continues to provide deep, durable responses in heavily pretreated patients, particularly those with CP-CML. One year after recommended dose reduction, responses were maintained and incidence of new AOEs was low. A dose-ranging trial of ponatinib in refractory CML to evaluate benefits and risks is currently planned.

P235

RESPONSES AT EARLY LANDMARK TIME POINTS ARE ASSOCIATED WITH OUTCOMES IN HEAVILY PRETREATED CP-CML PATIENTS (PTS) TREATED WITH PONATINIB

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Background: Ponatinib is a potent approved oral tyrosine kinase inhibitor (TKI) active against native and mutant BCR-ABL, with substantial activity in pts resistant or intolerant to dasatinib or nilotinib or with the T315I mutation. In pts treated with TKIs in the first-and second-line settings, responses at early (landmark) time points correlate with positive long-term outcomes.
Aims: Since landmark analyses are limited for populations treated with multiple prior TKIs, this analysis describes the impact of achieving early landmark responses with ponatinib on long-term outcomes in CP-CML pts in the ongoing PACE trial.
Methods: Retrospective landmark analyses of CP-CML pts (n=267) excluded pts who: dropped out before landmark time, had response at baseline, had missing or unevaluable assessments (<20 [13] metaphases examined for CCyR [MCyR]) at landmark time, or progressed before landmark time (for PFS). Pts gave informed consent. Molecular responses (International Scale; BCR-ABL ≤0.1% [MMR], ≤1%, and ≤10%) and cytogenetic responses (Ph+ metaphases ≤35% [MCyR] and 0% [CCyR]) at 3 and 6 months and their association with outcomes are reported. Log-rank test was used to calculate *P* values. Data cutoff: 6 Oct 2014; median follow-up: 38.4 (0.1–48.6) months.
Results: Of 267 CP-CML pts, 60% received ≥3 prior TKIs. Median age: 60 (18–94) years; median time from diagnosis: 7 (0.5–27) years. At 3 months, 48%, 39%, 49%, 34%, and 14% had achieved MCyR, CCyR, BCR-ABL ≤10%, BCR-ABL ≤1%, and MMR, respectively. These pts typically had better PFS and OS after 2 years, compared with those not achieving responses, although PFS and OS were high in general (**Table 1**). Molecular response at 3 months was also associated with achievement of MR4.5 over time. At 6 months, 62%, 52%, 55%, 49%, and 29% had achieved MCyR, CCyR, BCR-ABL ≤10%, BCR-ABL ≤1%, and MMR, respectively. Achievement of these responses was positively associated with PFS/OS after 2 years (**Table 1**).

Tabella 1. Association of Early Responses With 2-Year PFS and OS

	Based on 3-Month Response						Based on 6-Month Response					
	n	PFS	*P*	n	OS	*P*	n	PFS	*P*	n	OS	*P*
MCyR	97	87%	<0.0001	100	91%	0.0066	117	83%	<0.0001	118	91%	0.0617
No MCyR	98	55%		109	81%		57	55%		73	86%	
CCyR	78	87%	0.0002	80	90%	0.0238	96	87%	<0.0001	97	92%	0.0614
No CCyR	111	62%		123	83%		75	57%		91	86%	
BCR-ABL ≤10%	82	75%	0.0539	86	85%	0.7973	84	82%	0.0027	88	94%	0.0593
BCR-ABL >10%	79	61%		89	86%		56	58%		73	84%	
BCR-ABL ≤1%	75	87%	0.0003	77	90%	0.0357	96	86%	<0.0001	101	93%	0.0790
BCR-ABL >1%	133	62%		148	84%		87	61%		106	87%	
MMR	32	97%	0.0006	33	97%	0.0324	58	95%	<0.0001	61	95%	0.0428
No MMR	180	67%		197	84%		129	65%		150	88%	

Kaplan-Meier PFS/OS estimates calculated from landmark time; progression = death, AP/BP development, CHR loss in absence of CyR, MCyR loss, or increasing WBC without CHR

Summary and Conclusions: Rapid, deep reduction in BCR-ABL levels with ponatinib was associated with improved long-term outcomes in this heavily pretreated population. These data demonstrate that achieving early cytogenetic and molecular responses with ponatinib treatment has a significant prognostic value in heavily pretreated pts.

P236

LONG-TERM FOLLOW-UP OF PONATINIB EFFICACY AND SAFETY IN PATIENTS (PTS) WITH THE T315I MUTATION IN THE PHASE 1 AND PHASE 2 (PACE) TRIALS

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Background: Ponatinib is the only oral tyrosine kinase inhibitor (TKI) approved for use in refractory CML or Ph+ ALL pts that inhibits the BCR-ABL T315I mutant, which is uniformly resistant to other approved TKIs.
Aims: The long-term efficacy and safety of ponatinib in pts with the T315I mutation is reported based on pooled data from the ongoing phase 1 and PACE trials.
Methods: The phase 1 trial (NCT00660920) evaluated safety and antileukemic activity of ponatinib (2–60 mg qd) in pts with resistant/refractory hematologic malignancies (N=81), and the PACE trial (NCT01207440) evaluated efficacy and safety of ponatinib (45 mg qd) in pts with CML or Ph+ ALL who were resistant/intolerant to dasatinib/nilotinib or who had the T315I mutation (N=449). All pts gave informed consent. The T315I mutation was detected at baseline by Sanger sequencing at a central laboratory. PFS and OS data were collected in PACE only.
Results: Pooled data from 147 pts with T315I in the phase 1 (n=19) and PACE (n=128) trials are reported. Among 76 CP-CML, 19 AP-CML, 26 BP-CML, and 26 Ph+ ALL pts, median age was 50, 52, 47, and 62 y, respectively; median time since diagnosis was 4.6, 6.3, 2.3, and 1.3 y; and 86%, 84%, 96%, and 73% received ≥2 prior TKIs. At the time of analysis (26 September 2014 for phase 1; 6 October 2014 for PACE), median follow-up for pts with T315I was 36 (1.5–70) mo, 21 (3.1–45) mo, 2.9 (0.4–9) mo, and 2.7 (0.1–40) mo for CP-CML, AP-CML, BP-CML, and Ph+ ALL pts, respectively; 50%, 26%, 0%, and 4% remained on study. Pts discontinued primarily for disease progression (13%, 26%, 62%, and 46% in CP-CML, AP-CML, BP-CML, and Ph+ ALL, respectively) and AEs (12%, 16%, 15%, and 4%). Median dose intensity was 34, 38, 44, and 45 mg/d in these groups. Among CP-CML pts, 75% achieved MCyR; 72%, CCyR; 61%, MMR; 45%, MR4; and 37%, MR4.5. Responses were durable; 83% and 81% of CP-CML pts were estimated to maintain MCyR and CCyR, respectively, for 3 y. Among AP-CML, BP-CML, and Ph+ ALL pts, MaHR rates were 58%, 27%, and 38%, respectively. In phase 1, 11/12 CP-mo and median OS was not reached (3-y OS rate was 63%); for 24 BP-CML and 22 Ph+ ALL pts in PACE, median PFS/OS was 4.1/6.9 mo and 2.6/6.5 mo, respectively. Treatment-emergent AEs in ≥30% of all T315I pts were generally grade 1/2 and included: rash, 42%/55% (of total/CP-CML pts); abdominal pain, 39%/42%; headache, 39%/46%; nausea, 36%/41%; dry skin, 34%/49%; fatigue, 34%/39%; constipation, 33%/36%; and pyrexia, 32%/26%. Arterial occlusive events (AOEs) occurred in 21%/32% of total/CP-CML pts and included cardiovascular (13%/18%), cerebrovascular (8%/12%), and peripheral vascular (8%/13%) events. Venous thromboembolic events (VTEs) occurred in 8%/7% of total/CP-CML pts. Exposure-adjusted incidence rates of new AOEs and VTEs (events per 100 pt-years) were 13.0 and 5.0, respectively, among total pts, and 12.8 and 2.7, respectively, among CP-CML pts.
Summary and Conclusions: Ponatinib continues to provide deep and durable responses with up to 6 y of follow-up in CP-CML pts with the T315I mutation, in whom effective treatment options are limited. The response rates and safety profile observed among T315I pts were comparable to, if not better than, those observed among all pts with refractory CML or Ph+ ALL in the phase 1 and PACE trials.

P237

MINIMUM FOLLOW-UP OF 4 YEARS FOR ONGOING PATIENTS WITH CHRONIC-PHASE CHRONIC MYELOID LEUKEMIA (CP-CML) IN A PHASE 1 TRIAL OF PONATINIB

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Background: Ponatinib, an approved oral tyrosine kinase inhibitor (TKI), has potent activity against native BCR-ABL and resistant mutants, including the T315I mutant.
Aims: The safety and antileukemic activity of ponatinib were evaluated in patients with resistant/refractory hematologic malignancies in this phase 1 trial. This analysis includes a minimum follow-up of 4 years for ongoing patients, the longest follow-up of ponatinib-treated patients to date.
Methods: Patients (N=81) received ponatinib (2–60 mg once daily) in this ongoing, open-label, dose-escalation trial (NCT00660920; enrollment, 2008–2010; data cutoff, 26 September 2014). All patients gave informed consent. The median follow-up for all 43 CP-CML patients was 49.9 (1.7–69.9) months; minimum follow-up for the 22 CP-CML patients who remained on study at data cutoff was 50.2 months.
Results: Data for CP-CML patients are reported. Median age was 55 years;

time since diagnosis was 6.6 years. 60% of patients had received ≥3 prior TKIs; 63% had BCR-ABL mutations (28% had T315I). At the time of analysis, 22 (51%) of 43 CP-CML patients remained on study. The most common reasons for discontinuation were adverse events (AEs; 26%) and progression (9%). Among the 43 treated CP-CML patients, major cytogenetic response (MCyR) was 72%; complete cytogenetic response (CCyR), 65%; major molecular response (MMR), 56%; MR4, 42%; and MR4.5, 28%. Responses were durable (**Table 1**). Median durations of response (MCyR, CCyR, and MMR) were not reached. Of the 22 ongoing patients at data cutoff, 18 (82%) were in CCyR, and 17 (77%) had MMR or better (6 had MMR, 1 had MR4, and 10 had MR4.5). The most common treatment-emergent AEs were rash (65%), fatigue (60%), abdominal pain (58%), headache (58%), and arthralgia (53%). Rates of arterial occlusive events (AEs/serious AEs) were 40%/30%, including cardiovascular (30%/21%), cerebrovascular (9%/7%), and peripheral vascular (14%/9%) events. Rates of venous thromboembolic events (AEs/serious AEs) were 5%/0%.

Tabella 1. Stability of Response in CP-CML Patients Treated With Ponatinib.

	Responders n	Lost Response na	Maintain Response at 4 Years,% (95% CI)b
MCyR	31	8	68 (44–84)
CCyR	28	8	70 (48–84)
MMR	25	11	52 (30–70)

[a]Failed to meet criteria for response at any single time point after initial response
[b]Kaplan-Meier estimate

Summary and Conclusions: These data in CP-CML patients represent the longest follow-up with ponatinib. With 4-year minimum follow-up for ongoing patients, ponatinib continues to provide benefit to CP-CML patients with prior TKI failure. Risks and benefits should be evaluated when using ponatinib in this population.

Myelodysplastic syndromes - Clinical 1

P238

HYPOPLASTIC MYELODYSPLASTIC SYNDROME (H-MDS): DISTINCT CLINICO-BIOLOGICAL FEATURES AND PROGNOSTIC RELEVANCE

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Background: Myelodysplastic syndromes (MDS) are a heterogeneous group of hematologic malignancies characterized by clonal expansion of bone marrow (BM) myeloid precursors with impaired differentiation. It remains unclear whether hypoplastic MDS (h-MDS) is a distinct clinicopathologic entity that differs from normo-/hypercellular MDS (NH-MDS) in terms of clinical and biological features.
Aims: We aim to investigate the clinic-biologic features of h-MDS and its prognostic relevance in a large cohort of MDS patients.
Methods: A total of 369 *de novo* MDS patients diagnosed according to the 2008 World Health Organization (WHO) criteria at the National Taiwan University Hospital who had complete clinical data and cryopreserved BM cells for study were recruited into the analysis. Hypoplasia was defined as less than 30% of the cellularity in the BM biopsy specimen. The clinical features, cytogenetics, molecular mutations of 17 genes, and treatment outcomes were compared between h-MDS and NH-MDS patients.
Results: Among the 369 patients, 103 (27.9%) were diagnosed as having h-MDS. The patients with h-MDS had lower WBC count, peripheral blood and BM blast percentage than those with NH-MDS (P=0.024, P=0.001 and P<0.0001, respectively). Refractory anemia (RA) was more common in h-MDS patients, whereas RA with excess blasts (RAEB) occurred more frequently in NH-MDS patients. h-MDS was associated with lower risk MDS, including low and intermediate-1 risk by International Prognostic Scoring System (IPSS) (34.2% *vs* 14.8%, P<0.0001), or very low and low risk by revised IPSS (IPSS-R) (41.1% *vs* 20.9%, P<0.0001). Clonal chromosomal abnormalities were detected in 154 (45.2%) of 341 patients who had chromosomal data. There was no difference in the distribution of karyotypes between patients with h-MDS and NH-MDS. To investigate the interaction of genetic alterations in the pathogenesis of h-MDS, a mutational screening of 17 genes was performed. The patients with h-MDS had lower incidences of *RUNX1, ASXL1, DNMT3A, EZH2* and *p53* mutations than those with NH-MDS (3.9% *vs* 14.4%, P=0.003; 6.9% *vs* 22%, P<0.0001; 2.9% *vs* 12.8%, P=0.003; 0% *vs* 5.3%, P=0.013, 2.9% *vs* 11%, P=0.013, respectively).
With a median follow-up duration of 46.9 months (range, 0.1-250.7 months), the cumulated incidence of acute leukemic transformation at 5 years was 18.5% for h-MDS patients and 41.1% for NH-MDS patients (P=0.001). Further, the patients with h-MDS had longer median overall survival (OS) than those with NH-MDS (113.7 months *vs* 29.1 months, P<0.001). In subgroup analysis, the survival difference between h-MDS and NH-MDS patients was more significant in lower-risk group. In multivariate analysis, h-MDS was an independent favorable prognostic factor for OS (relative risk 0.651, 95% CI, 0.427–0.992, P=0.046) irrespective of age, IPSS-R, and gene mutations.
Summary and Conclusions: Our findings provided evidence that h-MDS is a distinct clinico-biological subgroup of MDS and is inversely associated with RUNX1, DNMT3A, ASXL1, P53 and EZH2 mutations. Patients with h-MDS have better leukemia-free survival and OS.

P239

LONG-TERM OUTCOME OF LOWER RISK MDS PATIENTS RECEIVING ESA, AND IMPACT OF POST ESA TREATMENTS ON SURVIVAL: A RETROSPECTIVE STUDY FROM THE GFM, DUSSELDÖRF, AND GESMD REGISTRIES

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Background: Up to-50% of lower risk MDS patients respond to ESA, with a median duration of response of 2 years, most responders relapsing and becoming RBC transfusion dependent (TD). In a previous work on 253 such patients, we found that outcome was largely dependent on whether ESA failure occurred

early (primary failure or relapse <6 months), or later (Kelaidi, Leukemia 2013). However, only few patients in that cohort had received, after ESA failure, treatments other than RBC transfusions.
Aims: In this larger study, gathering the data from the French (GFM), German (GMDS) and Spanish (GESMD) registries, we also analyzed whether second line treatments could influence survival.
Methods: 991 lower risk MDS patients included in French (GFM), German (GMDS) and Spanish (GESMD) registries between 1999 and 2013, and treated by ESA were analyzed. Median age was 76.5y, with 55.9%males, WHO diagnosis: RA (11.6%), RCMD (31.8%), (12.9%) RAEB-1, RARS(24.7%),RCMD-RS (7.9%), (3,2%) MDS-U, del 5q (1.9%), Median Hb level at onset of ESA was 9.4g/dl, and 36.8% patients were RBC-TD, median EPO level was 57.8 IU/l (range 0-28000). IPSS-R was very low (20.2%), Low (57,8%), Int (18.7%) High (0.03%) pts. OS was evaluated from ESA failure.
Results: Erythroid response (HI-E, IWG criteria) was 66.5%. 331 pts had primary resistance and 277 relapsed after a response. Median response duration in relapsing patients was 17 months. 27 patients out of 108 had progressed at ESA failure (26%). After ESA failure, 195 patients received second, third and fourth line treatments other than RBC transfusions, including azacytidine (AZA) (n=131), lenalidomide (LEN) (n=62) thalidomide (n=27), LD araC (7), ATRA+EPO (n=18), HSCT and ATG in 4 pts each. Considering only the second line treatments after ESA failure (called TX1), 82 pts received AZA (ORR 41,6%) and 37 LEN (ORR 29,7%). 18 pts received AZA and (after AZA failure) LEN (seq 1), and 10 LEN and (after LEN failure) AZA (seq2). There was an ORR of 8/18 (44%) in seq 1 and 4/10 (40%) in seq 2 (with no response to LEN but subsequent response to AZA). Among the patients who progressed at TX1 (n=26), 18 received AZA, 2 LEN and the others low dose chemotherapy. Baseline WHO diagnosis (RARS and RARS-T *vs* others, HR 0.58, p=0.002) and IPSS-R (very low and low *vs* int/high, HR 0.71, p=0.04) predicted better OS. In a multivariate Cox model, receiving a second line treatment (considered as time-dependent variable) did not influence OS, but second line treatment with AZA (and not with LEN) was associated with improved OS and lower AML progression risk in patients with ESA response >1 year (HR 0.158, p=0.02 for OS, HR 0.18, p=0.03 for AML progression risk). Among the patients receiving AZA at TX1 (n=82), 18 had progressed either to RAEB-2 or AML.
Summary and Conclusions: Baseline RARS(-T) and lower IPSS-R predicted better outcome after ESA failure. AZA, as second line treatment, improved OS and AML risk in pts with ESA response >1 year, a finding requiring confirmation in prospective studies.

P240

EVALUATION OF DYSPLASIAS IN 1979 PATIENTS WITH MYELODYSPLASTIC SYNDROMES COLLECTED IN A GERMAN-AUSTRIAN DATA SET TO APPROACH THE HETEROGENEITY OF THE DISEASE

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Background: Dysplasias are a hallmark of MDS, they are mandatory for diagnosis, define classifications, and may play a role in prognosis and prediction. Their relative and combined impact regarding other and newer parameters also on different endpoints (leukemic *vs* non-leukemic mortality) remains to be defined.
Aims: Aim of the study was to redefine the role of dysplasias in MDS using a larger multicenter database by evaluating correlations with other parameters, characterising diagnostic and prognostic entities according to dysplasias including their specific prognostic impact.
Methods: Patients: 1979 primary MDS patients without disease modifying treatment were analysed retrospectively: median age: 73 years, 53% male, median survival: 32 months, median follow up: 66 months. Statistical methods: besides correlations, relative dominances of each dysplasia are described by odds of discordant pairs as they are less sample dependent than marginal proportions. Multiple regressions and Cox-models including interactions were used to quantify their impact on cytopenias and prognosis, particularly on non-leukemic mortality.
Results: Dysplasias were found in 90.3 % for erythroid, 75.7% myeloid and 70.4% for megakaryocytic lineages, respectively. Unilineage dysplasia was found in 18.0%, bilineage in 27.6%, trilineage in 54.4% of patients. Correlations between dysplasias were generally weak, most prominently a positive correlation between dysmeylo-and dysmegakaryopoiesis was noted. Anemic patients had significantly more erythrodysplasia whereas myeloid and megakaryocytic dysplasia coincided with neutropenia and thrombocytopenia, respectively, although details regarding age, sex, and monocyte count remain to be explored. Dominance of erythroid dysplasia becomes less pronounced in subentities (FAB, WHO) associated with higher risk. Dysplasias are evenly distributed among different LDH and Ferritin levels as well as in cytogenetic risk groups (IPSS 1996 and IPSS-R 2012 definitions) and IPSS. Bone marrow blast categories and IPSS-R, IPSS-R-age, and WPSS risk groups show fading dominance of erythrodysplasia with higher risk. Regarding prognosis, dysplasias without adjustment for other parameters showed marked differences between uni-, bi-and trilineage dysplasias (Dxy =0.15), especially in low risk cases and with normal cytogenetics. In Cox models myeloid and megakaryocytic dysplasias had more impact on leukemic transformation than on overall survival, erythrodysplasia was dominant regarding non leukemic mortality. Inclusion of an interaction term for joint erythroid and myeloid dysplasias enhances prognostic models. Inclusion of dysplasias in Cox models using patients stratified according to IPSS-R shows no significant impact for single or combined dysplasias or dysplasia interactions.
Summary and Conclusions: Dysplasias play an important independent role in MDS, which is more pronounced in low risk and normal karyotype cases. Dichotomisation in uni-*vs* multilineage dysplasia may not be justified given the differences between bi-and trilineage cases. Although dysplasias do not provide additional prognostic power for IPSS-R stratified patients as their prognostic value is already included in parameters and cutpoints used in this system, they may support specific models for different mortality risks (leukemic *versus* non leukemic) and consequently provide more meaningful classifications for this heterogeneous disease. Dysplasias may contribute to a multidimensional model of MDS composed of anemina, proliferation, and infection/bleeding.

P241

RANDOMIZED, PLACEBO (PBO)-CONTROLLED, PHASE I/II TRIAL OF THE THROMBOPOIETIN RECEPTOR AGONIST ELTROMBOPAG (EPAG) IN THROMBOCYTOPENIC PATIENTS WITH ADVANCED MYELODYSPLASTIC SYNDROMES (MDS)

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Background: EPAG, an oral thrombopoietin receptor agonist, increases platelets (PLTS) in chronic immune thrombocytopenia (TCP), hepatitis C virus-associated TCP, and severe aplastic anemia. In patients (pts) with advanced MDS or acute myeloid leukemia (AML) and severe TCP, EPAG has shown to improve PLTS compared to PBO, leading to potential improvements in morbidity of severe TCP, progression-free survival (PFS), and overall survival (OS).
Aims: To explore the safety, tolerability, and efficacy of EPAG in pts with advanced MDS (baseline bone marrow [BM] blasts <20%) from study PMA112509.
Methods: Informed consent was provided. Pts with relapsed/refractory MDS, ineligible for antileukemic therapies, with 10–19% BM blasts and PLTS <30×10^9/Lwere randomized to EPAG 50 mg once daily (increased every 2 weeks in pts without PLT response, up to 300 mg [East Asian pts, 150 mg]) or PBO for 6 months. Standard supportive care and disease-modifying treatments were allowed.
Results: Overall, 32 pts with MDS (EPAG, n=18; PBO, n=14) were enrolled. All pts received ≥1 prior therapy, including hypomethylating agents (HMAs) or induction chemotherapy. Fewer EPAG than PBO pts had poor prognosis karyotype, but median baseline BM blast % was similar (Table). Most pts received the maximum dose (EPAG, n=10 [56%]; PBO, n=8 [57%]). Adverse events (AEs) (EPAG, n=17 [94%]; PBO, n=13 [93%]), Grade ≥3 AEs (EPAG, n=13 [72%]; PBO, n=8 [57%]), drug-related AEs (EPAG, n=10 [56%]; PBO, n=6 [43%]), and AEs leading to withdrawal (EPAG, n=5 [28%]; PBO, n=4 [29%]) were reported. The most common AEs on therapy or <30 days from the last dose were diarrhea (n=6; 33%), constipation (n=5; 28%), and pyrexia (n=5; 28%) for EPAG pts and pyrexia (n=6; 43%), back pain (n=4; 29%), epistaxis (n=4; 29%), and febrile neutropenia (n=4; 29%) for PBO pts. The most common Grade ≥3 AEs on therapy or <30 days from the last dose in either arm were febrile neutropenia, anemia, pneumonia, and sepsis. A total of 5 EPAG (28%) and 6 PBO (43%) pts died on therapy or <30 days from the last dose; primary cause of death in both arms was disease under study. Of 14 (EPAG, n=9; PBO, n=5) pts with post-baseline BM exam results, 8 (5 [56%] and 3 [60%], respectively) developed ≥20% BM blasts during treatment. PLT transfusion independence for ≥8 weeks was reported for 5 EPAG (28%) and 3 PBO (21%) pts. Grade ≥3 hemorrhages were reported in 2 EPAG (11%) and 3 PBO (21%) pts. More EPAG *vs* PBO pts started HMAs during the study (4 [22%] *vs* 2 [14%], respectively). Median PFS (Figure) was 16.1 weeks for EPAG *vs* 7.7 weeks for PBO (hazard ratio [HR]=0.61; *P*=0.1531). Median OS was 34.0 weeks for EPAG *vs* 15.4 weeks for PBO (HR=0.70; *P*=0.3844).

Tabella 1. Baseline Characteristics.

	EPAG (N=18)	PBO (N=14)
Poor prognosis karyotype, n (%)	5 (28)	8 (57)
	Median (Range)	
BM blast, %	11 (10–18)	12.8 (10–19)
Platelets,a×109/L	19.8 (2–60)	13.5 (5–38)

[a]Baseline PLT count was derived using an average of PLT counts during screening, except within 3 days of PLT transfusion.

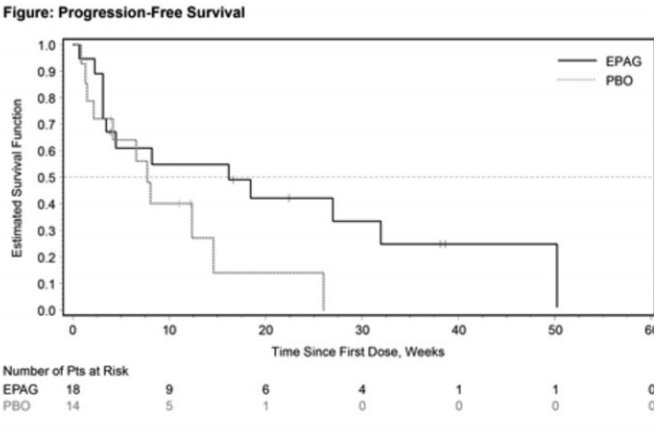

Figure 1. Progression-Free Survival

Summary and Conclusions: EPAG ≤300 mg was well tolerated in pts with advanced MDS. No obvious differences in blast % change were noted in pts receiving EPAG *vs* PBO. Pts receiving EPAG showed a trend toward fewer Grade ≥3 hemorrhages. Differences in PFS and OS may be influenced by the direct effect of EPAG, MDS treatments initiated, or imbalances in the proportion of pts with poor prognosis karyotype. Additional studies with EPAG in MDS and AML are ongoing. This study (NCT00903422) was funded by GlaxoSmithKline.

P242

THE EFFECT OF LENALIDOMIDE ON HEALTH-RELATED QUALITY OF LIFE IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES: RESULTS FROM THE MDS-005 TRIAL

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Background: Health-related quality of life (HRQoL) is compromised among patients with transfusion-dependent anemia due to myelodysplastic syndromes (MDS). In the phase 3 MDS-005 study, significantly more lenalidomide-treated patients with MDS achieved RBC transfusion independence (TI) *versus* placebo (26.9% *vs* 2.5%; *P*<0.001; Santini V, *et al.* Blood 2014;124:abstract 409); assessment of HRQoL was a pre-specified secondary endpoint.
Aims: The current analysis evaluated changes in HRQoL between treatment arms in the MDS-005 study.
Methods: In MDS-005, transfusion-dependent patients with International Prognostic Scoring System-defined Low or Intermediate-1-risk MDS without del(5q), unresponsive or refractory to erythropoiesis-stimulating agents, were randomized to lenalidomide (n=160) or placebo (n=79). Patients with RBC-TI ≥56 days or erythroid response by Day 168 (24 weeks) continued double-blind treatment until erythroid relapse, disease progression, unacceptable toxicity, or consent withdrawal. HRQoL was assessed using the European Organization for Research and Treatment of Cancer QLQ-C30 questionnaire at baseline, Week 12, Week 24, every 12 weeks thereafter, and at discontinuation. Analyses were performed among patients who completed a baseline HRQoL assessment and had ≥1 post-baseline assessment (lenalidomide, n =122; placebo, n =56). Changes from baseline were analyzed; 5 HRQoL domains were pre-selected as clinically relevant: Fatigue, Dyspnea, Physical Functioning, Emotional Functioning, and Global Quality of Life. Between-group comparisons were limited to weeks 12 and 24 due to low patient numbers after 24 weeks. A post hoc analysis assessed the impact of RBC-TI on pre-selected HRQoL domains. Clinically meaningful improvement in HRQoL was defined as a score difference of at least 10 points *versus* baseline.
Results: Questionnaire compliance rates were high (>72% in almost all visits) and not significantly different across all assessment visits (*P*>0.05). At Week 12, mean changes in HRQoL scores from baseline were similar between treatment arms across pre-selected domains. At Week 24, lenalidomide was associated with benefit *versus* placebo in all pre-selected domains; after adjusting for baseline scores, benefit was only significant for Emotional Functioning (*P*=0.047). For lenalidomide patients with RBC-TI and/or erythroid response at Week 24 who continued treatment, an improving trend in all pre-selected domains was observed. In a post hoc analysis, RBC-TI was associated with significant improvement (*P*<0.01) across all pre-selected domains, with benefit also exceeding the pre-specified threshold for clinically meaningful improvement in all 5 domains.
Summary and Conclusions: At Week 24, treatment with lenalidomide was associated with benefit compared with placebo across all 5 pre-selected HRQoL domains relevant to MDS; however, after adjusting for baseline scores, benefit was only significant for Emotional Functioning. Further improvement *versus* baseline was observed after Week 24 for patients continuing treatment with lenalidomide. RBC-TI was associated with statistically significant (*P*<0.01) and clinically meaningful improvement in all 5 pre-selected domains. These data may be important given the superior effect of lenalidomide over placebo in achievement of RBC-TI and highlight the beneficial effects of lenalidomide on HRQoL in this patient population.

P243

IMPACT OF MDS ON HEALTH-RELATED QUALITY OF LIFE: A COMPARISON OF IPSS LOW-AND INT-1 RISK MDS PATIENTS FROM THE EUROPEAN LEUKEMIANET MDS (EUMDS) REGISTRY AND EUROPEAN REFERENCE POPULATIONS

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Background: A prospective, multicenter European Registry (EUMDS) for newly diagnosed IPSS low and intermediate-1 Myelodysplastic Syndromes (MDS) was initiated by the European Leukemianet (ELN) to assess demographic data, disease-management and clinical course. Health-related quality of life (HRQoL) has become an important patient reported outcome (PRO) to address patients' needs and to tailor individualized therapy planning.
Aims: To analyze the impact of MDS on HRQoL as compared to a reference population.
Methods: The EQ-5D (European quality group 5 dimensions) descriptive system was introduced in EUMDS at initial diagnosis and at follow-up visits every six months. EQ-5D data from MDS-patients were compared with population norms published by the Euroqol Group (www.euroqol.org).
Results: So far, 1814 EUMDS patients from 17 European countries diagnosed between December 2007 to December 2014 were included in the EUMDS. 1490 (82.1%) patients with a median age of 74.2 years (range 18.7-95.3) and 61.9% male, completed an EQ-5D questionnaire at the time of inclusion to the registry. Moderate or severe restrictions in HRQoL were observed in a significant proportion of MDS patients in the different dimensions of EQ-5D: mobility in 41%, self-care in 13%, usual activities in 36%, pain/discomfort in 49%, and anxiety/depression in 40%, respectively. Mean visual analog scale (VAS) in EUMDS patients was 69.6±20.3 (mean, standard deviation). Population norms were available for nine European countries: age and sex-matched analysis reveal a significantly (p<0.05) higher proportion of restrictions in MDS in the dimensions anxiety/depression in eight, in usual activities in six, and in mobility and in self-care in four out of nine countries. Remarkably, problems in the dimension pain/discomfort were significantly more often observed in the reference population than in MDS in two countries; in one country a trend towards

significance was observed (p=0.07). It was possible to compute an index score for seven countries, which translates the five dimensions of EQ-5D into a single value, ranging from a score of 1 indicating no problems to a score of 0 indicating death. Compared to age-and sex-matched simulated populations using the population norms, EUMDS patients from France (n=340; mean [sd]: 0.69 [0.29]; p<0.001), Germany (26; 0.82 [0.28]; p<0.05), the Netherlands (48; 0.79 [0.20]; p<0.001) and Spain (120; 0.75 [0.32]; p<0.001) were characterized by significantly lower EQ-5D index scores than the corresponding reference populations. Visual analogue scores (VAS) in MDS ranged from 62.1 [20.5) (mean [sd]) in France to 73.4 [16.8] in Denmark. Four out of nine countries revealed significantly (p<0.05) lower VAS-values in MDS as compared to age-and sex-matched simulated populations based on the reference values. Significant differences were observed in France: n=340; VAS 62.08 [20.52]; p=0.02; The Netherlands: 48; 71.19 [16.61]; p=0.04; Sweden: 100; 67.45 [20.91]; p<0.001 and the United Kingdom: 252; 71.31 [20.14]; p=0.01.

Summary and Conclusions: This study demonstrates for the first time profound restrictions in self-reported health in VAS as well as in all dimensions of the EQ-5D, with the exception of pain/discomfort, in MDS-patients at diagnosis as compared with reference populations. Thus, the relevance of HRQoL in treatment algorithms and as an endpoint in clinical studies and registries is supported. This analysis will form the basis for the design of MDS-specific assessment scores.

P244

SPRESAS (SPANISH REGISTRY OF ERYTHROPOIETIC STIMULATING AGENTS STUDY): THE LARGEST RETROSPECTIVE STUDY OF TREATMENT WITH ESAS IN LOWER RISK MDS

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Background: Anemia is the most frequent cytopenia in lower-risk MDS. Erythropoietic-stimulating agents (ESAs) are commonly used in these patients.
Aims: The aims were (1) to collect the largest lower risk (IPSS low and int-1) series of MDS patients with anemia treated with ESAs, describe their results, and (2) to compare the outcomes of this ESA cohort (in terms of progression to AML and OS) with those of a cohort of lower risk RBC transfusion dependent untreated patients from the Spanish MDS Registry (SUPP cohort).
Methods: Data from 959 patients with MDS according to FAB and WHO criteria were recorded in Spresas (SPanish Registry of Erythropoietic Stimulating Agents Study). 237 cases were excluded from the study (23 treated with potential modifier agents, 38 with secondary MDS, 24 with IPSS >int-1 risk, 97 patients with Hb level >11 g/dL, 31 diagnosed after Dec 31, 2011 (pre-fixed end of recruitment period), and 24 with incomplete data). The remaining 722 anemic patients with low/int1 risk IPSS de novo MDS and sufficient follow-up data available (530 in ESAs and 192 in SUPP cohort) were included in the analysis and are the basis of this report. Response to treatment was evaluated according to IWG 2006 response criteria and a multivariate logistic regression analysis was used to identify independent predictors of erythroid response. OS and AML evolution (WHO criteria) were defined as the time between EPO introduction (or diagnosis in SUPP arm) and the corresponding event or last follow up (July 2014) and were analyzed using univariable and multivariable Cox proportional hazards regression methods.
Results: In the ESAs-treated population median age was 77 years (interquartile range [IQR] 25%>75%: 21-95 y), according to WHO 2008 there were 48 RCUD (9.2%), 176 RCMD (33.8%), 101 RARS (19.4%), 18 RAEB-1 (3.5%), 2 RAEB-2 (0.4%), 32 CMML (6.2%) and 3 MDS-U (0.6%) and the IPSS risk group was low in 66% (N=308) and Int-1 in 34% (N=158). Median Hb level at start of treatment was 9.6 g/dL (4-10.9). Among 329 patients with this data available, 185 patients (56%) were RBC transfusion dependent before ESAs treatment. Median time from diagnosis to ESAs treatment was 92 (1-5683) days. Among 484 with this data available 27.5% of patients received epoetin α, 19.9% epoetin β, 49.6% darbepoetin α, and 3%other epoietin. Doses and schedules were those usually employed in MDS. Among 470 patients (88.6%) evaluable for response the rate of erythroid response was 64.6%. To determine whether treatment with ESAs had an impact on disease progression and OS, outcome of ESAs cohort was compared to that of the SUPP cohort. After a median follow up of 3.12 years, 72 patients had developed AML (14%) and 209 had died (40%). Median overall survival (OS) was 6.7 and 2.6 years, for ESAs and SUPP cohorts, respectively (p<0.001). Response to treatment significantly improved outcome (data not shown). Median time to progression to AML was similar in both cohorts (p=NS), with 1, 2 and 5 years AML-free survival (LFS) of 95%, 92%, and 82% for ESAs patients and 96%, 92% and 88% for the SUPP group.

Summary and Conclusions: The present study, the largest retrospective series regarding ESAs treatment in lower risk MDS patients reported so far, shows that, using conventional survival statistical analysis, treatment with ESAs results in improved OS and does not increase the risk of AML evolution. Spresas study was partly supported by Janssen

P245

MDS-005 STUDY: EFFECT OF BASELINE ENDOGENOUS ERYTHROPOIETIN ON RBC TRANSFUSION INDEPENDENCE IN LENALIDOMIDE-TREATED PATIENTS WITH LOW OR INTERMEDIATE-1-RISK MYELODYSPLASTIC SYNDROMES WITHOUT DEL(5Q)

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Background: In the phase 3 MDS-005 study, lenalidomide treatment was associated with significant achievement of RBC transfusion independence (TI) for ≥8 weeks in 26.9% of RBC transfusion-dependent patients with International Prognostic Scoring System (IPSS) Low or Intermediate-1-risk myelodysplastic syndromes (MDS) without del(5q), unresponsive or refractory to erythropoiesis-stimulating agents (ESAs; *P* <0.001 *vs* placebo) (Santini V, *et al.* Blood 2014;124:abstract 409).
Aims: This analysis evaluated the impact of baseline erythropoietin (EPO) levels on RBC-TI in lenalidomide-treated patients with MDS.
Methods: Patients were randomized 2:1 to oral lenalidomide 10 mg once daily (5 mg for patients with creatinine clearance 40–60 mL/min) or placebo. Rates of RBC-TI for ≥8 consecutive weeks were analyzed according to baseline EPO levels prior to randomization (≤500 *vs* >500 mU/mL) in patients treated with lenalidomide.
Results: Of 160 lenalidomide-treated patients, 5 had missing baseline EPO data, 97 had EPO ≤500 mU/mL, and 58 had EPO >500 mU/mL; 125 (78.1%) of all patients and 94 of 97 (96.9%) patients with EPO ≤500 mU/mL received prior ESAs. Patients with EPO ≤500 mU/mL were slightly older than those with EPO >500 mU/mL (median age: 72 *vs* 68 years), had a longer duration of MDS (median: 3.7 *vs* 2.2 years), and lower RBC transfusion burden (median: 3.0 *vs* 3.8 packed RBC units/28 days). Among patients with EPO ≤500 mU/mL and prior ESA use, 35.1% achieved RBC-TI ≥8 weeks, whereas only 9.4% of patients with EPO >500 mU/mL and no prior ESA use had a response. Overall, there was an increasing linear trend in rates of RBC-TI ≥8 weeks across patients with decreasing EPO levels: 15.5%, 23.3%, 33.3%, and 42.5% for EPO levels >500 (n =58), 200 to ≤500 (n =30), 100 to ≤200 (n =27), and ≤100 mU/mL (n =40), respectively (Fisher's exact *P* =0.023; linear trend test *P* =0.002). A similar trend was seen in patients who had received prior ESAs.
Summary and Conclusions: In patients with IPSS lower-risk MDS without del(5q), response to lenalidomide correlated with endogenous serum EPO levels in a linear fashion. Baseline EPO ≤100 mU/mL was associated with RBC-TI ≥8 weeks in >40% of patients. Patients with EPO ≤500 mU/mL had higher response rates *versus* EPO >500 mU/mL. Patients with higher EPO levels may be less responsive due to intrinsic defects in erythroid signaling pathways, including STAT5, JAK2, and lipid raft assembly, which are believed to be restored or stimulated by lenalidomide (Ximeri M, *et al.* Haematologica 2010;95:406-14; McGraw KL, *et al.* PLoS One 2014;9:e114249). These results suggest it may be possible to identify a subset of patients with lower-risk MDS without del(5q) who can be more sensitive to lenalidomide.

P246

PROGNOSTIC IMPACT OF CHROMOSOMAL TRANSLOCATIONS IN MYELODYSPLASTIC SYNDROMES AND CHRONIC MYELOMONOCYTIC LEUKEMIA PATIENTS. A STUDY BY THE SPANISH GROUP OF MYELODYSPLASTIC SYNDROMES

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Background: Chromosomal translocations are an uncommon finding in myelodysplastic syndromes (MDS) and chronic myelomonocytic leukemias (CMML). Whether translocations other than t(3q) have a specific prognostic significance in these entities has never been examined in detail.
Aims: To analyze the prognostic impact of translocations in the context of the IPSS-R cytogenetic scoring system.
Methods: We evaluated 2014 patients from the Spanish Registry of MDS with a diagnosis of MDS or CMML (WHO 2008) and an abnormal karyotype by conventional cytogenetic analysis. The main study outcomes were survival from diagnosis and transformation into acute myeloid leukemia (AML). Survival curves were drawn using the Kaplan-Meyer method and compared by the log-rank test. The Cox regression model was used for the multivariate adjustment of factors predicting survival. The cumulative incidence of AML was estimated by taking non AML-related death as a competing risk, and adjusted for other predictive factors by means of the Fine and Gray regression method. Statistical comparisons were done by the Mann-Whitney U-test for continuous data, and the chi-square test for categorical factors. Stata, version 11, software (www.stata.org) was used for the statistical analysis.
Results: Translocations were identified in 168 patients (T-group). As compared with the 1484 patients with abnormal karyotype without translocations (non-T group), patients in the T-group were younger, had lower Hb, neutrophils and platelets in peripheral blood and more blasts in bone marrow. The T-group included a significantly larger proportion of patients with refractory anemia with excess of blasts and higher scores in both the cytogenetic and the global IPSS-R (p<0.0001). At the time of analysis, 905 (55%) patients had died. Median follow-up for survivors was 1.3 years (range, 0.2 to 20 years). The actuarial survival for all patients was 2.6 years (95% C.I, 2.4 to 2.9). Median survival for the T-group was significantly shorter compared to the non-T group (0.98 years [95% CI, 0.82 to 1.2] *versus* 2.86 years [95% CI, 2.58 to 3.32]), respectively (p<0.001) (figure1A). After adjustment for the IPSS-R cytogenetic category, no significant difference between the two groups was found (1.16 years [95% CI, 0.95 to 1.42] *versus* 1.65 years [95% CI, 1.55 to 1.75]), (ns) (figure 1B). Three-hundred and forty-three patients (20.75%) had developed AML after a median follow-up of 1.52 years (95% CI, 0.2-11.2) from the diagnosis of MDS, while 605 patients had died without progression to AML. Seven hundred and five patients were censored alive at the time of analysis. Presence of translocations was associated with an increased incidence of AML at the univariate analysis (subhazard rate (SHR): 1.5, 95% CI: 1.1-2.1;p=0.008) (figure 1C). Nevertheless, this association dissapeared after the multivariate adjustment for the IPSS-R cytogenetic risk category (SHR: 0.95, 95% CI: 0.68-1.35; p=0.78) (figure1D).

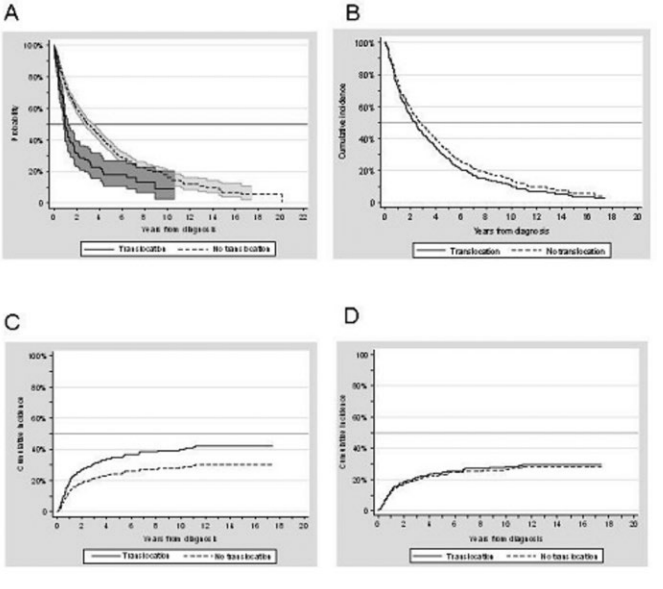

Figure 1.

Summary and Conclusions: In our study, the presence of translocations clearly identified a subgroup of patients with a more aggressive MDS and with a poorer IPSS-R cytogenetic prognosis. When a raw analysis of the prognostic impact of translocations was made, a significant worse survival and an increased risk of AML transformation were found in the T group. However, translocations lost their prognostic impact after adjustment for the IPSS-R cytogenetic category. This results suggest that translocations are a surrogate marker for other poor prognosis cytogenetic aberrations instead of being causally related to the poorer outcomes.

P247

SUPERIOR LONG-TERM OUTCOMES OF LOWER RISK MDS PATIENTS WHEN TRANSPLANTED IN RESPONSE COMPARED TO RELAPSED OR REFRACTORY TO HMA

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Background: Allogeneic hematopoieic cell transplantation (Allo-HCT) is generally recommended for lower-risk myelodysplastic syndrome (LR-MDS) patients with relapsed or refractory to HMA treatment. It is still under debate when LR-MDS patients should be transplanted in response or at the time of loss of response.
Aims: This study retrospectively evaluated the outcomes of allo-HCT who transplanted in response or relapsed/refractory to HMA treatment.
Methods: The data of 307 patients diagnosed with LR-MDS from Oct 1992 to Jul 2013 were retrospectively evaluated. To address the time dependence of allo-HCT, the methods of Simon and Makuch and the Mantel-Byar test was used.
Results: Among 307 patients with LR-MDS, 200 patients were treated with HMA. Sixty-three (31.5%) patients showed response (CR/PR/HI) to HMA and 119 (59.5%) were relapsed/refractory (SD/PD). Prior to Allo-HCT, 7 patients were in response and 26 patients in relapsed/refractory. Median time to allo-HCT were 2.8 years in responders and 1.9 years for patients with relapsed/refractory. Among HMA responders, the 5-year overall survival (OS) rate were 83.3% with allo-HCT (n=7) and 34.2% with continued HMA treatment (n=53) (p=0.07). Among patients with relapsed/refractory, the 5-year OS rate were 47.0% with allo-HCT (n=26) and 29.4% with BSC after HMA treatment (n=93) (p=0.45). There showed a trend in the benefits of OS for patients treated with allo-HCT in response compared to allo-HCT in SD/PD (p=0.17). In the multivariate analysis, ECOG-PS 2-3 (HR 3.030, p=0.006), IPSS blast ≥0.5% (HR 2.003, p=0.018), poor cytogenetic risk group (HR 12.362, p<0.001), and non-response to HMA (HR 2.487, p=0.001) were unfavorable factors for OS among LR-MDS patients.

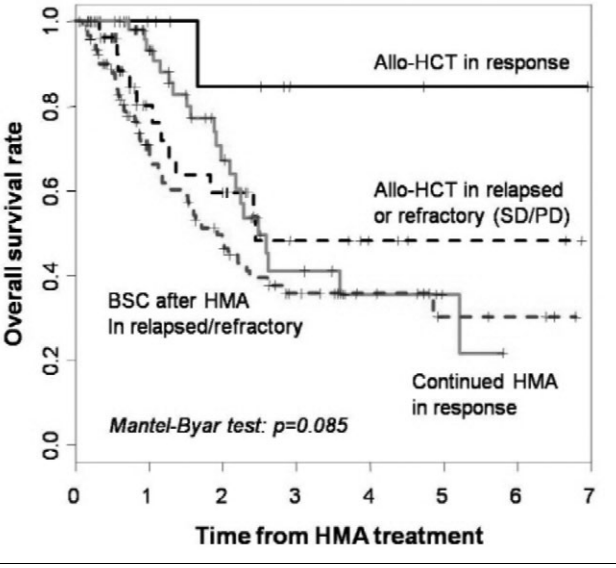

Figure 1.

Summary and Conclusions: Allo-HCT in response to HMA showed superior OS compared to allo-HCT in relapsed/refractory after HMA for patient with LR-MDS. To elucidate best time points of allo-HCT for LR-MDS patients, a large scale study will be needed.

BMF syndromes incl. PNH - Biology & Clinical

P248

ABERRANT EXPRESSION PROFILES OF HLA-G AND IMMUNOGLOBULIN-LIKE TRANSCRIPTS (ILTS) IN ACQUIRED APLASTIC ANEMIA

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Background: Human leukocyte antigen-G (HLA-G) is a non-classical MHC class I molecule characterized by low polymorphism, restricted tissue distribution and spliced transcripts which encode four membrane-bound (HLA-G1-G4) and three soluble (HLA-G5-G7) isoforms. HLA-G interacts with multiple cell subsets such as T cells and B cells, and exerts powerful immune suppressive effects by binding to its receptors immunoglobulin-like transcripts (ILTs). As a paradigm of bone marrow failure syndrome, acquired aplastic anemia (AA) is thought to be a specific autoimmune disease for the aberrant T-cell immune homeostasis, whereas the function of humoral immunity in acquired AA remains elusive. It is reported that humoral immune responses to hematopoietic antigens can be detected in AA patients, and these humoral immune responses may be useful in unraveling the disease pathophysiology of AA. However, there has been no data referred to the role of HLA-G in acquired AA.
Aims: The study was performed to investigate whether HLA-G and its two main receptors (ILT2 and ILT4) were involved in acquired AA.
Methods: Human bone marrow mononuclear cells (BMMCs) and bone marrow plasma were obtained from 20 severe AA (SAA) patients, 13 moderate AA (MAA) patients and 16 healthy control subjects. All the AA patients were newly diagnosed and had not received any specific treatment. The concentration of soluble HLA-G in bone marrow plasma was determined by enzyme-linked immunosorbent assay. The relative expression level of HLA-G, ILT2 and ILT4 in BMMCs was quantified by real time polymerase chain reaction. Besides, the percentages of membrane-bound HLA-G$^+$, ILT2$^+$ and ILT4$^+$ cells amongst CD4$^+$, CD8$^+$, CD14$^+$ and CD19$^+$ BMMCs were analyzed by flow cytometry.
Results: The concentration of soluble HLA-G in the marrow plasma of AA patients was much higher than that in healthy controls but no difference was found between SAA and MAA patients. At transcriptional level, the ILT2 mRNA expression on BMMCs in AA group was elevated compared with control group while the HLA-G mRNA level was similar in the two groups. In addition, the percentage of CD19$^+$lymphocytes among BMMCs was increased in AA group, and the proportion of ILT2-expressing cells among CD19$^+$ BMMCs in AA patients was significantly higher than that in healthy controls. In AA group the percentage of CD19$^+$ BMMCs was negatively correlated with the counts of neutrophils, platelets or reticulocytes in peripheral blood. No significant difference was observed for the expression of membrane-bound HLA-G, ILT2 and ILT4 on the surface of CD4$^+$, CD8$^+$ or CD14$^+$BMMCs between AA patients and healthy controls.
Summary and Conclusions: Together, our data demonstrated that HLA-G/ILT2 was aberrantly expressed in AA patients' marrow cells especially in the CD19$^+$ lymphocytes, which indicated HLA-G/ILT2 may play a role in the abnormal humoral immunity in AA. Blocking HLA-G/ILT2 interaction might be a novel therapeutic strategy for acquired AA.

P249

THE MTOR SIGNALING PATHWAY IS ACTIVIVED IN T CELLS OF APLASTIC ANEMIA

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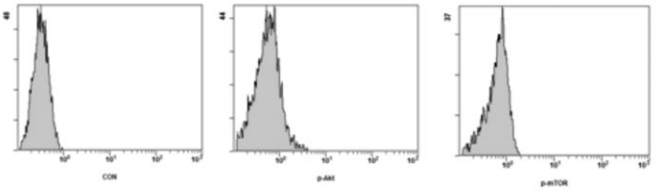
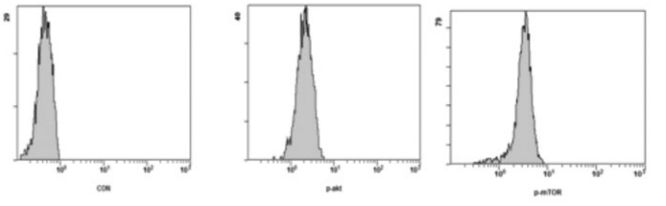
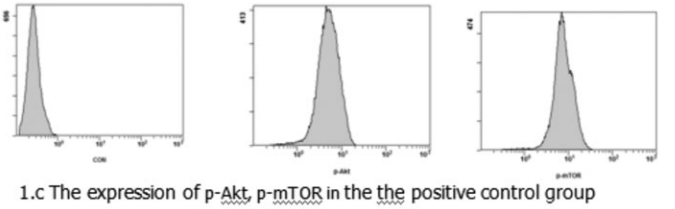
Background: Aplastic anemia (AA), an unusual hematologic disease, is the paradigm of the human bone marrow failure syndromes, The pathophysiology is immune mediated in most cases, with activated cytotoxic T cells implicated.As known to all, there are two signal transduction pathways for T cells' activation, TCR/CD3 as signal 1 heralds antigen recognition whereas cosignal molecules(such as CD28, CTLA-4) as signal 2 refers to the integrated sum of environmental cues. MTOR is an evolutionarily conserved PI3-kinase family member that plays a central role in integrating environmental cues in the form of amino acids, energy, and growth factors. Recently, an increasingly important role for mTOR in directing T cell activation and differentiation has become apparent.
Aims: To explore the roles of the positive and negative factors of mTOR (Mammalian target of rapamytin) signal pathway of the pathogenesis of aplastic anemia (AA).
Methods: (1) To analyze the expression of the positive factors (p-Akt, p-mTOR, p-TSC, p-P70S6K, p-S6, p-4EBP1) and the negative factor (Sirt1) in CD3$^+$ T cells selected by immunomagnetic beads of 21 aplastic anemia patients'(AA group) and 16 healthy persons' (control group) peripheral blood samples by flow cytometry. (2) To compare the difference of Sirt1/p-Akt, Sirt1/p-TSC, Sirt1/p-mTOR, Sirt1/p-4EBP1, Sirt1/p-P70S6K, Sirt1/p-S6 between the AA group and the control group. Analyze the correlation of absolute neutrophil count (ANC) and lymphocyte count (Ly) with the ratios.
Results: 1.The expression of p-Akt, p-TSC, p-mTOR, p-P70S6K, p-S6, p-4EBP1 in CD3+ T cells of the aplastic anemia patients were (8.31±2.28), (41.68±23.72), (14.02±11.51), (14.10±9.46), (20.17±11.75), (137.49±108.58), respectively. The expression of p-Akt, p-TSC, p-mTOR, p-P70S6K, p-S6, p-4EBP1 in CD3+ T cells of the control group were (3.11±0.85), (15.74±9.14), (3.17±1.06), (3.81±1.49), (3.02±0.79), (29.91±17.78), respectively. So, the expression of all the phosphorylated molecules in aplastic anemia patients were obviously higher than the control group, (P<0.05, Figure1, Table 1).2. The expression of the negative factor Sirt1 in CD3+ T cells of aplastic anemia patients was (10.85±6.15) which had no significant with the control group (9.67±2.91), (P=0.52, Table 1).3. The ratios of Sirt1/p-Akt, Sirt1/p-TSC, Sirt1/p-mTOR, Sirt1/p-4EBP1, Sirt1/p-P70S6K in the AA group were much lower than the control group, (P<0.05, Table 2).4. There were positive correlation between ANC and the ratios of Sirt1/p-TSC, Sirt1/p-mTOR, Sirt1/p-4EBP1, Sirt1/p-P70S6K and negative correlation between Ly and the ratios of Sirt1/p-mTOR, Sirt1/p-4EBP1 in AA group, (P<0.05, Table 3).



1.a The expression of p-Akt, p-mTOR in the healthy control group.



1.b The expression of p-Akt, p-mTOR in the AA group



1.c The expression of p-Akt, p-mTOR in the the positive control group

Figure 1.

Tabella 1. The expression of the factors in the three groups (Mean±SD)

	Control n=21	AA n=16	CEM
p-mTOR	3.17 ± 1.06	14.02 ± 11.51[ab]	31.64 ± 17.37[c]
p-Akt	3.11 ± 0.85	8.31 ± 2.28[ab]	19.50 ± 4.72[c]
p-TSC	15.74 ± 9.14	41.68 ± 23.72[ab]	103.9 ± 20.09[c]
p-P70S6K	3.81 ± 1.49	14.10 ± 9.46[ab]	35.36 ± 15.35[c]
p-4EBP1	29.91 ± 17.78	137.49 ± 108.58[ab]	264.74 ± 42.01[c]
p-S6	3.02 ± 0.79	20.17 ± 11.75[ab]	46.78 ± 14.70[c]

Compared with healthy controls, [a]P<0.05, [c]P<0.05,
Compared with healthy controls, [b]P<0.05;

Tabella 2. The rations of Sirt1/mTOR in the control and AA groups (Mean±SD)

	Control n=21	AA n=16	P
Sirt1/p-Akt	3.75 ± 0.98	1.05 ± 0.32	0.000
Sirt1/p-TSC	0.96 ± 0.89	0.24 ± 0.08	0.031
Sirt1/p-mTOR	3.89 ± 1.52	0.75 ± 0.34	0.000
Sirt1/p-4EBP1	0.72 ± 0.18	0.09 ± 0.05	0.010
Sirt1/p-P70S6K	3.14 ± 1.20	0.60 ± 0.19	0.000
Sirt1/p-S6	3.82 ± 1.32	0.67 ± 0.29	0.000

Tabella 3. The correlation of Sirt1/mTOR with ANC and Ly in AA group

	r	P
NE and Sirt1/p-TSC	0.700	0.011
ANC and Sirt1/p-mTOR	0.708	0.010
ANC and Sirt1/p-4EBP1	0.806	0.002
ANC and Sirt1/p-P70S6K	0.667	0.018
Ly and Sirt1/p-mTOR	-0.684	0.014
Ly and Sirt1/p-4EBP1	-0.620	0.032

Summary and Conclusions: (1) The mTOR signal pathway in T cells of aplastic anemia is activated, and the expression of Sirt1 in AA group relatively insufficient. (2) the ratios of Sirt1 with the phosphorylated molecules of mTOR signal pathway showed: the mTOR pathway in AA group migrated than the normal, which resulted in increased lymphocyte percentage and inhibition of hematopoiesis.

P250

THE PREVALENCE OF GATA-2 MUTATION AMONG PEDIATRIC PATIENTS WITH MYELODYSPLASTIC SYNDROME IN CZECH REPUBLIC

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Background: Germline mutation in GATA-2 transcription factor was recently identified in patients with immunodeficiency, familiar and sporadic myelodysplastic syndrome (MDS) and lymphoedema. Germline mutation in GATA-2 represents the most frequent genetic cause of MDS among children. Detailed prevalence of GATA-2 mutation among pediatric patients with MDS or aplastic anemia (AA) was not so far estimated.
Aims: Our aim was to define the prevalence of GATA-2 mutation among children with MDS or aplastic anemia in Czech Republic.
Methods: Since 1998 there were 27 Czech pediatric patients diagnosed with MDS refractory anemia with excess blasts (RAEB) or RAEB in transformation (RAEBt). The final diagnostic algorithm including histopathological investigation for discrimination of MDS subtype refractory cytopenia (RCC) and AA was introduced in 2005. Therefore only RCC/AA patients diagnosed in 2005-2014 were included: 32 RCC patients and 41 AA patients. Fanconi anemia as a cause of bone marrow failure was excluded in all patients. The coding part and the intronic enhancer region of *GATA2* gene was sequenced in all patients with available material, except for 2 patients with RAEB emerging on the background of Fanconi anemia and morbus Recklinghausen (in total 22 patients with RAEB/RAEBt, 31 RCC and 38 AA were analyzed). Immunophenotyping using flow cytometry was performed in bone marrow and peripheral blood. Levels of intronRSS-Kde recombination excision circles (KREC) were measured in peripheral blood and bone marrow with the aim to describe the level of B cell production.
Results: GATA-2 mutation was found in 9 patients with MDS: in 3 RAEB/RAEBt and in 6 RCC patients. As expected there was no AA patient with GATA-2 mutation. Patients with GATA-2 mutation frequently harbored cytogenetic abnormalities including monosomy 7 and trisomy 8. Interestingly one patient did not have any cytogenetic abnormality, however her father died of MDS harboring monosomy 7. In total 7 patients had monosomy 7, 1 patient had trisomy 8 and one patient had simultaneously monosomy 7 and trisomy 8. The prevalence of GATA-2 mutation within patients with monosomy 7 and trisomy 8 was 41% and 29%, respectively. Considering patients with monosomy 7 only, patients with GATA-2 mutation had significantly lower KRECs in peripheral blood and tend to have lower number of B cells in bone marrow. However, at the time of birth there was no major decrease in KRECs in 3 out of 4 analyzed Guthrie cards from GATA-2 mutated patients, indicating postnatal impairment of B cell production. The only one patient with low KRECs at birth developed MDS RAEB at the age of 4 and is the youngest patient in our cohort. Except for this patient, all other GATA-2 mutated patients were older than 11 years at time of diagnosis (median 17y). Two patients with GATA-2 mutation died: one of CMV pneumonitis, another one of AML with a phenotypic switch to BCP ALL.
Summary and Conclusions: So far published cohorts of patients were mainly identified based on clinical symptoms (*i.e.* immunodeficiency, familiar AML/MDS or lymphoedema). The incidence of GATA-2 mutation among pediatric MDS RCC, MDS RAEB/RAEBt and AA patients is 19%, 14% and 0%, respectively. However, the real prevalence could be higher since not all the introns are sequenced. Interestingly mutation in GATA-2 transcription factor can be identified even in patients without any cytogenetic abnormality. Diagnosis of GATA-2 mutation is important for genetic counselling and potential identification of GATA-2 mutation in a family donor before stem cell transplantation. *Supported by GAUK 802214, IGA NT/14534-3, UNCE 204012*

P251

EVALUATION OF BONE MARROW MICROVESSEL DENSITY IN PATIENTS WITH APLASTIC ANEMIA AND ITS ROLE IN PREDICTING OUTCOME TO THERAPY

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Background: Bone marrow (BM) microenvironment plays a crucial role in the growth of hemopoietic cells and BM function, which in turn depends on an intact microvasculature. Our study assesses the microvessel density (MVD) in the BM biopsy of aplastic anemia (AA).
Aims: To examine BM angiogenesis by assessing MVD in patients with aplastic anemia (AA), to compare these values with those obtained in control BM, to correlate the MVD values with the severity of AA and with the response to therapy (Anti Thymocyte Globulin (ATG), cyclosporine (CSA), and androgens).
Methods: Bone marrow biopsy specimens from 60 patients with AA and 17 controls were studied. There were 33 patients with non severe AA (NSAA), 12 patients with severe AA (SAA) and 15 patients with very severe AA (*VS*AA). MVD was calculated on sections stained immunohistochemically for CD34. 35 patients (58.33%) received only androgen (Stanazolol 2 mg/kg/day in three divided doses) due to poor socioeconomic status of patients, 22 patients (36.66%) were treated with androgen and CSA (5 mg/kg/day in two divided doses) while 03 patients (05%) were given ATG (40mg/kg for four days) and CSA.
Results: The patient age ranged from four to 72 years with median age at presentation being 22 years. Majority of the patients were males (40/20); male to female ratio was 2:1. The duration of symptoms ranged from one to sixty months (median =two months). The commonest symptom being weakness/fatigability noted in all cases followed by bleeding manifestations (66.66%) and fever (40%). 53 patients (88.33%) gave transfusion history at presentation with a range of one to 50 transfusions (median=four). Patients with AA exhibited reduced BM cellularity which ranged from 5% to 20%. As assessed by CD34 immunohistochemistry, the mean BM MVD in AA group was 1.28±0.36, being significantly lower than that in control group (6.80±1.59, P<0.001). MVD of SAA and NSAA patients were 1.16±0.35 and 1.49±0.27, respectively, being significantly different (P=0.003). MVD of *VS*AA was 0.93±0.25 and the difference with NSAA is significant, however there was no significant difference between SAA and *VS*AA. Response was assessed in those patients (n=31) who had a minimum follow up of six months. Of those cases, four had partial response (PR) while four patients had complete response (CR) at the end of six months follow up. It was found that MVD was higher among responders (mean MVD 1.96±0.16) as against non responders (mean MVD 1.26±0.25). The difference in mean MVD between these two groups was statistically significant with a p value of (P<0.001).
Summary and Conclusions: Our study is unique in the sense it is one of the largest to study the BM MVD in 60 cases of AA. In addition we compared the mean MVD count of those cases that had shown either PR or CR with mean MVD count of non responders. Our study shows that AA is associated with reduced BM angiogenesis. Upon quantification of angiogenesis, it is seen that the BM MVD reduces with the severity of the disease. Also the patients responded to therapy had significantly higher MVD than the non responders at presentation and hence may predict the behavior of the disease. This particular

finding was not reported in the earlier studies. This observation advances our knowledge about the role of microenvironment in the pathogenesis of AA and this critical finding may have implications for new concepts relating to therapy and outcome of this disease. It may guide future research towards looking at the option of using pro-angiogenic drugs in treatment of AA. The promotion of MVD in bone marrow and the improvement of microenvironment may induce hemopoiesis and create a new way to cure aplastic anemia.

P252

IMMUNOPHENOTYPIC PROFILING OF ERYTHROID PROGENITOR-DERIVED EXTRACELLULAR VESICLES IN DIAMOND-BLACKFAN ANAEMIA: A NEW DIAGNOSTIC STRATEGY

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Background: Diamond-Blackfan Anaemia (DBA) is a rare inherited pure red cell aplasia. Patients with DBA exhibit a macrocytic normochromic anaemia and reticulocytopenia. Erythroid progenitors (BFU-E and CFU-E) in bone marrow (BM) show a proapoptotic phenotype and their number is reduced. DBA is considered as the prototype of ribosomopathies. Heterozygous mutations in one of 13 ribosomal protein (RP) genes have been found in about 65% of patients. Diagnosis of DBA is hampered by the overlapping clinical presentation with other BM failure syndromes such as Fanconi Anaemia (FA), Shwachman-Diamond syndrome (SDS), Dyskeratosis Congenita (DC) and Transient Erythroblastopenia of Childhood (TEC). The absence of unique diagnostic features for the disease makes DBA a diagnosis of exclusion, finally confirmed by mutation analysis.
Aims: As an alternative strategy for developing a more inclusive assay for possible use in DBA diagnosis we turned to the study of extracellular vesicles (E*Vs*) whose presence may be altered as a consequence of the loss of erythroid progenitor cells in the marrow of these patients.
Methods: E*Vs* have been isolated from plasma of patients with DBA and appropriate controls by differential centrifugations and analysed by flow cytometry. Three markers have been used to characterise E*Vs* derived from cells of the erythroid lineage: CD34, CD71 and CD235a. EV immunophenotypic profiles of 8 patients with DBA, 22 healthy controls and 10 patients with other congenital or acquired anaemias (non-DBA) have been performed.
Results: Among the three EV clusters we found, only the CD34+/$CD71_{low}$ population showed a statistically significant difference between DBA patients and controls ($p<0.05$). This population, representing late erythroid progenitors, is substantially less represented in DBA patients as compared to controls. This finding is in agreement with the low level of erythroid progenitors in the patients' BM. The assessment of Receiver Operating Characteristic (ROC) curves demonstrated the potential use of the CD34+/$CD71_{low}$ population as a diagnostic tool. Area under the ROC curve (AUC) values suggest that this test has an excellent accuracy to discriminate DBA patients from healthy controls, from non-DBA patients and also from all other individuals under study.
Summary and Conclusions: These data overall suggest that the EV assay we devised may be useful to improve DBA diagnosis as a quicker and less invasive alternative to erythroid cultures. It should be noted that this assay is performed from peripheral blood, is amenable to transfused patients and requires two working days, whereas erythroid progenitors growth requires 15 working days and needs to be performed using a bone marrow sample in DBA patients.

P253

ALTERATION OF HEME METABOLISM IN A CELLULAR MODEL OF DIAMOND-BLACKFAN ANEMIA

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Background: Diamond-Blackfan anemia (DBA) is a congenital pure red cell aplasia often associated with skeletal malformations. Mutations in ribosomal protein coding genes, mainly in RPS19, account for the majority of DBA cases. The molecular mechanisms underlying DBA pathogenesis are still not completely understood. Alternative spliced isoforms of FLVCR1 (Feline Leukemia Virus subgroup C Receptor 1) transcript coding for non-functional proteins have been reported in some DBA patients. Consistently, a phenotype very close to DBA has been described in animal models of FLVCR1 deficiency. FLVCR1 gene codes for two proteins: the plasma membrane heme exporter FLVCR1a and the mitochondrial heme exporter FLVCR1b. Loss of FLVCR1a causes heme accumulation in cytosol while the lack of FLVCR1b leads to mitochondrial heme overload. The coordinated expression of both FLVCR1 isoforms regulates an intracellular heme pool, necessary for proper expansion and differentiation of erythroid precursors.
Aims: The aim of the work is the investigation of the role of FLVCR1 isoforms in a cellular model of DBA.
Methods: TF1 cells expressing two different inducible shRNAs against RPS19 were compared to that expressing an inducible shRNA against a scramble sequence. Cells were examined after 4 days of doxycycline treatment which causes the downregulation of RPS19. FLVCR1 isoforms levels and heme content were evaluated. K562 cells stably expressing a shRNA against FLVCR1a or both FLVCR1a/1b isoforms were compared to that expressing a shRNA against a scramble sequence. To stimulate erythroid differentiation, K562 cells were stimulated with 0.5 M sodium butyrate for 72 hours. Heme content, in vitro erythroid differentiation, cell proliferation, cell cycle, apoptosis and oxidative stress were analyzed.
Results: RPS19-downregulated TF1 cells show a reduction of both *FLVCR1a* and *FLVCR1b* mRNA levels. Consistently, RPS19-downregulated TF1 cells accumulated heme in both cytosol and mitochondria. It has already been reported that RPS19-downregulated TF1 cells showed reduced proliferation, a G0/G1 arrest and increased apoptosis. To investigate whether the loss of FLVCR1 isoforms could affect these processes, we took advantage of K562 cells with a specific downregulation of FLVCR1a or both FLVCR1a/1b. FLVCR1a-downregulated K562 cells showed cytosolic heme overload, reduced proliferation and increased *in vitro* differentiation whereas loss of both FLVCR1a and FLVCR1b causes mitochondrial heme overload, reduced proliferation and decreased *in vitro* differentiation. Both FLVCR1a-and FLVCR1a/1b-downregulated K562 cells showed cell cycle arrest at G0/G1 and increased apoptosis, following the stimulation of *in vitro* erythroid differentiation.
Summary and Conclusions: Taken together, these data indicate that the downregulation of FLVCR1 isoforms in K562 cells leads to a phenotype similar to DBA. Thus, we proposed that alteration of heme metabolism could contribute to defective proliferation and apoptosis of DBA erythroid progenitors. Further work is needed to completely define the role of FLVCR1 isoforms in the pathogenesis of DBA.

P254

AUTOIMMUNE NEUTROPENIA OF INFANCY: DATA FROM THE ITALIAN NEUTROPENIA REGISTRY

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Background: Neutropenia is a disorder characterized by a reduction of absolute neutrophil count (ANC) below the lower limit, which is $1.0x10^9$/L in Caucasian children up to age 1y, whereas from >1 year to adulthood it is 1.5 $x10^9$/L. Neutropenia is defined as mild if ANC is between 1.0 and $1.5x10^9$/L, moderate if between 0.5 and $1.0x10^9$/L, and severe if $<0.5x10^9$/L. Autoimmune neutropenia of infancy (AIN) is a disorder characterized by mild bacterial infections and a tendency to resolve spontaneously.
Aims: To provide clinical, biochemical and hematological insights through the analysis of a reasonably sized cohort of patients.
Methods: a retrospective analysis of all patients diagnosed with AIN, enrolled between 1.01.2002 and 1.06.2014 in the Italian Neutropenia Registry of AIEOP (Associazione Italiana Onco-Ematologia Pediatrica). The Indirect Granulocyte Immunofluorescence Test (GIFT) was used to detect circulating auto-antibodies reacting with purified granulocytes from donors who were not genotyped for Human Neutrophil Antigens. Italian Guidelines recommend repeating GIFT at

least 4 times if negative at first; after 4 negative tests neutropenia is defined as "idiopathic".
Results: We analyzed 157 AIN patients. The sensitivity of GIFT after 1, 2, 3, and 4 assays was 61.8%, 73.1%, 78.7%, and 81.8% respectively. The characteristics of the patients are shown in Table I. The median age at onset was 0.70y (range 0.005–4.59): 82% of the children were under 18 months of age at presentation. Three patients (1.9%) were under one month of age at onset, and 13.2% of all patients born preterm. Median ANC at onset were 0.45 1x10^9/L, with the following distribution: 56.0% ≤0.500x10^9/L, 38.2% between 0.501 and 1.0x10^9/L, and 5.7% >1.0x10^9/L. Diagnosis of AIN was made by chance in 29.3%, and in 70.7% on the basis of a suspected immunodeficiency or during a hospitalization due to fever or infections. 89% of the cohort recovered spontaneously and the median age of resolution was 2.14y, with a median disease duration of 1.30y: 85% of the children recovered at less than 5y, and 13 patients recovered spontaneously at 5-11y of age. Among all the analyzed parameters in the Cox model only age of appearance (p=0.00029) and absence of monocytosis (p=0.015) were statistically associated with higher recovery percentage. Lower age at presentation was also associated with reduced duration of disease (p=0.028). Recovery was abrupt in 67.4% of patients; in the remaining 32.5% there was an intermittent neutropenia, with a median time of definitive healing from the first normal blood count of 0.65 year. Bone marrow was examined in 53 children and was normal in all but 2 patients who showed a moderate decrease of myeloid cellularity. A transient positivity of direct antiglobulin test (DAT) and a selected IgA deficiency were present in 6.8% and 3% respectively: both associations did not impact the probability of spontaneous recovery. 44% of children were hospitalized for fever or infectious, but only 15 patients (9.6%) suffered from severe infections (sepsis, pneumonia, skin/*soft* tissue *abscesses, or meningitis/encephalitis),* without long-term consequences. G-CSF was administered "on demand" only in severe infections in 11 patients.

Tabella 1. Clinical characteristics of AIN cohort

	Patients (157)
Male	64.3%
Median age at onset (years)	0.70
Median age at diagnosis (years)	1.06
Median age at resolution	2.14
Median duration (years)	1.30
Recovery	89.1%
Median WBC at onset (x10^9/L)	6.1
Median ANC at onset (x10^9/L)	0.45
Leukopenia at onset	40.7%
Monocytosis at onset	19.3%
Increased IgG at onset	6.0%
Selective IgA deficiency	3%
Severe infections	9.6%
DAT positivity	6.8%
BM examination/normal appearance	34.4%/96.2%

Summary and Conclusions: This is the second largest cohort of AIN patients ever reported. Our analysis confirms that GIFT assay has a reasonable sensitivity on repeated testings and shows the following new findings that may affect clinical management of the disease: (i) AIN is frequently found in children born preterm; (ii) lower age at presentation is associated with reduced duration of the disease. (iii) AIN can be associated with other immunological abnormalities like transient positivity of DAT and low IgA serum level; (iii) AIN patients are often hospitalized for infections but severe infections occur in less than 10% of patients; (iv) recovery from AIN may be after age of 5 years and in one third of patients ANC normalizes alternating normal to neutropenic values over a timespan >6 months.

P255

PRELIMINARY DATA ON THE OUTCOME OF BONE MARROW TRANSPLANTATION IN CONGENITAL DISKERATOSIS. AN ANALYSIS FROM THE EUROPEAN GROUP FOR BONE MARROW TRANSPLANTATION (EBMT)

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Background: Dyskeratosis congenita (DC) is an inherited telomeropathy characterized by mucocutaneous features (oral leukoplakia, nail dystrophy, abnormal skin pigmentation), bone marrow failure and organs' compromision (mainlylung and gastrointestinal tract). Haematopoietic Stem cell Transplantation (HSCT) is the only definitive treatment to restore hematopoiesis, but increased organ toxicity has been reported after transplant. To the best of our knowledge, extensive reports on its outcome are lacking.
Aims: To collect data on the outcome of HSCT in DC by analyzing data from the European Society for Blood an Bone Marrow Transplantation (EBMT)
Methods: All patients registered in the EBMT data base and affected with DC were considered eligible to the study. Data about type of HSCT, characteristic of donors, source of cells, incidence of acute and chronic GvHD were collected.
Results: A total of 84 patients affected with DC who underwent HSCT from 1979 to 2013, entered the study. Females were 23% of the cohort. Median age at diagnosis of DC was 5.7 years (0-33y), while median age at HSCT was 12.3 years (range 0.56-41y). Median time from diagnosis to HSCT was 22 months (0.62-278 mo). The cell source was bone marrow (BM) in 60%, peripheral blood (PB) in 26% and cord blood (CB) 14%. Thirthy-three percent of patients were engrafted from a matched related and 50% from an matched unrelated donor. Fourteen percent were mismatched related and unrelated HSCT. Engraftment was documented in 88 % of subjects: 6.5 % had primary graft failure and 4% lost the engraftment. Overall 25 patients (33 %) died, and 59 were alive (67%). Causes of death were: rejection graft/ loss of graft 28%, infections 24%, non-infectious interstitial pneumonia 16%, multi-organ failure 12%, secondary malignancies 12%, GvDH in 8%. Lung injury was present in more than one third of the subjects who died. Transplant related mortality of the whole cohort was 18% . Acute GVDH occurred in 67% of the cohort, mainly of grade I and II (31%) and grade III in 6% of the cohort. No grade IV aGvHD has been documented. The 5-year OS and EFS (death, relapse, second tumours, primary and secondary graft failure being the events) were 63 % and 51% respectively. Of note EFS curve did not show a plateau. Analyzing data during the first 12 months of follow up, OS was 87% for patients aged 0-12 years and 75% for subjects who underwent transplant at age >12 years (p=0.23). The time between the diagnosis and transplant (>24 months and <24 months) did not significantly impact on the 5 years-OS which was assessed respectevely at 72% and 53% (p=0.14). Again, looking at the first 12 months of follow up, OS and EFS according to source of cells seemed not significantly differ, even if a precocious fall in the curve was observed for CB and PB rather than for BM.
Summary and Conclusions: HSCT may be an option in DC patients, but the never reached plateau for the EFS curves points out the high risk morbility of this disease. Lung involvement represents the main cause of death for both GvHD and infections. Lung status before HSCT, irrespective to bone marrow failure, has to be weighted regarding the choice of HSCT. Future data comparing outcome of DSK transplanted and non transplanted patients will be useful to definitely understand if any patients' risk stratification regarding HSCT can be applied.

P256

SOLUBLE CD163 AS A MARKER FOR MACROPHAGE ACTIVATION IN CHILDREN AND ADOLESCENTS WITH GAUCHER DISEASE: RELATION TO PULMONARY AND BONE INVOLVEMENT

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Background: Bone and lung involvement are two major causes of morbidity in Gaucher disease (GD). The soluble form of CD163 (sCD163) is a valuable diagnostic biomarker for monitoring diseases with increased macrophage activation.
Aims: We determined sCD163 levels in 30 children and adolescence with GD compared with 30 healthy controls and assessed the relation to phenotypes, disease severity and complications.
Methods: Thirty GD patients (10 had type 1 and 20 had type 3) were recruited from the regular attendants of the Pediatric Hematology Clinic, Pediatric Hospital, Ain Shams University. All patients were under regular enzyme replacement therapy (ERT) using recombinant glucocerebrosidase. Patients were studied stressing on skeletal, pulmonary or neurological manifestations and history of splenectomy. All studied patients were clinically asymptomatic for pulmonary hypertension. Screening for pulmonary hypertension was done by the non-invasive Doppler echocardiograpghy. Measurement of bone mineral

density (BMD) was performed by dual energy X-ray absorptiometry (DXA). Hematological profile, plasma chitotriosidase activity, D-dimer and sCD163 levels were assessed.
Results: The mean Z score for GD patients with normalBMD was-0.7±0.1. Patients with osteopenia had a mean Z score of-1.9±0.9 while the mean Z score for those with osteoporosis was-3.4±1.1. Patients with GD had significantly higher sCD163 than healthy controls (median [IQR], 7750 [4000] ng/mL *versus* 980 [460] ng/mL; p<0.001). Comparison between patients with type 1 and type 3 GD revealed that the incidence of squint, dysphagia, developmental delay and pulmonary hypertension risk was higher among type 3 GD patients (p<0.05). D-dimer, chitotriosidase activity and sCD163 levels were significantly increased in type 3 GD patients compared with type 1 (p<0.05). Severity score index (SSI) was higher among type 3 GD than type 1 (p<0.001). Other variables including DXA findings were similarly distributed among both GD groups (p>0.05). sCD163 was significantly elevated in GD patients with dysphagia (p=0.026), developmental delay (p=0.033), pulmonary hypertension risk (p<0.001) or abnormal BMD (p=0.031) than those without. GD patients receiving ERT every 2 weeks had lower levels than those under ERT more than 2 weeks (p<0.001). sCD163 was positively correlated to age, disease duration, SSI, WBC count, D-dimer and chitotriosidase activity. Multiple regression analysis showed that TRV, D-dimer and chitotriosidase activity were independently related to sCD163 in GD patients (r^2=0.768; p<0.001). The cutoff value of sCD163 at 9400 ng/mL could differentiate GD patients with and without pulmonary hypertension risk with a sensitivity of 90% and specificity of 95% %, area under the curve 0.975 and 95% confidence interval 0.681-1.0; p<0.001.
Summary and Conclusions: sCD163 is a biomarker for clinical assessment of macrophage proliferation and activity that would help in risk prediction of bone and lung involvement. Assessment of sCD163 levels may provide a potentially valuable tool for monitoring the response to ERT in GD patients.

P257

THE PROGNOSIS OF ACQUIRED HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS IN ADULTS IS HEAVILY AFFECTED BY A COEXISTING VIRAL INFECTION: ANALYSIS OF A SINGLE INSTITUTION SERIES OF 35 PATIENTS

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Background: Hemophagocytic lymphohistiocytosis (HLH) is a often fatal hyperinflammatory syndrome, mainly occurring on a genetic basis in the paediatric population. In adult patients (pts) acquired HLH can develop as a complication of many disorders, which may lead to similar deregulated immune response of the paediatric form. Early diagnosis is essential in order to avoid a fatal outcome. HLH-04 criteria have been validated in the paediatric setting, but are often difficult to apply to the adult population, since they include genetic and immunological parameters difficult to obtain in due time. Indeed, a readily applicable diagnostic score (HScore, Fardet 2014) has been recently proposed in order to facilitate the clinical diagnosis of acquired HLH.
Aims: To confirm the diagnosis of acquired HLH made in a single institution series of adult pts with HLH-04 criteria, by applying the HScore and to identify prognostic factors associated with clinical outcome.
Methods: Hscore diagnostic criteria were applied to a series of 35 pts with HLH diagnosed according to HLH-04 criteria during a 11-year period. The following variables of potential prognostic significance were considered: sex, age, underlying haematological disease, severity of anemia (<9g/dl), neutropenia (<1000/mcl) and thrombocytopenia (<100000/mcl), hypertrigliceridemia (>265 mg/dl), hypofibrinogenemia (<150 mg/dl), ferritin >10000 ng/ml, bilirubin >2 mg/dl, LDH>2N, morphological signs of HLH, concomitant viral infection, response to treatment.
Results: Median age of the 35 pts was 54 (range 17-81), M/F ratio was 20/15. In 26/35 (74.3%) pts an underlying haematological disease was present (2 Multicentric Castleman Disease, 10 B-cell Non Hodgkin Lymphoma [NHL], 14 T/NK-cell NHL); an autoimmune disorder was observed in 4 (11.4%) patients (1 Still Disease, 1 undifferentiated connective tissue disease, 2 haemolytic anemia); in 5 (14.3%) no underlying disease was identified. A concomitant infection by EBV was observed in 10 pts, CMV in 8, HHV8 in 6 and HIV in 1. Hyperferritinemia, fever and splenomegaly were present in more than 90% of pts, whereas bone marrow hemophagocytosis in 51% of cases only. According to HScore, 34/35 patients had a >75% and 32/35 >93% probability of HLH. Two pts did not receive any treatment; the remaining 33 pts were treated with different schemes, including steroids (30), i.v. Ig (20), antiviral agents (15), rituximab (9), chemotherapy (14), HLH-04 protocol (3), cyclosporine (2). Ten of the 29 evaluable pts responded. After a median follow-up of 649 days, 8/35 pts are alive. Four-year overall survival (OS) and HLH-free survival (HFS) were 17±7.9%SE and 23±8.2%SE, respectively. By univariate analysis, age >50y, treatment failure and a concomitant viral infection were predictive of fatal outcome. Notably, median OS of pts with and without a concomitant viral infection

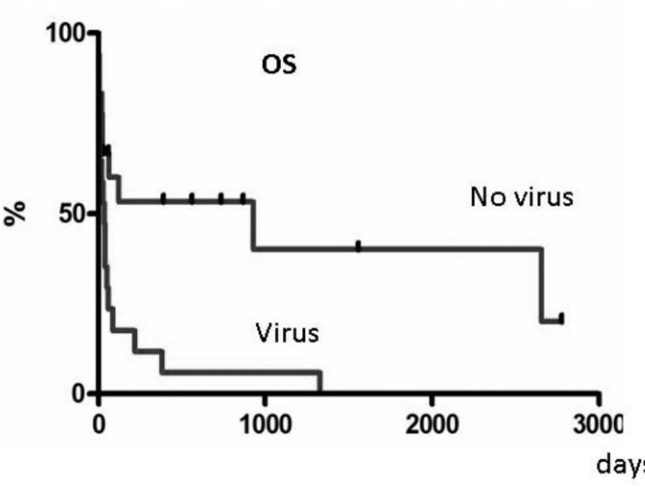

Figure 1.

Summary and Conclusions: Acquired HLH is not rare in adults and may be a dangerous but not uniformly fatal syndrome. HScore could be easily applied and showed high concordance with HLH-04 criteria for its diagnosis. An underlying haematological diseases, particularly T/NK-cell, but also B-cell NHL was the most frequent cause of acquired HLH. However a diagnosis of lymphoma did not influence HLH outcome, whereas, in addition to older age and lack of response to treatment, the presence of a concomitant viral infection, particularly herpetic, was a dismal prognostic factor and predicted a rapid fatal outcome. A better control of coexisting viral infections is warranted to improve the outcome of acquired HLH.

Multiple myeloma - Biology 1

P258

A COMPREHENSIVE GENOME-WIDE COPY NUMBER ALTERATIONS (CNAS) ANALYSIS OF HOMOGENEOUSLY TREATED, NEWLY DIAGNOSED MULTIPLE MYELOMA (MM) PATIENTS

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Background: Array-based technology has been showing a great impact on clinical cancer cytogenetic, especially on genetically heterogeneous disease, such as Multiple Myeloma (MM), where relevant lesions might be the hallmarks of different patients' subgroups, thus becoming of clinical relevance as well. The phase III EMN02 study was designed to compare Bortezomib, Melphalan, Prednisone with high-dose Melphalan and autologous stem cell transplantation after a bortezomib-based induction, for newly diagnosed MM patients.
Aims: Here we present the comprehensive, high throughput genomic profile performed at diagnosis in a subset of patients, in order to perform correlations with response to induction therapy.
Methods: Data obtained from 170 patients, treated with bortezomib-cyclophosphamide-dexamethasone (VCD) as induction therapy prior to randomization, have been analyzed. Highly purified CD138+ bone marrow plasma cells were profiled by SNPs array (Affymetrix 6.0 and CytoScanHD® chip). ChAS (Affymetrix) and Nexus Copy Number™ 7.5 (Biodiscovery) software were used to perform CNAs analyses and clinical correlations, respectively.
Results: Presenting MM cases were studied by SNPs array in order to compute CNAs and acquired loss of heterozygosity (LOH) in the tumor. The frequency distribution of the more relevant CNAs among the 170 MM cases is summarized in table 1. A subgroup of 13/170 (7.6%) cases is characterized by the absence of any macro CNAs (either gains or losses): these cases are mainly characterized by LOH events on chr. 1, 8 and 16, where putative tumor suppressor genes are located (*e.g. PLEKOH1* and *SIAH1* on chr.1 and 16, respectively). In order to identify novel chromosomal lesions impacting the response to therapy, we compared the CNAs profile of the extreme response categories, *i.e.* ³CR and SD. Either the absence of CNAs, or the presence of any of the renown prognostically relevant ones does not significantly impact on the response to induction therapy. On the contrary, two novel lesions resulted highly significant: (1) a 42.9Kb CN gain on chr.11q22.1-22.2, which only includes the Hippo pathway mediator *YAP1*, which significantly characterizes 6% of ³CR patients, as compared to 54% of SD ones (p=0.002); (2) an extended CN loss on chr.14q13.1-13.3, including genes implicated in the progression on cancer (*e.g. NKX2-8*), which significantly characterizes 62.5% of £CR patients, as compared to 4% of SD ones (p<0.001).

Tabella 1.

CNA	locus	gene (Kb)	positive	full gene	partial	LOH	response prediction
TP53 CN loss	17p13.1	25	15/170 (8.8%)	13/170	2/170	4/170	no
Rb1 CN loss	13q14.2	232	85/170 (50%)	74/170	11/17'	-	no
CKS1B CN gain	1q32.1	6	52/170 (30.5%)	52/170	-	12/170	no
MDM4 CN gain	1q32	144	57/170 (33.5%)	55/170	2/170	-	no
CDKN2C CN loss	1p32.3	7.7	20/170 (11.7%)	19/170	1/170	29/170	no
FAF1 CN loss	1p33	675	24/170 (14.1%)	16/170	8/170	30/170	no
FAM46C CN loss	1p12	29	37/170 (21.7%)	37/170	-	5/170	no
WWOX CN loss	16q23	1400	35/170 (20.6%)	26/170	9/170	12/170	no
CCND1 CNA	11q13	17	79/170 (46.5%)	-	-	1/170	no
CCND3 CNA	6p21	148	27/170 (15.9%)	-	-	3/170	no
MAFB CNA	20q11.2	4.4	23/170 (13.5%)	-	-	6/170	no
no CNAs	-	-	13/170 (7.6%)	-	-	+	no
YAP1 CN gain	11q22.1	160	71/170 (42%)	63/170	8/170	-	yes
CN loss	14q13.1	-	23/170 (13.5%)	-	-	3/170	yes

Summary and Conclusions: The reconstruction of high-throughput virtual karyiotype by SNPs array in a cohort of homogeneously treated, newly diagnosed MM cases offered the opportunity to obtain a comprehensive overlook of each patient's sub-chromosomal anatomy. This allowed both to perform a detailed patients' stratification at diagnosis and to identify, among the whole spectrum of CNAs, those having an impact on the response to therapy.

P259

RELATIONSHIP BETWEEN INHIBITION PROFILE OF B1, B2 AND B5 PROTEASOME SUBUNITS AND CYTOTOXIC ACTIVITY OF PROTEASOME INHIBITORS IN MULTIPLE MYELOMA

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Background: The use of proteasome inhibitors (PI) with the first-in class drug Bortezomib (Btz) has improved the outcome of multiple myeloma (MM) patients. However, Btz resistance frequently emerges in patients with advanced disease. The second generation PI Carfilzomib (Cfz) and additional PI (including ixazomib, delanzomib, oprozomib, ONX-0914 (PR-957)) are in advanced clinical development. All these PI by design target the β_5 subunit of the proteasome, the rate-limiting protease, except for ONX-0914, which targets the β_{5i} immunoproteasome subunit. The proteasome in addition contains β_1 and β_2 protease subunits that differ from β_5 in their substrate specificity. The degree of cytotoxicity of PI varies with the degree of co-inhibition of proteasomal subunits $\beta_{1/1i}$ and/or $\beta_{2/2i}$, in addition to $\beta_{5/5i}$. Btz-refractory MM cells upregulate activity of β_2, the subunit not targeted by Btz. The "optimum" pattern of proteasome inhibition to reach maximum cytotoxicity in MM, either Btz-sensitive or Btz-resistant is unknown, as well as the individual patterns of proteasome subunit inhibition(*i.e.* co-inhibition of β_1 and/or β_2) of the non-approved PI in MM.
Aims: (I) To assess the impact of co-inhibition of proteasome subunits activity (β_{1i}, $\beta_{1/1i}$, $\beta_{2/2i}$, β_{5i}, $\beta_{5/5i}$) on cytotoxicity in Btz-sensitive and Btz-resistant MM cells; (II) to analyse the subunit inhibition pattern of PI in clinical development in relation to their cytotoxic activity.
Methods: We developed a set of subunit-selective proteasome inhibitors as well as fluorophore-labelled activity-based probes (ABP) capable to visualize the individual activities of the β_1, β_2, β_5-subunits of the constitutive and immunoproteasome in intact cells. Equimolar concentrations of PI were used to assess the inhibition profiles of the different drug candidates. Btz-resistant MM cell lines were generated by continuous drug exposure from AMO-1 cells (AMOaBtz,). Viability was estimated by MTS and plotted against the inhibition of the individual β-subunits (alone or with various combinations of proteasome inhibitors). In addition, recently synthetized inhibitors with dual specificity for $\beta_{2/2i}/\beta_{5/5i}$ (Xin550, Xin551) were used to improve the efficacy of proteasome inhibition in PI resistant cells.
Results: Table 1: Cytotoxic effect of selective inhibition of individual subunits of the proteasome (β_1, β_2, β_5) or immunoproteasome (β_{1i}, β_{5i}) alone or in combination, in intact MM cells, either sensitive or resistant to Btz (wt/aBtz); no (-) low (+), intermediate (++), high (+++) effect.

Tabella 1.

AMO (wt /aBtz)	O	ß1i	ß5i	ß1/1i	ß2/2i	ß5+5i
O	- / -	- / -	- / -	- / -	+ / -	+++ / -
ß1i			- / -	- / -	+ / -	+++ / -
ß5i				- / -	++ / -	+++ / -
ß1/1i					++ / -	+++ / -
ß2/2i						+++ / +++
ß2/2i + ß1/1i + ß5i	+++/+					
ß2/2i + ß1/1i + ß5/5i	+++ / +++					

Summary and Conclusions: ABP is a reliable tool for analysis of proteasome subunit activity in intact cells. Viability of Btz-sensitive MM cells can be maximally reduced by selective inhibition of β_5-type activity, so that additional β_1 and/or β_2-targeted proteasome inhibition does not increase cytotoxicity. In contrast, co-inhibition of $\beta_{2/2i}$ in addition to β_5 is required to achieve meaningful cytotoxicity in Btz-resistant MM cells. Inhibition of $\beta_{1/1i}$, either with β_5 or $\beta_{2/2i}$, has no additional cytotoxic effect (Table 1). Further, Xin550/ Xin551, inhibitors of β_2/β_5 are able to overcome resistance to Btz *in vitro*. Novel PI drug candidates showed considerably less co-inhibition of either β_1 or β_2 subunits, compared to Cfz or Btz, which correlated with a lower cytotoxic activity, in particular against Btz-resistant MM cells.

P260

LSD1 IMPAIRS OSTEOCLASTOGENESIS AND EPITHELIAL-MESENCHYMAL TRANSITION IN MM AND ENHANCES HDAC INHIBITORS ACTIVITY

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Background: Lysine-specific demethylase 1 (LSD1), a FAD-dependent histone demethylase which selectively removes mono-and di-methyl groups from H3K4, H3K9 residues leading to either repression or activation of transcriptome. LSD1 forms a co-repression complex with HDAC1, HDAC2, mSin3a and MMSET, while the IMIDs cause cell cycle arrest in Multiple Myeloma (MM) by modifying the chromatin structure of the p21WAF-1 promoter through LSD1 demethylation.
Aims: Here, we study the functional role of LSD1 in MM pathogenesis and its contribution in aggressive traits of myeloma disease.
Methods: The expression level of LSD1 transcript and its prognostic role was evaluated in publically available datasets of MM patients (GSE2113, GSE16122). We utilized MM.1S, OPM2, LP1 and RPMI-8226 human myeloma cell lines (HMCLs). Osteoclastogenesis was evaluated by using double TRAP/ALP staining and by estimating the mRNA expression levels of osteoblast markers (APL, BSP, OC). Cell viability and proliferation was evaluated by CellTiter Glo and 3[H]Thymidine uptake assays. Immunoblot analysis was performed using antibodies against LSD1, HDAC1,2, H3K4me2/3, H3K9me2/3. We evaluated the expression of LSD1 in bone marrow paraffin embedded specimen from 10 newly diagnosed MM patients. The epithelial-mesenchymal transition (EMT) was evaluated by using the transwell migration assay, invasion and wound healing assays.
Results: First, we observed significant overexpression of LSD1 in patients with symptomatic MM and Plasma Cell Leukemia (PCL) (p<.001). The expression of LSD1 in a panel 45 HMCLs was also pronounced. We confirmed the expression and both its nuclear and cytoplasmic localization in HMCLs and primary bone marrow plasma cells from newly diagnosed, relapsed MM and PCL patients (N=10). LSD1 was highly expressed in bone marrow specimen of MM patients and the higher expression was positively correlated with presence of bone fractures at diagnosis. LSD1 knockdown in LP1 and MM1S cells resulted in modest cytotoxicity. After a combination silencing of JARID1 members and LSD1 we were able to observe a further significant decrease in survival of MM cells lacking JARID1C and LSD1, indicating that the overlapping demethylation of H3K4 is of high importance for the cell survival. We examined the post-translational histone modifications after LSD1 knockdown and as expected, we observed significant increase of K4me2/3 and K9me2 marks, but more interestingly, alteration of acetylation status of K9. Moreover, we observed that LSD1 depletion enhances significantly the cytotoxicity effect of LBH589. LSD1 depletion resulted in significant reduction of mRNA levels by using real-time PCR and protein expression by immunoblotting of HDAC1 and HDAC2. Furthermore, we sought to investigate the impact of LSD1 in EMT. LSD1 depletion in MM1S and LP1 cells inhibited significantly the migratory potency. More importantly, MM cells lacking LSD1 expressed significant lower levels of E-cadherin, N-cadherin and Vimentin evaluated by immunoblotting and immunocytochemistry. We confirmed the suppression of EMT-involved gene expression by performing a PCR-microarray assay. Finally, given the presence of osteolytic lesions as a hallmark of disease, and consequent impact on outcome, we evaluated the impact of LSD1 on osteoblast differentiation and osteoclastogenesis. LSD1 depletion and pharmacological inhibition (S2101) resulted in significant inhibition of osteoclastogenesis and RANKL-induced resorption and survival of OCs. In contrast, LSD1 overexpression confirmed the upregulation of Wnt/b-catenin pathway suggesting a possible underlying mechanism for the osteoclastogenesis potency in MM patients with high expression of LSD1.
Summary and Conclusions: Our data suggest a promising epigenetic approach in myeloma therapeutics by targeting the deregulated LSD1-methylome in MM patients.

P261

S1P MODULATOR FTY720 TARGETS OSTEOCLASTOGENESIS IN CXCR4-BASED MULTIPLE MYELOMA SYSTEMIC XENOGRAFT MODEL

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Background: Bone disease is one of the hallmarks of multiple myeloma (MM) leading to substantial morbidity and disability. Bone lesions result from abnormally increased osteoclast (OC) formation and activation. Studying the underlying mechanism may help to develop new therapeutic targets to treat MM associated osteolytic lesions and related complications.
Aims: Sphingosine-1-phosphate (S1P) was shown to play important role in osteoclast biology. Previously we reported that S1P modulator FTY720 exhibits potent anti-myeloma effect *in vitro* and *in vivo* in novel disseminated xenograft model of MM. We report now the effect of FTY720 on formation and activation of OCs and their functional sequel in MM model.
Methods: The *in vitro* effect of FTY720 was tested onOCsandOCprecursors from healthy donors, and on bone marrowmesenchymalstromal cells (BMSCs). *in vivo* model of BM-disseminated humanmyelomawas used to evaluate FTY720 anti-MM andboneactivities.
Results: The generated OCs expressed the genes encoding for S1P1 and S1P2 receptors and enzyme SPHK1, tested by RT-PCR. Treatment with FTY720 significantly reduced *in vitro* formation of TRAP+ OCs, resulting in 90% inhibition following 1 μM treatment, and complete abolishment of OC formation at 2.5 μM FTY720 (p<0.0001). Furthermore, FTY720 significantly reduced the expression of genes associated with OC activation. Thus, expression of osteoactivin, cathepsin K, NFATc1, OSCAR, RANK, RANTES, MT1-MMP and MMP9 genes were significantly down-regulated in FTY720-treated OC cultures (p<0.001). Mechanistically, FTY720 abrogated RANKL-induced Erk1/2 phosphorylation in OC progenitors. In addition, FTY720 targeted the microenvironment components-MM cells and BMSCs, suppressing the expression of osteoclastogenic factors. mRNA levels of MIP1a, RANTES, MCP-1 and RANKL were significantly reduced in both MM cells (RPMI8226, CAG, OPM-2) and BMSCs upon FTY720 treatment (p<0.001). Moreover, FTY720 altered the ability of myeloma and stroma cells to promote OC formation, and concomitantly FTY720 overcame OC-mediated support and drug resistance of MM cells. Thus, FTY720 was able to disrupt the deleterious cross-talk between the MM tumor cells and the OCs. Next, we evaluated the effect of FTY720 on OC activation *in vivo* taking advantage of our novel xenograft model of CXCR4-overexpressing MM cells that results in typical BM involvement accompanying by significant increase in number of TRAP+ murine OC. Treatment of MM-bearing mice with FTY720 (10 mg/kg) effectively targeted the MM cells in the BM milieu. Correspondingly, FTY720 significantly reduced mRNA levels of murine OC differentiation marker genes in BM, including those encoding cathepsin K, integrin β3, OSCAR, RANTES and RANKL (p<0.001). This effect correlated with increased numbers of circulating CD11c+ and F4/80+ monocytes, with OC precursors in both cell populations. Concomitantly, significantly increased numbers of TRAP+ OCs were generated *in vitro* from PBMCs of FTY720-treated animals in comparison to vehicle-treated controls (p<0.001). To investigate whether OC precursor migration is affected by FTY720, we evaluated the *in vitro* migration of human monocytes toward CXCL12, well-known chemo-attractant of OC precursors. FTY720 completely blocked CXCL12-induced migration of CD14+ cells and significantly reduced their surface CXCR4 expression. These results suggest novel mechanism of action of FTY720, affecting both S1P and CXCR4 pathways, reducing the attachment of the OC precursors to the bone and thus leading to their mobilization to the blood.
Summary and Conclusions: Our observations uncover new roles of S1P pathway in OC formation and activation in MM, delineating a novel mechanism of FTY720 targeting OC formation and migration *in vitro* and *in vivo* and providing preclinical rationale for its therapeutic application in patients with MM bone disease.

P262

PTC-209, A TRANSCRIPTIONAL SMALL MOLECULE INHIBITOR OF BMI-1, DEMONSTRATES POTENT ANTI-MYELOMA ACTIVITY *IN VITRO*

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Background: BMI-1 is a putative oncogene reported to be overexpressed in multiple myeloma (MM) compared to normal bone marrow plasma cells. Silencing of BMI-1 was shown to induce apoptosis, to impair proliferation and to sensitize malignant plasma cells to bortezomib. However, therapeutic agents specifically targeting BMI-1 were not available so far.
Aims: Here we investigated PTC-209, a novel transcriptional repressor of BMI-1, for its activity in MM.
Methods: We confirmed overexpression of BMI-1 in MGUS (n=44), SMM (n=12) and MM (n=414) compared to normal BM plasma cells (n=22) in a publically available gene expression dataset (Zhan *et al.*, 2006). Preclinical activity of PTC-209 was analysed in a panel of 8 human MM cell lines (HMCLs) as well as in human umbilical vein endothelial cells (HUVECs) and during osteoblast development.
Results: BMI-1 expression was significantly elevated in all 7 GEP-defined molecular subgroups analysed (median expression value 12409 in MM *vs* 8183 in BM plasma cells, *P*<0.0001). Interestingly, BMI-1 expression was especially increased in the CD2 (median expression value: 15965) and lower in the poor prognostic proliferation (median expression value: 9759) and MMSET (median expression value: 10860) associated molecular subgroups. Treatment with PTC-209 significantly decreased viable cell numbers in MM cells with IC50 values <2 μM in 6 of 8 HMCLs tested (range: 0.21-5.68 μM). The combination of PTC-209 with either pomalidomide or carfilzomib led to additive and synergistic drug activity, especially in the PTC-209 resistant HMCL U266. Mechanistically, PTC-209 induced a G1 cell cycle arrest in MM cells which was accompanied by a significant downregulation of cyclin D1 and MYC as well as upregulation of p16, p21 and p27. Induction of apoptosis was confirmed by Annexin-V/7-AAD staining and increased levels of PARP cleavage. We observed upregulation of NOXA after 5h (up to 3.23±1.06 fold induction at 2.5μM, *P*=0.02) and downregulation of MCL-1 after 20h (up to 0.84±0.07 fold reduction at 1 μM, *P*<0.0001) suggesting that these molecules mediate the apoptotic pathway of PTC-209. Importantly, the pro-apoptotic activity of PTC-209 was upheld in co-

culture of MM cells with BM stromal cells. In the MM microenvironment, PTC-209 impaired tube formation (>90% reduction at 1 μM, *P*<0.0001) of HUVECs in a dose dependent manner. Similarly, PTC-209 led to a dose-dependent decrease of osteoblast formation evidenced by a reduction in alkaline phosphatase activity (62% reduction at 1 μM, *P*<0.0001) and matrix mineralization. The latter might be attributed to the close interaction of BMI-1 and the Wnt signaling pathway. We indeed observed a strong induction of the Wnt signaling inhibitor DKK-1 after treatment of MM cells with PTC-209 for 5h (up to 6.86±3.23 fold induction at 2.5 μM, *P*=0.03).

Summary and Conclusions: Our data reveal therapeutic targeting of BMI-1 by PTC-209 as a promising novel therapeutic intervention for MM. We confirmed overexpression of BMI-1 in MM highlighting its role as attractive drug-target. In line with this, PTC-209 showed potent anti-MM activity in 7 of 8 HMCLs tested. Moreover, PTC-209 demonstrates synergistic activity with pomalidomide and carfilzomib, overcomes BM stromal support, impairs angiogenesis and affects the expression of several prominent "MM-genes" known for their essential role in the proliferation and survival of MM (*e.g. CCND1*, *MYC*, *MCL-1*). Upregulation of *DKK-1* suggests that the osteoblast suppressive effect of PTC-209 might be overcome by concurrent antibody treatment. Further studies will clarify the role of PTC-209 in bone remodelling and corroborate its potent anti-MM activity.

P263

POMALIDOMIDE ENHANCES THE ACTIVATION OF ANTI-MYELOMA SPECIFIC T CELLS IN HEALTHY DONORS AND MULTIPLE MYELOMA PATIENTS

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Background: Pomalidomide is an IMiD® (immunomodulatory drug)that maintain the immune modulating as well as anti-tumor effects similar to the parental compounds thalidomide and lenalidomide. It has been reported to be the most potent IMiD® compoundso far. The immune modulating effects of pomalidomide include activation of NK cells, enhancement of dendritic cell function, and control of regulatory T cells. Pomalidomide has been used in clinic for the treatment of multiple myeloma (MM). However, the exact mechanism of this IMiD® is still unclear.

Aims: The aim of our study was to investigate the immunmodulatory effects of pomalidomide especially on anti-myeloma specific T cells, which are able to recognize and kill the myeloma cells.

Methods: For our study we have applied an *in vitro* model using autologous dendritic cells to facilitate the expansion of antigen-specific T cells against the MM-antigen HM1.24. The expansion of the antigen-specific T cells from 16 healthy donors and 6 patients with plasma cell dyscrasias (symptomatic MM, smoldering MM and monoclonal gammopathy of undetermined significance) were conducted in the presence or absence of pomalidomide. The cells were incubated with pomalidomide for 12 or 21 days. We analysed the number of the anti-myeloma specific T cells by IFN-y ELISpot, and quantified the secretion of granzyme B and IL-6 from the specific T cells by ELISA.

Results: We found that pomalidomide was able to enhance significantly the number of anti-myeloma specific T cells of healthy donors (p ≤.05) and patients with plasma cell dyscrasias (p ≤.05). In addition, a longer duration of the IMiD®-exposure on T cells (21 days *vs* 12 days) induced a higher frequency of the specific T cells. We observed a significantly enhanced secretion of granzyme B in 7 healthy donors (p ≤.05) and in 4 patients with plasma cell dyscrasias (p ≤.05); thus providing evidence for pomalidomide´s effects on cytotoxicity of anti-myeloma specific T cells. Exploring the role of immune modulating cytokines by ELISA, we found that pomalidomide significantly enhanced the IL-6 secretion from anti-myeloma specific T cells of 16 healthy donors (p ≤.05) and 4 patients with plasma cell dyscrasias. Interestingly, the IMiD® lenalidomide has been reported to induce a blockade of IL-6 release. Accordingly, our previous work showed that IL-6 inhibited the activation of anti-tumor specific T cells. However, in the present study, pomalidomide demonstrated an enhancement of IL-6 release from anti-myeloma specific T cells.

Summary and Conclusions: In conclusion, we have provided evidence that pomalidomide was able to enhance activation and cytotoxicity of anti-myeloma specific T cells. In addition, we have defined the role of pomalidomide on the enhancement of IL-6 secretion. This knowledge might explain partly the mechanism of activity of this drug in patients with MM.

P264

LENALIDOMIDE INCREASES HUMAN DENDRITIC CELL MATURATION MODULATING BOTH MONOCYTE DIFFERENTIATION AND MESENCHYMAL STROMAL CELL INHIBITORY PROPERTIES

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Background: The immunomodulatory drugs (IMiDs®) have several mechanisms of action including the activation of T lymphocytes and natural killer cells, direct anti-tumor effect on myeloma cells and anti-angiogenic and osteoclastogenic effects. However their use to reverse tumor-mediated immune suppression and amplify multiple myeloma (MM)-specific immunity is currently being explored and particularly the effects of Lenalidomide (LEN) on antigen presenting cells is not yet fully known.

Aims: In order to clarify this issue in this study we investigated the potential effect of LEN on maturation of both human progenitors and mature Dendritic Cells (DCs) and on the immunosuppressive properties of human mesenchymal stromal cells (hMSCs) on DCs.

Methods: DCs were differentiated *in vitro* from $CD14^+$ cells, purified either from peripheral blood (PB) of 6 healthy donors (HDs) or both PB and bone marrow (BM) samples of a cohort of 9 patients with MM including 5 with symptomatic MM and 4 with smoldering MM (SMM). Differentiation of monocyte-derived DCs (mo-DCs) were induced by the treatment with granulocyte macrophage colony-stimulating factor and Interleukin (IL)-4 for 8 days and tumor necrosis factor (TNF) α for the last 24hrs. We added LEN at different concentrations (0,1-2 μM) during all the differentiation period or for the last 48hrs of culture. LEN concentrations were chosen according to the serum levels found in pharmacokinetic studies on patients treated withLEN ranging from 5 mg to 25 mg. At the end of culture period, non-adherent cells were analyzed for DC maturation markers (CD83, HLA-DR, CD80, CD86 and CD209) by flow cytometry. Circulating mature DCs were also obtained by an immunomagnetic kit and treated with LEN for 48hrs. Moreover mo-DC differentiation was performed in the presence of conditioned media (CM) of hMSCs treated for 5 days with LEN (0,1-2μM). The expression of soluble factors involved in DC development was tested in hMSCs treated with LEN for 24hrs by both gene expression profiling (Affymetrix® gene chips) and real time PCR. Finally PB $CD3^+$ cells were *in vitro* activated by ionomycin and phorbol myristic acetate for 72hrs, and then treated with LEN for 48hrs. CM was collected either to measure pro-survival DC cytokine levels or to treat precursors or mature mo-DCs for 48hrs.

Results: LEN treatment induced a reduction of both number and % of mature mo-DC as compared to untreated controls, either in HD's and in patient's samples. On the other hand, LEN treatment significantly increased the median intensity expression of CD86 (LEN 0,1μM *vs* control, *p*=0,008) and CD209 (LEN 0,1μM *vs* control *p*=0.0004) but not of CD80 by mo-DCs derived from BM. This effect was observed either in symptomatic MM or SMM patients but not in HDs. Similarly increased CD209 expression has been also seen in mo-DCs derived from PB (LEN 0,1μM *vs* control *p*=0,0014) in both MM and SMM patients. The possible indirect effect of LEN on DCs was further investigated. Interestingly LEN blunted the inhibitory effect of hMSCs on mo-DC differentiation and down-regulated *IL6* but not *TGFB1*, *IL8*, *TNF* and *HGF* gene expression. Moreover LEN increased the production of the pro-survival DCs factor receptor activator of nuclear factor κB ligand (RANKL) by activated T cells compared to controls.

Summary and Conclusions: LEN reduces the number of DCs but increases the expression of mature DC markers both directly and indirectly, reducing the immunosuppressive properties of hMSCs. These evidences underline a possible new effect of IMiDs® on the alloreactivity against MM cells.

P265

P21-ACTIVATED KINASE 4 (PAK4) REGULATES MULTIPLE MYELOMA (MM) CELL GROWTH AND SURVIVAL BY MODULATION OF CRITICAL TRANSCRIPTIONAL PATHWAYS: THERAPEUTIC APPLICATION OF PAK4 ALLOSTERIC INHIBITORS

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Background: PAK4 is a member of p21-activated kinase (PAK) family of serine/threonine kinases. It is activated in cancer cells, where it regulates a wide range of cellular processes including cytoskeleton reorganization, cell proliferation, survival and motility. However, the role of PAK4 in multiple myeloma (MM) is not established yet.

Aims: To investigate the expression and functional role of PAK4 in MM cells and to evaluate impact of orally bioavailable PAK4 allosteric modulators (PAMs) on MM cell growth and survival, providing evidences for a novel therapeutic approach for the treatment of MM

Methods: The expression of PAK4 and phosphorylated(p)-Pak4 Ser474 was assessed by immunoblot analysis. Both gain of-and loss of function studies

were performed to investigate functional and molecular impact of PAK4 on MM cells. Apoptotic cell death was analyzed by flow cytometry following Annexin-V and propidium iodide (PI) staining. Cell proliferation and viability were measured by [3H]-thymidine and CellTiter-Glo (CTG) assays, respectively. TOP/FOP-flash reporter assays were performed to examine B-catenin-mediated TCF/LEF transcriptional activity.
Results: We have demonstrated a differential expression and sub-cellular localization of PAK4 and (p)-Pak4 Ser474 in different multiple myeloma cell lines, primary MM cells and PBMCs. In a gain-of-function study, over-expression of PAK4 in deficient MM cells (RPMI8226 and U266) significantly increased cell proliferation and survival *in vitro* and *in vivo*. Conversely, in a loss-of-function study, conditional knock-down of PAK4 expression decreased MM cell proliferation and survival. With a significant impact of PAK4 on MM cell growth, we identified a class of orally bioavailable PAK4 allosteric modulators (PAMs). We observed inhibition of MM cell growth and survival after treatment with PAMs even in the presence of bone marrow microenvironment with no significant effect on normal PBMCs, suggesting a favorable therapeutic index in MM treatment. Furthermore, time and dose dependent induction of both intrinsic and extrinsic apoptotic pathways and G2/M arrest was observed after treatment with PAMs. Importantly, orally bioavailable PAMs given daily were able to inhibit tumor growth and prolong overall survival *in vivo* in two murine models of human myeloma. PAK4 is a nucleo-cytoplasmic shuttling protein that is imported into the nucleus, promoting TCF/LEF transcriptional activity and leading to B-catenin stabilization. Furthermore, nuclear import of PAK4 accompanies the import of B-catenin and increases TCF/LEF transcriptional activity. TOP/FOP-flash reporter assays were performed to examine whether B-catenin-mediated TCF/LEF transcriptional activity was modulated through PAK4 in myeloma cells. The results showed that PAK4 silencing significantly reduced TCF/LEF transcriptional activity in MM cells, highlighting the vital role that PAK4 plays in mediating the nuclear localization and oncogenic activity of B-catenin. Furthermore, after PAK4 inhibition we also observed a decrease expression levels of two B-catenin targets, cyclin D1 and c-myc, which are known to play a critical role in the myeloma pathobiology.
Summary and Conclusions: PAK4 plays an important cellular and molecular function in myeloma and its inhibition with a new class of PAK4 allosteric modulators provides a novel therapeutic approach for the treatment of MM. Further experiments are ongoing, to clarify the pathway and downstream effectors of PAK4 both in the nucleus and cytoplasm.

P266

BONE MARROW-MEDIATED DRUG RESISTANCE IS PROMOTED BY JAGGED-INDUCED NOTCH PATHWAY IN MULTIPLE MYELOMA

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Background: Multiple myeloma (MM) rapresents 10% of all hematological malignancies and is caused by the accumulation of malignant plasma cells in the bone marrow (BM). Although treatments with new drugs (immunomodulators and proteasome inhibitors) are increasing patients' survival, MM is still incurable because of the development of endogenous or BM mediated drug resistance. Therefore it is crucial to find new therapeutic targets. The dysregulated expression of two Notch ligands, Jagged1 and 2, hyperactivates Notch pathway both in MM cells and in bone marrow stromal cell (BMSC). Several Notch downstream effectors support MM cell growth, survival and proliferation, *i.e.* IL-6, SDF-1alfa, CXCR4, NF-kB, VEGF and IGF.
Aims: The aim of this study was to investigate the role of Notch signaling in endogenous and BMSC-promoted drug resistance in MM.
Methods: U266 and OPM2 MM cell lines were cultured alone in complete RPMI-1640 medium or co-cultured with murine (NIH3T3) or human (HS5) BMSC cell lines in DMEM medium supplemented with 10% V/V FBS. MM cells were either kept in suspension for 24 hours or plated on BMSC monolayer for 24h, then the MM cell lines were treated with drugs (Mitoxantrone, Bortezomib or Melphalan) for additional 24 hours. Apoptosis assay: HS5 cells were stained with PKH26 red fluorescent dye (Sigma-Aldrich) before co-culturing to allowed flow-cytometric detection of MM cells co-cultured with BMSCs. Cells were stained with Annexin V-FITC and processed with Cytomics FC500 software (Beckman Coulter). RNA interference: Silencing of Jagged1 and Jagged2 was obtained by transient expression of two specific siRNAs (Stealth Select RNAiTM siRNA system, Life Technologies). Quantitative PCR reactions were carried out on a 7500 Fast Real-time PCR system (Applied Biosystems) using the MaximaTM SYBR Green/ROX qPCR Master Mix (Dasit). Anti-apoptotic protein such as SDF-1alfa, CXCR4, Bcl-2, Survivin and ABCC1 were analyzed by flow cytometry using appropriate antibodies.
Results: Jagged1 and 2 silencing in OPM-2 and U266 cells is able to reduce the expression of anti-apoptotic genes *i.e.* SDF-1alfa, CXCR4, Bcl-XL, Bcl-2, Survivin and ABCC1. At the same time, MM cells with reduced levels of Jagged1 and 2 showed an increased sensivity to different drugs commonly used in MM therapy such as Bortezomib, Mitoxantrone and Melphalan. We investigated the underlying mechanism showing that MM cells and BMSC interaction resulted in the activation of Notch signaling in both cell types. When co-coltured with human or murine BMSCs, we observed that MM cells showed increased drug resistance due to: first of all, an increased expression of anti-apoptotic genes in MM cells among which SDF-1alfa, CXCR4, Bcl-XL, Bcl-2, Survivin and ABCC1; secondary, BMSC release of soluble factors, *i.e.* SDF-1alfa and VEGF, relevant for MM cell growth and survival. Interestingly, Jagged1 and 2 silencing in MM cells could reverse all gene and protein expression changes and BMSC protective effect. All these results were confirmed both at gene expression and protein level.
Summary and Conclusions: The evidence that Jagged-1 and 2 silencing affect endogenous and BMSC-induced drug resistance in MM cells supports the use of a Jagged-targeted approach in MM therapy alone or in a combination with common drugs.

Multiple myeloma - Clinical 1

P267

FDG-PET/CT FOCAL LESIONS IN THE ABSENCE OF OSTEOLYSES AS A NEW MARKER OF PROGRESSION OF SMOLDERING MULTIPLE MYELOMA INTO SYMPTOMATIC DISEASE

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Background: The probability of progression of smoldering multiple myeloma (SMM) into symptomatic disease (MM) is highly variable and therefore the identification of sub-groups of patients (pts) at different risk of progression is a relevant end point. FDG-PET/CT is a reliable technique for assessing early skeletal involvement and the presence of osteolytic lesions identified by this imaging technique has been recently incorporated in the updated criteria for the diagnosis of MM. However, no data are available regarding the impact of PET/CT FLs in the absence of underlying osteolyses in SMM on time to progression (TTP) into MM.
Aims: To address this issue, we prospectively studied a cohort of 120 pts with SMM with PET/CT.
Methods: By study design, all the pts were studied with PET/CT at presentation of the disease. Bone marrow involvement was described as negative, diffuse or focal. The number of FLs, as well as size and associated standardized uptake values (SUV) were recorded. For each FL, the presence of eventual underlying osteolytic lesion was investigated by the CT part of the scan; pts with osteolytic lesions were excluded from the study, as they were considered as having MM. Laboratory follow-up took place every 3-4 months. Skeletal progression was defined by the appearance of one or more sites of osteolytic bone destruction, pathological fractures and/or soft masses at PET/CT or MRI. The start of systemic therapy was defined as the date of event for the analysis of TTP.
Results: Baseline patient characteristics were as follows: median age 61 years, IgG isotype 73%, median M component 2.4 g/dL, ISS stage I 77%, median bone marrow plasma cell (BMPC) 27%, median serum involved/uninvolved FLC ratio 14.27. PET/CT was negative in 101/120 pts (84%) and positive in 19/120 (16%) of them; 8 pts had 1 FLs, 3 pt 2 FLs, 6 pts more than 3 FLs and 2 pt a diffuse bone marrow involvement. Median SUV max value was 4.7 (range 2.5-10.7). BMPC was significantly superior for patients with positive PET/CT (p=0.024). In 91 pts a baseline axial or WB-MRI was available as well and resulted positive in 27% of them. Ten per cent, 11% and 13% of the pts were having more than 60% BMPC, FLC ratio ≥100 and more than 1MRI FL, respectively. With a median follow-up of 2.2 years, 62% of the pts remained in the asymptomatic phase while 38% of them progressed to MM, in a median time of 4 years, including 21% with skeleton involvement, with/without the appearance of other CRAB symptoms, and 17% with exclusive serological signs of progression. The relative risk of progression of the pts with a positive PET/CT was 3.00 (95% CI 1.58-5.69, P=0.001). Moreover, the relative risk of skeletal progression was 4.44 (95% CI 1.97-10.02, P<0.001), with a median TTP of 2.2 years for pts with positive PET/CT *vs* 7 years for those with negative PET/CT (Figure 1). The probability of progression within 2 and 3 years for pts with positive PET/CT was 58% and 66%, respectively, *vs* 33% and 42% for negative pts. Results did not significantly differ after pts with early MM according to the updated criteria were excluded from the analysis. A multinomial logistic regression analysis showed that PET positivity was significantly related to progression with skeletal involvement while BMPC superior than 60% to the exclusive serological one.

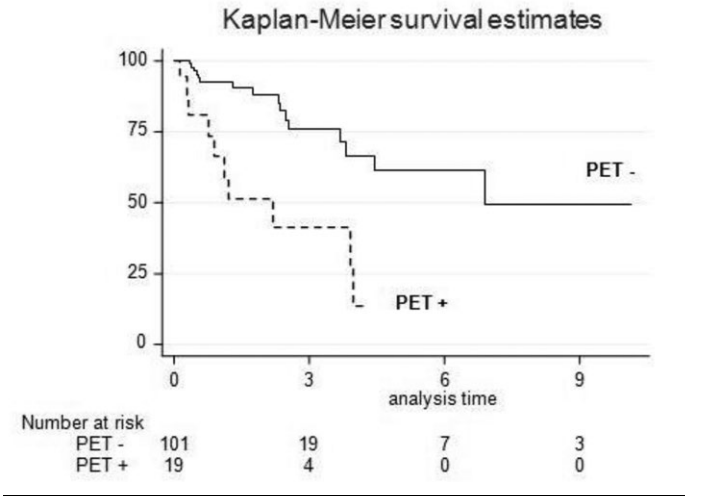

Figure 1.

Summary and Conclusions: In conclusion, approximately 16% of the pts with SMM have a positive PET/CT, mainly with few FLs, with a low FDG uptake. PET/CT positivity significantly increased the risk of progression of SMM into MM. PET/CT could become a new risk factor to define high risk SMM. Further studies are warranted to find and optimal cut off point of FLs and/or SUVmax to capture the higher risk of progression at 2 year and to merge with other prognostic factors.

P268

ANALYSIS OF OUTCOMES BY RESPONSE FOR PATIENTS WITH RELAPSED OR RELAPSED AND REFRACTORY MULTIPLE MYELOMA IN THE PHASE 3 PANORAMA 1 STUDY OF PANOBINOSTAT OR PLACEBO PLUS BORTEZOMIB AND DEXAMETHASONE

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Background: Panobinostat (PAN) is the first pan-deacetylase inhibitor (pan-DACi) to demonstrate a statistically significant and clinically relevant increase in median progression-free survival (PFS) in a phase 3 clinical trial of patients (pts) with relapsed or relapsed and refractory multiple myeloma (MM). In the PANORAMA 1 trial, pts randomized to receive PAN + bortezomib (BTZ) and dexamethasone (Dex; PAN-BTZ-Dex) demonstrated a median PFS of 12.0 months *vs* 8.1 months for pts who received placebo (Pbo) + BTZ and Dex (Pbo-BTZ-Dex; hazard ratio [HR], 0.63; *P*<.0001).
Aims: To determine the effect of clinical outcomes based on the level of response achieved.
Methods: Overall response rate, including rate of near complete/complete response (nCR/CR) and partial response (PR) per modified European Society for Blood and Marrow Transplantation (mEBMT) and stringent CR (sCR)/CR/very good PR (VGPR) per International Myeloma Working Group (IMWG) and duration of response (DOR) were analyzed. A landmark analysis at 6, 12, 18, and 24 weeks by response status using a Cox regression model was conducted to determine PFS in pts who achieved nCR/CR *vs* PR per mEBMT or sCR/CR/VGPR *vs* PR as per IMWG criteria.
Results: The rate of nCR/CR was significantly higher for PAN-BTZ-Dex *vs* Pbo-BTZ-Dex (27.6% *vs* 15.7%; nominal *P*=.00006). Per IMWG criteria, the rate of sCR/CR/VGPR was also higher for the PAN-BTZ-Dex arm (36% *vs* 24%; nominal *P*=.00001). The DOR per mEBMT criteria for pts with nCR/CR, ≥PR, and PR was 18.4, 13.1, and 9.0 months, respectively, in the PAN-BTZ-Dex arm and 14.5, 10.9, and 8.8 months, respectively, in the Pbo-BTZ-Dex arm. The landmark analysis by mEBMT and IMWG criteria is shown in the Table. At the 12-week landmark, a median PFS of 16.5 months was observed

for pts with CR/nCR and 10.3 months for pts with PR in the PAN-BTZ-Dex arm (hazard ratio [HR], 0.40; 95% CI, 0.25-0.65) and a median PFS of 14.1 months for pts with CR/nCR and 9.7 months for pts with PR in the Pbo-BTZ-Dex arm (HR, 0.62; 95% CI, 0.36-1.07). By IMWG criteria, the landmark analysis at 12 weeks demonstrated a median PFS of 15.9 months for pts with ≥VGPR and 8.1 months for pts with PR in the PAN-BTZ-Dex arm (HR, 0.36; 95% CI, 0.21-0.62) and a median PFS of 14.4 months for pts with ≥VGPR and 7.6 months for pts with PR in the Pbo-BTZ-Dex arm (HR, 0.39; 95% CI, 0.22-0.68).

Tabella 1. Landmark Analysis of Progression-Free Survival

Modified EBMT Criteria			
Landmark time	Number of responses at landmark time: ≥ nCR/PR	Median PFS at landmark time: ≥ nCR/PR, months	HR (95% CI)
6 weeks			
PAN-BTZ-Dex	12/57	Not estimable/12.6	0.33 (0.12-0.89)
Pbo-BTZ-Dex	3/57	15.8/10.2	0.85 (0.19-3.90)
12 weeks			
PAN-BTZ-Dex	49/107	16.5/10.3	0.40 (0.25-0.65)
Pbo-BTZ-Dex	23/122	14.1/9.7	0.62 (0.36-1.07)
18 weeks			
PAN-BTZ-Dex	76/104	16.5/10.9	0.43 (0.29-0.65)
Pbo-BTZ-Dex	41/126	14.6/10.4	0.54 (0.35-0.82)
24 weeks			
PAN-BTZ-Dex	84/96	19.0/12.0	0.40 (0.27-0.59)
Pbo-BTZ-Dex	46/112	14.9/11.8	0.57 (0.38-0.87)
IMWG Criteria			
Landmark time	Number of responses at landmark time: ≥ VGPR/PR	Median PFS at landmark time: ≥ VGPR/PR, months	HR (95% CI)
6 weeks			
PAN-BTZ-Dex	12/36	16.5/6.9	0.29 (0.08-1.03)
Pbo-BTZ-Dex	8/28	Not estimable/7.6	0.35 (0.10-1.22)
12 weeks			
PAN-BTZ-Dex	57/52	15.9/8.1	0.36 (0.21-0.62)
Pbo-BTZ-Dex	32/74	14.4/7.6	0.39 (0.22-0.68)
18 weeks			
PAN-BTZ-Dex	82/51	16.5/8.8	0.33 (0.21-0.52)
Pbo-BTZ-Dex	62/71	13.1/9.5	0.50 (0.33-0.75)
24 weeks			
PAN-BTZ-Dex	93/47	17.0/9.5	0.31 (0.20-0.49)
Pbo-BTZ-Dex	69/58	13.4/11.2	0.60 (0.39-0.92)

Summary and Conclusions: A higher proportion of deep responses (≥nCR or ≥VGPR) was achieved with PAN-BTZ-Dex, and the DOR was longer and was associated with a prolonged PFS. Results were consistent by mEBMT or IMWG. Overall, these data further support achievement of deeper responses as a treatment goal in pts with relapsed/refractory MM.

P269

WEEKLY CARFILZOMIB WITH DEXAMETHASONE FOR PATIENTS WITH RELAPSED OR REFRACTORY MULTIPLE MYELOMA: UPDATED RESULTS FROM THE PHASE 1/2 STUDY CHAMPION-1 (NCT01677858)

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Background: Carfilzomib is a selective proteasome inhibitor that is approved in the United States for the treatment of relapsed and refractory multiple myeloma. The approved dosing schedule for carfilzomib is a 2–10 minute IV infusion on days 1, 2, 8, 9, 15, and 16 of a 28-day cycle (starting dose: 20mg/m^2 [cycle 1]; escalated to a target dose of 27mg/m^2 in cycle 2).
Aims: Herein we present updated results from a multicenter, single-arm, phase 1/2 study (CHAMPION-1; NCT01677858) evaluating the safety and efficacy of weekly carfilzomib with dexamethasone (Kd) in patients with relapsed or refractory multiple myeloma (RRMM).
Methods: Patients who received 1−3 prior regimens were eligible. All patients provided informed consent. In the phase 1 portion, patients received carfilzomib as a 30-min IV infusion on days 1, 8, and 15 of a 28-day cycle using a 3+3 dose-escalation scheme. All patients received carfilzomib at 20 mg/m^2 on day 1 of cycle 1; subsequent dose cohorts received 45, 56, 70, or 88 mg/m^2 in successive cohorts until the maximum tolerated dose (MTD) was reached for use in the phase 2 portion of the study. Patients received dexamethasone 40 mg (IV or oral administration) on days 1, 8, 15, and 22 of cycles 1–8; dexamethasone was omitted on day 22 in cycles ≥9. In the phase 2 portion, patients are receiving carfilzomib at the recommended dose from the phase 1 portion (carfilzomib dose of 20 mg/m^2 on cycle 1, day 1 escalating to the MTD for subsequent doses) with the same dose and schedule for dexamethasone. Kd is being administered until disease progression or unacceptable toxicity.
Results: Results are presented for patients treated at the MTD (70 mg/m^2) in the phase 1b and phase 2 portions of the study. As of January 7, 2015, 104 patients (phase 1, n=15; phase 2, n=89 patients) were enrolled at the MTD; the study is fully enrolled. Median patient age was 68.5 years (range, 41–88). Patients received a median of 1prior regimen (range, 1–3); 48% of patients had received 2 or more prior regimens. A total of 82% of patients had received prior bortezomib (BTZ), 48% of patients were BTZrefractory, 28% were lenalidomide (LEN) refractory, and 16% were refractory to BTZ and LEN. Preliminary median carfilzomib treatment duration was 5.3 months (range, 0.03–18.8). The overall response rate (≥partial response) was 72% (95% confidence interval [CI]: 63%–81%); the clinical benefit rate (≥minimal response) was 80% (95% CI: 71%–87%). Kaplan-Meier median PFS was 10.6 months (95% CI: 7.2–not estimable). Seven patients (7%) discontinued treatment due to an adverse event (AE). The most common hematologic AEs (all grade) were anemia (24%), thrombocytopenia (18%), and neutropenia (8%). The most common nonhematologic AEs (all grade) were fatigue (48%), nausea (32%), and insomnia (30%). The most common grade ≥3 hematologic AEs were thrombocytopenia (6%), anemia (5%), and neutropenia (4%). The most common grade ≥3 nonhematologic AEs were fatigue (9%), dyspnea (6%), and back pain (6%). Four patients died on study: 1 patient had sepsis, respiratory distress, pneumonia, and acute renal failure; and 1 patient each had acute renal failure, cardiopulmonary arrest, and disease progression.
Summary and Conclusions: At the MTD (70 mg/m^2), weekly Kd is demonstrating acceptable safety and tolerability with promising efficacy in patients with RRMM. Updated results will be presented at the meeting.

P270

EARLY MORTALITY IN ELDERLY NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS TREATED WITH NOVEL AGENTS: A POOLED ANALYSIS OF TWO LARGE RANDOMIZED PHASE III TRIALS

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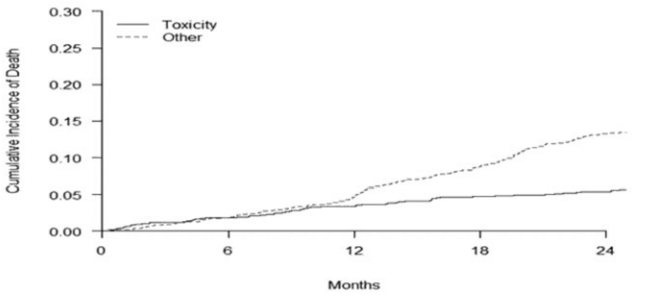
Background: Early mortality in elderly multiple myeloma (MM) patients is usually attributed to combined effects of active disease and co-morbid factors. Before the introduction of novel agents, toxic deaths within 60 days from start of conventional treatment occurred in 10% of patients (Augustson BM. J Clin Oncol 2005, 36:9219), mainly due to infection and renal failure. The use of novel agents has considerably improved MM outcome at the expense of newer toxicity.
Aims: The aim of this analysis is to study early deaths not related to disease progression during treatments with lenalidomide or bortezomib. We analyzed individual patient data from two large multicenter randomized trials to assess the rate and the causes of death, their predictability and whether current management strategies have reduced their frequency.
Methods: A total of 1,173 newly diagnosed MM patients ineligible for autologous transplantation due to age or co-morbidities enrolled in the Gruppo Italiano Malattie EMatologiche dell'Adulto (GIMEMA) and European Myeloma Network (EMN) trials from May 2006 to September 2012, were studied. Patients in the GIMEMA MM-03-05 trial (N=511) received bortezomib-containing regimens (Palumbo A. J Clin Oncol 2014, 32:634) and those in the EMN01 trial (N=662) lenalidomide-containing regimens (Magarotto V. Blood 2014, abs ASH).
Results: A total of 1146 patients could be evaluated for this analysis. Within 24 months from start of therapy, 207/1146 patients (18%) died for any causes, 61/1146 (5%) died due to adverse events. Toxic deaths within 60 days occurred in 12 patients (1%) with a linear increase over time of 1% every 6 months (Figure 1). There was no difference in the incidence of toxic deaths between patients receiving bortezomib-containing regimens (31 pts, 6%) and those receiving lenalidomide-containing regimens (30 pts, 5%, p=0.32). The incidence of toxic deaths was significantly higher in patients older than 80 years (11/107 [10%], p=0.005). Twenty-nine percent of deaths were attributable to cardiac complications (18 pts), 18% to infections (17 pts) and 15% to vascular complications (9 pts). By comparing the cause of toxic deaths between the 2 different treatment regimes, there was no significant difference in the proportions of cardiac events, infections, vascular events or other causes. In a multivariate analysis, age (HR 1.09 per 1 year increase, p=0.002) and ISS score (HR 3.81, p=0.01 ISS 2 *vs* ISS 1; HR 5.69, p=0.002 ISS 3 *vs* ISS 1) did increase the risk of death but poor performance status did not (HR 1.25, p=0.59). Greater tumor burden and activity (ISS) increased the risk of death because such deaths occurred before the maximal beneficial effect of therapy in reducing tumor load: 92% of patients dying from toxicity within 2 months of start of therapy had achieved a suboptimal response (8 not available, 3 SD, 1 PR, ORR 8%).



	2	6	12	18	24
Toxicity	1.1(0.5 to 1.6)	1.9(1.1 to 2.6)	3.4(2.3 to 4.5)	4.7(3.5 to 6)	5.4(4 to 6.7)
Other	0.6(0.2 to 1.1)	2(1.1 to 2.8)	4.8(3.5 to 6.1)	8.7(7 to 10.4)	13.2(11.2 to 15.3)

Figure 1. Cumulative incidence of death due to AEs or due to any other causes

Summary and Conclusions: Novel, more effective and more rapid therapies have reduced the risk of toxic deaths as compared to conventional treatments. Nevertheless one-third of early deaths occurred primarily due to cumulative specific drug-related toxicities. Improvement in supportive therapy together with prevention and prompt recognition/treatment of complications are urgently needed to reduce the risk of toxic deaths. The 2-fold higher risk of toxic mortality in octogenarians indicates the need for a careful assessment of frail patients who may benefit from a gentle or even palliative approach.

P271

MAINTENANCE THERAPY WITH LENALIDOMIDE IN ELDERLY PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA: A POST-HOC ANALYSIS OF THE EMN01 TRIAL

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Background: Lenalidomide maintenance showed to be effective in terms of progression free survival (PFS) in patients with newly diagnosed multiple myeloma (NDMM) ≥65 years or not eligible for autologous stem cell transplantation. Recently, the geriatric assessment has emerged as a fundamental strategy to evaluate patients' frailty and consequently to select the most appropriate treatment for elderly MM patients [Palumbo A, *et al.* Blood. 2015].
Aims: In a post-hoc analysis, we classified patients enrolled in the EMN01 trial according to the new geriatric assessment in order to evaluate safety and efficacy of Lenalidomide-containing maintenance in fit, unfit and frail NDMM patients.
Methods: In the EMN01 phase III trial, patients with NDMM were randomized to receive induction treatment with Lenalidomide-dexamethasone or Melphalan-Prednisone-Lenalidomide or Cyclophosphamide-Prednisone-Lenalidomide. After induction, patients were randomized to receive maintenance with Lenalidomide alone (10 mg on days 1-21 in 28-day cycles) or plus Prednisone (25 mg every other day in 28-day cycles), until disease progression or intolerance. The geriatric assessment was performed and included age, comorbidities (according to Charlson Comorbidity index), Activity of daily living and Instrumental Activity of Daily Living scores.
Results: Six-hundred-forty-three patients started induction treatment. Patients were classified as fit (n=280), unfit (n=202) and frail (n=161). During induction, 30 patients (11%) discontinued treatment due to adverse events (AEs) in the fit group and 38 patients (23%) in the frail group (p=0.0006). Deaths not related to progressive disease were 4 in the fit group (mainly due to stroke [n=2]) and 17 in the frail group (mainly due to cardiologic AEs [n=7] and infections [n=3]). Four-hundred-two patients started maintenance treatment: 192 in the fit, 121 in the unfit, and 89 in the frail groups. During maintenance, at least one grade ≥3 hematologic AE was reported in 24 patients (13%) in the fit and 13 patients (15%) in the frail groups. At least one grade ≥3 non-hematologic AE was recorded in 12 patients (6%) in the fit and 12 patients (13%) in the frail groups (p=0.04), and were mainly cardiologic AEs and infections. After a median follow-up of 30 months since the beginning of maintenance, median PFS was 24 months in fit and 27 months in frail patients (HR=1.136, CI 0.808-1.596, p=0.464). Median overall survival (OS) was not reached; at 24 months, OS was 90% in fit and 76% in frail patients (HR 2.804, CI 1.665-4.723, p=0.0001). In the frail group, 101 patients (63%) did not reduce any drug doses during induction, and 49 (49%) of them started maintenance; whereas 60 patients (37%) reduced drug doses during induction, and 40 (67%) of them started maintenance. The difference between the two groups of frail patients who began maintenance was statistically significant (P=0.02).
Summary and Conclusions: The benefit of maintenance therapy in frail patients is controversial. Frail patients who reduced doses at induction tolerated therapy better and had a higher probability to start maintenance. Therefore, an induction treatment tailored to the frailty status allows a higher number of patients to complete induction, and to start and benefit from lenalidomide maintenance.

P272

OUTCOMES FOR OLDER PATIENTS WITH REFRACTORY OR RELAPSED AND REFRACTORY MULTIPLE MYELOMA TREATED WITH POMALIDOMIDE + LOW-DOSE DEXAMETHASONE IN THE STRATUS (MM-010) TRIAL, A SINGLE-ARM, PHASE 3B STUDY

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Background: The survival of patients (pts) with multiple myeloma (MM) decreases with increased age (Pulte et al, *Oncologist*, 2011), and those with advanced disease who have exhausted treatment (Tx) options with newer agents have a poor prognosis (Kumar et al, *Leukemia*, 2012). The oral agent pomalidomide (POM) has anti-myeloma, stromal cell inhibitory, and immune modulatory effects (Quach et al, *Leukemia*, 2010; Mark et al, *Leuk Res*, 2014). In the pivotal MM-003 trial, Tx with POM + low-dose dexamethasone (LoDEX) prolonged progression-free survival (PFS) and overall survival (OS) *vs* high-dose DEX and had a tolerable safety profile in pts with refractory or relapsed and refractory MM (RRMM; San Miguel et al, *Lancet Oncol,* 2013). In MM-003 and in the pivotal phase 2 trial, MM-002, Tx with POM + LoDEX provided significant PFS and OS benefits across various age groups; tolerability and dose intensity were unaffected by age (Weisel et al, *Blood,* 2013; Jagannath et al, *J Clin Oncol*, 2013).
Aims: STRATUS is a multicenter, single-arm, open-label, European phase 3b trial designed to further evaluate safety and efficacy of POM + LoDEX in a large pt population. This analysis examined pt outcomes by age (≤65 *vs* >65 yrs and ≤70 *vs* >70 yrs).
Methods: RRMM pts with progressive disease (PD) during or within 60 days of their last line of Tx (refractory to their last prior Tx) were included. Other eligibility criteria included age ≥18 yrs, measurable M-protein in serum or urine, lenalidomide and bortezomib failure after ≥2 consecutive cycles of each (alone or in combination), and adequate prior alkylator therapy. Pts with absolute neutrophil count <800/μL, platelets <75,000 or <30,000/μL for pts in whom <50% or ≥50% of bone marrow nucleated cells were plasma cells, respectively, or creatinine clearance <45 mL/min were excluded. Pts received POM 4 mg D1-21 + LoDEX 40 mg (20 mg for pts aged >75 yrs) D1, 8, 15, and 22 of a 28-day cycle until PD or unacceptable toxicity. Thromboprophylaxis was required for all pts. Primary endpoint was safety; key secondary endpoints included overall response rate (ORR; ≥partial response), duration of response (DOR), PFS, and POM exposure. Outcomes were analyzed by pt age at baseline (≤65 *vs* >65 yrs and ≤70 *vs* >70 yrs).
Results: As of Sep 15, 2014, 604 pts have been enrolled. 599 received POM + LoDEX; 46% were ≤65 yrs, 54% were >65 yrs, 69% were ≤70 yrs, and 31% were >70 yrs. Pts were heavily pretreated (median 4-5 prior Tx). Median follow-up was 9.3 mos. Median relative dose intensity was similar independent of pt age (range, 0.93-0.94). The most frequent grade (Gr) 3/4 treatment-emergent adverse events (TEAEs) across age groups were neutropenia, anemia, thrombocytopenia, and infections (Table). Discontinuation of POM due to TEAEs was infrequent in pts aged ≤65 (7%), >65 (11%), ≤70 (7%), and >70 (14%) yrs. ORRs ranged between 32% and 38% for all age groups. Median PFS was also similar, regardless of age (4.1-4.7 mos; Table). Updated data will be presented at the congress.

Tabella 1. Safety and Efficacy Outcomes

Parameter	≤ 65 yrs (n = 274)	> 65 yrs (n = 325)	≤ 70 yrs (n = 415)	> 70 yrs (n = 184)
Grade 3/4 hematologic TEAEs in ≥ 10% of pts, %				
Neutropenia	43	41	43	41
Anemia	31	27	29	28
Thrombocytopenia	24	19	24	17
Grade 3/4 non-hematologic TEAEs in ≥ 10% of pts, %				
Infections	35	26	30	28
Pneumonia	14	9	11	11
Grade 3/4 TEAEs of interest, %				
VTE[a]	1	2	1	1
Peripheral neuropathy[b]	1	2	1	2
Efficacy Outcomes	**≤ 65 yrs (n = 277)**	**> 65 yrs (n = 327)**	**≤ 70 yrs (n = 418)**	**> 70 yrs (n = 186)**
ORR, %	38	32	35	33
Median DOR, mos	6.5	9.5	6.5	9.5
Median PFS, mos	4.2	4.2	4.1	4.7

[a] Includes the preferred terms deep vein thrombosis and pulmonary embolism; [b] Includes the preferred terms neuropathy peripheral, peripheral sensory neuropathy, paresthesia, hypoesthesia, polyneuropathy, peripheral motor neuropathy, peripheral sensorimotor neuropathy, and dysesthesia.

DOR, duration of response; ORR, overall response rate; PFS, progression-free survival; pt, patient; TEAE, treatment-emergent adverse event; VTE, venous thromboembolic event.

Summary and Conclusions: The results show that POM + LoDEX is efficacious and tolerable in RRMM pts regardless of age (≤65 *vs* >65 yrs and ≤70 *vs* >70 yrs). Across the age groups, the safety profile and relative dose intensity were similar. PFS and response rates were also similar and consistent with previous findings. The data support the use of 4 mg of POM as an effective starting dose, and the use of POM + LoDEX, regardless of age, in RRMM pts.

P273

THE STRATUS (MM-010) TRIAL: A SINGLE-ARM, PHASE 3B STUDY EVALUATING SAFETY AND EFFICACY OF POMALIDOMIDE + LOW-DOSE DEXAMETHASONE IN PATIENTS WITH REFRACTORY OR RELAPSED AND REFRACTORY MULTIPLE MYELOMA

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Background: Survival outcomes of patients (pts) with relapsed/refractory multiple myeloma (RRMM) have improved with introduction of newer agents (Kumar, *Leukemia*, 2014). However, there are few effective treatment (Tx) options for pts with RRMM who failed Tx with bortezomib (BORT) and lenalidomide (LEN). As a result, these pts have shortened overall survival (OS; Kumar, *Leukemia,* 2012). Pomalidomide (POM) is a distinct oral immunomodulatory agent with direct anti-myeloma, stromal cell inhibitory, and immune modulatory effects (Quach, *Leukemia,* 2010; Mark, *Leuk Res*, 2014). Pivotal trials demonstrated higher overall response rates (ORRs) with POM + low-dose dexamethasone (LoDEX) *vs* POM alone and significantly extended PFS and OS *vs* high-dose DEX (San Miguel, *Lancet Oncol*, 2013; Richardson, *Blood*, 2014). Refractoriness to LEN and/or BORT (including LEN as last prior Tx) did not affect outcomes with POM + LoDEX.

Aims: STRATUS is a multicentre, single-arm, open-label, phase 3b trial designed to further evaluate safety and efficacy of POM + LoDEX in RRMM pts.

Methods: Pts with refractory or relapsed and refractory disease (progressive disease [PD] on or within 60 days of last prior Tx), prior BORT and LEN Tx failure, and adequate prior alkylator therapy were eligible. Pts with absolute neutrophil count <800/μL, platelets <75,000 or <30,000/μL (for pts with <50% or ≥50% of bone marrow nucleated cells as plasma cells, respectively), creatinine clearance <45 mL/min, hemoglobin <8 g/dL, and peripheral neuropathy (PN) grade (Gr) ≥2 were excluded. POM 4 mg was administered D1-21 of a 28-day cycle in combination with LoDEX 40 mg/day for pts aged ≤75 yrs or 20 mg for pts aged >75 yrs on D1, 8, 15, 22 until PD or unacceptable toxicity. Thromboprophylaxis was required for all pts. Follow-up continued for Tx, OS, and second primary malignancy until 5 yrs post-enrollment. Primary endpoint was safety, and key secondary endpoints included ORR (≥partial response), duration of response (DOR), PFS, OS, POM exposure, and cytogenetic analyses. STRATUS is registered with ClinicalTrials.gov (NCT01712789) and EudraCT (2012-001888-78).

Results: As of Sep 15, 2014, 604 pts were enrolled. Median age was 66 yrs (range, 37-88); median time since initial diagnosis was 5.2 yrs (range, 0.5-27.1). Pts were heavily pretreated with a median of 5 prior Tx (range, 2-18); 95% of patients were LEN refractory, and 78% were refractory to both LEN and BORT. Median follow-up was 9.3 mos. The most frequent Gr 3/4 Tx-emergent adverse events (TEAEs) were hematologic, including neutropenia (42%), anemia (29%), and thrombocytopenia (22%); Gr 3/4 non-hematological toxicities included pneumonia (11%) and fatigue (5%). Gr 3/4 venous thromboembolic events were infrequent (1%), and Gr 3/4 PN was 1%. 5% of pts withdrew due to adverse events. Median PFS and OS were 4.2 and 11.9 mos, respectively (Figure). ORR was 35%; 7% pts achieved ≥very good partial response, and 1% achieved a complete response. Median DOR was 6.8 mos. In pts refractory to prior LEN or LEN and BORT, similar PFS (4.2 and 4.1 mos), OS (12 mos each), and ORR (34% and 35%) were observed. Updated data will be presented at the congress.

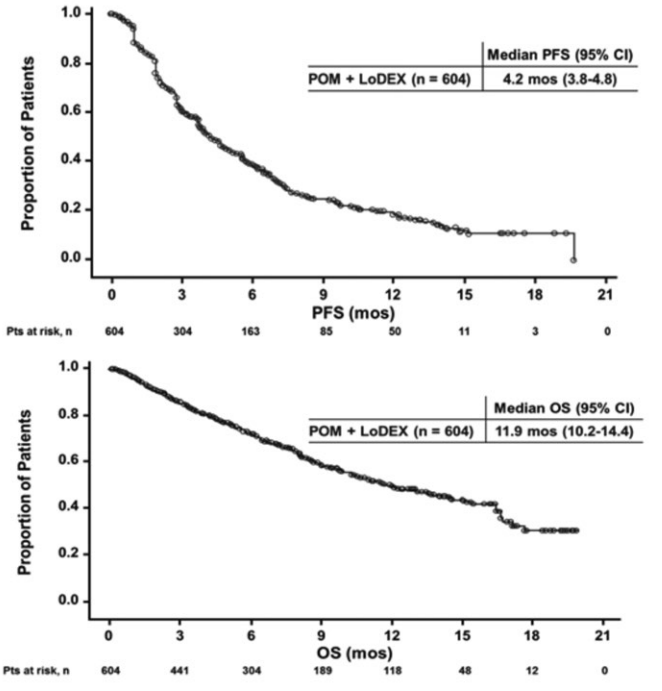

Figure 1.

Summary and Conclusions: Safety and efficacy of POM + LoDEX in the STRATUS trial was consistent with results from pivotal trials of POM + LoDEX. Efficacy outcomes were similar regardless of prior refractoriness to LEN, BORT, or LEN + BORT. This study confirms previous analyses demonstrating that POM + LoDEX is a standard of care for RRMM pts in whom LEN and BORT Tx has failed.

P274

IMPACT OF RENAL IMPAIRMENT ON OUTCOMES AFTER TREATMENT WITH LENALIDOMIDE AND LOW-DOSE DEXAMETHASONE IN PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA: FIRST TRIAL RESULTS

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Background: Renal impairment (RI) is reported in ≈ 20% to 40% of patients (pts) with multiple myeloma (MM) at diagnosis and is associated with a negative

impact on survival (Rajkumar, *Mayo Clin Proc,* 2005). The pivotal phase 3 FIRST trial represents the largest data set in transplant-ineligible newly diagnosed MM (NDMM) pts with various levels of RI. In this pt population, continuous lenalidomide plus low-dose dexamethasone (Rd continuous) improved progression-free survival (PFS; HR =0.72; *P* <0.01) and provided an overall survival (OS) benefit (HR =0.78; *P* =0.02) *vs* melphalan-prednisone-thalidomide (MPT; Benboubker, *N Engl J Med,* 2014).

Aims: This subanalysis was conducted to determine the impact of RI on PFS, OS, and time to second anti-myeloma Tx (AMT) in NDMM pts treated with Rd continuous or MPT.

Methods: NDMM pts ineligible for transplant were randomized to 3 Tx arms: Rd continuous until progression (n =535); Rd for 18 cycles (Rd18; 72 weeks; n =541); or MPT for 12 cycles (72 weeks; n =547). Enrolled pts were categorized according to their renal function; pts requiring dialysis were excluded. The starting dose of lenalidomide was modified based on renal function: 25 mg once daily (QD) for normal renal function (creatinine clearance [CrCl] ≥80 mL/min) or mild RI (CrCl ≥50 and <80 mL/min), 10 mg QD for moderate RI (CrCl ≥30 and <50 mL/min), and 15 mg QD for severe RI (CrCl <30 mL/min). The melphalan dose was reduced by 50% in pts with moderate or severe RI. The primary endpoint was PFS in pts treated with Rd continuous *vs* MPT. Secondary endpoints included OS, time to second AMT, safety, and improvement in renal function. Improvement in renal function was defined as a positive shift in renal function subgroup from baseline to the most extreme post-baseline CrCl value during active Tx. Renal response as defined by IMWG criteria (Dimopoulos, *J Clin Oncol*, 2010) was also assessed to show similar results across both CrCl and estimated glomerular filtration rate (eGFR).

Results: The median follow-up was 37 mos (data cutoff, May 24, 2013). PFS benefit was seen with Rd continuous *vs* MPT (HR =0.66-0.76) or Rd18 (HR =0.66-0.82) in all RI groups. OS results showed a benefit of Rd continuous *vs* MPT (HR =0.59-0.81) or Rd18 (HR =0.74-0.88) in all groups except severe RI (HR =1.03 *vs* MPT and 1.31 *vs* Rd18). Rd continuous compared with MPT extended time to second AMT in all renal groups (HR =0.54-0.83). Improvement in renal function was observed across all Tx groups, but appeared greater in pts treated with Rd continuous than those treated with Rd18 or MPT. Per IMWG criteria 24%, 28%, and 20% of pts had a renal response with Rd continuous, Rd18, and MPT Tx, respectively. Rate of complete renal response was 20.0% with Rd continuous, 27.4% with Rd18, and 14.3% with MPT. Most common grade 3/4 adverse events for these Tx were anemia, neutropenia, and thrombocytopenia.

Tabella 1. PFS, Interim OS, and Time to Second AMT in Renal Subgroups

	Normal Renal Function[a] (CrCl ≥ 80 mL/min) n (%) = 389 (24)	Mild RI[a] (CrCl ≥ 50 and < 80 mL/min) n (%) = 715 (44)	Moderate RI[a] (CrCl ≥ 30 and < 50 mL/min) n (%) = 372 (23)	Severe RI[a] (CrCl < 30 mL/min) n (%) = 147 (9)
Median PFS, mos				
Rd continuous	37.7	26.7	18.7	17.2
Rd18	24.6	21.0	18.7	12.1
MPT	24.9	21.4	17.5	11.7
3-y PFS, %				
Rd continuous	51	42	37	31
Rd18	35	22	13	12
MPT	31	24	17	11
PFS, HR; *P* Value[b]				
Rd continuous vs MPT	0.71 *P* = 0.05	0.74 *P* = 0.02	0.66 *P* < 0.01	0.76 *P* = 0.31
Rd continuous vs Rd18	0.67 *P* = 0.02	0.72 *P* < 0.01	0.66 *P* < 0.01	0.82 *P* = 0.48
Rd18 vs MPT	1.06 *P* = 0.72	1.04 *P* = 0.76	1.02 *P* = 0.89	1.00 *P* = 1.00
Median OS (at interim analysis), mos				
Rd continuous	NR	NR	43.7	33.2
Rd18	53.6	NR	41.8	42.6
MPT	NR	NR	38.5	42.9
OS, HR; *P* Value[b]				
Rd continuous vs MPT	0.59 *P* = 0.04	0.79 *P* = 0.14	0.81 *P* = 0.26	1.03 *P* = 0.92
Rd continuous vs Rd18	0.74 *P* = 0.26	0.88 *P* = 0.43	0.83 *P* = 0.34	1.31 *P* = 0.37
Rd18 vs MPT	0.79 *P* = 0.31	0.89 *P* = 0.48	0.98 *P* = 0.90	0.84 *P* = 0.54
Time to second AMT, mos				
Rd continuous	43.7	39.1	36.7	17.5
Rd18	31.2	29.9	24.1	23.7
MPT	31.3	27.8	21.8	18.1
Time to second AMT, HR; *P* Value[b]				
Rd continuous vs MPT	0.70 *P* = 0.05	0.67 *P* < 0.01	0.54 *P* < 0.001	0.83 *P* = 0.53
Rd continuous vs Rd18	0.79 *P* = 0.22	0.79 *P* = 0.07	0.56 *P* < 0.01	0.98 *P* = 0.95
Rd18 vs MPT	0.88 *P* = 0.46	0.84 *P* = 0.16	0.92 *P* = 0.62	0.94 *P* = 0.80

[a] According to Cockroft-Gault formula.

[b] Nominal *P* values are presented for multiplicity assessments, which need to be taken into consideration before making clinical interpretations.

Summary and Conclusions: PFS, interim OS, and time to second AMT outcomes generally improved across all Tx groups in pts with normal renal function and those with mild or moderate RI. Rd continuous was well tolerated, with a consistent safety profile across renal subgroups. Renal function also improved more in pts treated with Rd continuous *vs* Rd18 or MPT. Results of this analysis support the use of Rd in NDMM pts in most RI subgroups.

P275

AN OPEN-LABEL, MULTICENTER, PHASE 1B STUDY OF DARATUMUMAB IN COMBINATION WITH POMALIDOMIDE-DEXAMETHASONE AND WITH BACKBONE REGIMENS IN PATIENTS WITH MULTIPLE MYELOMA

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Background: Daratumumab (DARA) a human anti-CD38 IgG1κ MAb has shown robust, durable, single agent activity with deep responses and favorable safety in patients with multiple myeloma (MM) relapsed from or refractory (RR) to ≥2 prior therapy lines as well as in combination with lenalidomide (LEN) and dexamethasone (D) in relapsed or RRMM.

Aims: This ongoing open-label, 4-arm, multicenter, phase 1b study (NCT01998971) evaluated the safety and tolerability of DARA combined with pomalidomide (POM)-D and three backbone MM treatments: bortezomib (V)-D, V-thalidomide(T)-D, V-melphalan (M)-prednisone (P).

Methods: Patients (up to 100) in the DARA+POM-D arm were RR to ≥2 lines of therapy including 2 consecutive cycles of LEN and V. Newly diagnosed patients were included in the DARA+VD (n=6) and DARA+VTD (n=12) arms (irrespective of transplant eligibility) and DARA+VMP arm (transplant ineligible; n=12). Patients received DARA 16 mg/kg and approved label or standard of care of each treatment: POM-D (DARA qw, 2 cycles; q2w, 4 cycles; q4w remaining cycles); VD and VTD (DARA qw, 2 cycles; q3w, 16 cycles); VMP (DARA qw, 1 cycle; q3w, 8 cycles). Data were evaluated by an independent data safety monitoring board.

Results: Data from 49 patients with RRMM (DARA+POM-D [n=24]) and newly diagnosed MM (DARA+VD [n=6], DARA+VTD [n=11], DARA+VMP [n=8]) are presented. Median (range) durations of follow-up in days: DARA+POM-D, 29 (1-286); DARA+VD, 193 (151-218); DARA+VTD, 164 (9-274); DARA+VMP, 267 (63-295). There was little additional toxicity when DARA was added other than DARA-specific infusion related reactions (24/49 patients). Most occurred on Cycle 1 Day 1 and were mainly grade 1/2 (3/53 grade 3, no grade 4). All infusions were completed except 1 discontinuation (grade 3 hypoxia possibly DARA-related). Median (range) numbers of DARA infusions were: DARA+POM-D, 4 (1-20); DARA+VD, 13 (8-15); DARA+VTD, 8 (2-13); DARA+VMP, 16 (6-18). Other adverse events (AEs) were consistent with the non-DARA agents. 6 patients had serious AEs: pneumonia (n=3; possibly DARA-related; 1 likely POM-related), indirect Coombs test interference (n=1; DARA-related), and 1 each of non-DARA-related soft tissue infection, diarrhoea, pre-renal failure, hyperviscosity syndrome, cardiac failure, mental status change. The most common AEs were hematologic toxicity, likely related to POM or backbone therapies. Study discontinuations were 3 in the DARA+POM-D arm (death after disease progression [PD], physician decision, disease progression) and 1 in the DARA+VD arm (death; neutropenic sepsis, 60 d post-ASCT). Patients were not on study treatment at time of death. Efficacy dataset (n=35) had >1 post-baseline assessment: DARA+POM-D (n=11); DARA+VD (n=6); DARA+VTD (n=10); DARA+VMP (n=8). The overall response rates were 54.5% in the DARA+POM-D arm and 100% in the newly diagnosed groups. Responses deepened with continued treatment. In the DARA+POM-D arm there were 2 stringent complete responses, 1 very good partial response (VGPR), 3 partial responses (PR), 2 minimal responses, 2 stable disease, and 1 PD. In the DARA+VD arm there were 3 VGPRs and 3 PRs and in the DARA+VTD arm there was 1 complete response, 2 VGPRs and 7 PRs. In the DARA+VMP arm there were 4 VGPRs and 4 PRs. Median (range) times to first responses in days were: DARA+POM-D, 31 (29-57); DARA+VD, 23.5 (22-44); DARA+VTD, 22 (21-43); DARA+VMP, 22.5 (22-135).

Summary and Conclusions: DARA plus POM-D or with backbone MM therapies was well tolerated, with little significant additional toxicity, encouraging efficacy and deepening responses.

P276

OUTCOMES OF PATIENTS WITH PRIMARY REFRACTORY MULTIPLE MYELOMA IN THE ERA OF NOVEL THERAPIES

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Background: With the introduction of new drugs and use of multidrug combinations, lack of initial response to therapy in patients with newly diagnosed multiple myeloma (NDMM) has decreased considerably. Previous studies have shown that high dose therapy, in those eligible, is able to overcome resistance to conventional drugs, though long-term outcomes remain inferior among the rest of the patients. It is not clear if the outcomes of patients with primary refractory disease have changed with uniform introduction of newer therapies.
Aims: We performed this retrospective review to compare outcomes of patients with primary refractory multiple myeloma with outcomes of primary responders, specifically in the era of novel therapies. We also sought to identify characteristics predisposing to primary refractory disease.
Methods: We identified 816 patients with NDMM who started treatment after January 1, 2006, were seen at Mayo Clinic within 30 days of initiating treatment, and in whom details of the initial treatment regimen were available. Only patients who had consented to participation in research were included, and the study was performed with the approval of the Mayo Clinic IRB.
Results: The median age of the study cohort was 67 years; 59% were male. The median estimated follow up for the entire group was 4.3 years; 41% had died at the time of the analysis. The median overall survival (OS) for the entire group was 5.7 years from the beginning of treatment. Either a proteasome inhibitor or an IMiD was part of the initial induction therapy in 84% patients, with both an IMiD and a proteasome inhibitor combination used in 9%. The remaining 7% received steroid-based treatment, in most cases along with melphalan or cyclophosphamide. Response could be assessed in 669 (82%) patients, with a PR or better seen in 557 (83%) patients. Primary refractory disease (SD or progression) was seen in 112 patients (17%) overall, and was seen in 16% of those receiving a novel agent compared with 28% of those not receiving a novel agent (P <0.01). The median OS from start of therapy was significantly shorter for the primary refractory group at 3.6 years (95% CI 2.8-4.8) compared with 7.6 years (95% CI 6-8.6) for the responding patients (P <0.001). In a multivariable analysis including age >65 years, ISS stage 3, abnormal LDH, BMPC% >60%, PCLI >1%, serum creatinine >=2 mg/dL, high-risk FISH and primary refractoriness, only primary refractory disease, age >65, high-risk FISH and high LDH were independently prognostic for OS. Other than the lack of use of a novel agent, only a high-risk FISH was associated with primary refractoriness.

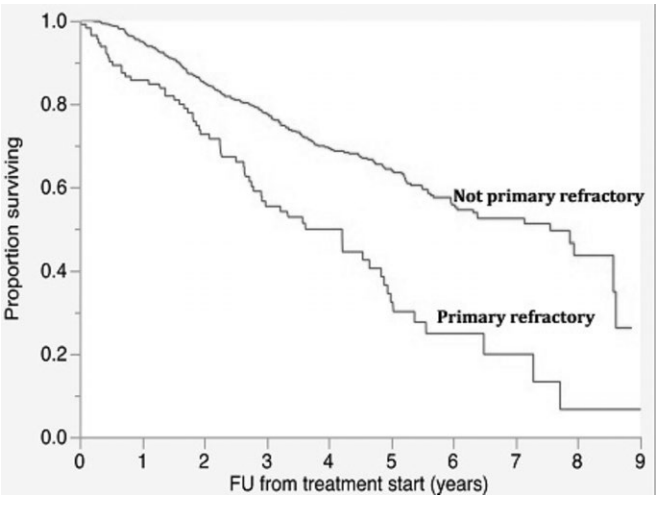

Figure 1.

Summary and Conclusions: In the current era, primary refractoriness to induction therapy with proteasome inhibitor or IMiD containing regimens is associated with significantly inferior outcomes among patients with NDMM. These patients, when identified, should be studied in clinical trials specifically designed for this high-risk condition.

Multiple myeloma - Clinical 2

P277

ASSESSING THE BENEFIT OF CONTINUOUS TREATMENT IN THE FIRST TRIAL (MM-020): IMPACT OF RESPONSE IN PATIENTS WITH TRANSPLANT-INELIGIBLE NEWLY DIAGNOSED MULTIPLE MYELOMA

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Background: Treatment (Tx) with melphalan, prednisone, and thalidomide (MPT) is a standard option for patients (pts) with newly diagnosed multiple myeloma (NDMM) who are ineligible for autologous stem cell transplant (SCT; Facon, *Lancet Oncol*, 2007; Fayers, *Blood*, 2011; NCCN Guidelines, Multiple Myeloma, V3.2015). The pivotal phase 3 FIRST trial assessed the efficacy of lenalidomide plus low-dose dexamethasone administered until disease progression or unacceptable toxicity (Rd continuous) *vs* Rd for 18 cycles (Rd18) and MPT in pts with NDMM ineligible for SCT. Rd continuous improved overall survival (OS), progression-free survival (PFS), and response rate *vs* MPT (Benboubker, *N Engl J Med,* 2014).
Aims: This analysis examined PFS and OS in pts treated with Rd continuous *vs* MPT and Rd18 by quality of response (ie, ≥very good partial response [VGPR]).
Methods: Pts with symptomatic NDMM who were not candidates for SCT were randomized 1:1:1 to Tx with Rd continuous (n =535), Rd18 (n =541), or MPT for 12 cycles (72 weeks; n =547). The primary endpoint was PFS. Secondary endpoints included OS, response rate (as assessed by International Myeloma Working Group criteria), time to response, duration of response (DOR), time to Tx failure, time to next antimyeloma Tx, health-related quality of life, and safety.
Results: As of the data cutoff date of May 24, 2013 for the final PFS analysis, the median follow-up was 37.0 mos. In pts who achieved a complete response (CR), greater benefit was observed with Rd continuous compared with MPT or Rd18 (Table); median PFS with Rd continuous was not reached *vs* 44.6 mos with MPT (HR =0.28; *P* <0.01) and 45.2 mos with Rd18 (hazard ratio [HR] =0.29; *P* <0.01). For pts who achieved ≥VGPR, PFS with Rd continuous (NR) was longer than with MPT (34.7 mos; HR =0.55; *P* <0.01) or Rd18 (31.0 mos; HR =0.46; *P* <0.01). Across all response categories (including CR), pts treated with Rd continuous *vs* MPT or Rd18 achieved longer DOR. The DOR in pts who achieved ≥partial response in the intent-to-treat population was 35 mos with Rd continuous, nearly a year longer than the DOR observed with MPT (22.3 mos; HR =0.63; *P* <0.01) or Rd18 (22.1 mos; HR =0.60; *P* <0.01). Pts who achieved a CR with Rd continuous had a longer DOR than whose who achieved a CR with Rd18 (NR *vs* 43.6 mos; HR =0.29; *P* <0.01), and results were similar for pts who achieved ≥VGPR (NR *vs* 29.9 mos; HR =0.46; *P* <0.01). A higher proportion of pts who achieved a CR were progression-free after 36 mos with Rd continuous (88%) *vs* Rd18 (55%) or MPT (62%). OS data for pts treated with Rd continuous *vs* MPT or Rd18 showed no significant differences by response.

Tabella 1.

PFS and DOR in Pts With NDMM	CR (n = 209)	≥ VGPR (n = 618)	≥ PR (n = 1140)	≤ SD (n = 483)	ITT (N = 1623)
Median PFS (mos)					
Rd continuous	NR	NR	37.7	3.7	25.5
Rd18	45.2	31.0	24.0	4.6	20.7
MPT	44.6	34.7	26.3	4.9	21.2
PFS, HR (95% CI); *P* value					
Rd continuous vs MPT	0.28 (0.13-0.61) *P* < 0.01	0.55 (0.40-0.76) *P* < 0.01	0.67 (0.55-0.82) *P* < 0.01	1.60 (1.22-2.11) *P* < 0.01	0.72 (0.61-0.85) *P* < 0.001
Rd continuous vs Rd18	0.29 (0.15-0.58) *P* < 0.01	0.46 (0.34-0.60) *P* < 0.01	0.60 (0.49-0.72) *P* < 0.01	1.10 (0.82-1.48) *P* = 0.53	0.70 (0.60-0.82) *P* < 0.001
Median DOR (mos)					
Rd continuous	NR	NR	35.0	–	35.0
Rd18	43.6	29.9	22.1	–	22.1
MPT	41.9	31.8	22.3	–	22.3
DOR, HR (95% CI); *P* value					
Rd continuous vs MPT	0.27 (0.13-0.59) *P* < 0.01	0.54 (0.39-0.74) *P* < 0.01	0.63 (0.51-0.76) *P* < 0.01	–	0.63 (0.51-0.76) *P* < 0.001
Rd continuous vs Rd18	0.29 (0.15-0.59) *P* < 0.01	0.46 (0.35-0.61) *P* < 0.01	0.60 (0.50-0.72) *P* < 0.01	–	0.60 (0.50-0.72) *P* < 0.001

CR, complete response; DOR, duration of response; HR, hazard ratio; ITT, intent-to-treat; MPT, melphalan, prednisone, and thalidomide; NR, not reached; PFS, progression-free survival; PR, partial response; Rd, lenalidomide plus low-dose dexamethasone; Rd18, lenalidomide plus low-dose dexamethasone for 18 cycles; SD, stable disease; VGPR, very good partial response.

Summary and Conclusions: For all responders, PFS and DOR were longer in pts treated with Rd continuous *vs* MPT or Rd18. The benefits of Rd continuous were more pronounced in pts who achieved a greater depth of response (≥VGPR). These data suggest that continuing Tx after achieving best response aids in delaying disease progression, particularly for those pts achieving a VGPR or CR. Longer follow-up may be required to assess the impact of continuous treatment on OS for good responders in pts receiving Rd continuous *vs* MPT or Rd18.

P278

LACK OF RESPONSE IMPROVEMENT BEYOND DAY +100 AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION IN ABSENCE OF MAINTENANCE THERAPY IN MULTIPLE MYELOMA

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Background: Patients with multiple myeloma (MM) achieving complete remission (CR) have a significant longer survival than patients who obtain less than CR. This is particularly established after high-dose chemotherapy followed by autologous stem-cell transplantation (ASCT). The response evaluation is usually done at day +100 after ASCT. Of interest, recent observations have shown that improvement in the response can be observed beyond day +100 after ASCT and that these late responders would have a better outcome than patients without this improvement.
Aims: The objective of the present study has been to evaluate the rate and outcome of patients who improve their response beyond day +100 after ASCT, with and without maintenance therapy.
Methods: One hundred and forty-four patients (63M/81F, median age 55 years; range 29 to 69) from Hospital Clínic de Barcelona (90) and Hospital Universitario de Salamanca (54) who underwent ASCT with melphalan-based conditioning between October 1992 and November 2013 were studied. The entry criteria were: 1) chemosensitive disease at the time of ASCT, 2) have received single ASCT, 3) less than CR at day 100 post-ASCT, and 4) minimum follow-up of one year. Medical records of all patients were reviewed. Responses were determined at 3, 6, 9 and 12 months after ASCT, according to the EBMT criteria. 27 (18.8%) of the patients had extramedullary disease at diagnosis.
Results: At day +100 post ASCT, 52.1% of the patients had achieved partial response (PR), 39.6% very good partial response (VGPR) and 8.3% were in minimal response. After a median follow-up of 66 months, the median OS for the whole series was 6.8 years (IC 95% 4.5 to 9) and the median PFS was 2.9 years (IC 95% 2.4 to 3.4). There were no significant differences in outcome between the two centers. Seventy four patients (51.4%) did not receive any maintenance and only one of them showed any upgrade in her response. This patient improved their response from VGPR to CR beyond 100 days after ASCT. The remaining 70 patients (48.6%) received maintenance therapy, mainly interferon (53; 36.8%), with glucocorticoids in 29 cases (20.1%); thalidomide (4.9%) or thalidomide plus bortezomib (6.9%). Twelve patients (8.3%), improved response beyond day +100 after ASCT: five of them with interferon (two associated with glucocorticoids), 4 bortezomib plus thalidomide and 2 single agent thalidomide. The outcome of these patients was better than those who did not upgrade their response in terms of PFS and OS (p=0.024 and p=0.01, respectively).
Summary and Conclusions: A minority of patients receiving maintenance therapy after ASCT upgrades their response and this finding is associated with better outcome. In contrast with previously published data, in our series the improvement in response beyond day +100 after ASCT in patients non receiving maintenance therapy is exceedingly rare. This fact must be taken into account when making treatment decisions based on the response status after ASCT.

P279

RICOLINOSTAT (ACY-1215) THE FIRST SELECTIVE HDAC6 INHIBITOR IN COMBINATION WITH POMALIDOMIDE AND DEXAMETHASONE IN PATIENTS WITH RELAPSED & REFRACTORY MULTIPLE MYELOMA: PHASE 1B & EARLY PHASE 2 RESULTS

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Background: Ricolinostat, an oral selective HDAC6 inhibitor, is well tolerated as monotherapy (Raje *Blood* 2012;120:4061) and demonstrates potent synergistic activity with lenalidomide (Len) and pomalidomide (Pom) in preclinical models (Quayle *Blood* 2013;122:1952). Pan-HDAC inhibitors vorinostat (Dimopoulos *Lancet Oncology* 2013;14:1129-40) and panobinostat (San-Miguel *Lancet Oncology* 2014;15:1195-206) are active in multiple myeloma (MM) in combination with bortezomib (Btz) and Len, but have toxicities (thrombocytopenia, fatigue, and gastrointestinal events) which limit dosing exposure. Ricolinostat, in combination with Len and dexamethasone (Dex) demonstrates clinical activity and a manageable safety profile at doses up to 160 mg twice daily (BID) for 21 consecutive days (Yee *Blood* 2014;124:4772).
Aims: ACE-MM-102 is a phase 1b/2 trial in relapsed and refractory MM patients who have received at least two cycles of a proteasome inhibitor and Len containing regimen and who have progressed on or within 60 days of the last therapy.
Methods: Phase 1b was a 3+3 design which explored ricolinostat 160 mg daily (QD) orBID combined with Pom (4 mg) for 21 days of a 28 day cycle with Dex (40 mg) on days 1, 8, 15 and 22. Patients had measurable disease, adequate bone marrow reserve and hepatic function with creatinine clearance >45 mL/min. Patients with non-secretory MM, prior Pom or HDAC inhibitor therapy were excluded. Bone marrow cytogenetics were obtained at entry. Peripheral blood samples were obtained for pharmacokinetic (PK) and pharmacodynamic (PD) assessment of acetylated tubulin and histones. Patients with serum free light chain-only disease were excluded from phase 2.
Results: 7 patients were treated in phase 1b: 3 at 160 mgQD and 4 at 160 mgBID. Median age was 66 and the majority received >3 lines of prior therapy. No dose limiting toxicities (DLTs) were observed. At 160 mg QD, 1 patient had grade 3 fatigue and 1 patient had grade 3 neutropenia both attributed to Pom. In the 160 mgBID cohort, all 4 patients had grade 2 diarrhea and 2 of 4 patients had grade 4 neutropenia attributed to Pom. 1 patient continues on study after 10 cycles; the other 6 phase 1b patients were withdrawn for progressive disease at 2-4 cycles. Following safety review committee (SRC) review, phase 2 opened at a dose of 160 mg BID, with SRC review to occur after 6 patients had completed one cycle of therapy. 3 of the 6 patients had grade 3 diarrhea requiring ricolinostat dose reduction in cycle 2. Other treatment emergent toxicities included fatigue, neutropenia, hypertension, thrombocytopenia and anemia that were mostly low grade. Although no DLTs were observed, reduction of the dose from 160 mgBID toQD for ongoing and future patients was recommended to maintain treatment at a therapeutic exposure for as long as possible. PK of ricolinostat was similar to that observed in combination with Btz and Len. There was no evidence of ricolinostat accumulation or drug-drug interaction with Pom.As of February 2015, 27 patients have been enrolled in phase 2; 11 were dose reduced toQD after initial 160 mgBID dosing and 16 started at 160 mg QD. 6 patients have withdrawn due to progressive disease (2), low-grade AEs (2), physician decision (1) or patient decision (1). 21 phase 2 patients remain on study after a median of 2 (1-5) cycles.
Summary and Conclusions: Ricolinostatappears well tolerated and can be safely administered in combination with Pom and Dex in patients with relapsed and refractory MM.Updated safety, PK, PD and preliminary clinical activity data will be presented as phase 2 enrollment continues.

P280

PROGRESSION-FREE SURVIVAL 2 (PFS2) IS THE MOST SIGNIFICANT PROGNOSTIC FACTOR OF LONG-TERM SURVIVAL IN THE ERA OF NOVEL AGENTS: A SINGLE GREEK MYELOMA CENTER EXPERIENCE

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Background: The term "long-term survivor" (LTS) in Oncology refers to patients alive for more than 10 years and according to published studies conducted before the use of novel agents, 3-4% of Multiple myeloma (MM) patients belong to this group. The current literature include very limited data regarding LTS in the era of novel agents.
Aims: The aim of this study was to described the incidence, disease characteristics and prognostic factors of LTS MM patients.
Methods: We checked the database of a single Greek myeloma center for the presence of LTS. Pearson's χ^2test, Mann-Whitney *U* test, and One-way ANOVA were used for comparisons; $p<0.05$ was considered statistically significant. Progression Free Survival (PFS) was estimated from the time of MM diagnosis until progression or death from any cause; OS was estimated from the time of MM diagnosis until the last follow up or death; PFS2 was defined as the time from MM diagnosis to second objective disease progression, or death from any cause whichever first. Prognostic factors for LTS were determined with a cox regression model.
Results: Among 648 consecutive MM patients diagnosed and treated between 1989-2013, we identified 50 LTS (7.7%; M/F: 23/27, median age: 65 years,

range: 47-72 years). LTS were younger and most of them presented with ISS stage I/II compared to all others who served as controls (p<0.001). In addition, LTS had higher hemoglobin, lower creatinine and lower β2 microglobulin at diagnosis compared to controls (p<0.05). Bence Jones proteinuria and immunoparesis at diagnosis presented less frequently in LTS; recovery of immunoparesis after 1st line treatment was more common in LTS (p<0.05); 72% of LTS received novel agent-based combinations (NAC) during the course of MM. The number of patients treated with NAC and the response rate after 1st line therapy, did not differ between LTS and the control group; LTS received more frequently autologous transplantation at 1st line, NAC at 2nd line and IMiD-based regimens at any line, compared to controls (p<0.05). After a median follow up of 12 years (range: 2-180 months), 28/50 LTS *vs* 84/598 MM patients were alive (p<0.001); 13 LTS patients died from MM and 9 patients died from irrelevant causes. The median PFS and OS for LTS *vs* controls was 81 months (95% CI: 56-105) *vs* 17 months (95% CI: 15-19) and 200 months (95% CI: 180-219) *vs* 33 months (95% CI: 30-36), respectively (p<0.05). In the univariate analysis PFS, PFS2, period of diagnosis (*i.e.* before or after 2000), 1st line treatment, 2nd line treatment and treatment at any line with conventional therapy *vs* NAC were independent predictors of long-term survival (p<0.05). Baseline disease characteristics or quality of response did not predict for LTS. In the multivariate analysis, PFS2 was the only positive predictor for long-term survival (p<0.001; HzR: 0.98. 95% CI: 0.96-0.99); 28% of patients with PFS2 ≥4 years were alive after 10 years compared to 0.2% of those with PFS2 <4 years.

Summary and Conclusions: The percentage of LTS has increased in the era of novel agents. LTS present with more favorable disease characteristics at diagnosis compared to other MM patients, but none of those predicted for long-term survival. PFS2 proved to be the most significant prognostic factor of long-term survival, whereas quality of response failed to show a prognostic value, suggesting that disease control during the initial phase of MM, where sensitive clones still prevail and there is no drug resistance, should be the main goal of the treatment strategy. Finally, 1/3 of patients who displayed disease control of ≥4 years after 2 treatment lines, enjoyed a long-term survival and most importantly, more than 20% of them could be considered as "cured" from MM, challenging the dogma of incurable disease.

P281

OBESITY AND RISK OF MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE: A POPULATION-BASED STUDY

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Background: All multiple myeloma (MM) cases are preceded by the premalignant state, monoclonal gammopathy of undetermined significance (MGUS), an asymptomatic condition that needs no treatment. The etiology of MGUS and MM is to a large extent unknown. Two studies on the association between obesity and MGUS have been conducted with conflicting results, despite a reported association between obesity and MM.

Aims: The aim of this study was to determine if obesity is associated with an increased risk of MGUS and light-chain MGUS (LC-MGUS) in a population-based screened cohort of individuals above the age of 65 years using extensive number of markers for current and early life obesity. Secondly, the aim was to examine whether progression to MM was affected by obesity.

Methods: This study was based on participants from the Age, Gene/Environment Susceptibility-Reykjavik Study (AGES-RS), consisting of 5764 men and women above the age of 65 years. Serum protein electrophoresis (SPEP) and serum free light-chain assay were performed on all subjects to identify MGUS and LC-MGUS. Obesity measures were performed at baseline, and participants were additionally asked about their weight at the age of 55 and 25 years and their lifetime max weight. The measures at baseline included were weight (kg), body mass index (BMI) (kg/m^2), percent body fat, fat (kg), fat-free mass (kg), waist circumference (cm), total body fat area (cm^2), and visceral and subcutaneous fat area (cm^2). Weight was measured using a digital scale and height using a stadiometer, measures were performed multiple times and a mean number found. Percent body fat, fat and fat free mass were calculated from bioelectric impedance, a commonly used method for estimating body composition. Computed tomography imaging of the abdomen was performed to calculate total body fat area, visceral fat area, subcutaneous fat area, and abdominal circumference. Abdominal circumference was additionally measured multiple times and a mean number found. The participants were followed prospectively until 2013 and information on MM diagnosis was collected through hospitals, nursing homes, and mortality records. The association with MGUS and LC-MGUS was analyzed using logistic regression and Cox proportional-hazard regression was performed to test whether progression to MM was affected by obesity. Adjustment was made for age and gender.

Results: A total of 299 (5.2%) MGUS cases and 33 (0.6%) LC-MGUS cases were identified. No association was found between any of the obesity markers and MGUS or LC-MGUS. Results for MGUS, presented as OR with 95% CI, were: $BMI_{baseline}$ 0.99 (0.97-1.02), BMI_{55y} 1.01 (0.97-1.05), BMI_{25y} 0.99 (0.94-1.04), weight 1.00 (0.99-1.01), max weight 1.00 (0.99-1.01), percent body fat 0.99 (0.97-1.02), fat 1.00 (0.98-1.02), fat free mass 1.00 (0.98-1.02), total body fat 1.00 (1.00-1.00), visceral fat 1.00 (1.00-1.00), subcutaneous fat 1.00 (1.00-1.00), CT waist circumference 1.00 (0.99-1.01), and measured waist circumference 1.00 (0.99-1.01). BMI ≥25 at the age of 55 affected MM progression (HR 3.20; 95% CI 1.15-8.88) (Table).

Tabella 1. Effect of obesity on MGUS and risk of progression

	No MGUS	MGUS	LC-MGUS	OR* (95%CI)	OR^L (95%CI)	HR¶ (95%CI)
BMI (n)						
<25	1795	100	16	Ref.	Ref.	Ref.
25-30	2331	146	11	1.15 (0.88 - 1.49)	0.55 (0.25 - 1.21)	1.88 (0.59 - 6.00)
≥30	1206	49	6	0.81 (0.57 - 1.16)	0.75 (0.29 - 1.95)	1.53 (0.34 - 6.92)
BMI 25y (n)						
<25	4019	217	19	Ref.	Ref.	Ref.
≥25	828	44	6	0.87 (0.62 - 1.22)	1.13 (0.44 - 2.89)	0.77 (0.17 - 3.49)
BMI 55y (n)						
<25	2849	152	15	Ref.	Ref.	Ref.
≥25	1932	105	10	0.95 (0.73 - 1.24)	0.80 (0.35 - 1.80)	3.20 (1.15 - 8.88)
BMI, mean (kg/m^2)	27.05	26.73	26.01	0.99 (0.97 - 1.02)	0.96 (0.88 - 1.05)	1.03 (0.92 - 1.15)
BMI 25y, mean (kg/m^2)	22.76	22.92	22.84	0.99 (0.94 - 1.04)	0.91 (0.76 - 1.08)	1.08 (0.97 - 1.25)
BMI 55y, mean (kg/m^2)	24.64	24.88	24.34	1.01 (0.97 - 1.05)	0.94 (0.82 - 1.07)	1.12 (0.98 - 1.29)
Weight, mean (kg)	75.38	75.51	75.44	1.00 (0.99 - 1.01)	0.99 (0.96 - 1.02)	1.02 (0.99 - 1.06)
Max weight, mean (kg)	80.81	82.46	84.40	1.00 (0.99 - 1.01)	1.00 (0.97 - 1.03)	1.03 (1.00 - 1.06)
Percent body fat, mean (%)	28.93	26.71	21.89	0.99 (0.97 - 1.02)	0.95 (0.88 - 1.03)	1.05 (0.92 - 1.19)
Fat, mean (kg)	21.93	20.59	17.47	1.00 (0.98 - 1.02)	0.96 (0.90 - 1.04)	1.05 (0.96 - 1.13)
Fat free mass, mean (kg)	53.47	55.53	60.05	1.00 (0.98 - 1.02)	1.00 (0.94 - 1.06)	1.08 (0.99 - 1.17)
Total body fat area, mean (cm^2)	494.41	483.00	459.06	1.00 (1.00 - 1.00)	1.00 (1.00 - 1.00)	1.00 (1.00 - 1.00)
Visceral fat area , mean (cm^2)	172.58	175.24	182.58	1.00 (1.00 - 1.00)	1.00 (0.99 - 1.00)	1.00 (1.00 - 1.01)
Subcutaneous fat area, mean (cm^2)	256.66	241.07	205.22	1.00 (1.00 - 1.00)	1.00 (0.99 - 1.00)	1.00 (1.00 - 1.01)
CT waist circumference, mean (cm)	125.83	125.78	123.21	1.00 (0.99 - 1.01)	0.99 (0.97 - 1.01)	1.02 (0.98 - 1.05)
Waist circumference, mean (cm)	100.84	100.81	99.99	1.00 (0.99 - 1.01)	0.99 (0.96 - 1.02)	1.03 (0.99 - 1.07)

*Effect of obesity on MGUS. Logistic regression, adjusted for age and gender

^{L}Effect of obesity on LC-MGUS. Logistic regression, adjusted for age and gender

¶Effect of obesity on progression from MGUS to MM. Cox survival analysis, adjusted for age and gender

Abbreviations: BMI - Body Mass Index, OR - Odds Ratio, HR - Hazard Ratio, CI - Confidence Interval.

Summary and Conclusions: In this large population-based cross-sectional study aimed at evaluating the association between obesity and MGUS, we did not find an association between several obesity markers and MGUS or LC-MGUS. However, BMI ≥25 at the age of 55 years seemed to affect progression from MGUS to MM. Future studies are needed to clarify underlying mechanisms for this finding. Taken together, we were unable to confirm the previously reported association between MGUS and obesity.

P282

POST AUTOLOGOUS STEM CELL TRANSPLANTATION MAINTENANCE IN MULTIPLE MYELOMA. SINGLE CENTER EXPERIENCE OF 18 YEARS: A MARKED SURVIVAL ADVANTAGE WITH INTERFERON

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Background: Interferon (IFN) maintenance was widely used in multiple myeloma (MM) during the 90s and early 2000s but then was mostly abandoned. The reasons of its decline were side effects, trials showing only marginal or no benefit and the emergence of new, highly effective drugs. However maintenance still has a very significant role in MM, and in this setting, drugs modulating the immune system play the most important part.

Aims: To study the effect of maintennce on post ASCT survival we analyzed the outcome of post-ASCT maintenance in 548 MM patients transplanted in our centre over 18 years.

Methods: Progression free survival (PFS) was calculated from the day of ASCT and overall survival (OS) from the first chemotherapy. The analyses were carried out using the SPSS (version 20.0) software package.

Results: 93 (17%) patients had IFN, 127 (23.2%) thalidomide (thal) at the discretion of the treating physician. 42 (7.7%) had both drugs as maintenance for a period of time mostly subsequently, but sometimes parallel. Maintenance strategies changed over the years. Between 2001 and 2008 half of the patients had maintenance. IFN was used from 1998 to 2011, but the majority of the patients had it between 2002 and 2008. Thal was increasingly used from 2000 until 2008, and then it was only occasionally employed in patients with poor response. Post-ASCT maintenance was more common after no-novel agent and thal based protocols. Thal was used in 34.1% after thal based, 12.2% after bortezomib and in 33.1% following no-novel agent based induction. IFN was employed in 20.5% after thal, 9.2% after bortezomib and 24.8% after no novel agent containing protocols. Patients with any maintenance had a 7 month PFS benefit [33 (28.9−37.1) *vs* 25.9 (22.6−29.2) months; p=0.044] and a 15 month OS advantage [101.4 (91.3−111.5) *vs* 86.1 (71.5−100.7); p=0.070]. With thal the difference was not significant, however with IFN there was a significant PFS [35.5 (31.3−39.7) *vs* 25.9 (22.6−29.2); p=0.017] and an even more pronounced OS [122.4 (79.6−165.1) *vs* 86.1 (71.4−100.8); p=0.006] benefit (Figure

1). The patients having the best results were those who had both maintenance drugs [PFS 38.7 (15.5−61.9), OS 150.4 (102.2−198.6)]. The positive effect of IFN maintenance was more prominent in patients with PR (OS benefit 52.8 months; p=0.01) than those in CR (OS difference 39.7 months; p=0.088). IFN maintenance was confirmed as a highly significant independent protective factor in multivariate analysis (PFS p=0.030; OS p=0.003) while thal was not (PFS p=0.736; OS p=0.165).

Fig 1.

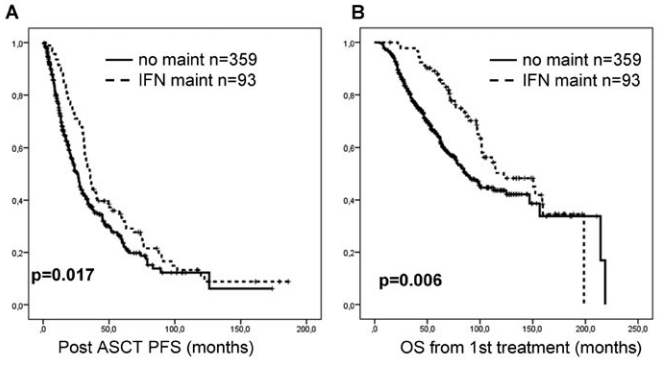

Figure 1.

Summary and Conclusions: The marked survival advantage in the IFN treated patients was beyond what the randomized interferon trials conducted back in the nineties indicated. Although, patient selection might have had a role in this, analyzing the distribution of presentation prognostic markers and post ASCT responses revealed no detectable imbalances. One possible explanation could be an interplay between thal and IFN, two drugs both exploiting the immune system in different ways.

P283

WHOLE BODY DIFFUSION WEIGHTED MRI SCANNING IN PATIENTS WITH MYELOMA. INCIDENTAL FINDINGS-THE ROYAL MARSDEN EXPERIENCE

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Background: The recent consensus statement from the International Myeloma Working Group has introduced the role of whole body MRI in the management pathway for patients with multiple myeloma. Whole body MRI is now recommended for all patients suspected of having smouldering or asymptomatic myeloma (Dimopoulos JCO 2015). The speed, coverage and high sensitivity of whole body diffusion weighted MRI (WB DW-MRI) and the capability to quantify both burden of disease and response to treatment has led to increasing implementation at leading centres worldwide for imaging malignant marrow disease. We have been offering a WB DW-MRI service for patients with myeloma since 2011. However the high sensitivity of this imaging technique will undoubtedly lead to detection of incidental findings.
Aims: The aim of this study was to determine the incidence of incidental and extramedullary findings from WB DW-MRI studies performed in myeloma patients.
Methods: Between January 2012 and October 2014 177 WB DW-MRI examinations were performed for 80 patients with myeloma at the Royal Marsden Hospital, UK. Scans and reports were retrospectively reviewed to determine whether the patient had active marrow involvement, extramedullary disease and if incidental findings were identified. Advice for management of equivocal findings was also documented.

Tabella 1.

Indeterminate findings	Number of examinations
Cutaneous nodule	2
Liver lesion	3
Lymphadenopathy	2
Hydronephrosis	1
Paratesticular mass query hernia	1
Adrenal nodule	1
Solid thyroid nodule	1
Rectal lesion	1
Colitis	1
Lung abnormality	1
Summary of non-equivocal findings	**Number of examinations**
Vertebral / sacral abnormality ie fracture or haemangioma	9
Other benign visceral or pulmonary abnormality ie pyelonephritis, cysts, gallstones	18
Other MSK / spinal abnormality ie avascular necrosis or degenerative	29

Results: 132/177 (75%) WB DW-MRIs showed evidence of active marrow involvement (80 focal, 45 diffuse, 7 both). 18/177 (10%) examinations in 9 patients identified extramedullary myeloma in the liver in 7 examinations, in muscles in 12 examinations, in the retroperitoneum in 4 examinations and in the dura in 1 examination. This was confirmed by response to treatment at follow up in all cases. 67/177 (38%) examinations in 47 patients had incidental findings. Some examinations identified multiple incidental findings so a total of 70 incidental findings were documented. 14/70 incidental findings (20%) were indeterminate. Guidance on further management of indeterminate lesions was given in 13/14 examinations in 14 patients (93%). Half of these indeterminate findings were addressed by clinical correlation and the remaining half required further investigation. The incidental findings are summarised in the table:

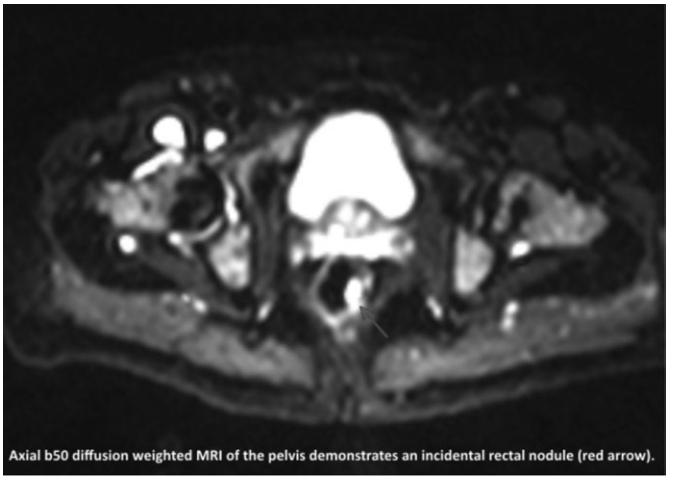

Figure 1.

Summary and Conclusions: Incidental findings are common in WB DW-MRI examinations but only a minority are equivocal requiring further action. The use of WB DW-MRI in patients with myeloma is likely to increase and hence we will need to develop strategies to manage incidental findings. The reporting radiologist should facilitate this by specifically expressing a level of concern and making suggestions regarding the need for further investigations. WB MRI is also likely to increase detection of asymptomatic extramedullary myeloma which may change our perspective of the disease.

P284

PATTERNS OF TOTAL COST OF CARE BY AGE GROUP FOR PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA (MM)

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Background: Clinical evidence supporting longer treatment (Tx) durations for patients (pts) with newly diagnosed multiple myeloma (NDMM) raises economic questions. Previous analysis showed that relapsed myeloma pts incurred higher monthly costs once they advanced to later lines of Tx (Gaultney, *J Clin Pharm Ther*, 2013). There is limited information on the cost patterns of MM pts by age group, before and after their first relapse.
Aims: This claims analysis evaluated patterns of total costs of care, from Tx initiation until progression, for NDMM pts and for newly relapsed pts by age group, across Tx, using time to next therapy (TTNT) as a proxy measure for progression.
Methods: Using a large US claims database, NDMM pts were identified based on the first claim associated with a diagnosis of MM (ICD-9-CM code 203.0X) between 2006 and 2013. Pts with claims for stem cell transplant (SCT) were excluded. This cost analysis focused on NDMM and relapsed MM pts, aged <75 and ≥75 yrs, receiving lenalidomide (LEN)-or bortezomib (BORT)-based Tx, where complete claim history was available from Tx onset to initiation of subsequent Tx. Using methods similar to those described by Gaultney (*J Clin Pharm Ther,* 2013), pts' average monthly drug and medical costs were determined, and differences in baseline measures between pt groups were evaluated using the Charlson Comorbidity Index (CCI).
Results: 2843 NDMM pts (1862 pts aged <75 yrs; 981 pts aged ≥75 yrs) and 1361 second-line MM pts (978 pts aged <75 yrs; 383 pts aged ≥75 yrs) were identified. For 41% and 29% of NDMM and second-line pts, respectively, complete data through initiation of subsequent Tx was available and used for cost determination. Monthly total costs for NDMM pts were $15,734 in the first 3 mos of Tx and declined each quarter to approximately $5000/mo at 18+ mos. For second-line therapy, initial monthly costs were more than $13,876 and also declined quarterly (Figure 1). Quarterly cost reduction patterns were consistent

across Tx, lines of therapy, and age groups. TTNT for NDMM pts was 28.1, 24.7, and 33.6 mos for the overall, age <75 yrs, and age ≥75 yrs populations, and TTNT was longer in pts initiated on LEN-based regimens compared with those initiated on BORT. We compared cohorts of pts initiated on each Tx and followed them for a common time period equal to the longer median TTNT of the 2 Tx (LEN): 35 mos in pts aged <75 yrs and 39 mos in pts aged ≥75 yrs. Monthly costs averaged $7941 in the LEN cohort *vs* $12,261 in the BORT cohort of pts aged <75 yrs. For pts aged ≥75 yrs, monthly costs averaged $6879 in the LEN cohort *vs* $7544 in the BORT cohort. Cost differences were due to higher average monthly medical costs for pts treated with BORT; average monthly drug costs were similar. CCI scores were similar across Tx.

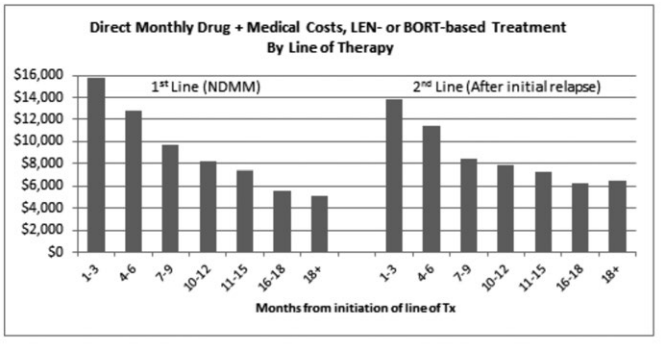

Figure 1.

Summary and Conclusions: For a population of NDMM pts receiving either LEN-or BORT-based Tx without SCT, followed until TTNT, total monthly drug and medical costs per pt declined steadily over time, decreasing by two-thirds over the TTNT period. Costs returned to near-baseline levels when pts began second-line therapy, then declined similarly. This pattern of cost decline was consistent regardless of age, Tx, or line of therapy. This may suggest that further extending the time to progression for NDMM pts may yield economic benefits for each month of extension before relapse. Average monthly total costs over 3 years were lower for NDMM pts initiated on LEN-based Tx due to longer periods with below-average costs prior to initiating second-line therapy.

P285

ENCOURAGING PRELIMINARY DATA IN ONGOING OPEN-LABEL PHASE 1/2 STUDY OF SAFETY AND EFFICACY OF MELFLUFEN AND *DEXAMETHASONE FOR PATIENTS WITH RELAPSED AND RELAPSED-REFRACTORY MULTIPLE MYELOMA

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Background: Melflufen is a potent and highly lipophilic alkylator designed for efficient targeting of tumor cells. In contrast to other alkylating agents that are hydrophilic, the lipophilicity of melflufen leads to rapid and extensive distribution into tissues and cells where it can bind directly to DNA or is readily metabolized by intracellular peptidases into melphalan. With targeted delivery of alkylating metabolites to tumor cells (such as multiple myeloma) melflufen exerts a higher anti-tumor activity compared with standard of care alkylator melphalan but with a similar safety profile.
Aims: To study the safety and efficacy of melflufen and dexamethasone (dex) in combination for patients with relapsed-refractory multiple myeloma (RRMM).
Methods: Melflufen is evaluated in combination with low dose dex in a Phase 1/2 study in RRMM. Phase 1 evaluated 4 dose levels of melflufen on day 1 with dex on days 1, 8 and 15 of 21 day cycles in a standard 3+3 design, with an additional 20 patients added at the MTD in Phase 2.
Results: Phase I was completed in September 2014. No dose limiting toxicities (DLTs) were observed in the first three dose cohorts (15, 25 and 40 mg melflufen). 4 of 6 patients at the highest dose, 55 mg, experienced DLTs of prolonged and severe neutropenia and thrombocytopenia, manageable with dose delays and appropriate treatment. The maximum tolerated dose (MTD) was established at 40 mg melflufen every 21 days combined with 40 mg dex weekly. As of 16 Jan 2015, 16 patients were treated at the MTD. Median time from initial diagnosis to first dose of melflufen was 5.5 years (1-15). Median number of prior therapies was 4 (2-11). All patients had been exposed to IMiDs, proteasome inhibitors and alkylators and 15 were at least single-refractory. 8 were double-refractory and 6 were alkylator-refractory. Treatment-related Grade 3 and 4 adverse events were reported in 69% of patients; neutropenia 50%, thrombocytopenia 44%, fatigue and leukopenia 12% each, anemia, febrile neutropenia and lymphopenia each occurred in 6% of patients. 10 patients were evaluable for response (defined as received ≥2 cycles with appropriate assessments). The overall response rate (≥PR) was 60%. The clinical benefit rate (≥MR) was 70%. Time to initial clinical benefit and response was rapid with 70% of patients achieving MR or better after only 1-2 cycles, and 50% of patients responding (≥PR) after 1-3 cycles. Of note, 1 patient that only achieved SD to previous melphalan 200 mg/m^2 with ASCT responded with a PR following only 1 cycle of 40 mg melflufen. Four patients discontinued treatment following 10, 6, 4 and 2 cycles for reasons other than progression. Three continue to maintain response off therapy, at time of data cut for 9.5, 9.2 and 4.6 months respectively. The fourth patient continues to maintain SD, 9.6 months at time of datacut. Median time on treatment was 3.4 months (1.3-7.8) with 5 patients still ongoing.
Summary and Conclusions: Melflufen plus low dose dex is tolerated and has promising activity in this advanced RRMM population. The MTD was established at 40 mg every 21 days with dex 40 mg weekly. In the first 10 evaluable patients treated at the MTD, all heavily pre-treated, 6 responded to treatment with PR. Responses are rapid and durable. Based on these early results the protocol will be amended to include 55 patients in Phase 2 to further characterize response. Updated information will be presented at the time of the conference.

P286

ANALYSIS OF PATIENTS WITH REFRACTORY OR RELAPSED AND REFRACTORY MULTIPLE MYELOMA AND RENAL IMPAIRMENT TREATED WITH POMALIDOMIDE + LOW-DOSE DEXAMETHASONE IN THE PHASE 3B STRATUS TRIAL (MM-010)

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Background: Overall survival (OS) of patients (pts) with refractory or relapsed and refractory multiple myeloma (RRMM) has been extended by treatment (Tx) with newer agents, such as lenalidomide (LEN) and bortezomib (BORT); however, shortened OS is observed in pts who relapse on or who become refractory to Tx (Kumar et al, *Leukemia*, 2012). Renal impairment (RI), a major cause of death in this pt population, (Korbet et al, *J Am Soc Nephrol,* 2006) occurs in ≈ 20% to 40% of MM pts (Kastritis et al, *Haematologica,* 2007). In RRMM pts who failed LEN and BORT Tx, pomalidomide + low-dose dexamethasone (POM + LoDEX) extended progression-free survival (PFS) and OS *vs* high-dose dexamethasone in the phase 3 MM-003 trial. This included pts with moderate RI (creatinine clearance [CrCl] <60 mL/min; Weisel et al, *J Clin Oncol,* 2013). Efficacy of POM + LoDEX was similar across renal function subgroups in the MM-002 trial, in which pts with serum creatinine ≥3 mg/dL were excluded (Vij et al, *Clin Lymphoma Myeloma Leuk*, 2013).

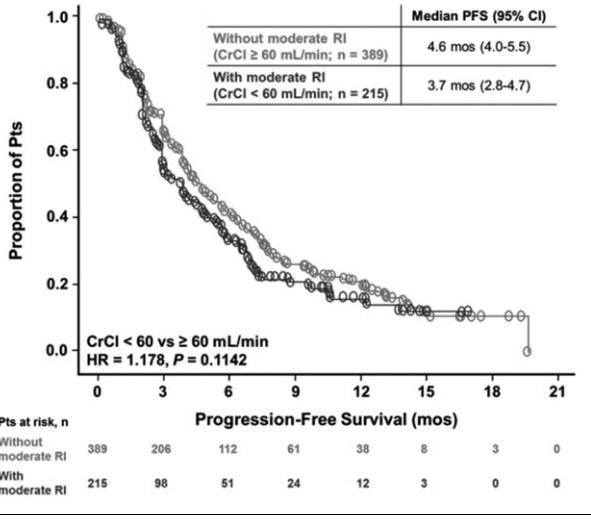

Figure 1.

Aims: STRATUS is a multicenter, single-arm, open-label phase 3b trial designed to further evaluate the safety and efficacy of POM + LoDEX in RRMM pts, including those with varying degrees of renal insufficiency.
Methods: RRMM pts (progressive disease [PD] during or within 60 days of last line of Tx), aged ≥18 yrs, with measureable M-protein levels in urine or serum and ≥2 prior Tx, who failed LEN and BORT after ≥2 cycles of each (alone or in combination), and who received adequate prior alkylator Tx were eligible. Pts with CrCl <45 mL/min were excluded. POM 4 mg was administered D1-21/28-day cycle and LoDEX 40 mg/day (20 mg for pts aged >75 yrs) on D1, 8, 15, and 22 until PD or unacceptable toxicity. Thromboprophylaxis was required for all pts. Primary endpoint was safety, and key secondary endpoints included, overall response rate (ORR; ≥partial response), duration of response (DOR), PFS, OS, and POM exposure.
Results: As of Sep 15, 2014, 604 pts were enrolled; 599 received POM + LoDEX, and 36% had moderate RI (CrCl <60 mL/min) at baseline. After a median follow-up of 9.3 mos, the most frequently reported grade (Gr) 3/4 hematologic treatment-emergent adverse events (TEAEs) in pts with moderate RI *vs* those without moderate RI (CrCl ≥60 mL/min) were neutropenia (39% *vs* 44%), anemia (33% *vs* 27%), and thrombocytopenia (22% *vs* 21%). The most frequent Gr 3/4 non-hematologic TEAE was infection (29% *vs* 30%). Dose reductions due to TEAEs were similar in pts with moderate RI *vs* those without moderate RI (19% *vs* 17%); 12% *vs* 7% discontinued POM due to TEAEs. Gr 3/4 venous thromboembolic events and peripheral neuropathy were infrequent, regardless of renal status. Median relative POM dose intensity was similar between subgroups (0.94 in pts with moderate RI *vs* 0.93 in pts without moderate RI). In pts with moderate RI *vs* those without moderate RI, ORR was 37% *vs* 33%, median DOR was 6.0 *vs* 7.9 mos, and median PFS was 3.7 *vs* 4.6 mos (*P* =0.1142; Figure). The median Tx duration for pts with moderate RI *vs* without moderate RI was 3.7 *vs* 4.3 mos. Updated data will be presented at the congress.
Summary and Conclusions: This analysis demonstrated that in pts with moderate RI, POM + LoDEX has a manageable safety profile and is efficacious. Tolerability and efficacy were similar in pts with or without moderate RI and consistent with results from the pivotal MM-002 and MM-003 trials. There was a trend toward longer PFS in pts without moderate RI. Two ongoing trials, MM-008 (US) and MM-013 (EU), are evaluating RRMM pts with severe RI.

Multiple myeloma - Clinical 5

P287

PROGNOSTIC VALUE AND USEFULNESS OF SERUM-FREE-LIGHT-CHAINS-RATIO (SFLCR) AND SERUM HEAVY-LIGHT-CHAINS-RATIO (SHLCR) IN SYMPTOMATIC MULTIPLE MYELOMA IN THE CONTEXT OF THREE GEM/PETHEMA CLINICAL TRIALS

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Background: Several studies have analysed the prognostic impact of high values of sFLC or sHLC and their abnormal ratios in Multiple Myeloma (MM), most of them retrospectives. Currently, the normalization of sFLC or HLCr after treatment has been proposed as a good response biomarker.
Aims: To investigate the prognostic impact of sFLCr and sHLCr at diagnosis and after treatment in MM patients in the context of three GEM/PETHEMA clinical trials (GEM2005MENOS65, GEM2005MAS65 & GEM2010MAS65).
Methods: Among a total of 819 patients, treated according to the GEM2005MENOS65, GEM2005MAS65 & GEM2010MAS65 GEM/PETHEMA trials, serum samples for sFLC and sHLC analyses were available at diagnosis in 623 and 183 of the patients, respectively. After induction, regardless of the achieved response, it was available in 308 cases for sFLCr and 89 for sHLCr. All patients in which sHLC assay was analysed were IgG or IgA-MM. The sHLC and sFLC assays (HEAVYLITE® and FREELITE®, The Binding Site, Birmingham, UK) were performed on an automated nephelometer (BNII, Dade Behring/Siemens, Marburg, Germany). The sPE assay was performed by capillary electrophoresis (V8, Helena Biosciences Europe), and sIFE was performed for γ, α, μ, κ, and λ Ig chains (SAS-3 & SAS-4, Helena Bioscience Europe).
Results: At diagnosis, 92% of the sFLCr and sHLCr were abnormal, which had no impact in prognosis; even when we considered "very pathological" (VP) sFLCr values (0.03-32)[FIGURE 1A]. Establishing several cut-offs, we note that VP-sHLCr values (<0.29 or >73) at the time of diagnosis showed an increased risk of progression (p=0.006)[FIGURE 1B], confirmed by multivariate analysis — age [p=0,003; OR 1.04 (1.01-1.06)]; LDH [p=0.03; OR 0.4 (0.26-0.94)]; VP-sHLCr [p=0.01; OR 1.78 (1.14-2.78)]; high *vs* low risk FISH [p=0.02; OR 1.75 (1.11-2.74)]. Interestingly, absolute HLC-involved-Ig values show linear correlation with absolute values of MP by sPE (p=0,000; Pearson's r=0.676) and the presence of uninvolved HLC pair suppression had no prognostic impact in our series (OS p=0.89; PFS p=0.57). After treatment, regardless of the achieved response, a normal sFLCr or a normal sHLCr do not imply a better prognosis in our series. Concerning the sFLCr, among the 130 patients in CR after induction no prognostic differences were observed between patients with a normal (0.26-1.65) *vs* pathological sFLCr.

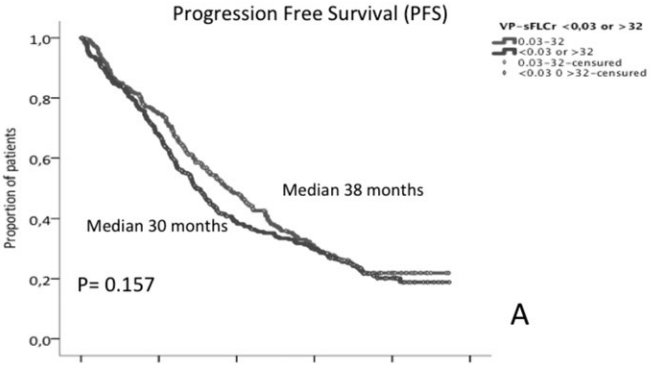

Figure 1 A.

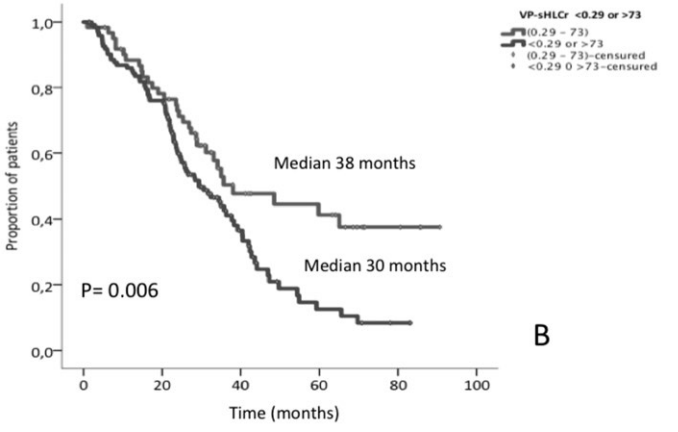

Figure 1 B.

Summary and Conclusions: VP-sHLCr values (<0.29 or >73) at diagnosis could suggest a greater risk of progression. Since the HLC-involved-Ig values show a strict linear correlation with values of MP by sPE,its clinical utilityis debatable.The sHLC non-involved pair suppression at diagnosis wasn't related to worse prognosis, nor did a normal sFLCr or sHLCr at diagnosis seem to imply differences in prognosis. However, in CR, a normalization of the sFLCr doesn't correlate with a better prognosis in our series, what opens up controversy about the value of the current IMWG criteria for sCR.

P288

RISK OF VARICELLA-ZOSTER VIRUS INFECTION IN MYELOMA PATIENTS WITHOUT BORTEZOMIB TREATMENT

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Background: Patients with multiple myeloma have an increased risk of varicella-zoster virus (VZV) infection, which has been established, especially with the use of bortezomib. However, few studies have discussed the risk of VZV infection in myeloma patients without bortezomib treatment.
Aims: We conducted a nationwide population-based study to investigate the risk of VZV among myeloma patients and the association between non-proteasome inhibition therapies and development of VZV.
Methods: We recruited patients with newly diagnosed multiple myeloma from Taiwan's National Health Insurance database between January 1, 2000 and December 31, 2011. Patients who were aged under 20 years or with antecedent VZV were excluded. Each myeloma patient was matched with four age-, sex-, and comorbidity-matched individuals. All participants were followed up until VZV development, death, dropout from the National Health Insurance program, initiation of bortezomib treatment, or the end of 2011. The Kaplan-Meier method was employed for estimation of cumulative incidence. Hazard ratios (HRs) of VZV risk compared between the two cohorts were calculated by Cox proportional regression analysis. In addition, therapeutic agents were put into Cox models as time-dependent covariates to avoid immortal time bias.
Results: This study consisted of 3,677 patients with multiple myeloma and 14,708 matched individuals, with a median age of 67 years (interquartile range 58–75). Four hundred eighteen VZV infections developed among 3,677 myeloma patients, with a follow-up of 6083.8 person-years. The incidence of VZV infection was 5.93 times higher in the myeloma cohort than in the matched cohort (68.7 *vs* 12.0 per 1,000 person-years), with an age-, sex-, and comorbidity-adjusted HR of 6.02 (95% confidence interval [CI] 5.30–6.83; *P* <0.001). The multivariable Cox proportional hazard models showed thalidomide treatment (adjusted HR 1.33; 95% CI 1.04–1.69; *P* =0.022), cytotoxic chemotherapy (adjusted HR 1.34; 95% CI 1.02–1.77; *P* =0.036) and hematopoietic stem cell transplantation (adjusted HR 2.80; 95% CI 2.09–3.75; *P* <0.001) as independent risk factors determining the subsequent VZV in myeloma patients.
Summary and Conclusions: Our study reveals an increased risk of VZV infection among myeloma patients without bortezomib therapy. Treatment with thalidomide, cytotoxic chemotherapy, or hematopoietic stem cell transplantation is associated with a higher risk of VZV infection in myeloma patients.

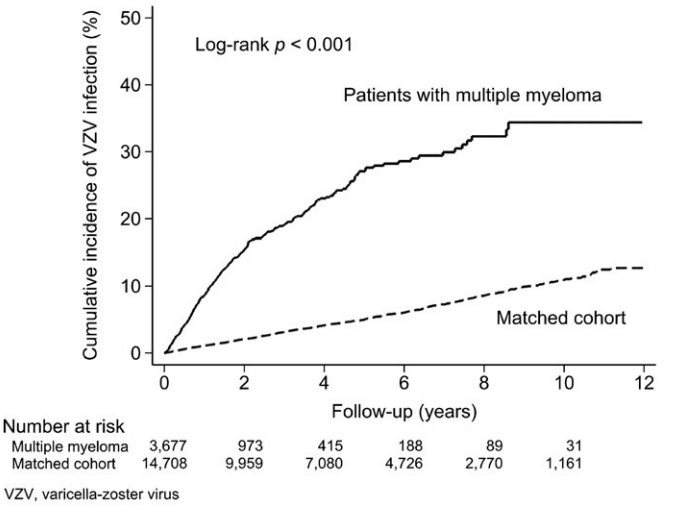

Figure 1.

P289

VENETOCLAX (ABT-199/GDC-0199) IN COMBINATION WITH BORTEZOMIB AND DEXAMETHASONE IN RELAPSED/REFRACTORY MULTIPLE MYELOMA: PHASE 1B RESULTS

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Background: The anti-apoptotic proteins BCL-2 and MCL-1 promote multiple myeloma cell survival. Bortezomib (BTZ) can inhibit MCL-1 activity by increasing the MCL-1 antagonist, NOXA. Venetoclax is a selective, orally bioavailable, small-molecule BCL-2 inhibitor, which enhances BTZ efficacy in multiple myeloma xenograft models. This Phase 1 study evaluates venetoclax with BTZ and dexamethasone (Dex) in patients with relapsed/refractory multiple myeloma.
Aims: Objectives include safety, pharmacokinetics, preliminary efficacy, and maximum therapeutic dose of venetoclax with BTZ and Dex.
Methods: Patients received venetoclax (50-500 mg PO daily) in cycles 1-11 per designated dose escalation cohorts (continual reassessment method); BTZ (1.3 mg/m^2 SC, days 1, 4, 8, 11) and Dex (20 mg PO, Days 1, 4, 5, 8, 9, 11, 12) in cycles 1-8 (21 days), BTZ+Dex days 1, 8, 15, 22 in cycles 9-11 (35 days), and venetoclax alone in cycle 12 and beyond.
Results: 32 patients were enrolled as of 12/18/2014: median age 65; 12/20 female/male. 12 were ISS stage I, 7 stage II, 10 stage III. Median (range) prior therapies: 5 (1–15). 26 patients received prior BTZ (10 were refractory), 26 had prior lenalidomide, and 20 had autologous hematopoietic stem cell transplant. Adverse events (AE; all grades) occurring in ≥20% patients: constipation (41%), diarrhea (38%), peripheral edema (28%), thrombocytopenia (31%), peripheral neuropathy (28%), insomnia (28%), dyspnea (25%), and anemia (22%). Grade 3/4 AEs (≥10%): thrombocytopenia (25%) and anemia (13%). 14 patients had serious AEs: none were assessed as venetoclax-related. Reasons for discontinuation (n=17): disease progression (PD; n=14), AEs (n=2: adenocarcinoma, cardiac and respiratory decompensation), consent withdrawal (n=1). 3 deaths occurred (due to PD); 1 dose-limiting toxicity at 300 mg (cardiac decompensation attributed to Dex). No tumor lysis syndrome occurred. Dose-normalized venetoclax exposure when given with BTZ+Dex (n=30) was similar to venetoclax alone.
Summary and Conclusions: Venetoclax with BTZ and Dex has an acceptable safety profile in heavily pretreated multiple myeloma patients. This combination targeting BCL-2 and MCL-1 resulted in anti-tumor activity and longer time on study in patients naïve or sensitive to prior BTZ. Dose escalation continues at 600 mg.

Tabella 1.

Preliminary efficacy (best response) by BTZ status

n (%)	Refractory (n=10)	Sensitive (n=16)	Naïve (n=6)
Stringent complete response (sCR)	0	1 (6)	0
Complete response (CR)	0	0	2 (33)
Very good partial response (VGPR)	0	2 (13)	1 (17)
Partial response (PR)	0	7 (44)	2 (33)
Minimal response (MR)	1 (10)	0	0
Stable disease (SD)	3 (30)	4 (25)	0
Disease progression (PD)	4 (40)	1 (6)	1 (17)
Discontinued	2 (20)	1 (6)	0
Overall response rate (sCR+CR+VGPR+PR)	0	10 (63)	5 (83)
Median (range) time on study, months	1.3 (0.3–4.8)	5.0 (0.7–9.5)	5.0 (1.4–9.4)

P290

RETROSPECTIVE REFINING OF DIAGNOSIS IN 286 MGUS AND SMM PATIENTS ACCORDING TO THE UPDATED IMWG CRITERIA. CLINICAL IMPLICATIONS

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Background: With the recent publication of updated criteria for the diagnosis of multiple myeloma by the IMWG, a subset of patients formerly diagnosed as having asymptomatic MM (SMM) or monoclonal gammopathy of undetermined significance (MGUS), should be reclassified.
Aims: To retrospectively discriminate the subset of patients that present today diagnostic discrepancy and to evaluate eventual damage, if any.
Methods: 286 patients (147 diagnosed as SMM and 139 as MGUS), diagnosed and followed-up in our section, were studied. Patients were re-diagnosed according to factors susceptible to change the diagnosis according to the new IMWG criteria, namely the existence of IgA or IgG >30g/L or urinary moclonal protein of 500 mg/24h or more, that could shift MGUS to SMM and, with regard to an eventual shift from SMM to MM, the existence of plasma cell infiltration equal or more than 60% and the presence of at least one osteolytic lesion on more sensitive methods than x-rays. Anemia, hypercalcemia and renal impairment were already taken in account initially and are not reassessed. Statistical analysis was performed by standard methods with the SPSS v21.0 software.
Results: No MGUS patient had IgA or IgG >30g/L or urinary moclonal protein of at least 500 mg/24h, so no change were made. The new criteria did not taken into account serum free light chain (FLC) measurements or ratio (FLCR). Five patients had an FLC value >100mg/L and 6 an FLCR >8 but although Kaplan-Meier curves separated well, the statistical difference in evolution rates for patients with the aforementioned FLC/FLCR values was not significant perhaps due to the small number of patients. With regard to SMM patients, 25 of them had at least one osteolysis by sensitive radiology methods (CT, MRI) in the absence of bone pains or other end-organ damage-related symptomatology; they should however be considered as having symptomatic MM. Their median time to treatment initiation was 34 months (range 15-53) and their median overall survival since diagnosis was 80 months (range 36-124). In addition, other 9 patients had more than 60% bone marrow plasma cell infiltration and should also be considered as suffering from symptomatic MM, although presenting no other symptoms. This second patients' subgroup had a shorter median time to treatment initiation of 6 months (range 5-7) and their median overall survival since diagnosis was 60 months (range 10-110). In total, the diagnosis of 34 patients (23%) of the present series should change according to the IMWG updated criteria for the diagnosis of multiple myeloma.
Summary and Conclusions: The diagnosis of MGUS patients did not change with the application of the new IMWG updated MM criteria, on the contrary the diagnosis of 23% of SMM patients changed. This was totally justified for patients with more than 60% bone marrow plasma cell infiltration, but is somewhat controversial in the case of patients with existing but not extensive skeletal disease.

P291

IS A DEEP RESPONSE THE KEY TO SUCCESSFUL TREATMENT OF MULTIPLE MYELOMA?

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Background: Several authors have highlighted the importance of a deep response to chemotherapy in multiple myeloma (MM), especially in first line.
Aims: The objective was to assess which patient/treatment/disease characteristics are prognostic for a deep response. Also, to assess whether the deep response is prognostic for overall survival (OS) independent of treatment, treatment line and patient/disease characteristics.
Methods: A retrospective analysis was performed on 2960 MM-patients from 24 hospitals in Denmark, Finland, Norway and Sweden. The database contained information on patient baseline characteristics such as age, gender, ISS stage, albumin, creatine, and MM type, which were recorded at start of first line therapy. The following outcomes were considered; response, time to next line of treatment (TTNT) and OS. The following categories of response were differentiated: progressive disease (PD), no response (NR), partial response (PR), very good PR (VGPR) and equal or better than near complete response (>=nCR). To identify prognostic factors for response, univariate and multivariate multinomial regression were conducted with response as dependent and patient baseline characteristics and type of treatment as independent variables. To assess whether response is an independent predictor of OS, multivariate cox-proportional hazard models were run for the first four lines of treatment.
Results: Patients in the dataset were on average 67 years old, 48% were male, 28%, 41% and 31% in ISS stages I, II and III, respectively. Multinomial regression showed that type of treatment, age, ISS type and MM type were significant prognostic factors for response in first line. In second line, first line response, type of treatment and age were significant prognostic factors for response in second line. Multivariate cox-regression showed that in first line patients with NR, PR, VGPR and >=nCR had significant lower hazard ratio's (HRs) 0.61 (0.43-0.85), 0.56 (0.41-0.78), 0.34 (0.22-0.51) and 0.36 (0.24-0.54) respectively compared to PD. Age, Albumin, Calcium and Beta-2-microglobulin levels were also significant prognostic factors for OS with HRs of 1.03 (1.01-1.04), 0.98 (0.96-0.99), 1.41 (1.11-1.79) and 1.02 (1.01-1.03) respectively. The following categorical variables also were significant prognostic factors for first line OS; type of treatment, ISS-stage and MM type. For second line OS multivariate cox-regression showed that patients with PR, VGPR and >=nCR had significant lower HR's 0.58 (0.46-0.73), 0.42 (0.3-0.58), 0.4 (0.27-0.6) compared to PD respectively. Age also had a significant HR of 1.02 (1.01-1.03). For third line OS multivariate cox-regression showed that patients with NR, PR, VGPR and >=nCR had significant lower HR's 0.67 (0.5-0.89), 0.37 (0.27-0.51), 0.32 (0.21-0.5), 0.18 (0.1-0.34) compared to PD respectively. Age also had a significant HR of 1.01 (1.00-1.02). For fourth line OS multivariate cox-regression showed that patients with PR, VGPR and >=nCR had significant lower HR's 0.45 (0.31-0.64), 0.31 (0.19-0.52) and 0.39 (0.21-0.73) compared to PD respectively. Age was also identified as a significant prognostic factor.
Summary and Conclusions: Type of treatment, age, ISS type and MM type were significant prognostic factors for response in first line. For second line response, the significant prognostic factors were response in first line, type of treatment and age. Moreover, multivariate cox-regressions shows that in the first four lines of treatment, response is an independent prognostic factor for OS. Future research should include genetic prognostic factors, which were not collected in our dataset and could therefore not be assessed.

P292

FAMILY HISTORY OF LYMPHOPROLIFERATIVE DISEASE ASSOCIATED WITH A SUPERIOR SURVIVAL IN MULTIPLE MYELOMA: A POPULATION-BASED STUDY

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Background: Multiple myeloma (MM) is characterized by a neoplastic proliferation of plasma cells in the bone marrow and overproduction of monoclonal immunoglobulins in serum or urine. MM is preceded by a premalignant condition called monoclonal gammopathy of undetermined significance (MGUS). Familial aggregation of MM and MGUS has been reported by several authors. The role of genetic factors in the pathogenesis of multiple myeloma is also indicated by emerging data. However, the underlying genetic cause of the disease is uncertain and the impact of family history on survival is unknown.
Aims: The aim of our study was to compare survival in MM patients with family history of lymphoproliferative disorders (LPD) to MM patients without family history of LPD.

Methods: MM patients and their first-degree relatives were identified using the nationwide Swedish Registries. Information on malignancies in relatives of MM patients was obtained by record-linkages to the Swedish Cancer Registry. A positive family history of LPD was defined as MM patients having at least one first-degree relative diagnosed with MM, chronic lymphocytic leukemia (CLL), non-Hodgkin's lymphoma (NHL), Waldenström's macroglobulinemia (WM), or Hodgkin's lymphoma (HL). We performed a cox proportional hazard model and adjusted for age, sex, and year at diagnosis.
Results: A total of 13,947 MM patients diagnosed in 1958-2005 were included of whom 332 had a linkable first-degree relative with LPD. When compared to MM patients without family history of LPD, MM patients with a family history had a borderline significant decreased risk of death (HR 0.89; 95% CI 0.79-1.00, p=0.0549). When stratified by age there was a significant difference in patients 70 years of age or younger (0.83; 0.71-0.98, p=0.0298), but not older (Figure 1). A better survival was also found in male MM patients with family history than with sporadic disease (0.83; 0.71-0.98, p=0.0297). There was no statistical difference by family history among female MM patients (Figure 1).

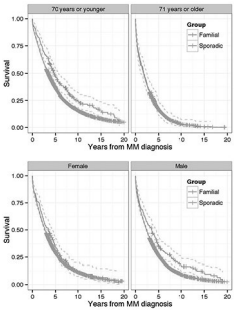

Figure 1.

Summary and Conclusions: In this large population-based study we showed for the first time that there is a trend toward superior survival in MM patients with family history of LPD, compared to MM patients without family history. Interestingly, survival in MM patients younger than 70 years was significantly better in the familial group (compared to sporadic). Lastly, male patients with family history of LPD had a significantly lower risk of death. Our findings suggest that familial MM may be associated with less agressive nature or better response to therapy, at least in subgroups of patients. The underlying molecular explanations for our findings are yet unknown and need to be established by further research.

P293

PARENTAL LONGEVITY AND SURVIVAL IN PATIENTS WITH MULTIPLE MYELOMA AND MGUS

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Background: In the general population, parental longevity is associated with an increased life expectancy. However, studies on specific diseases and parental longevity have shown conflicting results. A few studies on multiple myeloma have identified host characteristics that have an influence on the patient's prognosis, such as socioeconomic status, poor performance status, and co-morbidity. Longevity as a host factor among patients with multiple myeloma has never been studied in relation to outcome. Recent studies focusing on the myeloma precursor, monoclonal gammopathy of undetermined significance (MGUS), have implied that host biology plays an important role in relation to malignant transformation.
Aims: Our aim was to study the effect of parental longevity on the survival of patients with multiple myeloma and MGUS.
Methods: A total of 1,815 patients with multiple myeloma, 1,407 MGUS patients as well as 8,267 population-based controls for multiple myeloma patients and 5,595 controls for MGUS were included in the study. We compared the risk of death, using Cox's proportional hazards regression (adjusting for age, gender, and year of diagnoses), among multiple myeloma and MGUS patients with a history of parental longevity to those patients without a long-lived parent. Longevity was defined as exceeding 90 years of age or living to the 90th percentile.
Results: Among multiple myeloma patients, a history of parental longevity was not associated with a decreased risk of death (hazard ratio (HR=0.92; 95% confidence interval (CI) 0.81-1.05, Table). Having one long-lived parent or both did not have an effect (HR=0.91; 95% CI 0.80-1.04 and HR=1.02; 95% CI 0.72-1.44, respectively). A history of parental longevity among MGUS patients was associated with a significant decrease in risk of death (HR=0.69; 95% CI 0.53-0.91, Table). The risk of death decreased when one parent was long-lived (HR=0.69; 95% CI 0.52-0.91). However, based on few numbers, when both parents were long-lived the risk of death was decreased but not statistically significant (HR=0.72; 95% CI 0.34-1.53).

Tabella 1. Relative risk of death for patients with multiple myeloma and MGUS in relation to parental longevity.

	Multiple Myeloma		MGUS	
Category	HR (95% CI)	p-value	HR (95% CI)	p-value
Parental history of longevity	0.92 (0.81 – 1.05)	0.21	0.69 (0.53 – 0.91)	0.0079
One parent long-lived*	0.91 (0.80 – 1.04)	0.18	0.69 (0.52 – 0.91)	0.0098
Both parents long-lived*	1.02 (0.72 – 1.44)	0.92	0.72 (0.34 – 1.53)	0.39

*HR = Hazard Ratio, CI = Confidence Interval

Summary and Conclusions: In this first, to our knowledge, study on parental longevity and survival among multiple myeloma and MGUS patients, we conclude that a history of parental longevity is associated with superior survival among MGUS patients, which may reflect the impact of host biology in relation to overall survival. However, parenteral longevity does not decrease the risk of death for patients with multiple myeloma. This finding probably reflects the poor outcome of the malignancy itself which seems to outweigh the positive effects of parental longevity.

P294

SERUM B-CELL MATURATION ANTIGEN IS A NOVEL PROGNOSTIC INDICATOR FOR MULTIPLE MYELOMA PATIENTS AND CORRELATES WITH CLINICAL STATUS AND SURVIVAL

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Background: B-cell maturation antigen (BCMA) is a tumor necrosis factor receptor family member that is expressed on normal and malignant B-cells. Our group has recently shown that BCMA is present in the serum of multiple myeloma (MM) patients (pts) and that its levels may correlate with their clinical status and overall survival (OS).
Aims: We analyzed the relationship between serum BCMA levels and monoclonal (M)-protein levels as well as the relationship of BCMA to response status, progression-free survival (PFS) and OS in a large cohort of MM pts.
Methods: Serum was obtained on 227 MM pts following informed consent. Enzyme-linked immunosorbent assay (ELISA) was used to determine serum BCMA levels (R&D Systems). The Mann-Whitney test was used to assess the differences between clinical status groups. Kaplan-Meier analysis and multivariate Cox regression models were also used. Kaplan-Meier survival of MM pts was determined from the time of initial serum BCMA measurement to death or the date of last follow-up. PFS of MM pts was evaluated from the time of serum BCMA measurement to date of first disease progression. Cox-proportional hazards regression was utilized to determine the predictive influence of serum BCMA and various other factors including age, creatinine, hemoglobin, ISS stage, and bone disease on OS and PFS. *P*-values less than 0.05 were considered statistically significant. Changes in serum BCMA levels were correlated with serum M-protein levels for 44 MM pts during their course of disease. Similarly, serum BCMA levels were correlated with bone marrow and PET scan findings for non-secretory disease (NSD) pts.
Results: Serum BCMA levels correlated with the patient's clinical status at the time of its determination (P <0.0001). Specifically, pts with ≥PR had significantly lower serum BCMA levels (median, 52.49 ng/mL) than those with stable and progressive disease (median, 124.8 ng/mL; P <0.0001). Changes in serum BCMA levels highly correlated with changes in serum M-protein levels among 44 consecutive MM pts that had multiple determinations made during their course of disease. Notably, pts with NSD showed a direct correlation between changes in serum BCMA levels and their clinical status as reflected by PET scan and bone marrow findings during their course of disease. PFS was markedly longer among pts with serum BCMA levels below than among those with levels above the median (P =0.006). We divided pts into quartiles based on their serum BCMA levels. Pts in quartiles 1-3 had a longer PFS compared to pts in quartile 4 (P <0.0001). Among all 227 pts, the 36-month OS of pts whose serum BCMA levels were above the median (≥102 ng/mL) was significantly lower than among those whose levels were below the median (P =0.02). In the multivariate regression analyses, serum BCMA levels significantly correlated with only OS (P =0.0003). In contrast, age, bone disease status, serum creatinine, hemoglobin, and ISS staging did not correlate with OS. Lastly, serum BCMA levels were independent of ISS staging and serum creatinine levels.

Summary and Conclusions: BCMA is a novel serum marker that can be used to follow the course of disease in MM patients. Serum BCMA levels are elevated in patients with MM. Levels of this marker also correlate with clinical status, predict PFS and OS, and provide patients with NSD a way to follow their disease course.

P295

THE NOTCH LIGANDS JAGGED1 AND 2 ARE A POTENTIAL THERAPEUTIC TARGET IN MULTIPLE MYELOMA-ASSOCIATED BONE DISEASE

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Background: Bone disease is still a relevant issue in multiple myeloma (MM), since it not only affects patients quality of life but it also promote tumor growth and survival, finally contributing to the fatal outcome of this disease. The Notch oncogenic pathway is dysregulated in MM, due to the hyperexpression of the Jagged 1 and 2 ligands. This alteration improve the ability of MM cells to establish pathological interactions with the bone marrow (BM) niche, finally promoting tumor progression. Notch is also a key regulator of bone tissue remodeling and skeletal development.
Aims: The aim of this work was to provide a rationale for the targeting of the two Notch ligands Jagged 1 and 2 in MM-driven bone disease. To address this issue we assessed: the role of the Notch pathway in MM-associated osteoclastogenesis; the effects of Jagged1/2 silencing on the ability of MM cells to activate the Notch pathway in OCLs and boost bone resorption.
Methods: OCL differentiation of Raw264.7 cells was induced by treating them with 50ng/ml mRANKL or co-culturing with MM cells or their conditioned medium (CM). After 5-7days cells were stained using the TRAP Kit and counted. DAPT was used at a concentration of 50μM. Select RNAi^{TM} siRNA system (Invitrogen) was used according to the manufacturer's guidelines for the selective knockdown of Jag1 and Jag2. Jagged1 recombinant peptide was used at 0.5μg/ml. anti-RANKL neutralizing antibody was used at 0.1μg/ml.Total RNA was isolated using TRI-Reagent. cDNA was prepared through MMLV reverse transcriptase, then quantitative PCR (qPCR) was performed by Maxima SYBR Green qPCR Master Mix.RANKL was quantify by ELISA Assay and flow cytometry.
Results: Our findings indicate that the autonomous release of RANKL (Receptor activator of nuclear factor kappa-B ligand) by MM cells is essential for their ability to boost osteoclastogenesis. Interestingly, RANKL release is Notch-dependent, since Jagged1/2 silencing in MM cells causes the inhibition of the Notch pathway and impairs their ability to secrete RANKL and to stimulate osteoclasts (OCLs) differentiation and activity. MM-derived Jagged are also able to directly activate the pro-osteoclastogenic Notch signaling in neighboring pre-OCLs, boosting their differentiation. Moreover, Jagged1/2 are essential for the interaction of MM cells with BM stromal cells (BMSCs), that can further enhance the osteoclastogenic potential of tumor cells. Jagged1/2 withdrawal blocks the cross-talk between MM cells and the surrounding BMSCs and pre-OCLs, finally causing a decrease in the formation of mature OCLs and in bone resorption.
Summary and Conclusions: Our study provided the first evidence that two Notch ligands dysregulated in MM, Jagged 1 and 2, play an essential role in myeloma-induced osteoclast differentiation and bone resorption activity. Jagged ligands can trigger Notch signaling in the same MM cells, resulting in the release of the key osteoclastogenic factor RANKL, and may also activate Notch signaling in the neighboring osteoclast progenitor further promoting their differentiation. Finally, we demonstrated that MM cells are able to crosstalk with BMSCs which are able to stimulate low-RANKL expressing myeloma cells, to release higher amount of RANKL and acquire osteoclastogenic ability. Importantly, BMSC support can be prevented by silencing Jagged ligands on myeloma cells. All together, our results demonstrate that the two Notch ligands Jagged1 and 2 represents two new promising therapeutic targets in MM-associated bone disease.

LB296

BORTEZOMIB, DEXAMETHASONE, THALIDOMIDE AND MELPHALAN (VDT-MEL) PREPARATIVE REGIMEN RESULTS IN A VERY HIGH STRINGENT CR (SCR) RATE IN MULTIPLE MYELOMA (MM)

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Background: The role of autologous stem cell transplant (ASCT) in the treatment of myeloma has been well established and Melphalan 200mg/m^2 has been the standard preparative regimen of choice. Although the introduction of novel agents improved the treatment of myeloma significantly, data about their role in preparative regimens are very scarce.
Aims: The purpose of this study is to understand the toxicity and efficacy of novel agents used in combination with high-dose melphalan as a new preparative regimen.
Methods: Retrospective analysis was performed on all patients who received an ASCT with the VDT- Mel during 2012- 2014. IRB approval was obtained. To determine which variables were significantly associated with the odds of achieving a sCR post-transplant, logistic regression models were applied. All statistical testing was two-sided and assessed for significance at the 5% level using SAS v9.4 (SAS Institute, Cary, NC). Clinical end points were treatment- related toxicity and quality of response
Results: 97 patients underwent 149 transplants and toxicity was analyzed at 100 days; 45 patients underwent single and 52 had tandem transplants (TT), respectively; 66 patients received early and 31 salvage transplantation. Median age was 59 y. Best responses were 58% sCR, 20%CR, and 9.5% VGPR. For patients receiving early transplantation, the sCR rate was 61%, and for TT patients, 62%. Only early *versus* salvage transplantation was significant for achieving sCR with a hazard ratio: 3.18 (P =0.02). Grade 3 and 4 non-hematologic toxicities were related to infections in 54%; 100 day mortality was 2% and 3.8% for single and TT, respectively. Median time to ANC>500/μL was 12 days in both early and late transplantation.
Summary and Conclusions: This study shows that VTD-Mel is a well-tolerated preparative regimen. Importantly, the sCR rate was significantly higher than in other published studies. Since sCR is an early surrogate marker for progression-free and overall survival, it appears likely that this regimen will be superior to melphalan alone and may become the new standard preparative regimen for ASCT in myeloma. This is the largest study performed to date evaluating this novel preparative regimen.
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LB297

SIGNIFICANT IMPACT OF MINIMAL RESIDUAL DISEASE (MRD) STATUS ON SURVIVAL OUTCOMES IN PATIENTS WITH MULTIPLE MYELOMA (MM) WHO ACHIEVE COMPLETE RESPONSE (CR): A META-ANALYSIS

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Background: Most patients with MM eventually relapse, suggesting that a residual disease which cannot be detected by conventional assessment persists, even in patients achieving CR with treatment.
Aims: To assess the impact of MRD status on survival outcomes in patients with MM who achieved CR.
Methods: A PubMed search was performed to identify studies published between January 1990 and September 2014, with ≥20 newly-diagnosed patients with MM. Patients requiring treatment and who had MRD evaluation results were included in this meta-analysis. Analysis was restricted to techniques with a limit of detection of 0.01% or lower. If primary data were not accessible, survival graphs were used to derive individual survival and censoring times. Data were adjusted for the different proportions of MRD patients in different studies using a method that considers time-dependent effects (Gregory WM, Br J Cancer 1988). *P*-values are for adjusted log-rank χ^2 tests.
Results: A total of 302 published articles were retrieved and 25 articles were identified through the reference sections of recently published articles. Of these, 18 reported overall survival (OS) or progression-free survival (PFS) results, as well as MRD status. Overall, 2,208 pts were evaluated for MRD by methods including multiparameter flow cytometry (n=1,606), PCR (n=492), or high-throughput sequencing (n=110). Nine publications reported conventional CR at the time of MRD measurement; however only 4 represented unique data sets. In total, there were 496 evaluable patients (362 were MRD-negative and 134 MRD-positive). The presence of MRD predicted shorter PFS (odds ratio [OR] 0.36; 95% confidence interval [CI] 0.26–0.50; $P < 0.0001$). Median PFS was 60 months for MRD-negative patients *versus* 36 months for MRD-positive patients; PFS at 3 years was 72% *versus* 50%, respectively and at 5 years was 50% *versus* 29%. MRD-negativity reduced the odds of death by 59% (OR 0.41; 95% CI 0.26–0.63; $P < 0.0001$); median OS was not reached for MRD-negative patients *versus* 82 months for MRD-positive patients. OS was higher for MRD-negative patients *versus* MRD-positive patients at 3-years (93% *vs* 79%) and 5-years (78% *vs* 60%). Presence of MRD was predictive of outcome in patients with adverse cytogenetic profiles. Overall, 37% of MRD-negative patients *versus* 7% of MRD-positive patients were progression-free at 8 years. There was no significant difference between studies in the impact of achieving MRD-negativity on outcome, indicating that the predictive value of MRD status does not depend on the type of treatment.
Summary and Conclusions: These results show that MRD negativity, as determined by various high-sensitivity methods, predicted for substantially bet-

ter PFS and OS in patients with MM who had achieved CR. Furthermore, nearly all MRD-positive patients had disease progression within 8 years, whereas a third of MRD-negative patients remained progression-free. This large cohort, meta-analysis confirms that MRD status is a crucial marker of long-term outcomes in patients with MM. It is, therefore, a key endpoint in MM clinical trials and clearly also has an important role as a surrogate marker of OS.

Biology of MPN

P296

INCREASED AND PROLONGED STAT5 ACTIVATION DETECTED FOR A JAK2 E846D GERMLINE MUTATION IN THE SPECIFIC CONTEXT OF EPO RECEPTOR

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Background: Somatic mutations of a gene that encodes the *Janus kinase 2* (JAK2) are the most observed disease-causing events of the myeloproliferative neoplasms (MPN). Recently, germline JAK2 mutations have been described in cases with familial MPN, exhibiting hereditary thrombocytosis.
Aims: To characterize a novel JAK2 germline mutation found in a patient with erythrocytosis.
Methods: The effect of JAK2 E846D mutation was functionally analyzed using Ba/F3-EPOR cells (immunoblots, BrdU, MTT, inhibitors assays) and JAK2-deficient γ2A cells (reporter luciferase assay).
Results: The patient was diagnosed with increased red cell mass, facial plethora and splenomegaly at the age of 15. The bone marrow aspirate showed features typical for MPN. Two heterozygous mutations were found by targeted analyses; E846D in JAK2 inherited from the mother and Q157H in PHD2 (SNP rs61750991) inherited from the father. Erythroid progenitors derived from the patient and his parents were hypersensitive to erythropoietin (EPO), however only the patient exhibited the erythrocytosis phenotype. *In silico* modeling of JAK2 showed that the substitution of Glu by a residue one CH2 shorter, namely Asp, in the kinase domain might result in a formation of a tighter salt bridge to residue K926, more difficult to disrupt upon returning of JAK2 to its inactive state. This leads to a prolonged activation of JAK2 kinase.
Ba/F3 JAK2 E846D stable transfectants have revealed EPO-induced increased and prolonged JAK2 and STAT5 activation detected by immunoblots assessing active forms of JAK2 and STAT5. This was subsequently confirmed by luciferase assay in γ2A cells that detected higher and prolonged cytokine-induced levels of STAT5 transcriptional activity. Surprisingly, among the STAT family members, the E846D substitution significantly increased only STAT5 transcriptional activity and only in the presence of EPO receptor but not with TPO or G-CSF receptors. Two JAK2 inhibitors (Ruxolitinib, AZ-960) reduced the prolonged STAT5 activation; the E846D variant exhibited similar sensitivity as oncogenic JAK2 V617F. Although the E846D did not support growth factor independence of Ba/F3 cells as V617F, increased STAT5 activity was detected also in the condition without cytokines in transcriptional assays. In addition, Ba/F3 JAK2 E846D transfectants showed improved survival in EPO-limiting conditions when compared to JAK2 wild-type (wt) cells. Cell cycle studies demonstrated that upon growth factor starvation JAK2 E846D cells re-enter faster into S phase in low EPO concentrations, when compares to JAK2 wt.
Summary and Conclusions: The germline JAK2 E846D mutation emerged as the main disease causing event in our patient. It causes EPO hypersensitivity and erythroid hyperproliferation due to increased and prolonged JAK2/STAT5 signaling. This mutation gives this phenotype only in the context of the EPO receptor, explaining the erythrocytosis *in vivo*. We propose that the disease is a hereditary myeloproliferative disorder with incomplete penetrance; we observed full clinical phenotype in the patient and intermediate phenotype in the mother, the genetic background likely contributing to the extent of disease expression. *Grants support: P301/12/1503, NT13587-4, CZ.1.07/2.3.00/20.0164, CZ.1.07/2.3.00/30.0041 from Czech Republic; MEXP31C1, ARC10/15-027, FRIA PhD fellowship from Belgium.*

P297

PH-NEGATIVE MPN RED BLOOD CELLS DISPLAY DEREGULATION OF IQGAP1-RHO GTPASE SIGNALING DEPENDING ON CALR/JAK2 STATUS

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Background: Polycythemia Vera (PV), Essential Thrombocythemia (ET) and Primary Myelofibrosis are myeloproliferative neoplasms (MPN) characterized by a clonal proliferation of hematopoietic progenitors that results in an overproduction of mature blood cells. Several molecular abnormalities have already been described in progenitor cells such as *JAK2* or *CALR* mutations which activate the STAT signaling pathway causing an enhanced and uncontrolled proliferation of progenitor cells. In parallel, leucocyte and platelet functional alterations have been reported in MPN patients. Nevertheless, little is known about functional and protein alterations of erythrocytes despite their implications in PV. Initial studies showed that abnormal red blood cell (RBC) adhesion to endothelial cells in PV, which can cause vascular complications, could be due to the activation of Lu/BCAM by the JAK2V617F/Akt/Rap1 pathway.
Aims: In this context, we have developed an integrative study of RBC proteome in PV and ET looking for protein deregulations that could be related to functional RBC alterations and MPN physiopathology.
Methods: Seeing as hemoglobin is a big impediment to the study of the RBC proteome, we developed one technique based on the capture of low abundant proteins by ferromagnetic beads surrounded by chemical surfaces. This hemoglobin depletion was used on 48 samples (PV, ET and controls) before MS analysis which allowed us to select, according to their proteomic profile, the five most representative patients of each subgroup for LC MS/MS analysis (LTQ Orbitrap).
Results: Quantitative comparative analysis distinguished 1019 proteins among which 51 were upregulated in PV and 86 in ET compared with controls. Furthermore, functional comparison using *Ingenuity Pathway Analysis* software showed that the Ras GTPase family pathway was deregulated in both MPN patients. Specifically, *Ras GTPase-activating like protein IQGAP1* was overexpressed in ET and PV compared with controls ($p<0.05$). Additionally, mass spectrometry result verification by westernblot not only highlighted IQGAP1 overexpression in PV and ET compared with controls ($p<0.01$) but also depending on patient genotype (*JAK2V617F>CALR+*,$p<0.05$; *JAK2->CALR+,* $p<0.05$). IQGAP1 is a scaffold protein that regulates several pathways including Ras GTPase ones. Therefore, we explored connections between IQGAP1 and Rho GTPases (Ras GTPase subfamily) demonstrating direct links between IQGAP1 and Rac1, Cdc42 and RhoA in patient RBCs. Moreover, we found that the Rho subfamily protein recruitment profile by IQGAP1 was different between *JAK2+* patients where it linked activated Rac1 but not RhoGDI (Rho GTPase inhibitor). Conversely, in *CALR+* patients, IQGAP1 co-precipitated with RhoGDI but didn't with Rac1, underlining a different impact of IQGAP1 overexpression between *CALR+* and *JAK2+* patients. We also found that calreticulin protein was expressed in erythrocytes independently of *JAK2* or *CALR* mutation but it didn't interact with IQGAP1. Finally, we showed that p21 activated kinase 1 (PAK1), a Rho GTPase effector implicated in cell motility, and its phosphorylated form (PAK1-P), co-precipitated with activated Rac1 as well as with IQGAP1.
Summary and Conclusions: Our data showed a deregulation of IQGAP1-Rho-GTPase signaling that could be implicated in erythrocyte adhesion alterations and thereby in vascular complications via the activation of the cytoskeleton motility protein PAK1. Differential recruitment of RhoGDI and Rac1 between *CALR+* and *JAK2+* patients might induce variations of PAK1 activation and thereby take part in variations of vascular risk among different genotypes.

P298

CRITICAL ROLE OF HIS499 IN REGULATING THE DIMERIZATION AND FUNCTION OF THE HUMAN THROMBOPOIETIN RECEPTOR AND RESTRAINING THE REPERTOIRE OF ASPARAGINE PATHOGENIC MUTATIONS

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Background: The thrombopoietin receptor (TpoR) is a member of the type-I cytokine receptor family that regulates the proliferation and differentiation of megakaryocytes, as well as stem cell homeostasis. Tpo binding mediates dimerization of the extracellular domain of the receptor, which imparts a specific orientation on the receptor's transmembrane (TM) and intracellular (IC) domains. TpoR can autonomously dimerize in the presence of asparagine substitutions in its TM domain leading to its activation, as it is the case with the Essential Thrombocythaemia associated mutant «TpoR S505N».
Aims: We aim to determine the molecular mechanisms regulating TpoR dimerization and function in the context of the asparagine mutant S505N, and whether TM asparagine mutations other than S505N could be identified in MPNs.
Methods: First, we took advantage of differences in the murine and human TpoR sequences and we performed asparagine-scanning mutagenesis on the receptors TM domain. We used a combination of transcriptional activity and cellular growth to map out the inactive and active orientations of the transmembrane helices. Second, we assess TpoR dimerization by using a split luciferase reporter. Third, we used protein segments corresponding to the receptor transmembrane and juxtamembrane domains in order to complete polarized IR and solid-state NMR spectroscopy**.**
Results: Asparagine mutants in the transmembrane domains of the murine and human TpoR did not display the same biologic effects; while mTpoR exhibited several active conformations; only one variant of hTpoR (S505N) was consistent with constitutive signaling. Our studies indicate that this discrepancy could be attributed to the eltrombopag (Promacta/Revolade; GlaxoSmithKline, London, UK) target, His499, only present in the human TpoR TM domain. Indeed, by point mutational studies, we showed that His499 was responsible for protecting the human receptor from activation by several Asn mutations. By spectroscopy, using short peptides, we showed that His499 accounts for a change in the conformation and secondary structure of the transmembrane helix and shifts the monomer-dimer equilibrium toward monomer for the human TpoR. Strikingly, replacement of His499 by Leu, the residue at the corresponding position in the murine TpoR induces dimerization of these peptides. When His499 is replaced by Leu in the human TpoR, asparagine substitutions at several different TM positions activate oncogenic signaling, like in the case of the murine TpoR.
Summary and Conclusions: We show that His499 is critical for dimerization of human TpoR and orientation-dependent activation. Using alignments of TpoRs from different species and other cytokine receptors we show His499 in TpoR evolved from primates and we discuss its possible role in evolution in preventing oncogenic activation by multiple asparagine substitutions.

P299

COMBINED CONSTITUTIVE CANONICAL AND NON-CANONICAL HH SIGNALING CAUSED BY DEPLETION OF PATCHED 2 INDUCES A MYELOPROLIFERATIVE PHENOTYPE AND TRANSFORMS CHRONIC MPNS INTO ACUTE LEUKEMIAS

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Background: MPNs frequently transform into leukemias, but the factors driving this process are not understood. Aberrant Hedgehog (HH) ligand secretion, as frequently found in MPN and CML, not only induces constitutive Smoothened-dependent canonical HH-signaling causing GLI1 transcription, but also causes PTCH1-dependent non-canonical HH signaling leading to constitutive ERK activation. HH-ligands do not only act on malignant cells, but also stimulate the surrounding niche cells, driving dual pathway activation in both compartments. Previous results in CLL show, that HH ligand induced dual pathway activation causes primary resistance to SMO-inhibitors. Combined canonical- and non-canonical pathway activation was so far never investigated regarding hematopoietic development and leukemic transformation.
Aims: Evaluate the effects of combined activated canonical and non-canonical HH signaling within hematopoietic and niche cells in normal and malignant hematopoiesis, especially regarding acceleration of myeloproliferative diseases into leukemias.
Methods: Human PB samples of CML (BCR-ABL), AML and MPN (Jak2V617F$^+$) patients were analyzed concerning canonical-and non-canonical HH signaling activation. The phenotype of Ptch2$^{-/-}$mice mimicking over-activated canonical and non-canonical HH signaling was described and this model was used for *in vivo* transplantation experiments. Therefore hematological or niche Ptch2$^{-/-}$was analyzed and combined with Jak2V617F or BCR-ABL regarding disease development.
Results: Our investigations show, that excess HH ligand secretion is present in MPNs and CMLs, while absent in AML, and constitutively activates canonical-and non-canonical HH-signaling. Furthermore 70% of MPN-and CML-cases display loss of the Ptch1-competitive Ptch2 receptor. Depletion of the Ptch2 receptor *in vitro* and *in vivo* recapitulates overactivation of both pathways (canonical-and non-canonical HH signaling), while depletion of Ptch1 only reflects constitutive Gli1 activation (canonical). Ptch2$^{-/-}$mice develop a pronounced hematopoietic phenotype with leukocytosis driven by an increase in neutrophils and monocytes, anemia, loss of B-and T-cells combined with a strong increase of cKit$^+$ progenitors in the peripheral blood and increased extramedullary hematopoiesis causing splenomegaly reflecting a MPN phenotype. LKS (lin$^-$cKit$^+$Sca1$^+$) cells residing within the BM (bone marrow) showed enhanced cycling properties causing exhaustion and loss of LKS cells over time, but improved stress hematopoiesis after 5-FU treatment. Niche change experiments show that cytopenias and loss of LKS cells are caused by overactivated HH signaling within the niche cells, causing depletion of osteoblasts and alterations of essential niche factors like *Cxcl12, Scf, Angiopoietin* or *Jagged1*. In contrast, the hematopoietic Ptch2$^{-/-}$is responsible for leuko-

cytosis and even promotes LKS expansion and replating capacity *in vitro*. Interestingly, depletion of Ptch2 in the niche or within hematopoietic cells dramatically altered Jak2V617F driven pathogenesis causing transformation of a non-lethal chronic myeloproliferative disease into an agressive AML-like disease with up to 30% blasts in the peripheral blood. In contrast, the BCR-ABL-driven leukemia was exclusively accelerated by the cell intrinsic Ptch2$^{-/-}$, but not by cell extrinsic HH activation.

Summary and Conclusions: Combined constitutive canonical and non-canonical HH activation induced by depletion of Ptch2 causes a MPN phenotype driven by cell intrinsic, but mainly cell extrinsic mechanisms and accelerates myeloproliferative diseases caused by Jak2V617F and BCR-ABL into acute leukemias. These finding suggest combined canonical-and non-canonical HH pathway inhibition as a potential treatment option to prevent disease progression in myeloproliferative diseases.

P300

PROSPECTIVE ISOLATION OF NOVEL POPULATIONS OF MEGAKARYOCYTE-AND ERYTHROID-PRIMED MEGAKARYOCYTE-ERYTHROID PROGENITORS DEMONSTRATES MEGAKARYOCYTE-BIASED LINEAGE COMMITMENT IN PRIMARY MYELOFIBROSIS

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Background: In Primary Myelofibrosis (PMF), mutations in the JAK-STAT pathway result in clonal myeloproliferation and abnormal megakaryopoiesis. Increased low-ploidy/immature megakaryocytes (MKs) are a diagnostic hallmark, and aberrant MK expression of fibro-angiogenic cytokines mediates BM fibrosis. The increased MKs in PMF have been attributed to increased MK proliferation, inhibited endoreduplication and apoptosis. MKs and erythroid (E) cells arise from a common progenitor (MEP). Due to lack of precise immunophenotypic definitions of both MEP and early committed MK progenitors (MKP), whether biased commitment to the MK lineage also contributes to aberrant megakaryopoiesis in PMF has not been properly explored.

Aims: Using current strategies, immunophenotypic MEP are a negatively defined, heterogeneous population. To enable detailed investigation of megakaryopoiesis in PMF we sought 1) To determine whether MEP cells primed/poised for MK differentiation and early committed MKs could be identified by their surface antigen expression 2) To prospectively isolate and compare these populations in PMF and controls.

Methods: Peripheral blood CD34+ progenitors from healthy mobilized donors (n=7) and JAK2+PMF (n=10) were analysed using previously validated immunophenotyping (Weissman *et al*). A novel strategy was then developed to examine expression of MK and E-associated antigens CD71, CD41 and CD42 within CD123-CD45RA-CD34+CD38+MEP. Specific MEP populations were FACS-isolated for functional/molecular studies at population and single-cell level using lineage-specific liquid cultures, clonogenic assays and transcriptional profiling (Taqman/Fluidigm).

Results: To capture the earliest stages of megakaryopoiesis, we first examined myeloid progenitor populations and found a higher % of immunophenotypically-defined MEP in PMF *vs* controls (P<0.05). Three MEP fractions were identified by their expression of CD71 and CD41. Prospective isolation of these populations demonstrated that CD71+CD41+MEP had highest MK colony forming activity ("MK-MEP"), while CD71+CD41-MEP showed highest E colony forming activity ("E-MEP"). Notably, MK-MEP, although MK-"primed", retained the ability for erythroid differentiation, giving rise to BFU-E colonies and GlyA+CD71hi mature erythroblasts, and CD71+CD41-MEP gave rise to MKs. Isolating populations from early*in vitro* MK cultures confirmed that CD71hiCD41-CD42-and CD71+CD41+CD42-cells were bipotent and could give rise to either MK or E, and CD71loCD41hiCD42+MKP were the first identifiable *committed* MKP with no E potential. Single cell and population transcriptional profiling with a panel of 43 mega-erythropoiesis genes confirmed that MK-MEP and E-MEP possessed MK and E gene expression signatures. Bivariate Pearson correlation analyses suggested that gene expression was strongly correlated between single cell E-and MK-MEP replicates (>0.7), with greater heterogeneity among total MEP. Comparing PMF and control MEP *ex vivo* demonstrated higher %MK-MEP in PMF (P=0.01). Further, a higher % of MK-MEP expressed CD42 in PMF(P=0.003) and MK-MEP from PMF patients showed more pronounced MK bias with reduced E colony forming activity and higher expression of MK-*vs* E-associated genes (increased Fli1/PF4 in tandem with reduced EKLF).

Summary and Conclusions: Co-expression of CD71 and CD41 allows for prospective identification of bipotent MEP cells that are primed/poised for MK *vs* E differentiation, while CD42 marks commitment to the MK lineage. Functional/molecular profiling indicate that MK-MEP and E-MEP represent homogeneous populations within the more heterogeneous MEP. In PMF, although the frequency of immunophenotypically-defined MEP appears higher, this is due to increased MK-committed CD42+MKPs. Together with reduced BFU-E output, this suggests a bias towards megakaryopoiesis at the expense of erythropoiesis at the level of the MEP. Abnormal regulation of mega-erythropoiesis in PMF may underlie the pathologically expanded MKs and refractory anaemia, and the ability to prospectively identify these populations enables further detailed molecular characterization in PMF and related disorders.

P301

HIGH RESOLUTION CYTOGENETIC MAPPING AND WHOLE EXOME SEQUENCING REVEAL A COMPLEX PATTERN OF CHROMOSOME 6P ABERRATIONS IN PATIENTS WITH MYELOID MALIGNANCIES

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Background: Chromosomal aberrations, together with somatic mutations, contribute to disease onset and progression in myeloid malignancies. The detection of these aberrations in patients has allowed identification of genes involved in the disease pathogenesis. Chromosome 6 shows high complexity of aberrations detected in patients. We previously showed that *JARID2* is a target for deletions amplified to homozygosity by 6pUPD (uniparental disomy). However, *JARID2* is not mutated in the rest of the patients with 6pUPD. This implies that other genes are possibly targets of chromosome 6p (chr 6p) aberrations.

Aims: Identification of target genes of chromosome 6p aberrations in patients with myeloid malignancies, using cytogenetic mapping and whole exome sequencing.

Methods: Peripheral blood samples (n=913) were collected from patients with myeloproliferative neoplasms (MPN), post-MPN acute myeloid leukemia (AML), myelodysplastic syndrome (MDS) post-MDS AML, de novo AML (dnAML) and chronic myeloid leukemia (CML), along with written informed consent, in compliance with local ethics committees. Genomic DNA was isolated from whole blood, granulocytes or mononuclear cells. Affymetrix Genome-Wide Human SNP 6.0 arrays and Genotyping Console software were used for detecting deletions, gains and UPDs. Whole exome sequencing was performed, using the TruSeq DNA sample prep kit and exome enrichment kit (Illumina), on the Illumina HiSeq2000 instrument. Data was analyzed with the GATK Haplotype Caller code. Variants were filtered for exonic and splice site variants, located in the affected region of chr 6 (individually defined for each sample). An allelic frequency filter of >50% was introduced in samples with UPD and gains of chr 6p. All variants were validated by Sanger sequencing. For 2 patients T lymphocyte DNA was used as control tissue DNA.

Results: We combined all chr 6 aberrations, detected by SNP microarrays, from 913 patient samples. A total of 41 chromosomal aberrations were detected on chr 6p in 25 patients (7 MPN, 2 progression phase MPN, 7 post-MPN AML, 2 post-MDS AML, 6 dnAML, 1 CML). Deletions represented 54% of events (n=22). Six patients harbored 6pUPD, two of which had focal deletions of *JARID2* amplified to homozygosity by UPD. An additional small deletion mapped to *JARID2*. Two independent UPD events were detected in one patient. The aberration map was complemented with exome sequencing data from 4 selected patients: 1 with a deletion (post-MPN AML), 2 with a UPD (post-MPN AML, dnAML) and 1 with a chr6 trisomy (secondary myelofibrosis). One somatic, 8 germline and 9 mutations of unknown origin were validated. None of the mutations were found in multiple patients. The somatic L521R mutation in *FAM65B* was amplified by the trisomy. Overlap of the mutations with chromosomal aberrations identified a commonly affected region containing genes *NCR3* and *PSORS1C1*. Trisomy amplified a germline *PSORS1C1* P38 frameshift mutation, whereas the *NCR3* R96Q mutation was amplified by UPD. A focal gain overlapped with a UPD-amplified *UHRF1BP1* S506P mutation.

Summary and Conclusions: Intersecting microarray data from a large pan-myeloid cohort of patients with exome sequencing data of 3 accelerated phase MPN/post-MPN AML and 1 dnAML patient resulted in identification of 4 novel genes, in addition to *JARID2*, as putative targets of chr6p lesions. As the position of the genes does not overlap with all 6pUPDs, it remains possible that mutations in regulatory regions or epigenetic events are targets amplified by 6pUPD in patients with myeloid malignancies.

P302

DNA METHYLATION PROFILING OF SORTED CELLS FROM MYELOFIBROSIS PATIENTS REVEALS ABERRANT EPIGENETIC REGULATION OF IMMUNE PATHWAYS AND IDENTIFIES EARLY MPN DRIVER GENES

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Background: Primary myelofibrosis (PMF) belongs to the heterogeneous group of chronic myeloproliferative neoplasms (MPN) together with essential thrombocytosis (ET) and polycythemia vera (PV). It has been suggested that these neoplasms represent a biological continuum from early cancer stage (ET, PV) toadvancedMF. Multiple studies report frequent mutations in epigenetic regulators. However, the association to epigenetic changes and the role of epigenetic aberrations in different cell populations is still unknown.
Aims: We therefore performed DNA methylation profiling of sorted cells from MF patients to unravel pathways contributing to disease phenotype and gain insight into MF pathogenesis. As an aberrant DNA methylation pattern may be an early event in tumorigenesis and may be crucial for progression of the malignant clone towards the more aggressive forms of MPN, we further aimed to identify candidate driver genes.
Methods: Peripheral blood samples from 16 MF patients and BM (bone marrow) and peripheral blood from 3 healthy age matched controls were sorted in CD34+ cells, granulocytes and mononuclear cells, and analysed for differential methylated regions using Illumina Infinium HumanMethylation 450K BeadChip. Candidate genes were validated by pyrosequencing in a second cohort of 30 MF patients where DNA was extracted from full blood (PB). To identify potential driver genes, the DNA methylation status of candidate genes was likewise analyzed in PB from a larger cohort consisting of 60 ET and PV patients.
Results: The number of differentially methylated CpG sites between MF cells and the respective counterparts from healthy donors differed extensively among the three cell populations analyzed. In MF CD34+ cells 1628 CpG sites were differentially methylated compared to normal CD34+ cells, and 519 and 213 differential methylated CpG sites were observed in MF granulocytes and MF mononuclear cells, respectively (Δβ was set to 0.2 with an adjusted p-value <0.05, T-test). Differentially methylated genes were mainly involved in cancer and embryonic development pathways in both the MF CD34+ cells and granulocytes, while mononuclear cells also showed aberrant methylation of genes involved in the inflammatory disease pathways. MF granulocytes showed significant aberrations in pathways involving immunological diseases, cell death and survival. Candidate genes have been identified and validation is ongoing. Interestingly, a gradual increase of the DNA methylation level of*TRIM59*was observed from the healthy controls (31%) over ET (53%) to PV (64%) and MF (65%). ET patients could be distinguished from both healthy controls (*P*=0.0004, Mann-Whitney test) and from the more progressed stages PV and MF (*P*=0.0132, Mann-Whitney test) based on the*TRIM59*DNA methylation level.*TRIM59*promoter methylation could, however, not discriminate between PV and MF (*P*=0.4721, Mann-Whitney test).
Summary and Conclusions: Genome-wide DNA methylation profiling of sorted MF blood cells provided an exclusive insight into the pathways that contribute to the MF phenotype at a cell specific level. The MF CD34+ cells had the highest number of differential methylated CpG sites (n=1628) when comparing to granulocytes (n=519) and mononuclear cells (n=213) and should be cells of choice when exploring new treatment strategies. Interestingly, the mononuclear compartment show aberrant methylation of inflammatory genes supporting a role of aberrant immune regulation in the pathogenesis of MPN. Earlier studies have failed to identify aberrant methylation in early ET and PV, however, our preliminary data on the methylation of individual genes (*TRIM59*promoter methylation) shows that early MPN driver genes may be identified. Further studies are ongoing to unravel the power by which DNA methylation can discriminate between reactive thrombocytosis and ET. These data will be presented at the meeting.

P303

SEQUENTIAL EVALUATIONS OF CALR MUTANT BURDEN IN PATIENTS WITH MYELOPROLIFERATIVE NEOPLASMS

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Background: Recently, somatic mutations of *CALR*, encoding calreticulin, have been found in most patients with essential thrombocythemia (ET) or primary myelofibrosis (PMF) with nonmutated *JAK2* and *MPL*. Previous studies have reported that most *CALR* mutations are heterozygous. Data regarding sequential evaluations of *CALR* mutant burden are still lacking.
Aims: We studied the variation of *CALR* mutant burden during follow-up in a large cohort of *CALR* mutated myeloproliferative neoplasms (MPN) compared to *JAK2* (V617F) mutated patients matched for diagnosis.
Methods: Inclusion in the current study required availability of clinical data at diagnosis and during follow-up and at least two DNA samples to assess variations of mutant allele burden. A total of 94 *CALR* mutated patients (60 ET, 29 PMF, 5 post-ET myelofibrosis) were collected from our database at the Department of Hematology Oncology Pavia, Italy. This cohort was compared with a cohort of 218 *JAK2* mutated patients with similar diagnosis (170 ET, 44 PMF, 4 post-ET MF). The evaluation of *CALR* mutant burden was done comparing the height of the mutated and wild type picks. A quantitative real-time polymerase chain reaction (qRT-PCR)-based allelic discrimination assay was employed for the quantification of the *JAK2* (V617F) allele. For each patient we calculated the variation from first to last evaluation and the slope between sequential assessments (*i.e.* the slope of the regression line with allele burden as outcome and time as covariate).
Results: 91 of 94 *CALR* mutated patients remained positive at the second evaluation, except for three of them who underwent bone marrow transplantation, that were indeed excluded from further analyses. All of the 3 leukemic evolutions were still *CALR* mutated. Statistical analysis was carried on ET and PMF, due the low number of post-ET MF.Considering first and last evaluation, we observed a significant increase in the median value of mutant *CALR* in ET patients (45.5% *vs* 49%, Wilcoxon signed-rank test *P*=0.007), while it remained stable over time in PMF (51% *vs* 51%, *P*=0.428). Median values of slope were positive both in ET (0.05% increase of *CALR* mutant alleles/month) and in PMF (0.02% increase of *CALR* mutant alleles/month). Mann Whitney U test showed a trend toward a difference between natural and therapy-related slope in ET (*P*=0.09) but not in PMF (*P*=0.126). Then we calculated the slope in a corresponding cohort of *JAK2* mutated ET and PMF: the slope was 0.01% (0.01% increase of *JAK2* V617F alleles/month) both in ET and in PMF, with a significant difference between natural and therapy-related slope in ET (*P*=0.031). To evaluate the effect of the type of mutation (*CALR vs JAK2*) on the slope of mutant burden adjusting for the effect of diagnosis (PMF *vs* ET), we performed a multivariate linear regression in all untreated patients (to avoid the potential influence of cytoreduction). Among patients with a positive slope (n=71, 14 ET *CALR*; 7 PMF *CALR*; 41 ET *JAK2*; 9 PMF *JAK2*), the increase of mutant burden was significantly higher in *CALR vs JAK2* (β=0.26, p=0.009) with no difference between diagnosis (p=0.603).
Summary and Conclusions: The increase of mutant burden during time is higher in *CALR* patients respect to *JAK2* regardless of diagnosis.

P304

NDEL1-PDGFRB FUSION GENE IN JUVENILE MYELOMONOCYTIC LEUKEMIA ASSOCIATED WITH RESISTANCE TO TYROSINE KINASE INHIBITORS

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Background: A novel fusion gene NDEL1-PDGFRB was identified in a child with juvenile myelomonocytic leukemia (JMML) with no mutations in NF1/RAS/PTPN11/cCBL. Malignant disorders displaying fusion genes involving PDGFRB are generally sensitive to imatinib (IM) (Chan *et al.*, 2014), but the patient relapsed on IM and subsequently on nilotinib (NIL) treatment after initial response.
Aims: Unravel molecular mechanisms underlying acquired resistance to tyrosine kinase inhibitors (TKIs), and determine *in vitro* sensitivity to different TKIs.
Methods: Cytogenetic, FISH and 5'-RACE analyses were used to identify the translocation. A point mutation in the PDGFRB TKD was detected by Sanger sequencing of overlapping amplicons in peripheral blood specimens from the time of relapses and diagnosis. Protein modelling was done on SWISS-MODEL and I-TASSER servers using available crystal structures of VEGFR2 and CSF-1R TKDs. To test the TKI responses, BaF3 cells constitutively expressing retrovirally introduced NDEL1-PDGFRB constructs (BaF3-N-Pb) were tested in viability assays against a panel of TKIs.
Results: Reciprocal translocation t(5;7)(q33;p11.2) encoding a novel fusion gene NDEL1-PDGFRB was identified in the patient. The chimeric mRNA contains the 5´exons 1-5 of NDEL1 encoding the dimerization domain fused in frame to the PDGFRB exons 10-22 encoding the transmembrane (TM) and

tyrosine kinase (TKD) domains. Point mutation converting Asp850 into glutamate (D850E) in the activation loop (A-loop) was identified from the time of both relapses, but not diagnosis. Modelling of PDGFRβ TKD suggested that the mutation D850E may result in higher activity of the TKD and resistance to type II TKIs, in line with the observed failure of IM and NIL treatment. However, the model suggested sensitivity to type I TKIs such as dasatinib (DAS) and midostaurin (PKC412). BaF3-N-Pb^{WT} cells were sensitive to IM (IC_{50} =60 nM), NIL (100 nM), sorafenib (SOR; 20 nM), DAS (5 nM) and PKC412 (20 nM). Conversely, BaF3-N-Pb^{D850E} cells exhibited high IC_{50} to the type II TKIs IM (>2500 nM), NIL (1000 nM) and SOR (2500 nM), but retained sensitivity to the type I TKIs DAS (20 nM) and PKC412 (5 nM). Activating mutations in the A-loop of RTKs such as PDGFRA (D842V), FLT3 (D835V) or c-Kit (D816V) associated with resistance to type II TKIs were described in different tumor entities. However, the mutation D850E in the PDGFRβ TKD with apparent insensitivity to IM, NIL, and SOR revealed a different pattern of resistance than the same amino acid exchange at the corresponding site of PDGFRα (D842E) in the FIP1L1-PDGFRA fusion gene which had been previously shown to be sensitive *in vitro* to IM (4 nM), NIL (12.5 nM), and SOR (0.25 nM) (von Bubnoff *et al*., 2011). Protein modelling of PDGFRβ TKD suggested that the distinct properties of PDGFRβ TKD carrying D850E may be attributed to the poorly conserved residue in +3 position to the mutated D850. In PDGFRβ, it is arginine (R853), as opposed to histidine (H845) in PDGFRα. Mutation R853H in PDGFRβ carrying D850E reduced its kinase activity and restored *in vitro* sensitivity to type II TKIs.

Summary and Conclusions: To our knowledge, this is the first observation of an exchange between two negatively charged amino acids in a tyrosine kinase PDGFRβ associated with a major change in responsiveness to TKI treatment. The ongoing further investigation of this finding may extend our understanding of structural interactions leading to TKI resistance and activation of tyrosine kinases in MPNs.

P305

IDENTIFICATION AND CHARACTERIZATION OF PUTATIVE NEOPLASTIC STEM CELLS IN PATIENTS WITH MAST CELL LEUKEMIA

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Background: Leukemic stem cells (LSCs) have been identified as an important therapeutic target in human leukemia and other related hematologic disorders. Systemic mastocytosis (SM) is a rare hematopoietic neoplasm characterized by abnormal growth and expansion of mast cells (MCs) in the bone marrow (BM) and other organ systems. Whereas patients with indolent SM (ISM) have a normal life-expectancy, patients with more advanced forms of SM have a grave prognosis. In these patients, neoplastic MCs are usually resistant against conventional drugs and various targeted drugs. MC leukemia (MCL) is the rare leukemic variant of advanced SM, defined by a rapid expansion of immature MCs in various hematopoietic organs and a poor prognosis with short survival. Although MCL is considered a stem cell disease, little is known about the origin and phenotype of MCL-initiating LSCs.

Aims: We examined the phenotypic and functional characteristics of putative LSCs in patients with aggressive SM (ASM, n=12) and MCL (n=6) by flow cytometry.

Methods: Highly enriched, sorted LSCs were injected into NOD-SCID-IL-2Rg$^{-/-}$mice exhibiting human membrane-bound SCF (NSG_{SCF}).

Results: We found that disease-initiating and propagating LSCs reside within a $CD34^+$fraction of the MCL clone. Whereas cell fractions containing $CD34^+$ cells as well as highly enriched $CD34^+$ cells engrafted in NSG_{SCF} mice with a MCL-like disease, no substantial engraftment was produced by MC-rich but stem cell-depleted, $KIT^+/CD34^-$ cell fractions obtained from the same patients. Moreover, we were able to confirm long-term engraftment by successful serial transplantations into secondary recipient mice. As assessed by flow cytometry, the $CD34^+/CD38^-$ MCL LSCs were found to co-express several stem cell markers, including aminopeptidase-N (CD13), leukosialin (CD43), Pgp-1 (CD44), the IL-3R alpha-chain (CD123), AC133 (CD133) and CXCR4 (CD184). In addition, in several of the patients examined, MCL LSCs were found to display IL-1RAP, a surface antigen that is otherwise expressed in CML LSCs but is not expressed in normal stem cells. In addition, MCL LSCs were found to express various cell surface targets, including CD33 and CD52. By contrast, MCL LSCs did not express CD2, CD25, CD26 and CLL-1. In patients with ISM and ASM, the $CD34^+/CD38^-$ stem cells exhibited a similar surface marker profile compared to MCL, but expressed higher levels of CD117, lower levels of CD133, and did never express IL-1RAP. Subsequently, we examined the effects of target-specific antibodies. As assessed by flow cytometry, the CD52-targeting antibody alemtuzumab was found to induce lysis of $CD34^+/CD38^-$cells in all MCL samples analysed. Furthermore, pre-incubation of MCL cells with alemtuzumab prior to injection into NSG_{SCF} mice resulted in a significantly reduced engraftment.

Summary and Conclusions: In conclusion, our data show that the MCL clone originates from a primitive hematopoietic stem cell. In addition, our data indicate that MCL LSC express a number of clinically relevant surface targets, including CD33, CD52 and CD117 (KIT). These observations may lead to the development of novel LSC-eradicating treatment concepts in this highly aggressive and drug-resistant form of leukemia.

P306

COMORBIDITY AND ITS IMPACT ON ALL-CAUSE MORTALITY IN DANISH PATIENTS WITH MYELOPROLIFERATIVE NEOPLASMS FROM 1994-2013: A POPULATION-BASED MATCHED COHORT STUDY

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Background: Comorbidities are frequent in patients with myeloproliferative neoplasms (MPNs), and studies show increased comorbidity already at time of diagnosis. Furthermore studies in different cancer types, show an impact of comorbidity on all-cause mortality. Neither the overall comorbidity, nor the impact of it on all-cause mortality, have been widely investigated in large epidemiological studies including both MPN patients and matched controls.

Aims: To describe comorbidity in myeloproliferative cancer patients in Denmark compared with matched controls and assess impact of comorbidity on all-cause mortality in different MPN subgroups.

Methods: We conducted a population based cohort study, including all patients, age 18+ or older, with a first listed diagnosis of MPN: essential thrombocythemia (ET), polycythemia vera (PV), myelofibrosis (MF), unclassifiable MPN (MPN-U) and chronic myeloid leukemia (CML) in the Danish National Registry of Patients, between 1994-2013. Follow-up status was determined through linkage to the Danish Civil Registration System, and started 30 days after the first diagnosis date of MPN =index date, and continued until death, emigration, or end of 2013, whichever came first. Comorbidity prevalence at the index date was measured using an adapted version of the Charlson comorbidity index (CCI), within a ten-year period preceding index date. Codes corresponding to MPN diagnosis were excluded to allow comparison of overall non-MPN cancer comorbidity. To compare CCI with the background population, we identified ten sex and age matched individuals, without MPN, for each corresponding MPN patient. Patients and matched controls were classified into four diagnostic calendar periods (1994-1998, 1999-2003, 2004-2008, 2009-2013) and in five groups by CCI score (0, 1, 2, 3, ≥4). Prevalence of comorbidity in MPN patients and controls were compared using chi-square test and t-tests for average CCI score. Impact of comorbidity on all-cause mortality was analysed by Kaplan-Meier plots and Cox regression. Patients and controls with CCI score 0 in each group was used as reference in all analyses. We adjusted for period of diagnosis, smoking related and alcohol related conditions (yes/no) and for CML patients before/after year 2000 to allow for the introduction of tyrosine kinase inhibitor treatment.

Results: We included 9,868 patients (ET=2,735, PV=3,324, MF=603, U-MPN=1,849, CML=1,357) and a total of 98,627 matched controls. Our results show a higher percentage of patients than controls with CCI score >0 and differences between MPN patients and controls were observed for mean CCI, with significant higher CCI score in all MPN subgroups (p<0.0001) (table 1). All-cause mortality for CCI score 0, was significantly increased in all MPN subgroups compared to controls, except for ET (p=0.12) (table 1). Furthermore data showed increased all-cause mortality with increasing CCI score for both MPN patients and controls, but the influence of increasing CCI had lower impact in all MPN subgroups than in controls (P<0.05) and ET and PV patients with comorbidity had lower mortality than corresponding controls.

Tabella 1. Percentage of CCI score >0, mean CCI (± standard deviation) and hazard ratios of all-cause mortality between patients and controls without comorbidity (95%-confidence intervals).

MPN group	% CCI score > 0		Mean CCI [SD]		All-cause mortality HR (95 % CI)	
	Patients	Controls	Patients	Controls	Patients / Controls	P-value
ET	47.5	28.8	0.95 [±1.43]	0.61 [±1.30]	1.09 (0.98-1.22)	0.12
PV	48.2	30.4	0.95 [±1.44]	0.64 [±1.31]	1.21 (1.11-1.31)	<0.001
MF	52.9	35.9	1.34 [±1.84]	0.78 [±1.43]	3.21 (2.73-3.76)	<0.001
U-MPN	56.4	35.8	1.27 [±1.69]	0.76 [±1.40]	2.20 (1.99-2.44)	<0.001
CML	45.6	26.4	1.16 [±1.81]	0.55 [±1.24]	2.43 (2.16-2.74)	<0.001

Difference in mean CCI between patients and controls: P value < 0.0001 for all analysis.

Summary and Conclusions: MPN was associated with significantly increased comorbidity compared to the general population and patients with no comorbidity had higher all-cause mortality, except for ET patients. Comorbidity was associated with increased all-cause mortality in all MPN groups and controls, but impact of increasing CCI was different within the groups. Remarkably ET and PV patients with comorbidity had lower mortality than controls.

Myeloproliferative neoplasms - Clinical 1

P307

IMPACT OF JAK2, CALR OR MPL MUTATION STATUS ON PREGNANCY OUTCOME IN PATIENTS WITH ESSENTIAL THROMBOCYTEMIA

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Background: Essential thrombocytemia (ET) may occur in women of childbearing age. Pregnancy may therefore be an issue in the clinical management of young women with ET. Previous studies have reported live birth rates of 50 to 70% and spontaneous abortion rates of 25% to 50%, mostly during the first trimester. The pathogenesis of pregnancy complications is poorly understood. Indeed, the association between *JAK2* (V617F) mutation and poor pregnancy outcome is uncertain, while data regarding the impact of the other MPN-associated driver mutations are lacking.
Aims: We studied the impact on pregnancy outcome of the three MPN driver mutations (*JAK2*, *MPL*, *CALR*) in a large cohort of pregnant ET patients.
Methods: Inclusion in the current study required availability of clinical data at diagnosis and during pregnancy, and at least one DNA sample to assess mutation status. A total of 155 pregnancies that occurred in 94 patients with ET were collected from 2 centers (Department of Hematology Oncology Pavia, and Division of Internal Medicine Padova). All patients were screened for *JAK2*, *MPL* and *CALR* mutations with previously reported methods.The 4 genotypic subgroups (*JAK2* mutated, *MPL* mutated, *CALR* mutated, and triple negative) were compared in terms of pregnancy outcome. Pregnancy complications included fetal loss (first-, second-or third-trimester miscarriage), intrauterine growth retardation (IUGR), and maternal complications.
Results: Of 94 ET patients studied, 59 (62.8%) carried *JAK2* (V617F), 19 (20.2%) carried *CALR* mutations, 2 (2.1%) carried *MPL* mutations, and 14 (14.9%) were triple-negative. The hemoglobin value at diagnosis was the only hematological parameter that differed according to mutation status, being higher in *JAK2* (V617F)-mutated patients (*P*<0.001). Three pregnancies that were terminated with voluntary abortion were excluded from this analysis. Overall, 72 of 152 pregnancies (47.4%) were complicated: 46 (30%) by fetal loss (37 during the first trimester, 6 during the second, 3 during the third), 18 (11.8%) by maternal complications, and 13 by IUGR (8.6%). The rate of complications (including both fetal and maternal complications) was not influenced by mutation status (Fisher's exact test *P* 0.134). When we restricted the analysis to late pregnancy losses, a relationship with *JAK2* (V617F) mutation (*P* 0.027) was found: second-and third-trimester miscarriage occurred in 9.4% of *JAK2* (V617F) pregnancies *vs* none of *MPL*/*CALR*/triple-negative pregnancies. Considering the potential role of calendar year at pregnancy, we observed that the rate of pregnancy complications before 2007, was higher than that reported after 2007 (54% *vs* 35%, *P* 0.028). Then, we performed a multivariate analysis considering calendar year (before or after 2007) and mutation status (*CALR vs JAK2*/*MPL*/triple negative). In multivariate analysis we found that the occurrence of pregnancy before 2007 correlated with a poorer outcome (OR 2.4, *P* 0.024), and the presence of *CALR* mutation showed a trend toward a lower rate of pregnancy complications (OR 0.4, *P* 0.059).
Summary and Conclusions: The presence of *JAK2* (V617F) mutation is associated with late pregnancy losses. The outcome of pregnancy seems to be influenced by calendar year, suggesting an improvement in our management strategy. Pregnancies occurring in *CALR*-mutated patients show a trend toward a better outcome than those occurring in *JAK2*-mutated or *MPL*-mutated or triple negative patients.

P308

QUALITY OF LIFE IN PATIENTS WITH MYELOPROLIFERATIVE NEOPLASMS COMPARED TO NORMAL INDIVIDUALS-CASE-CONTROL EVIDENCE OF SIGNIFICANT IMPAIRMENT

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Background: The myeloproliferative neoplasms (MPNs) including polycythaemia vera (PV), essential thrombocythaemia (ET) and primary myelofibrosis (PMF) arediseases of hemopoietic stem cell originthat causesignificant morbidity and mortality. Comparisons betweensymptom burdenof MPN cases and those of the general population have not previously been reported.
Aims: The aim of the study was to compare the quality of life experienced by patients with classic MPNs (PV, ET, PMF) with that of age and sex matchedindividuals with no evidence of MPN.
Methods: The myeloproliferative neoplasm symptom assessment form (MPN-SAF) is a reliable and validated clinical tool used for assessing MPN patient symptom burden. A pilot case-control study of MPN, called the 'MyelOproliferative neoplasmS: An In-depth Case-Control study' (MOSAICC), recruited MPN patients and controls in Belfast, Northern Ireland and Southampton, England. The MPN-SAF was completed by cases and, for the first time, by non-blood relatives andindividuals from primary care centres all without evidence of MPN to act as controls. Mean symptom scores were compared between cases (n=106) and controls (n=124). Mean scores in cases were then compared to published data on 1446 MPN patients from the Mayo Clinic, USA. Mean scores in cases from the UK and USA studies were then combined and compared with controls.
Results: MPN cases had significantly higher mean scores than controls for 26 of the 27 symptoms measured (p<0.05). Fatigue was the most common symptom in cases and controls (92.4% and 78.1% respectively). Patients with PMF reported the worst symptomatic burden (88.3%) which was significantly higher than that reported by PV cases (p<0.001). Female MPN patients suffered worse symptomatic burden than males (p<0.001). Compared to MPN cases in the USA, UK cases reported similar symptom burden in all categories exceptlower satiety (p=0.046).Combining the UK and USA cases resulted in an increase in significance of reported symptoms compared with the controls. Interference with work was reported in 77.4% of cases compared with 54.8% of controls (p<0.001). Abdominal pain occured in 46.4% of cases *versus* 20.3% of controls (p<0.001). Sexual problems were experienced by 60.9% of cases against 27.9% of controls (p<0.001). Bone pain occurred in 49.3% of cases compared with 19.7% of controls (p<0.001).Overall QoL was impaired in 78.4% compared with 57.4% of controls (p<0.001).
Summary and Conclusions: This novel study demonstratesthe significant morbidity experienced by MPN patients compared to controls and the similarities between patients in the UK and USA highlighting the common need to manage disease burden. For the first time the MPN-SAF has been shown to be a good discriminatory tool to assess the extent of symptoms in MPN patients compared to normal individuals.

P309

PREDICTORS OF SURVIVAL IN PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA

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Background: Standard risk stratification for overall survival (OS) in patients with essential thrombocythemia (ET) is based on advanced age and history of thrombotic events. Recently, International Prognostic Score for ET (IPSET) incorporated also leukocytosis in prognostic model.
Aims: was to establish additional risk factors for OS in ET patients.
Methods: The study was conducted on 244 consecutive patients with ET who were diagnosed according to WHO criteria and treated at the Clinic for Hematology, CCS, from January 2000 to January 2012. The following parameters at diagnosis of ET were taken into consideration to find prognostic risk factors for OS: age, sex, leukocyte and platelet count, hemoglobin level, *JAK2* mutational status, splenomegaly and thrombotic events (previous and during the follow-up). We also analysed influence of cardiovascular (CV) risk factors on survival. We considered as main CV risk factors presence of arterial hypertension, diabetes mellitus and hyperlipidemia while smoking attitude was considered as additional CV risk factor.
Results: Mean age at the time of diagnosis was 56 years (range: 18-85 years) and 68% of patients were women. After the median follow up of 7 years, 32 deaths were documented (13.2%). The 5-and 10-years OS was 95.9% and 79.7% respectively. Patients with CV risk factors had increased risk of death (HR=2.33). In present study, univariate Cox analysis identified following risk factors as unfavorable predictors of survival: age≥60 years (p<0.001), leukocyte count ≥$10x10^9$/L (p=0.004), previous thrombosis (p=0.002), presence of CV risk factor (p=0.048) and CV risk factor ≥1 plus active tobacco use (p=0.012). Accordingly, we assigned risk scores based on hazard ratios (HR) to age≥60 (HR=7.2; 2 points), 1 previous thrombosis (HR=1.19; 1 point), ≥2 previous thrombosis (HR=5.7; 2 points), leukocyte count ≥$10x10^9$/L (HR=3.15; 1 point), presence of main CV risk factor (HR=2,33; 1 point) and CV risk factor ≥1 plus active tobacco use (HR=2,08; 2 points). A final 4-tiered prognostic model named Cardio-IPSET was thus developed, as low (score 0-1), intermediate-1 (score 2-3), intermediate-2 (score 4) and high risk (score 5-7) with median survivals were: not reached for the low risk group (81 patients), not reached for the intermediate-1 risk group (92 patients), 122 months for the intermediate-2 (47 patients) and 97 months for the high risk group (24 pts) (log rank=52.154, *p*<0.001). Multivariate regression analysis confirmed the statistical significance of new Cardio-IPSET prognostic model (*p*<0.001, HR 3.169, 95%CI 2.186-4.594) as only independent predictor of OS in ET patients. This new model displayed a better hazard ratio profile compared to the standard risk stratification (log rank=23.262, p<0.001) and IPSET (log rank=40,010, *p*<0.001). In addition, patients who developed arterial thrombosis during the follow-up had significantly shorter survival then those who did not develop the arterial thrombosis (97 months *vs* 140 months, *p* <0.001).

Summary and Conclusions: The addition of CV risk factors allows better prognostic assessment by delineating the intermediate risk category and improved identification of the high-risk patients. Accordingly, inclusion of CV risk factors is essential for individualizing assessment and optimizing the treatment of ET patients in term to improve their outcome.

P310

ELEVATED HEMOGLOBIN CONCENTRATION AND RISK OF ACUTE MYOCARDIAL INFARCTION AND VENOUS THROMBOEMBOLISM

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Background: Patients with polycythemia vera (PV) and elevated hemoglobin (Hb) concentration are at an increased risk of cardiovascular disease and venous thromboembolism (VTE). In earlier studies, patients without PV but elevated Hb were at increased risk of cardiovascular events. However, these studies were carried out before the discovery of *JAK2*V617F in the year 2005 and a number of patients with idiopathic erythrocythosis were later diagnosed with PV. Thus, there is paucity of data on the risk of vascular events in persons with an elevated Hb concentration with no known PV diagnosis.
Aims: To assess the risk of acute myocardial infarction (MI) and VTE in relation to Hb concentration among healthy blood donors without PV.
Methods: The study was based on the Scandinavian Donations and Transfusions (SCANDAT2) database, which includes nationwide data on blood donations including donor identity, date of donation, as well as current Hb from Sweden and Denmark since the 1960's and 1980's, respectively. From SCANDAT2, all donors who had performed at least one donation between 1987 and 2012 were identified. Through the nationwide Swedish and Danish Patient and Cancer Registers, information on events of MI and VTE (deep venous thrombosis and pulmonary embolism) as well as relevant co-morbidities was obtained. Patients with a diagnosis of PV were excluded. The association between Hb concentration and risk of MI or VTE was assessed using pooled logistic regression adjusted for age, sex, calendar period, country of birth, and co-morbidity. We also fitted conditional, person-adjusted models, where each individual was compared to him-/herself. Hb concentration was considered time-dependently, allowing values to change with time. Separate analyses were carried out for men and women. Results are presented as odds ratios (ORs) with 95% confidence intervals (CIs).
Results: In total, 1.6 million blood donors with a total of 22.5 million Hb measurements were included. Median age at first donation was 33.2 years, 50% of donors were women and 65% were from Sweden. The risk of MI increased gradually with higher Hb in both men and women. In the standard, adjusted model, women with Hb ≥16.5 g/dL were at 3-fold increased risk of MI (OR=3.00; 95% CI 1.95-4.62) compared to women with an Hb of 12.0-13.4 g/dL (Table). Similarly, the OR for MI in men with Hb ≥17.5 g/L was 3.49 (95% CI, 2.81-4.33) compared to those with Hb 13.0-14.4 g/dL. In the conditional model, where each subject serves as his or her own control, risks were attenuated. A slight increase in risk of MI remained in men with Hb ≥17.5 g/L (OR=1.29, 1.05-1.57) but no significantly elevated risk in women with Hb ≥16.5 g/L (OR=0.91, 0.56-1.48). For VTE, the standard model revealed a pattern with elevated risks both at subnormal Hb in men with Hb <13.0 (OR=1.61, 1.33-1.95), and a gradually increasing risk with higher Hb (Table). Again, the association largely disappeared in the person-specific conditional model. For women there was no association between current Hb and VTE risk in the standard model and an inverse association in the conditional model.

Tabella 1. Relative risk of myocardial infarction and venous thromboembolism among female and male blood donors in relation to hemoglobin concentration.

Hemoglobin concentration (g/dL)	Myocardial infarction			Venous thromboembolism		
	Number of events	Standard, adjusted model*	Conditional adjusted model†	Number of events	Standard, adjusted model*	Conditional adjusted model†
		Odds ratio (95% confidence interval)			Odds ratio (95% confidence interval)	
Women						
0.80-11.9	64	1.21 (0.93-1.57)	1.12 (0.79-1.58)	131	0.94 (0.78-1.12)	1.16 (0.92-1.46)
12.0-13.4	397	1.00 (ref)	1.00 (ref)	937	1.00 (ref)	1.00 (ref)
13.5-14.9	384	1.23 (1.07-1.42)	0.99 (0.77-1.28)	737	1.14 (1.03-1.26)	0.83 (0.70-0.99)
15.0-15.9	110	2.84 (2.29-3.52)	0.96 (0.66-1.39)	82	1.23 (0.98-1.55)	0.55 (0.41-0.73)
≥16.0	22	3.00 (1.95-4.62)	0.91 (0.56-1.48)	9	0.93 (0.48-1.79)	0.27 (0.18-0.42)
Men						
0.80-12.9	133	1.01 (0.84-1.20)	0.89 (0.76-1.04)	118	1.61 (1.33-1.95)	1.11 (0.91-1.35)
13.0-14.4	1 684	1.00 (ref)	1.00 (ref)	991	1.00 (ref)	1.00 (ref)
14.5-15.9	3 198	1.36 (1.28-1.45)	1.13 (1.01-1.25)	1 672	1.15 (1.06-1.24)	1.20 (1.05-1.38)
16.0-17.4	1 098	2.14 (1.98-2.31)	1.20 (1.04-1.39)	385	1.26 (1.12-1.42)	1.09 (0.90-1.33)
≥17.5	90	3.49 (2.81-4.33)	1.29 (1.05-1.57)	18	1.38 (0.86-2.19)	0.89 (0.66-1.19)

*Adjusted for country, attained age, calendar year, and comorbidity (chronic obstructive pulmonary disorder, cancer, diabetes mellitus, atrial fibrillation, obstructive sleep apnea syndrome, hypertension, angina pectoris).
†Person-specific model with hemoglobin concentrations adjusted for country, attained age, calendar year, and comorbidity (chronic obstructive pulmonary disorder, cancer, diabetes mellitus, atrial fibrillation, obstructive sleep apnea syndrome, hypertension, angina pectoris).

Summary and Conclusions: These findings imply that an elevated Hb can serve as a marker of an increased risk of MI and VTE. In addition, a subnormal Hb can indicate an elevated risk of VTE. However, when each subject served as his/her own control, the risk elevations largely disappeared, indicating that the risk is most likely driven by additional underlying co-morbidities and cannot exclusively be attributed to the Hb elevation itself. Nonetheless, an elevated Hb signals the need for thorough assessment of cardiovascular risk factors.

P311

IMPACT OF DYNAMIC BLOOD COUNT FOLLOW-UP ON VASCULAR COMPLICATIONS IN 217 PV PATIENTS

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Background: Vascular complications and are risks for patients with polycythemia vera (PV).
Aims: To investigate if blood counts, with respect to different treatments, influence the complication rate and/or survival during the course of the PV disease.
Methods: 217 PV patients from Uddevalla Sweden, Luleå Sweden, Roskilde Denmark and Dijon France were included. The mean follow-up time was 6.2 years per patient. Blood values and co-morbidity were recorded at diagnosis, during treatment and at the time of any complication were retrieved.
Results: At the time of diagnosis the median age 70 years, hemoglobin (Hb) level was 17.6 g/dL, hematocrit (Hct) 55%, white blood count (WBC) 10.9 x10^9/L and platelets (Plt) 512x10^9/L. LDH was elevated in 136 of 156 patients. 38 patients (median age 77 years) experienced vascularevents during follow-up, 40 patients (median age 72 years) had non-vascular complications and 139 patients (median age 66) had no complications. Both complication groups had significantly higher age compared to the patients without complications (p<0.01). No significant differences were found in Hb, Hct, WBC or Plt. Elevated LDH at diagnosis was significantly more frequent in the patient group with vascular complications compared to the patients without (p<0.01). The main treatmentswere Hydroxyurea (HU) (139 patients), Phlebotomy (Phl) only (55 patients) and other (interferon, busulfan or pipobroman in 23 patients). No significant survival differences were found between the HU and Phl groups (p=0.6), even though the median age was 72 and 66, respectively. A total of 78 PV patients (36%) had recorded complications during the observation time. There were 35 vascular complications, 16in the139 HU patients compared to 15in the 55 patients on Phl (p=0.013). Blood values at the time for complication were compared to the mean for the yearly controls for the patients without complications, 41% with vascular events compared to 20 in the group without had WBC >10 x10^9/L(p=0.042). The differences between the groups as regards Hct and Plt were not significant.The survival for HU treated patients with need for, at least, one phl per year was significantly better than for HU treated patients without or need for less than one Phl per year (p=0.019). Fig 1

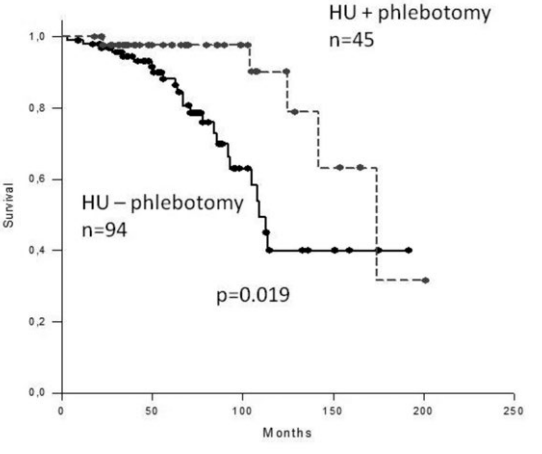

Figure 1. Survival of 139 Hydroxyurea treated PV patients, with and without need for phlebotomy.

Summary and Conclusions: We did not find any association between blood counts at the time of diagnosis and complication rate during follow-up, whereasLDH was found in a larger proportion of the patients with vascular events. Complications appeared in 36% of the patients. Vascular events were recorded in 16 % of the patients, being significantly more frequent in the group of patients treated with Phl only as compared to the patients treated with HU (p=0.013), despite the fact that that their median age was significantly lower (66 and 72 years, respectively). ELN criteria for HU resistance/intolerance have been established, including need for Phl, WBC >10 x10^9/L and Plt >400 x10^9/L. In

this study we found a significantly better survival for patients on HU with need for Phl compared to patients with less than one Phl per year, comparison with findings for "masked PV" could be made.We also found that elevated WBC seemed to be a risk factor for complications, whereas Plt levels did not influence complication rate. We conclude that elevated LDH at the time of diagnosis and high WBC during the corse are risk factors for future vascular complications in PV. HU therapy is associated with, at least, equal survival compared to treatment with Phl only. Need for complementary Phl to HU treated patients is safe.

P312

SPLEEN ENLARGEMENT IS A RISK FACTOR FOR THROMBOSIS IN ESSENTIAL THROMBOCYTHEMIA: EVALUATION ON 1097 PATIENTS AND VALIDATION ON 792 PATIENTS

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Background: Spleen enlargement is present in about 10-20% of Essential Thrombocythemia (ET) patients at diagnosis and is a feature clinically easy to assess, confirmable by echography and with a very low chance of misinterpretation. Nonetheless, the clinical and prognostic role of splenomegaly has been seldom evaluated.
Aims: The present study aimed to correlate spleen enlargement with baseline disease characteristics and outcome, with particular focus on thrombotic risk.
Methods: From 1979 to 2010, 1097 ET patients retrospectively collected in the database of the Lazio Cooperative Group were evaluable for spleen enlargement at diagnosis and were included in the primary analysis. The results of the main Lazio cohort were validated in 792 ET patients who were diagnosed and followed in the Bologna University Hospital between 1979 and 2013.
Results: In the Lazio cohort, ***s***pleen was enlarged in 213/1097 (19.4%) patients; in most cases (92%) splenomegaly was mild (<5 cm). Patients with splenomegaly were predominantly male, with higher platelet count, lactate dehydrogenase levels and JAK2V617F mutation load. Despite comparable use of cytoreductive/antiplatelet therapies in the two groups, the cumulative risk of thrombosis at 10 years was significantly higher in patients with baseline splenomegaly (23% *versus* 11% in patients without splenomegaly, p=0.007) (Figure 1a). In multivariate analysis exploring risk factors for thrombosis, splenomegaly retained its negative prognostic role, together with older age and previous thrombosis. In the validation Bologna cohort, 55 out of 792 (7%) ET patients carried baseline splenomegaly. Overall, 113 (14.3%) patients experienced a thrombotic event during follow-up. At 10 years, the cumulative incidence of thrombosis was 31.5% and 17.4% in patients with or without splenomegaly, respectively (p=0.039) (Figure 1b); splenomegaly confirmed its impact on thrombosis also in multivariable analysis.

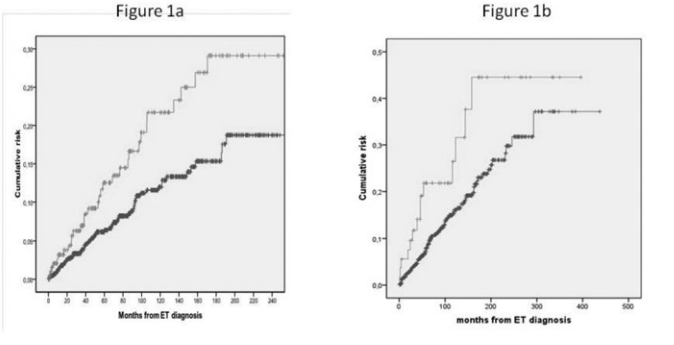

Figure 1 - Cumulative risk of thrombosis according to spleen enlargement at diagnosis in the 1097 patients of the leading Lazio cohort (Figure 1a) and in the 792 patients of the validation Bologna cohort (Figure 1b). In patients with spleen enlargement (green line), the cumulative risk of thrombosis at 10 years was 23% and 31.5% in the Lazio and in the Bologna cohorts, respectively, compared to 11% and 17.4% in patients without baseline spleen enlargement (blue line) (*p=0.007* and *p=0.039*).

Figure 1.

Summary and Conclusions: Baseline splenomegaly seems to be an independent additional risk factor for thrombosis in non-strictly WHO-defined ET patients. This data could be useful in the real-life clinical management of these patients.

P313

CLINICAL VALUE OF SPLENIC VOLUME MEASURED BY COMPUTED TOMOGRAPHY AS A NOVEL PROGNOSTIC FACTOR IN PATIENTS WITH PRIMARY MYELOFIBROSIS

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Background: Until now, due to less reliable method based on physical examination (PEx) to measure spleen size, prognostic clinical value of splenomegaly have been devalued. Therefore, several clinical studies failed to demonstratethe significant prognostic value of spleen enlargement in primary myelofibrosis (PMF), although splenomegaly is a major characteristic associated with the adverse clinical features.
Aims: We evaluated whether spleen volume (SV) in computed tomography (CT) would accurately predict clinical outcomes in PMF.
Methods: A total of 188 patients with PMF who received abdomen CT at diagnosis, were enrolled. SV was quantitated from the cross-sectional images by using 3-dimensional analytical *volume* software (*Voxar*, Ltd., Edinburgh, United Kingdom). The computer automatically contoured the spleen in each CT slice and calculated the SV in cubic centimeter (cm^3). In order to evaluate prognostic significance of SV by CT, we compared the prognostic values of several prognostic factors present at the time of diagnosis: sex (male or female), age (≥or <65 years), the presence of constitutional symptoms, hemoglobin (Hb) level (≥or <10 g/dL), platelet (PLT) counts (≥or <100×10^9/L), white blood cell (WBC) counts (≥or <25×10^9/L), presence of peripheral blood (PB) circulating blasts (≥or <1 %), red blood cell (RBC) transfusion dependency, the presence of JAK2-V617F mutation, MF grading according to the ECSS (MF-3 or <MF-3), and the presence of unfavorable cytogenetic abnormalities such as complex karyotype or sole or two abnormalities including +8,-7/7q-,-5/5q-, i(17q), 12p-, inv(3), or 11q23 rearrangement.
Results: In ROC curve, SV by CT more accurately predicted prognosis than spleen length by PEx (p <0.001). Ideal cut-off value, 378.1 cm^3 of SV divided with high and low volume status. Low SV status had superior leukemia-free survival (LFS) and overall survival (OS) compared with high status (p <0.001, p <0.001). In univariate and multivariate analyses, old age (LFS, HR =1.864, 95% CI =1.217-3.056, p =0.004; OS, HR =2.102, 95% CI =1.381-3.190, p =0.001), low Hb level (LFS, HR =1.793, 95% CI =1.302-2.923, p =0.023; OS, HR =1.786, 95% CI =1.053-3.029, p =0.021), high WBC counts (LFS, HR =2.051, 95% CI =1.287-3.269, p =0.003; OS, HR =1.892, 95% CI =1.197-2.989, p =0.006), PB circulating blasts ≥1 % (LFS, HR =1.590, 95% CI =1.024-2.469, p =0.029; OS, HR =1.699, 95% CI =1.064-2.598, p =0.020), unfavorable cytogenetic abnormalities (LFS, HR =1.808, 95% CI =1.371-1.987, p =0.025; OS, HR =1.802, 95% CI =1.104-2.945, p =0.028), and high SV status (LFS, HR =2.633, 95% CI =1.368-5.069, p =0.004; OS, HR =2.641, 95% CI =1.404-4.967, p =0.003) were independently associated with survivals. By using the independent prognostic factors, each patient was assigned according to sum of 0-6 adverse points. The six score groups were consolidated into 5 risk groups according to intergroup survival differences ; low risk group (0 risk factor), intermediate-1 risk group (1 risk factor), intermediate-2 risk group (2 risk factors), high risk group (3 risk factors), and very high risk group (≥4 risk factors, Figure A and B).

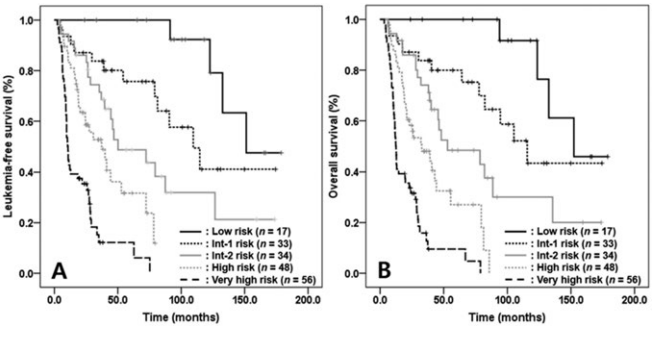

Figure 1.

Summary and Conclusions: High SV status measured by CT had a qualification as a remarkable risk factor at diagnosis to predict survivals in PMF. Therefore, we could develop the novel prognostic scoring system model including splenomegaly by the imaging modality in PMF.

LB314

PERSIST-1: A PHASE III STUDY OF PACRITINIB (PAC) VS BEST AVAILABLE THERAPY (BAT) IN PRIMARY MYELOFIBROSIS (PMF), POST-POLYCYTHEMIA VERA MF (PPV-MF) OR POST-ESSENTIAL THROMBOCYTHEMIA MF (PET-MF)

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New Information: Submitted to ASCO 2015. New information includes additional safety data such as dose reductions, dose interruptions and reason for dose modification.

Background: Treatment options for patients (pts) with MF are limited. A significant proportion present with disease-related thrombocytopenia, an independent survival risk factor. Others develop treatment-emergent thrombocytopenia on currently available therapies. These pts represent an underserved population. PAC is a potent multikinase inhibitor of JAK2/FLT3 and has exhibited minimal myelosuppression in early phase MF studies.

Aims: The open-label PERSIST-1 (NCT01773187) trial compared the efficacy and safety of oral PAC *vs* BAT in PMF, PPV-MF, and PET-MF.

Methods: JAK inhibitor naïve pts were randomized 2:1 to oral PAC 400mg once daily or BAT stratified by DIPSS risk (Int-1 or Int-2 *vs* High) and platelet (plt) count (<50,000/μL *vs* 50,000 to <100,000/μL *vs* ≥100,000/μL). Eligibility criteria included: DIPSS Int-1, Int-2, or High risk; ANC >500/μL; no restriction on plt or hemoglobin; palpable splenomegaly ≥5 cm; and baseline total symptom score (TSS) ≥13 using the MPN Symptom Assessment Form (MPN-SAF). The primary endpoint was the proportion of pts achieving ≥35% spleen volume reduction (SVR) at Week 24 (WK24) by centrally-reviewed MRI or CT. The secondary endpoint was the proportion achieving ≥50% reduction in TSS at WK24 using MPN-SAF.

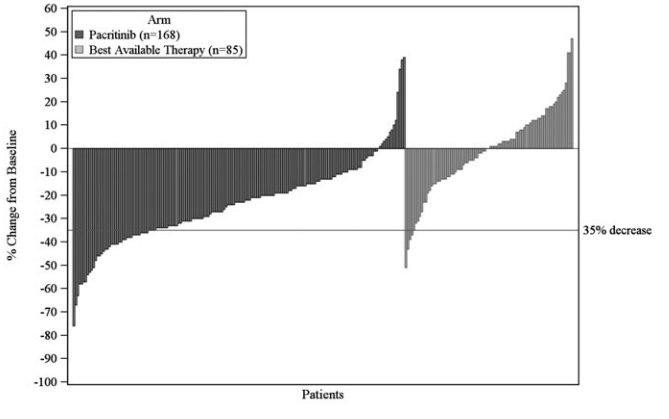

Figure 1.

Results: 327 pts were enrolled (PAC: 220, BAT: 107), 62% with PMF; 32% had plt counts <100,000/μL and 16% had plt counts <50,000/μL; 75% were JAK2V617F-positive. Median time from diagnosis was 1.0 y (PAC) and 1.6 y (BAT). Median baseline spleen volumes were 2006 cm^3(PAC) and 2153 cm^3 (BAT) and TSSs were 20 (PAC) and 23 (BAT).Estimated median duration of treatment was 16.2 mo (PAC) and 5.9 mo (BAT). SVR rates at WK24 were 19% for PAC *vs* 5% for BAT (p=0.0003) in intent-to-treat (ITT) and 25% *vs* 6% (p=0.0001) in pts evaluable at baseline and WK24 (figure). Progressive disease was not required for crossover after week 24. 79% of BAT pts crossed over to PAC (median 27 weeks). TSS (V1+V2) response rates were 25% for PAC *vs* 7% for BAT (p<0.0001) by ITT, and 41% *vs* 10% in evaluable pts (p<0.0001). Patient Global Impression of Change improvement on PAC (81%) was significantly higher than on BAT (21%) in evaluable pts (p<0.001). PAC significantly improved SVR rates irrespective of baseline plt counts. In pts with <100,000 and <50,000 plt/μL, SVR rates were 17% for PAC *vs* 0% for BAT (p=0.009), and 23% *vs* 0% (p=0.045) by ITT, respectively, and 24% *vs* 0% (p=0.007) and 33% *vs* 0% (p=0.037) in evaluable pts, respectively. In baseline RBC transfusion-dependent pts (PAC: 35, BAT: 15), 26% of PAC pts became RBC transfusion independent *vs* 0% of BAT pts (p=0.043). The most common adverse events (AEs) through WK24 for PAC were gastrointestinal (GI): diarrhea, nausea, and vomiting. Grade (Gr) 3 GI AEs rates were 5%, <1%, and <1% respectively (no Gr 4). Gr 3-4 hematologic AEs occurring in >2% were similar for PAC *vs* BAT: anemia (17% *vs* 15%) and thrombocytopenia (12% *vs* 9%). PAC AEs were similar in thrombocytopenic and non-thrombocytopenic pts. 90% of PAC pts did not require any dose reduction. AEs leading to dose reduction included diarrhea (3%) and anemia (2%). Dose interruptions occurred in 22% (median duration 7 days) with 6% due to diarrhea, 3% thrombocytopenia, and 2% anemia.

Summary and Conclusions: PAC dosed 400mg once daily was well tolerated with minimal dose modifications and induced clinically and statistically significant and meaningful SVR and meaningful symptom control even in pts with severe thrombocytopenia. PAC therapy also resulted in RBC transfusion independence in a significant proportion of pts.

Non-Hodgkin & Hodgkin lymphoma - Biology

P314

Abstract withdrawn

P315

DYNAMIC ROLE OF THE IRF4-PU.1 AXIS IN IBRUTINIB-RESISTANT WALDENSTRÖMS MACROGLOBULINEMIA

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Background: Waldenströms macroglobulinemia (WM) is a rare and incurable non-Hodgkin's B-cell lymphoma that is defined by lymphoplasmacytic infiltration of the bone marrow and lymphoid tissue with increased monoclonal IgM protein production. Aberrant activation of B-cell receptor (BCR) signaling, results in neoplastic transformation of B-lymphocytes. Recent investigations using small molecule inhibitors have validated Bruton's tyrosine kinase (BTK), one of the components of the BCR signaling hub, to be a druggable target. Ibrutinib (PCI-32765), an irreversible BTK inhibitor has shown clinical efficacy in CLL, mantle cell lymphoma (MCL) and Waldenströms macroglobulinemia (WM). Ibrutinib binds to cysteine-481 of the BTK protein and blocks its phosphorylation, resulting in termination of BCR-mediated activation of cells, leading to tumor cell death. Despite the clinical success of ibrutinib, WM patients achieve only partial response and invariably acquire resistance to the drug, resulting in aggressive relapse of the disease. A mutation of Cys^{481}-Ser in BTK (ibrutinib-BTK binding site) has been reported to be one of the reasons for the development of ibrutinib resistance (IR). To understand the mechanisms resulting in acquisition of IR, we established and characterized several models of IR-WM.
Aims: To evaluate the significance of IRF4-PU.1 axis in the development of ibrutinib resistance
Methods: Ibrutinib was obtained from Pharmacyclics, CA. Validated human WM models (BCWM.1, RPCI-WM1 and MWCL.1 cell lines) were used for the study. shIRF4 clones of WM cell lines. qRTPCR, Western blotting, MTS assay, Flow cytometry.
Results: Exposure of WM models for prolonged periods of time with progressively increasing concentrations of ibrutinib resulted in outgrowth of IR tumor clones. IR cells displayed 2-20 fold resistance to ibrutinib compared to their respective parental cell lines as determined by MTS assay. Sequence analysis of the BTK gene in all the IR cell lines revealed no mutation at Cys^{481} suggesting that in an acquired IR state, resistance to ibrutinib can be developed independent of BTK Cys^{481} mutation. BTK was constitutively phosphorylated at Y^{223} and Y^{551} in all the cell lines tested and this was inhibited by ibrutinib at a concentration as low as 1mM. Phosphorylation of other kinases in the cascade such as SYK (Y^{323} and $Y^{525/526}$) and PLCg2 (Y^{759} and Y^{1217}) were also inhibited while AKT phosphorylation at both Ser^{473} and Thr^{308} was consistently increased in presence of ibrutinib. Interestingly, we found p-BTK levels to be markedly reduced in IR cells. Ibrutinib reversal experiments suggested that while continuous presence of ibrutinib was needed for the inhibition of BTK phosphorylation, a stable IR state was maintained for ≥1 month with no loss of cell viability upon reintroduction of ibrutinib, suggesting the cells reliance on a parallel survival pathway, independent of BTK phosphorylation. Focused mRNA (Nanostring nCounter assay) and immunoblot analysis revealed significant changes in the expression profiles of several cellular elements. These included transcription factors such as PU.1, IRF4, STAT1 and 3 suggesting a reprogramming of critical cellular networks, with IRF4 emerging as central in IR cells, indicated by altered expression of its downstream targets. We assessed the significance of IRF4 in IR cells by silencing IRF4 expression through IRF4-shRNA. The results indicated that down regulation of IRF4 by itself has minimal effect on ibrutinib sensitivity of IR cells. However, treatment of WM cells with all trans retinoic acid (ATRA), which is known to upregulate PU.1, induced dose dependent cytotoxicity in these cells suggesting involvement of PU.1 (and its shifting partnership with IRF4) in ibrutinib-resistance.
Summary and Conclusions: Here we demonstrate that in the absence of BTK Cys^{481} (ibrutinib binding site) mutation, an IR state is associated with reprogramming of transcriptional networks to overcome ibrutinib-induced toxicity. Moreover, this results in activation of parallel survival pathways directed by an altered IRF4-PU.1 axis, which is associated with sensitivity of WM cells to Ibrutinib.

P316

KDM5 INHIBITION LEADS TO INCREASED H3K4ME3 LEVELS AND CELL DEATH IN GERMINAL CENTRE LYMPHOMA CELL LINES INDEPENDENT OF MLL2 MUTATION STATUS

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Background: Follicular lymphoma (FL) and the germinal centre B-cell (GCB) subtype of diffuse large B cell lymphoma (GCB-DLBCL) account for nearly 40-50% of cases of Non-Hodgkin lymphomas (NHL). Whole exome and targeted resequencing studies demonstrated an epigenetic addiction in these germinal centre lymphomas with many biopsies harbouring mutations in histone methyl (*MLL2*, *EZH2*) and acetyltransferases (*CREBBP*). Loss of function mutations in *MLL2* (also referred to as *KMT2D)* are detected in >30% of GCB-DLBCL and 90% of FL cases and are expected to lead to a reduction in levels of the chromatin mark H3K4me3 (histone 3 lysine 4 trimethylation), which is associated with active transcription. We therefore set out to assess whether stabilising this H3K4me3 mark, by directly targeting the KDM5 family of H3K4me3 demethylases (KDM5A/B/C/D), offers a means to ameliorate the effects of these mutations and provide a rationale for a therapeutic opportunity.
Aims: EpiTherapeutics have developed a specific inhibitor against the KDM5 family of demethylases (EPT001). The aim of this study was to determine the potency of KDM5 inhibition in GCB-DLBCL lymphoma cell lines based on their *MLL2* mutation status and restoration of H3K4me3 levels.
Methods: The mutational profile of 10 GCB-DLBCL cell lines was characterised via targeted deep sequencing, using Fluidigm-MiSeq and further validated by Sanger sequencing. Expression of the KDM5 isoforms and downstream targets of KDM5 were assessed by qRT-PCR. Cell lines were exposed to increasing concentrations of EPT001 and cell proliferation and apoptosis were assessed using the Guava EasyCyte Plus system. In parallel, cell cycle analysis was performed by propidium iodide staining and flow cytometry. Immunoblotting was used to establish the effect of treatment on a range of epigenetic histone marks (H3K4me1/2/3, H3K27me3/Ac, and H3k36me2).
Results: MLL2 mutations were detected in 6 of the 10 cell lines tested. All 4 KDM5 isoforms (A-D) are expressed in these cell lines with a predominance of A and C. Exposure to EP1001 in all 10 lines resulted in a concentration dependent increase in global H3K4me3 (range 1.4-4.8 fold at 48 hrs). Of note, elevation of H3K4me3 was independent of *MLL2* mutation status. A striking reduction in proliferation and an induction of apoptosis were observed in 2 cell lines (SU-DHL-6, OCI-LY-18) both at low nanomolar concentrations (GIC_{50}: <5nM) and this was accompanied by a concentration dependent accumulation of cells in the G_1/S-phase and a concomitant loss of G2/M phase. Treatment did not result in any measurable differences in expression of established KDM5 targets as assessed by qRT-PCR.
Summary and Conclusions: Inhibition of KDM5 family of demethylases is sufficient to induce cell cycle arrest and induction of apoptosis in at least some GCB-DLBCL cell lines. This appears independent of *MLL2* mutation status even in cell lines harbouring homozygous mutations. RNA-seq and ChIP-seq are in progress to assess changes in gene expression that accompany KDM5 inhibition and H3K4me3 elevation in sensitive and resistant cell lines.

P317

MICRORNA 203 CONTROLS T-CELL LYMPHOMAGENESIS BY TARGETING IL-2 RECEPTOR SIGNALING

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Background: The pathogenesis of primary cutaneous T-cell lymphoma (CTCL) is poorly understood, and early CTCL lesions are difficult to diagnose. We have previously identified and validated a 3 microRNA (miR) classifier (miR-155, miR-203 and miR-205), which can separate CTCL from benign inflammatory skin disorders with >95% accuracy. Subsequently, we showed that miR-155 enhances proliferation and is induced by STAT5a. MiR-203 has been shown to repress stemness during normal keratinocyte differentiation, and in solid tumors, miR-203 has been shown to be silenced by promoter hypermethylation.
Aims: The role of miR-203 in CTCL pathogenesis has not previously been described, however since miR-203 down regulation is a hall mark of CTCL,we have investigated the transcriptional regulation and function of miR-203 in primary CTCL biopsies and cell lines.
Methods: Two IL-2 independent CTCL cell lines (MyLa2059 and PDB2B), and two IL-2 dependent CTCL cell lines (SeAx and SeZ4) were analyzed in this study. In addition, biopsies from 21 primary CTCL (Mycosis Fungides (MF), Sezary syndrome, cutaneous T-cell lymphoma, NOS, and cutaneous anaplastic large cell lymphoma) from 21 patients were included. Promoter hypermethylation of miR-203 was studied by methylation specific melting curve analysis, and miR-203 expression by RT-qPCR. CTCL cell lines were treated by hypomethylating agents (HMAs), and were transfected with miR-203 mimic or non-template-control by electroporation. Proliferation was measured by 3H-

Thymidine and apoptosis by MMT assays. Cells transfected with miR-203 mimic and non-template-control were examined by Affymetrix RNA expression arrays (GeneChip Human Gene 2.0 ST). Cloning was done according to the manufacturers' recommendation and luciferase reporter assays were performed using the Dual-Glo system (Promega). Protein levels were analyzed by flow cytometry.
Results: We found that a member of our CTCL classifier, miR-203, is epigenetically silenced by DNA methylation in all examined CTCL cell lines and in 9 of 21 (43%) of primary CTCL samples. MiR-203 expression can be up-regulated by HMAs *in vitro*, and induction of miR-203 reduces cell viability and decreases cell proliferation. Affymmetrix array analysis of miR-203 mimic *vs* non-template-control cells identified 19 significantly differentially expressed genes (P<0.5/log fold change>2), including the novel miR-203 target molecule IL2RB, which is essential for IL-2 induced JAK/STAT signaling. QPCR and FACS analysis confirmed this up-regulation both at the mRNA and protein level. The IL-2 dependent cell line SeAx showed significantly more profound down-regulation of IL2RB in miR-203 transfected cell lines. Luciferase reporter assays of a construct containing the IL2RB 3' untranslated region confirm that IL2RB expression is regulated by miR-203. Thus, we suggest that targeted down regulation of miR-203 in CTCL promotes IL-2/STAT driven T-cell lymphomagenesis
Summary and Conclusions: We provide the first evidence that miR-203 is an epigenetically silenced tumor suppressor in primary CTCL, including early MF lesions. Our functional studies show that miR-203 keeps the level of IL2RB in check. Thus, we suggest that epigenetic down-regulation of miR-203 and IL2RB up-regulation are important early and driving events in CTCL pathogenesis.

P318

ANALYSIS OF MECHANISMS OF MALIGNANT TRANSFORMATION IN TP53 WILD-TYPE BURKITT´S LYMPHOMA BY INTEGRATION OF MOLECULAR AND FUNCTIONAL GENOMICS DATA

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Background: Burkitt´s lymphoma (BL) is an aggressive mature B-cell lymphoma. The hallmark genetic lesion of BL is a translocation of the *MYC* gene to an IG-locus. In addition, recent studies identified recurrent mutations of *TCF3* and *ID3* in a large proportion of BL. Oncogenic activation of MYC promotes cell growth, but also activates a stress response via the *TP53* pathway. Similarly, aberrant mitogenic signaling induces oncogene-induced senescence. Therefore, all BL depend on cooperating lesions that promote survival and cell growth. Up to 50% of BL carry mutations in the tumor suppressor p53 leading to an impaired response to the stress signals from oncogene activation. In the presence of functional *TP53*alternative mechanisms of *TP53*inactivation are frequently observed in cancer, including loss of $p14^{ARF}$ locus or overexpression of the main p53 inhibitors, MDM2 or MDM4. In BL, aberrations in the p53-pathway have not been systematically investigated and their role in the pathogenesis of MYC-driven lymphoma is not known.
Aims: Our aim was to identify driver lesions specific to BL without *TP53* mutation promoting tumor survival and growth.
Methods: We analyzed differential copy number changes (CNA) and gene expression in 67 BL and 205 diffuse large B cell lymphoma (DLBCL) from the Molecular Mechanisms of Malignant Lymphoma (MMML) consortium according to their *TP53* mutation status. For an unbiased functional analysis, we performed an RNAi drop-out screen on a panel of representative BL cell lines (n=8) with defined genetic background.
Results: In order to identify *TP53*-dependent viability genes, we chose 4 BL cell lines that carry mutations in *TP53* and show impaired p53 pathway response and 4 cell lines with wild-type *TP53* and an intact p53 pathway. These cells were infected with a pooled shRNA library targeting ~5000 genes with 5-6 shRNAs per gene. Toxic shRNAs were identified by comparison of the shRNA counts on day 2 and day 14 after infection as determined by high-throughput sequencing (Figure 1A). As expected, shRNAs targeting common viability genes as ribosomal and proteasomal proteins showed the strongest depletion in all cell lines. We identified 81 genes that showed increased toxicity in cell lines with wild-type *TP53* (Figure 1B) and 88 genes that were significantly enriched for toxicity in mutant *TP53* cell lines. The strongest genes classifying wild-type *TP53* were involved in the p53 pathway and cell cycle control. Integration of our RNAi viability data with CNA and gene expression analysis from BL patients showed, that the gene with the highest p53-dependent toxicity was located within a minimally gained region associated with wild-type *TP53* patients. Validation studies confirmed the p53-dependent cytotoxicity in single gene knock-down experiments and demonstrated that toxicity was caused by strong cell cycle arrest (Figure 1C).

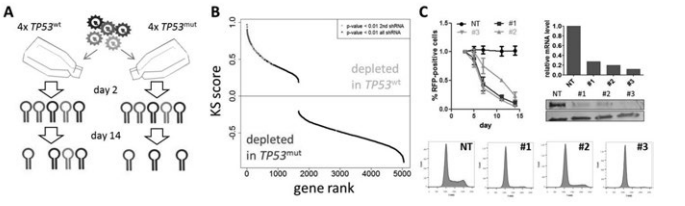

Figure 1.

Summary and Conclusions: BL is known to be driven by the oncogene MYC and mitogenic signaling. Cooperating lesions as mutation of *TP53* are needed to escape oncogene-driven apoptosis and senescence. In the presence of functional p53, we identified an alternative disease driver in BL with wild-type *TP53* controlling cell cycle progression, which may be exploited therapeutically.

P319

COMBINED 3D IMMUNO-FISH ANALYSIS OF PRIMARY HODGKIN (H) AND REED-STERNBERG (RS) CELLS REVEALS DISRUPTION OF TELOMERE-TRF2 INTERACTION AND IDENTIFIES HODGKIN'S LYMPHOMA SHELTERIN ASSOCIATED DISEASE

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Background: In Hodgkin's lymphoma (HL) derived H-cell lines the transition from H-to RS-cells is associated with progression of 3D telomere dysfunction, major changes in the telomere protecting shelterin complex, chromosomal rearrangements and formation of giant "zebra" chromosomes. Analogous findings are observed in a post-germinal center B-cell *in vitro* model for Epstein-Barr-virus (EBV)-associated HL. In this experimental system the EBV encoded oncogene LMP1, also expressed in EBV-associated HL, mediates multinuclearity through downregulation of TRF2. (Blood 2015 Jan 7.[Epub ahead of print]). Thus, the 3D interaction of telomeres and TRF2 appears to be primordial in the formation of H-and RS-cells.
Aims: Our previous findings obtained with H-cell lines and diagnostic, deparaffined lymph node biopsy slides were consistent with a shelterin-associated pathogenesis of HL (reviewed in Cancers 2013, 5:714). These findings were reinforced by our recent *in vitro* model for EBV-associated HL. The aim of the present investigation is to test our results and hypothesis on primary H-and RS-cells allowing the 3D analysis of the entire nuclear content.

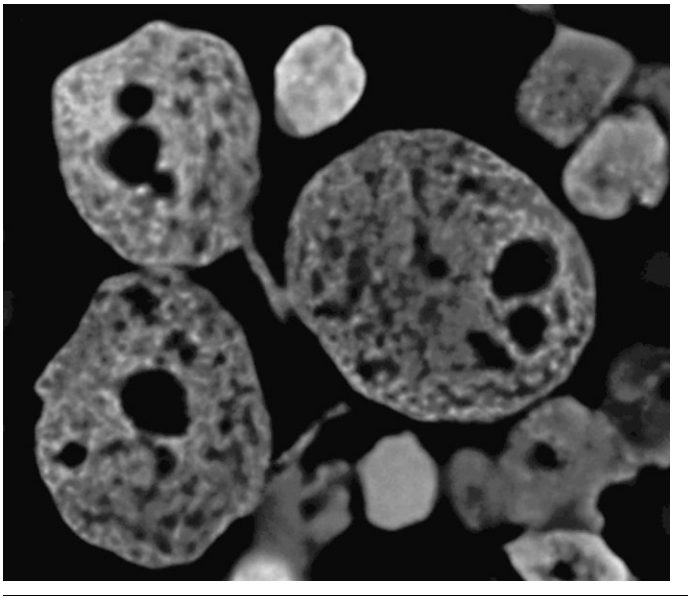

Figure 1.

Methods: Combined TRF2-Telomere 3D immuno-FISH analysis (Blood 2015 Jan 7. data supplement) was performed on cultured BJ-5ta normal diploid

fibroblasts and B-cell rich suspensions (negative selection) of diagnostic lymph nodes for HL, cultured for 24-36 hours after thawing. Quality of H-and RS-cells was assessed by Wright-Giemsa staining. Lymphocytes, H-and RS-cells of six classical HL were analyzed, one of them with LMP1 positive tumor cells.
Results: Whereas all BJ-5ta normal diploid control fibroblasts and many lymphocytes showed an intact 1:1 quantitative and qualitative tight 3D interaction of TRF2-Telomeres, with mainly mid-to large-sized telomeres, H-cells displayed a variable pattern of disruption of steric interaction ranging from few to many TRF2-deprotected and often short telomeres. These changes were more pronounced in RS-cells. By far the most important loss of TRF2 expression, gradually progressing from H-to RS-cells, was identified in the LMP1-expressing case. In one further case of clinically aggressive disease, some lymphocytes had already lost TRF2 signals. In this case huge multinucleated RS "ghost" cells without any telomeres but inter-nuclear DNA-bridges (image) were observed. Interestingly, in these RS cells TRF2 was still identified. In a third case bi-nucleated RS-cells with grossly different nuclear telomere content of nucleus one and nucleus two were present.
Summary and Conclusions: The 3D TRF2-Telomere interaction is disrupted in primary H-and RS-cells and this process starts in B-lymphocytes. The identified changes are major and analogous to those of the telomere-shelterin complex reported in H-and RS-cells of H-cell lines. Advanced TRF2-associated telomere deprotection is observed in EBV-associated HL and confirms the hypothesis of our *in vitro* model for EBV-associated HL. The unique 3D cytological appearance and behavior of H-and RS-cells is best explained by the designation of HL as a shelterin associated disease. The telomere-shelterin complex is a "plaque tournante" in the pathogenesis of HL and a potential therapeutic target.

P320

A NOVEL ULTRA-DEEP SEQUENCING METHOD FOR TRACKING OF DRIVER MUTATIONS IDENTIFIES THE MYD88 L265P MUTATION IN EARLY HAEMOPOIETIC PRECURSORS IN DIFFUSE LARGE B CELL LYMPHOMA
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Background: Activated B cell diffuse large B-cell lymphoma (ABC-DLBCL) is characterised by a poorer clinical outcome compared to patients with germinal centre (GC-DLBCL) with >50% patients failing standard first line chemo-immunotherapy. The NFkappaB signature is upregulated in patients with ABC-DLBCL with gain-of-function somatic mutations identified in genes involved in this pathway including the MYD88 gene. However, the exact point in ontogeny at which this mutation arises is not known.
Aims: We investigated the cellular origin of the MYD88 L265P mutation using a novel ultra-deep sequencing assay as the presence of somatic mutations in earlier haemopoietic precursors could potentially account for treatment failure and act as a reservoir for future relapses.
Methods: We identified an initial cohort of 46 DLBCL samples from the ACT Haematology Research Tissue Bank and screened them for somatic mutations using a custom next-generation sequencing approach. A Haloplex custom library preparation kit was used to develop an automated library and samples were sequenced using the MiSeq and HiSeq2500. We identified 5 patients with MYD88 L265P mutations. For each case, we then performed bulk cell sorting on 3 non-clonal populations-T cells characterised by CD3 expression, myeloid cells characterised by CD13/CD33 expression and non-clonal B cells with the opposite light chain to that expressed by the lymphoma clone. For all cases, single cell sorting and whole genome amplification was also performed. An ultra-deep sequencing assay was developed based on Kinde's approach to screen for rare cells bearing somatic mutations amongst normal non-clonal populations[Kinde et al,Proc Natl Acad Sci U S A,2011 Jun 7;108(23):9530-5]. By the addition of nucleotide barcodes in forward and reverse primers during the PCR amplification process, we have eliminated processing and sequencing errors, and have developed an assay capable of identifying very low numbers of non-lymphoma cells with gain-of-function MYD88 mutations (~ 0.001%)-cell line titration experiments.
Results: We have tested the assay on5 cases presenting with ABC DLBCL heterozygous for the MYD88 L265P mutation. In 3 cases, the MYD88 mutation was limited to the clonal population; it was not identified in bulk sorted non-clonal B cells, T cells or myeloid cells. In the remaining 2 cases, the mutation was identified in non-clonal B-cells and T cells but not in myeloid cells. Whole genome amplificationfrom non-clonal single B-cells in both cases was subjected to Sanger sequencing for MYD88 and IgHV.This analysisconfirmed thatnon-clonal B cells lacking expression of the lymphoma specific IgHV (IgHV 4-39*01) were heterozygous for the MYD88 mutation,indicating thepresence of the mutation in early precursors. We postulate that the MYD88 mutation arises in the CD20 negative common lymphoid progenitor pool in 2 casesfrom our cohort but is limited to the lymphoma clone in others.
Summary and Conclusions: Varying cellular origin of the MYD88 L265P mutation may account for some of the disease heterogeneity seen in MYD88-driven DLBCLs. Further, early origin of MYD88 L265P driver mutation in CD20 nagetive early precursors is a likely cause of treatment failure with Rituximab. Such cases may potentially respond better to Ibrutinib as the bruton's tyrosaine kinase is expressed in CD20 nagteive B-cell precursors.

P321

TRANSLOCATIONS AND CLONALITY DETECTION IN LYMPHOPROLIFERATIVE DISORDERS BY CAPTURE-BASED NEXT-GENERATION SEQUENCING. A PILOT STUDY BY THE EUROCLONALITY-NGS CONSORTIUM
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Background: Detection and characterization of clonal *IG/TR* rearrangements and translocations in lymphoproliferative neoplasms provides critical information in the diagnostic pathway in several clinical scenarios and is a valuable tool to address research questions around B and T cells. This includes ascertaining the clonal nature of lymphoid proliferations, characterization of translocations in lymphomas and leukemias, characterization of CDR3 regions for MRD target identification and stereotyping analysis, amongst others. Until now, collecting this information required a combination of different methodologies, such as Gene-scanning/heteroduplex analysis, FISH and Sanger sequencing.
Aims: This is a pilot study to assess the feasibility of a comprehensive *IG* and *TR*genes NGS protocol to detect *IG* and *TR* rearrangements in lymphoproliferative disorders as a new tool for clonality diagnosis. Additionally, the pilot aims to assess the feasibility of discovering novel translocation partners in lymphoma and leukemia patients, potentially leading to more accurate diagnostic classification and novel therapeutic options.
Methods: As part of the EuroClonality-NGS consortium, we have designed a capture-based protocol covering the coding V, D and J genes of the IG/TR regions, as well as switch regions in the IGH locus. The assay uses Nimblegen (Roche Molecular Systems) capture baits spanning a total of 180kb and the products are analyzed on a MiSeq Illumina sequencer and MiSeq v3 sequencing chemistry with 2 x 120bp pair-end reads. This design allows the identification of D-J and V(D)J rearrangements as well as chromosomal translocations involving *IG/TR* genes by Next-generation sequencing (NGS). We piloted this approach for clonality and translocation detection in a cohort of 21 peripheral blood, bone marrow and fresh-frozen samples (3 precursor B-cell acute lymphoblastic leukemias [B-ALL], 4 Burkitt lymphoma [BL], 5 chronic lymphocytic leukemias [CLL], 2 splenic marginal zone lymphomas [SMZL], 2 diffuse large B cell lymphomas [DLBCL], 2 follicular lymphomas [FL], 2 precursor T-cell lymphoblastic leukemias [T-ALL] and 1 T-cell non-Hodgkin lymphoma [T-HNL]) with well-characterized translocations by FISH/Karyotype and/or clonal rearrangements by PCR and Sanger sequencing.
Results: We were able to detect the described *IG/TR* translocation in 18/21 samples, including translocations into the J, D and switch regions of the *IG/TR* genes. Three samples failed to produce results, two concerned fresh-frozen lymphomas with low quality DNA, and one concerned a technical error in a B-ALL case. The translocation partner and the breakpoint was identified in 15/18 evaluable cases, including *CRLF2, MYC, BCL11A, BCL2, CCND3, IRF4, BCL11B,* and *TLX1*. In three cases with only karyotyping data available, a translocation involving the IGH locus was identified with no clear leukemia/lymphoma-related genes in the neighboring regions of the reciprocal chromosome, suggesting the potential for new translocation partners and/or mechanism of disease. In all 14 samples with well-characterized D-J/V(D)J rearrangements by PCR and sequencing, NGS was able to detect the same rearrangements. These included *IGH* and *IGK/IGL* rearrangements in B-cell proliferations and *TRB, TRG* and *TRD* in T-cell proliferations. Additionally, aberrant clonal rearrangements were seen that were not detected with conventional PCR-based approaches (*e.g.* IGKV to IGK intron).
Summary and Conclusions: The EuroClonality NGS *IG/TR* capture-based approach is a promising tool for the simultaneous detection and characterization of *IG/TR* translocation and rearrangements in a clinical setting. A formal pan-European validation study is underway within the EuroClonality-NGS consortium.

P322

MORAXELLA CATARRHALIS-A BACTERIAL INVOLVEMENT IN THE ORIGIN OF NODULAR LYMPHOCYTE PREDOMINANT HODGKIN LYMPHOMA OF IGD TYPE?
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Background: Nodular lymphocyte predominant Hodgkin lymphoma (NLPHL) is a rare type of Hodgkin lymphoma. In contrast to Hodgkin-Reed-Sternberg cells in classical Hodgkin lymphoma, lymphocyte predominant (LP) cells, the tumor cells in nodular lymphocyte predominant Hodgkin lymphoma, show functional variable region genes of immunoglobulins with ongoing somatic hypermutation. Chronic B cell receptor (BCR) stimulation has been proposed to play a central role in the pathogenesis of NLPHL. However, the mechanisms underlying this stimulation are poorly understood.
Aims: Aim of the study was to identify potential target antigens of B cell receptors of LP cells, with emphasis on autoantigens and bacterial structures.
Methods: Recombinant Fabs were constructed of corresponding variable region heavy and light chain genes from LP cells isolated by laser capture microdissection from cryopreserved specimens.
Results: Fabs of 9 cases were constructed. When tested on a protein macroarray derived from human fetal brain to detect autoantigenic structures as the targets of the LP cell BCR, ribosomal protein S27a and pyruvate carboxylase (PC) were identified as target antigens of two individual Fabs. When tested on the lysates from 10 bacterial strains, the recombinant BCR of 2 additional patients reacted with the lysate from Moraxella catarrhalis (MC), a common bacterium colonizing the upper respiratory tract, which expresses a 200 kD IgD-binding protein (MID/hag) enabling MC to bind to the Fc part of IgD and thus activate IgD carrying B-cells. Interestingly, the BCRs of the two patients reactive with MC were derived from LP cells of IgD+ NLPHL, which are known to represent a peculiar clinical subgroup of NLPHL, affecting the cervical lymph nodes of young people (median age: 21 years) with a strong male predominance (23:1). Analysis of additional IgD+ NLPHL cases revealed that the LP cell BCR of 4/5 IgD+ NLPHL cases reacted specifically with the DNA-directed RNA polymerase subunit beta' of MC (rpoC), a protein of 155 kD. Serum was available from 1 patient with rpoC-specific LP cell-BCR and was shown to contain polyclonal antibodies reactive against rpoC.
Summary and Conclusions: This study shows an association between MC and IgD+ NLPHL, suggesting a causal role of MC in the pathogenesis of IgD+ NLPHL. MC is capable of binding to the Fc fragment of LP-BCR of the IgD type via its IgD-binding domain and can mediate chronic antigenic stimulation of B-cells with specificity of MC-rpoC. The presence of polyclonal serum antibodies against MC-rpoC in patients with IgD+ NLPHL suggests that NLPHL is the result of a clonal evolution from a polyclonal B-cell response against MC-rpoC. Our findings are fundamental for the understanding of the pathogenesis of this peculiar clinical subtype of NLPHL and might have relevance for the prophylaxis and treatment of this disease.

P323

COMBINED CHEMOIMMUNOTHERAPY (R-CVP) IS AN EFFECTIVE TREATMENT FOR HIV-NEGATIVE, HHV-8 POSITIVE PATIENTS WITH MULTICENTRIC CASTLEMAN'S DISEASE

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Background: Castleman disease is a rare and heterogeneous lymphoproliferative disorder: the unicentric type includes hyaline vascular and plasma cell variants, while the multicentric disease (MCD) is predominantly of the plasma cell or mixed variant. The aetiology is poorly understood; the potential roles of HHV-8 are suggested in several studies. MCD is also associated with HIV infection. No standard treatment has been established for MCD patients: steroids, chemotherapy, rituximab, thalidomide, bortezomib, antiviral therapy, and IL-6 inhibitors have been used.
Aims: We have reported a complete hematologic remission and virologic response to chemoimmunotherapy R-CVP in a HIV negative, HHV-8 positive patient (Leukemia Lymphoma, 2008); in the following years we have treated MCD patients with R-CVP in order to assess the effectiveness of this treatment.
Methods: Six HIV negative, HHV-8 positive patients (5 men, 1 woman, median age 77 years, range 69-80 years) with MCD were diagnosed in our Hematology Unit over a eight-year period. The patients' characteristics are described in Table 1. Five patients were initially treated by chemoimmunotherapy R-CVP combination (cyclophosphamide 750mg/m2 on day 1, vincristine 2mg on day 1, prednisone 40mg/m2 per day orally on days 1 to 5, rituximab 375mg/m2 on day 8 of the first cycle and then on day 1 of each cycle) every 3 week for 6-8 cycles. Patient n° 3 was initially diagnosed with a associated thrombotic thrombocytopenic purpura (TTP) and treated with steroids, plasma exchange and rituximab.
Results: Four patients out of five, four completed the planned treatment. In patient n°1 to clear residual disease (on immunofixation electrophoresis a persistent monotypic IgG kappa was found) a maintenance treatment with rituximab 375mg/m2 once every 2 months for 4 cycles was made. In the 4 patients CT scan and PET scan documented a complete remission (CR); the HHV-8 clearance was also obtained. Patient n° 1 died after a 63 months follow up with a ischemic cardiopathy. Patients n°4, n°5, n°6 are alive with a follow up respectively of 2, 1 and 62 months. Patient n° 2 initially refused treatment; 1 year later chemoimmunotherapy was started because of the disease worsening with transfusion-dependent anemia. After the first cycle this treatment was stopped for hematologic toxicity. With his informed consent the patient was treated with bortezomib and valganciclovir, but she died one month later with pseudomonas aeruginosa sepsis. Patient n° 3 obtained a partial remission with 9 plasma exchange and rituximab, but refused further therapies and died one month later.

Tabella 1.

Patient	Sex	Age (years)	Histologic diagnosis	Manifestation	Laboratory	Associated diseases
1	M	78	Mixed cell type	Constitutional symptoms, edema and pleural effusion, hepatosplenomegaly	Anemia, monoclonal gammopathy	
2	F	79	Hyaline-vascular type	Constitutional symptoms, lymphoadenomegaly	Anemia	Kaposi's sarcoma HCV infection
3	M	76	Plasma cell variant	Constitutional symptoms, lymphoadenomegaly, hepatosplenomegaly	Anemia, thrombocytopenia	Thrombotic thrombocytopenic purpura
4	M	75	Mixed cell type	Constitutional symptoms, lymphoadenomegaly, hepatosplenomegaly	Anemia, monoclonal gammopathy	Diabetes mellitus
5	M	69	Mixed cell type	Constitutional symptoms, lymphoadenomegaly	Monoclonal gammopathy	Kaposi's sarcoma
6	M	80	Mixed cell type	Constitutional symptoms, lymphoadenomegaly, hepatosplenomegaly	Anemia, thrombocytopenia	

Summary and Conclusions: In HIV negative HHV-8 positive MCD patients the illness can be fatal if not or lately treated. Chemoimmunotherapy is an effective treatment for these patients and is able to attain a clinical response with associated undectectable HHV-8 viral load.

LB324

GENETIC ALTERATIONS OF IMPORTANCE FOR THE TRANSFORMATION OF FOLLICULAR LYMPHOMA TO DIFFUSE LARGE B-CELL LYMPHOMA

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New Information: CGH array data was published at Genes Chromosomes Cancer. 2014 Sep;53(9):750-68. New NGS data has never been submitted before. None of these results has ever been submitted or presented at another event.
Background: Transformation of low malignant follicular lymphoma (FL) to the more aggressive diffuse large B-cells lymphoma (DLBCL) has been associated with new genetic events during the disease course. The number of recent evidences suggests that mutations in several genes such as *p53, EBF1, MYD88* are acquired during transformation. In studies of genomic copy number alterations associated with transformation from FL to DLBCL, gains involving 2p, 12q13-14, 18q21, Xq as well as losses of 1p36, 6q, 17p were earlier described.
Aims: To identify further genetic alterations of importance for transformation by analysis of sequential tumor samples from FL-DLBCL transformation using array comparative genomic hybridization (array-CGH) and whole-exome sequencing (WES).

Methods: Material: 81 tumours from 60 patients í29 *de novo* DLBCL (dnDLBCL), 31 *transformed* DLBCL (tDLBCL), 21 concordant FL including 15 paired tumours (FL and tDLBCL). Methods:Immunohistochemistry, 1 Mb array-CGH, NGS Illumina HiSeq2000
Results: Using array-CGH, we identified a gain of 2p15-16.1 as a potential prognostic marker of transformation as it was more commonly found in tDLBCL in comparison with dnDLBCL (p<0,001). This chromosomal region encompasses, among others, genes involved in NFkB pathway such as *REL, USP34, COMMD1, OTX1,* and of known importance for lymphomagenesis as *BCL11A*. Notably, a high level amplification of 2p15-16 was also detected in FL prior to transformation, indicating an early event of importance for this process. Furthermore, we compared CGH-array data with WES results in a smaller group of patients with available consecutive lymphoma samples and had access to a multiple sample case with four subsequent FL (FL1-FL4) and two tDLBCL (D1-D2) tumours. We focused on alterations appearing in the FL tumour just prior to the DLBCL transformation *i.e.* FL4-D1, thus in the peri-transformation phase. A number of mutations were identified affecting genes such as *CHD4, WDR33, FOXO4, PPP1R15B, DOCK4, SERPINI2, NPY5R,* and *MROH5*.
Summary and Conclusions: Having access to subsequent samples from patients with consecutive FLs and tDLBCLs we were able outline the sequential order of genetic alterations of importance for the transformation process. Using array-CGH we found copy number changes that by WES were found to encompass mutated genes, showing that these two methods complement and strengthens each other in pinpointing crucial genetic events. Our studies revealed that clonal evolution of transformed tumours occurs rather according to branching than linear model.

LB325

THE TYK2 PATHWAY AS A NOVEL THERAPEUTIC INTERVENTION SITE IN AGGRESSIVE T-CELL LYMPHOMA

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Background: Anaplastic large cell lymphoma is a CD30 positive, aggressive Non-Hodgkin T-cell lymphoma mainly affecting children or young adults. Anaplastic lymphoma kinase (ALK) inhibition has shown remarkable success in targeted therapy of non small cell lung cancer and in ALCL where ALK kinase fusion proteins have initially been identified in 60% of ALCL patients (ALCL, ALK+). However, treatment with ALK inhibitors inevitably leads to resistance development. Moreover, the tumour drivers in ALK- ALCL remain still elusive making the identification of new therapeutic targets an imperative.
Aims: We have recently identified Tyrosine kinase 2 (TYK2) fusion proteins in the context of ALCL. Here we describe high expression of TYK2, a member of the Janus kinase family (JAK1, JAK2, JAK3, TYK2) in human ALCL samples and pathway dependence on TYK2 in all ALCL subtypes. By using TYK2 knock down as well as TYK2 kinase inhibitors we demonstrate the therapeutic relevance of our findings.
Methods: TYK2 immunohistochemical stainings were performed in human ALCL tissue microarrays (ALK+, ALK-). Lentiviral transduction mediated shRNA knock-down of TYK2, JAK1 and STAT1 was performed in human ALCL cell lines. Knock-down and activation of downstream targets was shown by Western blot and IHC. Efficacy of JAK1 and TYK2 inhibitors (Bay-18, Symansis; TYK2_1 and JAK1_1, Genentech) was analysed by XTT and cell titer glow assays as well as Western blot analysis. The *in vivo* relevance of TYK2 was assessed via straight TYK2 knockout (TYK2 -/-) in a *CD4*-NPM-ALK ALCL mouse model.
Results: Tumor cell specific IHC staining revealed strong TYK2 expression in both ALK+ and ALK- ALCL patient samples. ShRNA mediated TYK2 or JAK1 gene knockdown resulted in strong induction of apoptotic cell death. Analysis of TYK2 downstream targets (STAT1, STAT3, STAT5) revealed distinct p-STAT1 reduction after small molecule TYK2 inhibition. Accordingly, shRNA mediated gene knockdown of STAT1 revealed an essential function for ALCL cell survival. Treatment with selective small-molecule inhibitors of TYK2 resulted in dramatically reduced cell proliferation in ALCL cell lines but not in peripheral blood mononuclear (PBMC) and JURKAT T-ALL control cell lines, suggesting novel therapeutic options through TYK2-pathway inhibition in T-cell lymphomas. TYK2 knockout in *CD4*-ALCL mice resulted in a complete rescue of the early lethality phenotype.
Summary and Conclusions: Our results indicate a strong TYK2-pathway dependence in ALCL (ALK+ and ALK-). We identified a novel therapeutic strategy by taking advantage of recently developed small molecule inhibitors. Ongoing *in vivo* experiments using *CD4*-NPM-ALK transgenic ALCL mice in which TYK2 is conditionally knocked out in T-cells as well as treatment of patient derived ALCL tumor cells with these inhibitors will systematically assess the therapeutic potential of our findings.

Aggressive lymphoma - Therapy and Prognostication

P324

SINGLE ARM NCRI FEASIBILITY PHASE II STUDY OF CHOP IN COMBINATION WITH OFATUMUMAB IN INDUCTION AND MAINTENANCE FOR PATIENTS WITH NEWLY DIAGNOSED RICHTER'S SYNDROME

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Background: Transformation of Chronic Lymphocytic Leukaemia (CLL) to diffuse large B cell lymphoma (DLBCL) (Richter's syndrome, RS) is rare (2-15%) (1–3) but associated with a very poor prognosis. Despite dose-intense chemotherapy (4–8), little has improved outcomes, with the median overall survival (OS) remaining approximately 8 months. These regimens can also cause significant toxicity. Patients are often elderly, immunosuppressed, and co-morbid. Ofatumumab is a fully human anti-CD20 IgG1κ monoclonal antibody that targets a B-cell epitope which is distinct from that targeted by rituximab. It displays increased binding affinity and a longer dissociation time compared to rituximab, improving complement dependent cellular cytotoxicity (9,10); a mechanism with the potential to overcome apoptosis-resistance. Given the prevalence of TP53 disruption in RS (11), ofatumumab is a non-toxic agent with a sound rationale to test (off label) with CHOP (CHOP-O).
Aims: To evaluate the safety, feasibility and activity of CHOP-O in induction and maintenance for newly diagnosed RS. The primary objective was overall response rate (ORR) according to standard criteria (12) after 6 cycles of CHOP-O. Secondary objectives include progression free survival (PFS), OS, safety and tolerability.
Methods: This single arm, multi-centre, non-randomised phase II NCRI feasibility study recruited 43 patients with newly diagnosed RS across 10 UK centres. Eligibility included: ≥18 years, PS 0-3, known or newly diagnosed CLL and newly diagnosed, untreated RS. Eligible patients received CHOP-O for 6 cycles, followed by 6 cycles of O-maintenance (8 weekly). The trial was run in accordance with the Declaration of Helsinki. All patients gave informed consent. Histology results were reviewed locally for study inclusion to replicate 'real life', and later for central review.
Results: Characteristics: 43 patients were recruited of which 36 patients are currently evaluable (1 pending). Table 1 summarises in detail their main characteristics. 73% were over 60 years, and most were male. Over half of patients had received a fludarabine and cyclophosphamide based regimen as prior treatment for CLL. Baseline PET CT was performed in 42% of enrolled patients, with no clear change of scanning frequency occurring over recruitment. **Response:** The ORR to induction was moderate. 66% responded (PR or CR) after four cycles of CHOP-O, although this reduced to 44% ORR (CR 25%, PR 19%) at the end of induction. 17 patients started maintenance with 8 completing maintenance, and 5 currently still continuing. The median PFS was just over six months (95% confidence interval (CI) 4.87-14.96 months), with no clear plateau on the survival curve. The current median OS was 11.4 months (95% CI 6.38 months-NR), consistent with outcomes described in the literature using R-CHOP and platinum-based regimens (6–8,13). Five patients received platinum-containing salvage at progression, but none completed a stem cell transplant. **Safety:** CHOP-O was typically well tolerated. 15 episodes of neutropenic fever were reported and grade 3-4 neutropenia was noted on 14 occasions. 46 additional non-neutropenic infections were noted, of which 21 were serious adverse events (SAE). A single SAE as the result of an ofatumumab-induced infusion reaction was reported. There were no treatment related deaths. **Further Evaluation:** The diagnostic tissue is currently undergoing further molecular genetic testing, and central review analysis. This data will be presented alongside an update on patient outcome. Within this study we also plan to prospectively validate the two scoring systems devised in RS (1,7). **Feasibility:** This was the first UK-wide prospective study in RS and is the largest ever prospective study performed to date. It therefore proves the feasibility of studying novel combinations in a rare disease when national collaboration is successful. The study was completed in 3.5 years, only just behind the predicted schedule. It is important that further studies are performed in a collaborative manner to study novel agents in this difficult-to-treat disease.

Tabella 1. Patient Characteristic at baseline

Patient Characteristics	All evaluable patients N = 37 n (%)
Mean Age (range, SD)	66.2 (43.9-90, 11.3)
<60y	10 (27%)
>60y	27 (73%)
Gender	
Male	26 (70%)
Female	11 (30%)
ECOG performance status (PS)	
0-1	29 (78%)
2-3	8 (22%)
Rai Staging	
0	8 (22%)
I	10 (27%)
II	4 (11%)
III	3 (8%)
IV	2 (5%)
Not assessed to date	10
Binet Staging	
A	17 (46%)
B	12 (32%)
C	6 (16%)
Not assessed to date	2
Ann Arbor Stage:	
I-II	12 (32%)
III-IV	23 (62%)
Not assessed to date	2
B symptoms	
Yes	22 (59%)
No	15 (41%)
Platelet count at baseline	
< 100 x 10^9/L	10 (27%)
≥100 x 10^9/L	27 (73%)
Tumour bulk > 5cm	
Yes	18 (49%)
No	19 (51%)
LDH level at baseline	
<ULN	13 (35%)
>ULN	22 (60%)
>1.5 x ULN	11 (30%)
Not tested	2
Beta-2 Microglobulin level at baseline	
<ULN	12 (32%)
>ULN	18 (49%)
Not tested	7
Extranodal Sites	
0	19 (51%)
1	13 (35%)
>1	5 (14%)
IPI (LDH>ULN; >1 ENS, >60y, Ann Arbor III-IV, PS>1)	
0-2	19 (51%)
3-5	13 (36%)
Awaiting completion	5
Previous line of chemotherapy for underlying CLL	
0	17 (46%)
1	12 (32%)
>1	8 (22%)
Prior treatments – frequency received (patients with ≥1 prior treatment included)	
Fludarabine, cyclophosphamide, rituximab	
Fludarabine, cyclophosphamide	12
Chlorambucil ± rituximab	9
Alemtuzumab ± Steroid (High dose)	5
Rituximab	4
Other	2
	10
TP53 disruption (in PB, tumour, prior CLL both):	
Yes	13 (35%)
No	20 (54%)
Undefined or Incomplete to date	4
PET Scans at diagnosis (all 43 evaluable & non-evaluable patients)	
Yes	18 (42%)
No	25 (58%)
Average SUV max range	mean: 21.5 (range 5.85-69.4)
Rossi et al Score:	
ECOG PS ≤1 and no *TP53* disruption plus CR to induction (low risk)	4 (11%)
ECOG PS ≤1 with either *TP53* disruption or <CR to induction (intermediate)	21 (57%)
ECOG PS >1 (high risk)	7 (19%)
Incomplete at present	5 (13%)
Tsimberidou et al Score: (1) ECOG PS >1; (2) LDH>ULN; (3) Plts<100x109/L; (4) tumor >5 cm; (5) number of prior therapies for CLL >1	
0-1 (low)	19 (51%)
2-3 (intermediate)	15 (41%)
4-5 (high)	1 (3%)
Incomplete at present	2 (5%)
CR rate post induction	
CR	9/36 (25%)
<CR	27/36 (75%)
Ongoing induction	1

Summary and Conclusions: This study proved that it is feasible to perform clinical trials in RS and answer important questions. It is likely that CHOP-O does not considerably improve outcomes compared with R-CHOP in RS.

P325

A PHASE 1 TRIAL OF CUDC-907, AN ORAL, FIRST-IN-CLASS, DUAL INHIBITOR OF HDAC AND PI3K, IN PATIENTS WITH REFRACTORY OR RELAPSED LYMPHOMA AND MULTIPLE MYELOMA

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Background: The importance of histone deacetylase (HDAC) and phosphatidylinositol 3-kinase (PI3K) pathways in lymphoma coupled with anti-tumor synergy observed in preclinical studies by concurrent inhibition of both enzymes support clinical testing of CUDC-907, an oral inhibitor of class I and II HDAC as well as class I PI3K enzymes. Anti-tumor effects of CUDC-907 have been demonstrated in cultured cells and xenograft models of B-cell lymphoma and multiple myeloma via inhibition of PI3K/AKT, JAK/STAT and MAPK pathways as well as modification of the tumor microenvironment.
Aims: The primary objective of this ongoing trial is to determine the maximum tolerated dose (MTD) or biologically effective dose (BED) and recommended Phase 2 dose (RP2D) of CUDC-907 in subjects with relapsed/refractory (RR) lymphoma and multiple myeloma (MM). The secondary objectives are to assess the safety and tolerability, pharmacokinetics, biomarker activity, and preliminary anti-cancer activity of CUDC-907.

Methods: This first-in-human trial used a standard Phase 1 3+3 design to examine CUDC-907 in patients with RR lymphoma or MM. CUDC-907 was orally administered using 3 different dosing schedules; once daily (QD), intermittent twice (BIW) or thrice weekly (TIW), and five days on/two days off (5/2) in 21-day cycles. Dosing started at 30 mg for the QD schedule and 60 mg for the other schedules, escalating in 30 mg increments.
Results: 51 subjects received CUDC-907 up to a maximum of 60 mg for the QD and 5/2 schedules and 150 mg for the intermittent schedules. Dose limiting toxicities occurred in 3 subjects: G4 hyperglycemia and G3 diarrhea (both in the same patient) at 60 mg QD, G3 hyperglycemia at 150 mg BIW and G3 diarrhea at 150 mg TIW. The most common treatment-related adverse events (AEs) were diarrhea (45%), fatigue (31%), nausea (20%) and thrombocytopenia (14%). The most common treatment-related AEs of Grade ≥3 intensity included thrombocytopenia (14%), neutropenia (4%) and diarrhea (4%). Among 41 subjects evaluable for disease response, 4 objective responses were observed: 1 subject with diffuse large B-cell lymphoma (DLBCL) achieved complete response (CR) in Cycle 2 (60 mg 5/2), 2 subjects with transformed follicular lymphoma (t-FL/DLBCL) achieved partial response (PR) in Cycles 4 and 26 (30 mg QD and 150 mg BIW, respectively), and 1 subject with DLBCL achieved PR in Cycle 4 (60 mg QD). To date, stable disease (SD) lasting for a median of 101 days (40-717) has been observed in 22 (54%) subjects including Hodgkin Lymphoma (HL) (n=9); DLBCL and t-FL/DLBCL (n=3); and MM (n=3).

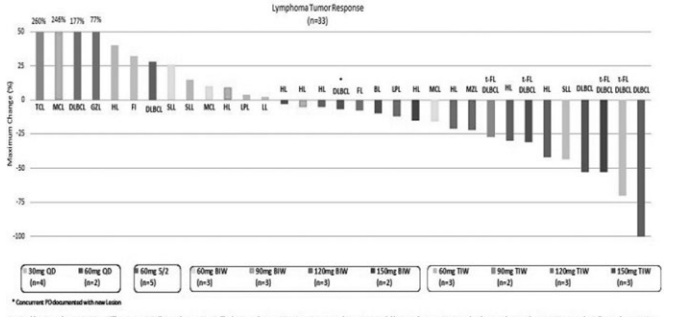

Figure 1.

Summary and Conclusions: The safety profile of CUDC-907 is similar to that of other HDAC and PI3K inhibitors. Reversible gastrointestinal, hematologic, endocrine and other AEs have been managed with standard interventions or dose interruption. In this first-in-human trial, CUDC-907 has achieved objective responses and durable disease control across multiple tumor types and dosing schedules. MTD was not reached in the 5/2 schedule, which was selected for expansion in patients with DLBCL and MM based upon CUDC-907's toxicity and efficacy profile across various schedules tested in this study population.

P326

A PHASE 1 STUDY OF PATIENTS WITH RELAPSED OR REFRACTORY B-CELL MALIGNANCIES TREATED WITH A PI3K DELTA INHIBITOR (INCB040093) ALONE OR IN COMBINATION WITH A SELECTIVE JAK1 INHIBITOR (INCB039110)

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Background: Inhibition of the PI3K and JAK-STAT pathways may be efficacious in B-cell malignancies because of their contribution to tumor growth and survival both directly and through modulation of the tumor microenvironment. Furthermore, inhibiting both pathways may result in synergistic efficacy due to JAK-STAT augmentation of B-cell receptor activation of the NFκB pathway.
Aims: To determine the safety, efficacy, and pharmacodynamics of INCB040093 alone or in combination with INCB039110 in patients with relapsed or refractory B-cell malignancies.
Methods: Adults with relapsed/refractory B-cell malignancies were eligible for this ongoing dose escalation study with expansion cohorts. Patients received INCB040093 monotherapy (100 mg once daily, 100 mg twice daily, 150 mg twice daily, or 300 mg once daily) or INCB040093 in combination with INCB039110 (INCB040093: 150 mg daily, 100 mg twice daily, or 150 mg twice daily; INCB039110: 400 mg or 600 mg once daily). Safety, efficacy, and pharmacodynamics were evaluated.
Results: A total of 83 patients have been enrolled, including patients with follicular lymphoma (n=19), classical Hodgkin lymphoma (cHL; n=17), diffuse large B-cell lymphoma (DLBCL; n=15), chronic lymphocytic leukemia/small lymphocytic lymphoma (n=13), and other subtypes (n=19). At baseline, the median age was 61 years and 70% of patients were men. The median number of prior regimens received was 4 and 24% of patients had undergone prior hematopoietic stem cell transplantation. The median exposure during the study was 185 days (range: 5–491+ [ongoing]) for INCB040093 alone and 99 days (range: 6–337+ [ongoing]) for INCB040093 + INCB039110. The most common adverse events were fatigue(28%), headache(19%), and pyrexia(19%) and the most common grade ≥3 adverse event was pneumonia(6%). The most common laboratory abnormalities were liver enzyme elevations and cytopenias. One patient had a dose-limiting toxicity of gastrointestinal bleed secondary to gastric DLBCL regression on INCB040093 100 mg twice daily. INCB040093 100 mg twice daily and INCB040093 100 mg twice daily + INCB039110 400 mg once daily were selected as the dosing regimens for expansion cohorts based on the incidence of liver enzyme elevations with INCB040093 and cytopenias with INCB040093 + INCB039110 at higher doses. At the selected doses, pAKT was decreased by ≈90% at trough on INCB040093 and IL-6–induced pSTAT3 was decreased an average of 65% on INCB039110. Of 75 evaluable patients, 28 responses have been reported. In evaluable patients with cHL (n=15), the objective response rate was 60%, which included 3 complete responses. Both patients enrolled with the non-germinal center B-cell–like subtype of DLBCL experienced complete responses.
Summary and Conclusions: Treatment with INCB040093 alone or in combination with INCB039110 was tolerable and resulted in partial and complete responses in this heavily pretreated population of patients with relapsed or refractory B-cell malignancies. Based on these results, the study was expanded to enroll additional cohorts of patients with relapsed/refractory B-cell malignancies such as DLBCL and cHL, and a phase 2 study was initiated in patients with relapsed/refractory cHL.

P327

UBLITUXIMAB + TGR-1202 DEMONSTRATES ACTIVITY AND FAVORABLE SAFETY PROFILE IN RELAPSED/REFRACTORY B-CELL NHL AND HIGH-RISK CLL

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Background: Ublituximab (UTX) is a novel anti-CD20 mAb that has been glycoengineered for enhanced ADCC. TGR-1202 is a novel once daily oral PI3Kδ inhibitor with demonstrated clinical activity in B-cell lymphoma and notably absent hepatotoxicity associated with similar agents. The combination of UTX + TGR-1202 showed strong synergistic activity *in-vitro* (Lugano 2013).
Aims: This Phase 1 trial evaluates the safety and efficacy of the combination of UTX + TGR-1202 in patients with heavily pre-treated rel/ref NHL and CLL.
Methods: A 3+3 designwas utilized with rel/ref NHL and CLL patients accruing independently. Informed consent was obtained on all patients. There were no limits on number of type of prior therapies. Patients refractory to prior PI3K or BTK inhibitorswere eligible. UTXwas administered D 1, 8, 15 of Cyc 1 & 2, followed by D 1 of Cyc 4, 6, 9 & 12. TGR-1202was administered orally once-daily. Primary endpoints: Safety and dose limiting toxicities (DLT). Secondary endpoints: Efficacy (ORR, CR rate) with criteria as follows: CLL per Hallek 2008 and NHL per Cheson 2007.

Tabella 1.

Type	TGR-1202 Higher* Dose					Type	TGR-1202 Lower** Dose				
	Pts (n)	CR (n)	PR (n)	ORR n (%)	PD (n)		Pts (n)	CR (n)	PR (n)	ORR n (%)	PD (n)
CLL/SLL	3	-	3	3 (100%)	-	CLL/SLL	7	-	4	4 (57%)	-
DLBCL	4	2	1	3 (75%)	1	DLBCL	3	-	-	-	2
FL/MZL	7	1	4	5 (71%)	-	FL/MZL	4	-	1	1 (25%)	-
Richter's	1	-	1	1 (100%)	-	Richter's	-	-	-	-	-
Overall	15	3	9	12 (80%)	1	Overall	14	-	5	5 (36%)	2

*Higher Dose = 1200 original formulation and 600 or > micronized
**Lower Dose = 800 original formulation and 400 micronized

Results: 37 patients were enrolled and evaluable for safety: 13 CLL/SLL, 12 FL, 9 DLBCL, 2 MZL and 1 Richter's transformation. Med age 64 yo (range 29-86); 24 M/13 F; median # prior treatment regimens =3 (range 1-9). Day 1 infusion reactions (3% G 3/4), neutropenia (32% G 3/4), diarrhea (3% G 3/4) and nausea (0% G 3/4) were the most commonly reported adverse events, regardless of causality. To date, TGR-1202-related hepatotoxicity has not been observed. One DLT occurred: a patient with Gr 3 neutropenia at study entry which worsened (cohort 1). A dose-response relationship was observed with

TGR-1202; greater clinical activity was observed at higher doses. 29/37 were evaluable for efficacy (7 too early and 1 was ineligible) with best response to treatment as follows: To date, of the 29 patients evaluable for response, 87% (13/15) in the higher dose cohorts remain progression-free compared to 43% (6/14) in the lower dose cohorts. All but 1 of the CLL patients remain progression-free with a median follow up time of 9 months (range 2-12+ mos).
Summary and Conclusions: The chemotherapy free combination of UTX + TGR-1202 is highly active and well tolerated in patients with both indolent and aggressive rel/ref NHL and CLL. Dose escalation continues with enrollment ongoing at the highest dose cohort and in recently opened expansion cohorts.

P328

BENDAMUSTINE COMBINED WITH RITUXIMAB (BR) IN ELDERLY FRAIL PATIENTS WITH NEWLY DIAGNOSED DLBCL

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Background: Rituximab (R) in combination with cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) is currently the most widely used first-line therapy for Diffuse Large B-cell Lymphomas (DLBCL). However, many patients, including elderly and/or frail patients, may not tolerate the toxicities associated with this regimen. Recent data suggested that bendamustine plus rituximab (BR) was superior in effectiveness and tolerability compared to R-CHOP in the treatment of indolent and mantle cell lymphomas. Preliminary data have shown a promising activity in DLBCL, both in the relapsing and upfront setting.
Aims: We investigated the safety and efficacy of BR combination in elderly patients affected by diffuse large B-cell lymphoma and defined as frail according to CGA.
Methods: Eligible patients were elderly patients (>70 years) with a newly diagnosed DLBCL not suitable for R-CHOP-based chemotherapy. All patients were evaluated by Comprehensive Geriatric Assessment (CGA) according to ADL, IADL and CIRS-G and were considered FRAIL if the following criteria were met: in patients aged 70-80 ADL<4 or IADL<5 or 1 grade 3 comorbidity or >8 grade 2 comorbidities; in patients older than 80 years ADL>5 or IADL>6 or 5-8 grade 2 comorbidities. Patients received bendamustine at a dose of 90 mg/m^2 daily on days 1 and 2 of each 28-day cycle along with rituximab on day 1 for up to 6 cycles. The study evaluated the complete response rate (CRR), and treatment safety. Secondary end points included progression free survival (PFS) and overall survival (OS).
Results: From February 2012 to February 2014, 49 patients were enrolled in 24 Italian centers. The majority (both 59%) were male and stage III-IV. The median age was 82. Overall, 83% (38) of the patients was older then 80 years and at CGA evaluation showed unfavourable 3 (68%, 26), 2 (26%, 10), and 1 (5%, 2) criteria. 25 patients completed all planned cycles of chemotherapy. The most frequent comorbidities were cardiovascular (37%) metabolic (11%) and respiratory diseases (7%). 24 patients discontinued the treatment (12 progressions of disease, 8 adverse events, 4 deaths). The adverse events that led to treatment discontinuation were: persistent cytopenia (3), worsening general condition (2), coronary acute syndrome (1), second tumor (1), febrile neutropenia associated to infection (1). Among four patients not receiving at least2 courses of BR, 2 progressed. The overall response rate in 47 evaluable patients was 64%, with 26 patients (55%) achieving a complete response. At the last analysis performed with a median follow up of 10 months (1-31), 13 progressions and 4 relapse have been observed. The 2-years PFS and OS were 43% and 59%.
Summary and Conclusions: Combination therapy with BR demonstrates low toxicity profile in this high risk population. The promising results on activity can encourage clinicians to considered BR for the treatment of FRAIL elderly patients with DLBCL not eligible for R-CHOP.
Disclosures: No relevant conflicts of interest to declare.

P329

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P330

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P331

CD56 NEGATIVE EXTRANODAL NK/T CELL LYMPHOMA SHOULD BE REGARDED AS A DISTINCT SUBTYPE WITH POOR PROGNOSIS

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Background: The majority cases of extranodal NK/T cell lymphoma (ENKTL) have a natural killer cell origin, and a small minority has clonal T cell receptor rearrangements and appears to be derived from cytotoxic T cells, which usually featured by CD56 negative expression. Previous results about the clinical and prognostic significance concerning CD56 expression status are controversial due to small sample size and the heterogeneity nature of this disease.
Aims: We aimed to define the prognostic value of CD56 expression in ENKTL.
Methods: The complete data of 288 patients with early stage upper aerodigestive tract ENKTL were retrospectively reviewed.
Results: 183 patients (63.5%) had stage I disease, and the primary tumor site of 204 patients (70.8%) was in nasal cavity. 60 patients (20.8%) were categorized to CD56 negative ENKTL group. There were more patients with primary tumor site in extranasal upper aerodigestive tract (43.3% *vs* 25.4%, P =0.011) and poor ECOG score (8.4% *vs* 2.2%, P =0.036) in CD56 negative group. There were no significant correlations between CD56 expression status and gender, age, lactate dehydrogenase (LDH) level, Ann-Arbor stage, B symptoms and IPI score (P >0.05). At a median follow-up time of 69 months, the 5-year and 10-year PFS rate were 52% and 41% respectively, and the 5-year and 10-year OS rate were 69% and 68% respectively. Patients with primary tumor site located in nasal cavity or CD56 positive expression had significantly superior PFS and OS (P <0.05). In multivariate Cox regression model that included age, Ann Arbor stage, LDH level, primary tumor site, and CD56 expression status, all these five factors remained to be independent prognostic factors. In subgroup analysis according to primary tumor site location, CD56 expression status significantly correlated with survival outcomes in patients with primary nasal cavity involvement, however, it lost the prognostic value in patients with primary extranasal upper aerodigestive tract involvement (P >0.05).
Summary and Conclusions: In this largest cohort of early stage ENKTL ever reported, we found that CD56 negative ENKTL had significantly inferior survival outcomes, indicating CD56 negative ENKTL should be regarded as a distinct phenotype and optimal treatment strategies need to be evaluated further for this entity.

P332

DEFINING IMMUNOGLOBULIN SOMATIC HYPERMUTATION IN DE NOVO DIFFUSE LARGE B-CELL LYMPHOMA PATIENTS: POTENTIAL APPLICATION FOR PROGNOSIS AND RISK STRATIFICATION

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Background: Diffuse large B cell lymphoma (DLBCL) is a heterogeneous group of diseases with variable clinical presentation, morphologic features, genomics, gene expression signature and prognosis. Some of the variability in patient course and response to therapy is likely to represent a function of developmental stage and/or specific pathway of transformation. We have been engaged in a detailed investigation of the molecular and clinical features of a large cohort of patients with DLBCL at the MD Anderson Cancer Center. Through these analyses we have begun to subcategorize this patient population based on these distinctive clinical and biological parameters. In this current aspect of our investigation we have explored the prevalence of somatic hypermutation (SHM) of the immunoglobulin loci in these de novo DLBCL patients using the platform of multiplex PCR and high-throughput sequencing (immunoSEQ) developed by Adaptive Biotechnologies, Inc. It has previously been established that the presence or absence of somatic hypermutation is an independent prognostic factor in patients with chronic lymphocytic leukemia (CLL). The ultimate goal of this collaborative effort is to determine if a similar biological mechanism between somatic hypermutation and prognosis exists within the population of DLBCL patients or subset and to relate the presence of SHM to clinical, pathological, and molecular aspects of this disease.
Aims: In this study, we investigated whether the immunoSEQ (Adaptive Biotech) assay could be used to reliably discriminate dominant clones in diagnostic specimens from patients with DLBCL with regard to rearrangement status of the immunoglobulin heavy and light (kappa and lambda) chains and the presence or absence of SHM.
Methods: The study group consisted of 200 DLBCL patients treated with R-CHOP. Patients with primary mediastinal large B-cell lymphoma, primary cutaneous DLBCL, primary central nervous system DLBCL, and DLBCLs transformed from a low-grade B-cell lymphoma or associated with HIV infection

were excluded. Genomic DNA was extracted from FFPE sections of diagnostic lymph node specimens of patients with DLBCL. Immunoglobulin heavy and light chain sequences were then independently amplified using multiplex PCR with optimized primer sets. Following high-throughput sequencing, a bioinformatics pipeline clusters the sequences into distinct clonotypes to determine overall frequencies and to identify diagnostic clones. V, (D,) and J genes are also identified for each clonotype, and point mutations that are not known germline allele variants are assigned as somatic hypermutation events.
Results: Using both the IgH and IgL (kappa and lambda) we have been able to identify an index trackable sequence in 90%+ of the samples (we identify an index diagnostic sequence or sequences in about 70% of the cases using each assay individually). Using a definition of SHM as >2% point mutations in the observed V gene, the samples can be split into three distinct categories: 1, V(D)J or VJ rearranged with SHM (50-55%); 2, V(D)J or VJ rearranged without SHM (10-25%) and 3, DJ only evident (20-40%). The vast majority of complete V(D)J rearrangements are in-frame.
Summary and Conclusions: The IgH and IgL immunoSEQ assays are robust in their ability both to identify dominant sequences in diagnostic lymph node specimens from patients with DLBCL and to distinguish those clones in which evidence of somatic hypermutation is present. The distribution of SHM in these samples lends itself to potential correlative and stratifying analyses on this well-characterized patient cohort, and likely have significant application in other aggressive B-cell lymphoma patients.

P333

A PHASE 1 STUDY OF PATIENTS WITH RELAPSED OR REFRACTORY CLASSICAL HODGKIN LYMPHOMA TREATED WITH A PI3K DELTA INHIBITOR (INCB040093) ALONE OR IN COMBINATION WITH A SELECTIVE JAK1 INHIBITOR (INCB039110)

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Background: Efficacy of treatment options for patients with relapsed or refractory classical Hodgkin lymphoma (cHL) is limited. Preclinical and early clinical evidence suggests that blocking the JAK-STAT or PI3K pathways may be efficacious in cHL, both directly and through modulation of the tumor microenvironment. Furthermore, blocking both pathways may provide synergistic efficacy.
Aims: To determine the safety, efficacy, and pharmacodynamics of INCB040093 alone or in combination with INCB039110 in patients with relapsed or refractory cHL.
Methods: Adult patients with relapsed or refractory B-cell malignancies, including patients with cHL, were enrolled in this ongoing, open-label, dose escalation study. Patients received INCB040093 monotherapy (100 mg once daily, 100 mg twice daily, 150 mg twice daily, or 300 mg once daily) or INCB040093 in combination with INCB039110 (INCB040093: 150 mg daily, 100 mg twice daily, or 150 mg twice daily; INCB039110: 400 mg or 600 mg once daily). Safety, efficacy, and pharmacodynamics were evaluated. Results from patients with relapsed or refractory cHL are reported herein.
Results: A total of 17 patients with relapsed or refractory cHL have been enrolled. At baseline, the median age was 34 years and 59% of patients were men. The median number of prior treatment regimens was 5, 82% of patients had undergone prior hematopoietic stem cell transplantation, and all patients had received brentuximab vedotin therapy prior to study entry. The median exposure to treatment in this study was 209 days (range: 22+ [ongoing]–388). The most common nonhematologic adverse events (all grades) in patients with cHL were fatigue (41%), headache(35%), and decreased appetite(35%). Pneumonia(12%) was the only nonhematologic grade ≥3 adverse event to occur in >1 patient. All-grade neutropenia, thrombocytopenia, and anemia occurred in 47%, 47%, and 41% of patients, respectively. Grade ≥3 thrombocytopenia occurred in 18% of patients. No patients with cHL experienced a dose-limiting toxicity. Of 6 evaluable patients receiving INCB040093 monotherapy, the objective response rate (ORR) was 50% (including 1 complete response [CR]); of 9 evaluable patients receiving INCB040093 + INCB039110, ORR was 67% (including 2 CRs). In this limited dataset of proximate dose cohorts, a dose response in efficacy was not evident. INCB040093 100 mg twice daily and INCB040093 100 mg twice daily + INCB039110 400 mg once daily were selected for expansion based on pharmacodynamics and the safety profile of higher dose levels in the overall study population. At the selected doses, ORR in the cHL cohort was 50% for INCB040093 monotherapy and 75% (including 1 CR) for INCB040093 + INCB039110.
Summary and Conclusions: INCB040093±INCB039110 was generally well tolerated in this heavily pretreated population of patients with relapsed or refractory cHL. Although the number of evaluable patients is limited, efficacy compares well to approved therapies and investigational agents for cHL. This activity warranted further investigation of INCB040093 alone and in combination with INCB039110 in patients with relapsed or refractory cHL, and a phase 2 study has been initiated.

P334

ROUTINE BONE MARROW BIOPSY IN PET/TC ERA FOR HODGKIN LYMPHOMA STAGING

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Background: Accurate staging is crucial in stratifying treatment in Hodgkin Lymphoma (HL). Bone marrow involvement (BMI) detection traditionally requires a BM biopsy (BMB), an invasive procedure. Some studies have shown that 18F-fluoro-2-deoxy-D-glucose Positron Emission Tomography/Computed Tomography (FDG-PET/TAC) scanning is more sensitive than BMB in detecting BMI in HL. Although baseline FDG-PET-CT is recommended, it is not considered mandatory as a staging tool.
Aims: To determine whether BMB is still providing useful staging information now that FDG-PET/CT is available at baseline.
Methods: We retrospectively identified all patients with newly diagnosed HL between January 2008 and December 2014 in our center and evaluated those with a baseline staging with FDG-PET/CT and BMB. 70/83 BMB were bilateral. We classified the bone FDG uptake patterns as: negative (negative and reactive, [those with diffuse symmetrical uptake]) and positive (focal). Clinical stage, risk evaluation and treatment plan were determined with and without the contribution of BMB results according to the Ann Arbor classification and the guidelines from the German Hodgkin Study Group.
Results: 91 new cases of HL were identified. Eighty three patients (91%) had both FDG-PET/CT and BMB at baseline. The median age was 36 years and the male/female ratio was 1.77. 43, 13 and 17 patients had stage II, III and IV, respectively. Fifteen (18%) had BMI by FDG-PET/CT and 5 (6%) by BMB. BMB underestimated 11 patients (13.2%) which had focal positive uptake by FDG-PET/CT. None of the patients with BMI by BMB was assigned to stage I to II disease by FDG-PET/CT. We identified one case with positive BMB but no FDG skeleton uptake by FDG-PET/CT, although this case was already stage IV on the basis of liver uptake. Therefore, none of the 83 patients would have been allocated to another treatment on the basis of BMB results. However, 3/15 patients assessed to stage Ito II by CT where upstaged to stage IV by FDG-PET/CT. Skeletal FDG-PET/CT lesions identified patients with positive and negative BMBs with a sensitivity and specificity of 80% and 85.9%, respectively. The positive and negative predictive values of skeletal FDG-PET/CT lesions for BMB results were 26.7% and 98.5%, respectively.
Summary and Conclusions: In this retrospective analysis of patients staged by FDG-PET/CT and BMB, the omission of BMB would not have changed the treatment strategy in any case. Our results suggest that FDG-PET/CT may be an appropriate method to replace BMB to assess bone marrow infiltration in newly diagnosed HL. We should confirm in further studies that BMB has little to offer in staging patients with HL in the FDG-PET/CT era and becomes an unnecessary and painful procedure.

P335

THE PROGNOSTIC VALUE OF BIOLOGICAL MARKERS IN PEDIATRIC HODGKIN LYMPHOMA

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Background: The outcome in pediatric classical Hodgkin's lymphoma (cHL) is excellent when combined modality therapy is used. Over the last two decades it has been tried to identify prognostic factors that allow the identification of children who are likely to benefit from reduced intensity treatment and patients at higher risk of treatment failure. Many biological and inflammatory markers at diagnosis have been proposed as having a prognostic value, but very few have been validated in childhood.

Aims: To explore the significance of biological and inflammatory markers in a large population of 769 children affected by cHL.
Methods: By using the database of patients enrolled in A.*i.e.*O.P. (Associazione Italiana di Emato-Oncologia Pediatrica) trial LH2004 for pediatric cHL, we identified 769 patients treated from June 1, 2004 to April 1, 2014 with ABVD (doxorubicin, bleomycin, vinblastine, and dacarbazine), or hybrid COPP/ABV (cyclophosphamide, vincristine, prednisone, procarbazine, doxorubicin, bleomycin and vinblastine) regimens. We considered the following data at diagnosis: age, sex, stage, white blood cell count, absolute lymphocyte count (ALC), absolute monocyte count (AMC), absolute eosinophil count (AEC), absolute neutrophil count (ANC), platelet count (Plts), 1-hour erythrocyte sedimentation rate, ferritin, albumin, hemoglobin; ALC/AMC ratio, ANC/ALC ratio, and AEC/ALC ratio were also considered. All prognostic factors were first analyzed as continuous variables, except for the A-B categorization, sex and stage. However, since these continuous variables are more likely to be of clinical significance if categorized in binary form, we performed a ROC analysis to find some reasonable cutoff value. Bivariate survival analysis was performed using Kaplan Meier curves and log-rank test. Multivariate survival analysis was performed using a Cox proportional hazards model. We also performed an Internal Bootstrap Validation (IBV) of the model with 800 repetitions.
Results: There were 361 female patients (46.9%), and 408 males (53.0%); 454 patients (59.0%) were in category A and 315 (40.9%) in category B. There were 27 stage I patients (3.5%), 397 stage II (51.6%), 177 stage III (23.0%) and 168 stage IV (21.8%). Histologically, 636 patients (82.7%) were classified as nodular sclerosis, 81 (10.5%) as mixed cellularity, 7 (0.9%) as lymphocyte depleted, 8 (1.0%) as lymphocyte rich, and in 37 (4.8%) no histological subtype was reported. There were 108 (14.0%) events: 71 were relapses, 37 progressions (pathologically-confirmed recurrence within 3 months of the end of therapy). On bivariate analysis with variables in binary form, under the identified cutoffs, 5-year freedom from progression (FFP) survival was significantly lower in patients with stage IV or elevated values of ferritin (≥209 ng/ml), AEC (≥0.493x10^9/L) and Plts (≥537 x10^9/L): 73.4% *vs* 83.9% for stage IV, 66.0% *vs* 86.5% for ferritin, 71.6% *vs* 84.9% for AEC and 66.3% *vs* 82.4% for Plts. Moreover in the Cox multiple regression model for FFP with variables in binary form the hazard ratios were 2.08, 2.72, 3.05 and 2.65 for Stage (IV *vs* others), Ferritin, AEC and Plts respectively. The IBV showed that the model based on these 4 Risk Factors (RFs) was not over-fitted. A different Cox regression model for the total number of RFs in each child is shown in table I.

Tabella 1. Cox regression model for total number of RFs from our categorical model (*vs* 0 risk factors).

Number of risk factors	Number of cases	p	hazard ratio	95% CI	5 years FFP	95% CI
0	227				90.4%	85.5 – 95.6
1	135	0.00989	2.457	1.241 - 4.865	81.5%	74.0 – 89.7
2	77	2.57e-05	4.578	2.254 - 9.298	71.1%	60.2 – 84.1
3	12	7.68e-14	29.303	12.087 - 71.042	0%	
Concordance=0.719 (se =0.035)						
Rsquare = 0.099 (max possible = 0.782)						
Likelihood ratio test = 47.21 on 3 df, p=3.129e-10						
Wald test = 59.1 on 3 df, p=9.144e-13						
Score (logrank) test = 100.4 on 3 df, p=0						

Summary and Conclusions: Using the combination of 4 simple RFs it is possible to classify the patients into subgroups with very different outcomes.

Stem cell transplantation - Clinical 1

P336

EVI1 EXPRESSION ASSOCIATES WITH HIGHER CUMULATIVE INCIDENCE OF RELAPSE IN PATIENTS WITH ACUTE MYELOID LEUKEMIA RECEIVING HEMATOPOIETIC CELL TRANSPLANTATION WITH NON-MYELOABLATIVE CONDITIONING

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Background: Prognosis of acute myeloid leukemia (AML) patients (pts) is still dismal. Therefore, more personalized therapeutic strategies are required. Allogeneic hematopoietic cell transplantation (HCT) represents a therapy offering potential cure. Non-myeloablative conditioning (NMA) is increasingly used in pts that are ineligible for conventional conditioning. The ecotropic viral integration site 1 (*EVI1*) gene maps to chromosome 3q26 & encodes a transcription factor that has an important role during embryogenesis. In AML, the gene is well known as a part of a fusionprotein in pts with inv(3)(q21q26.2) or t(3;3)(q21;q26.2) that are recognized as a distinctive entity in the WHO classification.
Aims: The presence of *EVI1* expression has been described as a predictor of poor outcome. Whether the expression of *EVI1* also associates with outcome in AML pts undergoing NMA-HCT, with a therapeutic approach mainly based on an immunological graft-*versus*-leukemia effect, remains unknown.
Methods: We analyzed 136 pts (median age, 64 years [y]; range 22–75y) who received NMA (Fludarabine 30mg/m^2 at day-4 to-2 & 2Gy total body irradiation [TBI] at day 0 [n=131; 96.3%] or Fludarabine 30mg/m^2, Cytarabine 2g/m^2 & Amsakrine 100mg/m^2 at day-12 to-9, Cyclophosphamide 40mg/kg at day-4 to-3, ATG/TG day-3 to-1 & 4 Gy TBI day-5 [n=4; 2.9%])-HCT at the University of Leipzig between May 2000 & August 2012, with pretreatment bone marrow material available. Donors were human leucocyte antigen (HLA)-matched related (n=23, 16.9%) or HLA-matched (n=81; 59.6%) or mismatched (>=1 antigen; n=32; 23.5%) unrelated. 26.5% (n=36) had acute graft-*versus*-host disease (GvHD; >=grade 2) & 44.1% (n=60) (14.3% (n=15) limited; 33.1% (n=45) extensive) chronic GvHD. The mutation status of *NPM1* & *CEBPA* gene & *FLT3*-ITD status were assessed at diagnosis. Pts were grouped according to the European LeukemiaNet (ELN) classification in favorable (n=32; 23.5%), intermediate-I (n=36; 26.5%), intermediate-II (n=26; 19.1%) & adverse (n=38; 27.9%). Presence of selected surface markers was assessed using flow cytometry at diagnosis. *EVI1* expression was measured by RT-PCR & normalized to *18S*. The normalized expression of *EVI1* in the cell line SKOV3 was used to define EVI1 positive (EVI1+) expressers, *i.e.* pts with an expression higher than 0.1 relative to the *EVI1* expression of SKOV3 in the ddCT method were labeled EVI1+.
Results: Median follow-up for pts alive was 4.3y. 21.3% (n=29) pts were EVI1+. At diagnosis EVI1+ pts' blasts were more often CD7 (*P*=.001) & CD56 (*P*=.023) positive. Regarding cytogenetics, EVI1+ pts had more often a monosomal karyotype (*P*<.001) & a monosomy 7/del7 (*P*=.003), but less frequent a normal karyotype (*P*=.024). Two pts in the set had an inv3 and were both EVI1+. EVI1+ pts were less often *NPM1* mutated (*P*=.014). Furthermore, EVI1+ associated with a lower white blood cell count at diagnosis (*P*=.016). Regarding the cumulative incidence of relapse (CIR), EVI1+ pts had a significant higher CIR (*P*=.010; Figure 1B). EVI1+ associated with shorter overall survival (OS; *P*=.063; Figure1B), by trend. The median 2y-OS was 34.7% for EVI1+ pts compared to 51.8% for EVI1 negative pts. Analyzing the ELN groups, these findings could only be recovered in the adverse group by trend (*P*=.089 and *P*=.095, respectively).

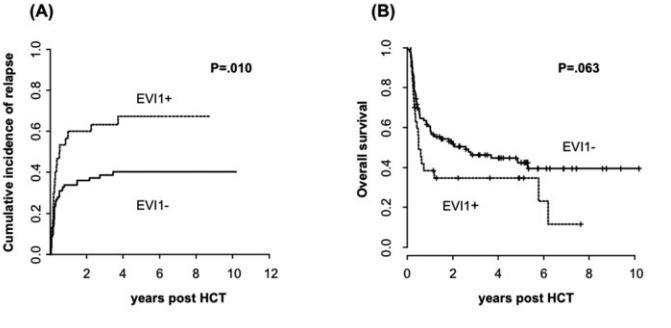

Figure 1.

Summary and Conclusions: In conclusion, the *EVI1* expression associated with worse outcome and higher CIR in NMA-HCT treated AML pts. Pretreatment *EVI1* expression may refine the risk stratification for AML pts undergoing NMA-HCT.

P337

SUPPORTIVE CARE AND INFECTIOUS COMPLICATIONS IN CORD BLOOD AND HAPLOIDENTICAL STEM CELL TRANSPLANTATION IN ADULTS WITH HIGH RISK HEMATOLOGIC DISEASES

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Background: Infections due to post transplant immune deficiency are a major problem following allogeneic stem cell transplantation, particularly in patients receiving cord blood (CBT) or haploidentical transplants (haplo-SCT).
Aims: To our knowledge, there are only some heterogeneous studies comparing this important issue in this setting.
Methods: We evaluated the incidence and type of infectious complications that occurred in these two types of transplant for 150 patients, 81 cord blood and 69 haploidentical, who received the same conditioning regimen: fludarabine (Flu), cyclophosphamide (Cy) and low dose TBI (2 Gy) combination in the two groups. The GVHD prophylaxis consisted of Cyclosporine A (CsA) and MMF in all patients in the two groups. In the Haplo group all patients received also 50 mg/kg Cy at day 3 and 4 post transplant. Of note, supportive care was the same during the whole study period. CMV infection management was also homogeneous.
Results: The median times to neutrophil and platelet recovery were 20 d (14-39) and 29 d (14-50) after Haplo and 22 d (6-67) and 41 d (18-80) after CBT. All supportive care measures included red blood cell, platelet transfusions, antibiotics days, parenteral nutrition days and hospitalization time were significantly increased in CBT group. (p=0.0001). While incidence of infections appeared similar in both types of transplant, viral infections were more frequent than bacterial or fungal infections and were the most common cause of death in both groups. During the first year after stem cell transplant, the CBT group had 154 episodes of infection (31 infection episodes per 1,000 patient-days) *versus* 125 of the haplo patients (39 per 1,000 patient days). Viral infections were most common in both groups (83 *vs* 60 episodes) in CBT *vs* haplo group respectively. Patients in the haplo group were 1.2 times (95% CI: 1.1 to 2.5) more likely to have a viral infection (p=0.07). Pneumonia was the most common clinical syndrome and was higher in the haplo group 28% *vs* 11% in CBT. Both pneumonia and bacteremia prevalence occurred within the first 100 days in the majority of CBT patients while haplo patients had a bimodal distribution with more than one third of bacteremia episodes after 6 months post-transplant. Survival analysis showed that transplant source (CBT *versus* Haplo) did not have a significant effect on the probability to have an infection episode. (p=0.10) Bacterial, fungal and CMV infections still quite frequent and contributed to higher mortality in CBT *vs* haplo (p=0.06). The first cause of mortality was the viral infection (12 (15%) 3 (4%) p=0.060) followed by the bacterial infection cause (4 (5%) *vs* 3 (4%)) and the fungal infection (1 (1%) *vs* 0) in CBT and haplo group respectively.
Summary and Conclusions: Our study is unique in comparing adult patients with advanced hematologic malignancies who received the same reduced-intensity conditioning regimen, which offers a common base for comparing the infections in these two types of transplant. In our hands, outcome is better for haplo donor decreasing the need of supportive measures and facilitating to perform the transplant in time. Haploidentical transplants are a good and promising alternative option for high risk hematologic patients who lack an HLA-matched donor. Collectively, the impact of infectious complications after haplo-SCT was different from that in CBT patients, suggesting that a more intensive strategy for infection control in haplo patients is required to reduce infectious mortality.

P338

AUGMENTED CONDITIONING WITH TARGETED MOLECULAR RADIOTHERAPY PRIOR TO AUTOLOGOUS STEM CELL TRANSPLANTATION IN MYELOMA: RESULTS OF A PHASE II RANDOMISED CONTROL TRIAL WITH IMPROVED CR RATE POST ASCT

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Background: Autologous stem cell transplantation (ASCT) remains important as consolidation therapy for patients with myeloma, improving rate and duration of response. The standard conditioning treatment with high dose melphalan has remained unchanged for 3 decades, further increase in the intensity of therapy is limited by toxicity to non-haematopoietic organs. We developed TMRT with a radiolabelled monoclonal antiCD66 that targets bone marrow, plasma cells and lymphoid tissue as a vector for delivering high dose radiation to sites of disease. We report the results of a Phase II RCT demonstrating improved sCR/CR responses using the radiolabelled mAb.
Aims: Trial Design:A randomised, multi-centre, non-blinded Phase II study comparing two treatment conditioning schedules prior to ASCT. Arm A TMRT plus high dose melphalan *vs* Arm B high dose melphalan alone. Primary objective:To determine the efficacy of TMRT delivered by an Yttrium-90 (^{90}Y)-radiolabelled murine antiCD66 monoclonal antibody given in addition to high dose melphalan (200mg/m^2) in terms of disease response in patients undergoing ASCT for multiple myeloma. Secondary objectives: Engraftment, time to disease progression, time to next treatment, overall survival, HAMA formation.
Methods: Twenty-five patients were recruited out of a planned 90. All patients had confirmed multiple myeloma, were in PR/vgPR following chemotherapy and were scheduled to receive autologous stem cell transplantation. Randomisation was stratified by disease risk (low/high). Patients randomised to receive TMRT received an infusion of indium-111 labelled antiCD66 to allow imaging and organ dosimetry calculation. Patients with favourable dosimetry received an infusion of ^{90}Y-labelled antiCD66, at an activity of 37.5MBq/kg lean body weight as outpatients 14 days prior to scheduled ASCT. Patients in both arms were admitted two days before ASCT and given high does melphalan. All patients were managed post ASCT as per centre protocols. Disease response was assessed pre-ASCT and at 3, 6, 9 and 12 months using EBMT response criteria. Logistic regression, adjusting for risk group, was used to analyse the primary endpoint (alpha 15% 1-sided).
Results: Twenty-five patients were randomised: 12 to Arm A; 13 to Arm B. Twelve patients in each arm were available for analysis. Myeloma sub-type, gender, patient's age and risk group numbers were similar in each arm. Median infused ^{90}Y-activity 2.31GBq, range 1.61-2.74GBq. Median radiation doses to organs BM 32.10Gy (17.2-34.7), liver 4.70Gy (1.7-12.0), spleen 28.55Gy (7.4-34.0), renal 2.90Gy (2.1-3.6), pulmonary 3.45Gy (1.2-4.0), whole body 0.85Gy (0.8-1.2). Toxicities were similar in each arm.

Tabella 1.

Disease status	Pre transplant		Post transplant	
	Arm A	Arm B	Arm A	Arm B
sCR/CR	0	0	6	3
vgPR	7	7	6	6
PR	5	5	0	3

All Arm A patients showed a response improvement, 3 Arm B patients had stable disease with no change (2 PR, 1 vgPR).
Summary and Conclusions: The study achieved its primary endpoint demonstrating a statistically significant improvement in the complete response rate for patients in Arm A compared to Arm B (50% *vs* 25%, odds ratio [85% 1-sided CI]: 0.277 [0.102, 0.753]). This is the first demonstration in an RCT of effective augmentation of the conditioning prior to ASCT that improves the response rate post transplant without additional toxicity. Eudract: 2006-003424-12. The study was managed by Southampton Clinical Trials Unit Sponsor: University of Southampton NHS Foundation Trust Funding: Leukaemia & Lymphoma Research;ECMC core funding.

P339

ALLOGENEIC STEM CELL TRANSPLANTATION RECIPIENTS REQUIRING INTENSIVE CARE: DELAY BETWEEN ORGAN DYSFUNCTION AND REFERRAL TO THE INTENSIVE CARE UNIT AND TIME SPENT ON ORGAN SUPPORT IMPACT SURVIVAL

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Background: Although the prognosis of patients with hematological malignancies admitted to the intensive care unit (ICU) has improved over the past years, it remains poor in allogeneic stem cell transplantation (SCT) recipients and thus admission of these patients to the ICU remains controversial.
Aims: We aimed to assess the prognostic factors of survival in ASCT recipients with a special interest on graft-*versus*-host disease (GVHD), the requirement for organ support, the timing between the occurrence of organ dysfunction and ICU referral, the time spent with each organ support, and the time since ICU admission at initiation of each organ support.
Methods: All allogeneic SCT recipients between 2002 and 2013 who were admitted to the medical ICU in our center were included. Details on patients, graft procedures, GVHD, ICU admission, and outcome were collected from individual medical records. The need for oxygen therapy, the presence of low blood-pressure (systolic arterial pressure <90 mmHg), altered mental status, elevated serum creatinine (≥1.5 times baseline or ≥0.3 mg/dL increase), and total bilirubin (>34 µmol/l) defined respiratory, hemodynamic, neurological,

renal, and liver dysfunctions, respectively. Potential prognostic factors were explored by logistic regression analysis for ICU and hospital discharge. Cox regression analysis was performed for overall survival (OS).
Results: Of 344 patients who underwent ASCT during the study period, 92 patients (27%) were admitted to the ICU. No demographic or SCT parameter was associated with ICU or hospital discharge, or with overall survival. 66% patients could be discharged from the ICU and 46% from hospital whereas one-year survival was 24%. At the time of referral to the ICU, 12 patients had 3 or 4 organ dysfunctions and only 4 of these patients (25%) could be discharged alive from the hospital *versus* 57/80 patients (71%) with 0 to 2 organ dysfunctions (OR: 12, 95% CI: 1.5-94, p=0.02). Patients who were admitted until 3 days following the first signs of organ dysfunction were more likely to be discharged alive from the hospital (41/80 patients, 51%) than those who were presenting with symptoms for more than 3 days before admission to the ICU (1/12 patients, 8%) (OR: 1.3, 95% CI: 1-1.8, p =0.03). Grade 2-4 acute GVHD (37%) had a significant impact on hospital discharge (11/34 patients alive, OR: 2.4, 95% CI: 1-5.8, p=0.05) but not with ICU discharge (20/34 patients alive, p=0.25). Mortality was greatly associated with the number of organ failures: one-year OS rates were 31%, 27%, 23%, and 0% among patients with 0 (26 patients, 28%), 1 (26 patients, 28%), 2 (26 patients, 28%), and 3 to 4 (14 patients, 15%) organ failures, respectively (HR: 1.7, 95% CI: 1.4-2.1, p<0.001). Neither time spent with invasive mechanical ventilation nor time from ICU admission to the initiation of this life-supporting intervention was associated with hospital survival. There were no survivors in patients for whom vasoactive drugs and/or renal replacement therapy were initiated after day 1. There were no survivors for those who received vasoactive drugs for more than 2 days and for those who received renal replacement therapy for more than 5 days. The Sepsis-related Organ Failure Assessment (SOFA) better distinguishes outcome of patients when performed day 2 onward. Patients who had a stable or worsening SOFA at day 2 had greater hospital mortality (OR: 2.8, 95% CI: 1.1-7, p=0.03).
Summary and Conclusions: ASCT recipients, even with GVHD, might be considered for transfer in the ICU as soon as they present first signs of organ dysfunction and before they have too many organ dysfunctions. A reappraisal should probably be performed after 48 hours of ICU.

P340

OUTCOME AND PROGNOSTIC FACTORS IN FLAMSA SEQUENTIAL CHEMOTHERAPY FOLLOWED BY RIC AND HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR HIGHER RISK AML AND MDS PATIENTS
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Background: Allogeneic hematopoietic stem cell transplantation (HSCT) is the only potentially curative approach in refractory or relapse acute myeloid leukemia (AML) and higher risk myelodysplastic syndrome (MDS). Sequential cytoreductive chemotherapy including fludarabine, aracytine and amsacrine (FLAMSA) followed by reduced intensity conditioning (RIC) and HSCT gives promising results in this unfavorable group. However, little is known concerning prognostic factor associated with this treatment approach.
Aims: This study aims to defined prognostic factors of overall survival (OS) and early relapse after FLAMSA-based conditioning in higher risk AML or MDS patients.
Methods: We retrospectively reviewed consecutive patients (pts) in our institution undergoing an SCT for high-risk myeloid malignancy after FLAMSA-RIC between March 2006 and June 2014. We evaluated transplantation related mortality (TRM) and early relapse defined before 90 days after transplantation. The uniformly used conditioning regimen was 4 days of Fludarabine (30 mg/m2/d), Amsacrine (100 mg/m2/d) and Aracytine (2 g/m2/d) followed by 4 days of Busulfan (13,6 mg/kg/day) after 3 days of rest. Antithymocyte globulin (Genzyme® 6 mg/kg), ciclosporin and mycophenolate mofetil were use for graft-*versus*-host disease (GvHD) prophylaxis.
Results: Among 73 pts with a median age of 54 years (19-69), 51 (70%) were AML and 22 (30%) pts were MDS. In AML pts, transplantation indication was refractory disease for 24 (47%), secondary AML for 19 (37%), relapse for 8 (16%). In MDS pts (including 7 RAEB-T), 18 (81,8%) were high or very high R-IPSS and 9 (41%) were previously treated with demethylating agents, 4 (18%) with intensive chemotherapy and 9 (41%) were untreated and transplanted upfront. Median leucocytosis and bone marrow blast before conditioning was 3,41 G/L (0,21-39) and 5% (0-90) respectively. Cytogenetics was adverse in 22 (30%) and intermediate in 51 (70%) pts and among them 15 had unfavourable genotype (FLT3-ITD). Sorror score before transplantation (HCT-CI) was higher or equal to 3 in 34 (47%) pts. Thirty-seven (51%) pts received HLA-identical sibling transplantation and 36 (49%) pts received transplantation from unrelated donors. Source of stem cells was mobilized peripheral blood stem cell for 64 (88%), bone marrow for 5 (7%) and cord blood for 4 (5%) pts. Eighteen (25%) pts experienced grade III-IV acute GvHD and 9 (12%) severe chronic GvHD according to NIH classification. After a median follow-up of 58,8 months, OS at 24 and 48 months was 38,4% and 27% respectively. The 2-years TRM was 31,5%. In univariate analysis, performans status >2 (p=0,041), adverse cytogenetics (according to MRC classification, p<0,001), pre transplant leucocytosis >10 G/L (p=0,015) and pre transplant bone marrow blasts (p=0,011) were associated with poorer overall survival. Neither age, HCT-CI nor number of CD34+ transfused were associated with outcome. Only intermediate cytogenetics (p=0,02) was associated with superior overall survival in multivariate analysis. Thirteen (18%) pts relapsed before the 90 days. In univariate analysis, leucocytosis >10 G/L, higher bone marrow blast and adverse cytogenetic were associated with early relapse (p=0,003; p=0,001; p=0,021 respectively). The pre transplant leucocytosis >10 G/L (p=0,042) and higher bone marrow blasts (p=0,01) remains prognostic factors of early relapse in multivariate analysis.
Summary and Conclusions: Despite median age of 54 years, high HCT-CI and high-risk diseases, FLAMSA-based HSCT was feasible with acceptable TRM. The 2-years overall survival (38,4%) is encouraging for this very high-risk population and only adverse cytogenetics remains prognostic factor for survival. Proliferative diseases before conditioning are associated with a high risk of early relapse. New therapeutic approaches, for those pts need to be tested in order to improve the HSCT outcomes.

P341

PRE-TRANSPLANT QUANTITATIVE MONITORING OF NPM1 MUTATION SIGNIFICANTLY PREDICTS OUTCOME OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN NORMAL KARYOTYPE AML IN COMPLETE REMISSION
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Background: detection and the level of minimal residual disease (MRD) affects prognosis of AML patients (pts) treated with intensive chemotherapy alone. But impact of MRD in the setting of allogeneic hematopoietic stem cell transplantation (alloHSCT) especially in pts with normal karyotype AML in complete remission (CR) is less clear.
Aims: with the aim to evaluate the potential role of pre-transplant MRD we studied impact of pre-transplant MRD level on outcome of alloHSCT in 60 consecutive pts with normal karyotype AML harboring NPM1 mutations in complete remission. MRD level was determined using quantitative real-time polymerase chain reaction (qPCR) for detection of NPM1 mutations.
Methods: from 2/2005 to 9/2014 60 consecutive pts with median of age 54 years (range, 30-66 years) with normal normal karyotype AML harboring NPM1 mutations (53% FLT3/ITD positivity) in 1st CR (45 pts) and 2nd CR (15pts) underwent myeloablative (16 pts) or reduced-intensity (44 pts) alloHSCT (27% HLA identical related, 50% HLA matched unrelated, 23% HLA mismatched unrelated). Source of stem cells was in 80% peripheral blood and in 20% bone marrow. MRD level was determined using real-time qPCR for detection of NPM1 mutations before start of conditioning regimen from bone marrow. Pre-transplant prognostic factors (age, type of donor, donor recipient sex combination, CMV status, type of conditioning regimen, source of stem cells, number of CR, FLT3/ITD status, MRD level) were included in the univariate and multivariate statistical analysis.
Results: all pts fully engrafted with ongoing CR after alloHSCT. 36 pts (60%) developed aGVHD and 24 pts (40%) developed chGVHD. With median follow-up 52 months (range, 4-101 months) 35 pts (58%) are alive. 16 pts (27%) relapsed and 13 of them died. 12 pts (20%) died due to NRM. The estimated probabilities of 3-years EFS and OS are 54% and 59%. Statistical analysis showed that only age over 63 years and MRD level affected alloHSCT outcome in multivariate analysis. Pre-transplant MRD level 10 NPM1mut/10000 ABL copies had the strongest statistical significance. The estimated probabilities of 3-years relapse incidence, EFS and OS were for pts with low MRD level (≤10 NPM1mut copies) 6%, 72% and 75% and for pts with high MRD level (>10 NPM1mut copies) 31%, 35% and 40%.
Summary and Conclusions: our data show that pre-transplant quantitative assessment of NPM1 mutation in pts with normal karyotype AML harboring NPM1 mutation in CR provides important prognostic information, which as an independent prognostic factor predicts transplant results.

P342

EARLY RELAPSE FOR FOLLICULAR LYMPHOMA (FL) AFTER AUTOLOGOUS STEM CELL TRASPLANTATION (HDT/ASCT) CARRIED OUT IN 1ST OR SUBSEQUENT COMPLETE RESPONSES (CRS) DEFINES PATIENTS AT HIGH RISK FOR DEATH
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Background: Despite gains in survival with immunochemotherapy and HDT/ASCT intensification, a considerable number of patients with FL die from the lymphoma. A strong correlation between quality of response and better progression-free survival (PFS) and overall survival (OS) in FL patients is well known. Early relapse of disease within 24 months of HDT/ASCT has been suggested as defining a group of patients with very low survival.
Aims: In this retrospective analysis, our interest is to study patients undergoing HDT/ASCT in CR at the moment of transplantation, in order to analyze survival and basal characteristics of patients relapsing early; within the first 24 months from the procedure.
Methods: FL patients undergoing HDT/ASCT in 1st or subsequent CRs from 1989 to 2007 and reported to the GELTAMO registry were studied .Two groups were defined: patients with relapse or and/or death ≤2 years from HDT/ASCT (early relapse group) and a reference group without relapse or death within 2 years of diagnosis. Survival probability was estimated by the Kaplan-Meier method. A Cox model evaluated the effect of early relapse on OS.
Results: A total of 405 patients were analyzed (mean age 47 years, female sex 53%). Two hundred and three patients received HDT/ASCT in 1st CR, 43% of them requiring more than one therapy line to achieve CR and 202 in subsequent CRs (174 in 2nd CR and 28 in 3rd CR). Median follow-up from HDT/ASCT was 11, 8 years. An early relapse was observed in 20, 5% (83/405) of patients. For patients undergoing HDT/ASCT in 1st CR, 16% (33/203) had early relapse compared to 25% (50/202) of patients who underwent ASCT in subsequent CRs (*P*=0.02). Early relapse following HDT/ASCT was dramatically and significantly related to worsened OS, both in transplanted in 1st CR (HR of 15.5; 95% CI 14.9—16.1), and in subsequent CRs (HR of 8; 95% CI 7.6—8.4), compared with those not progressing so soon. Median OS for patients with early relapse was 44 months compared to OS median not reached in the reference group ($P<10^{-5}$). OS was 39% *vs* 96% at 5 years, 28% *vs* 88% at 10 years and 21% *vs* 82, 5% at 15 years for the early relapse and the reference group, respectively. OS curves for patients transplanted in 1STCR and in subsequent CRs are shown in the figure. Multiple logistic regression analysis showed that older age (*P*=0.05), male sex (*P*=0.05), high LDH (*P*<0.1), high B_2 microglobuline (*P*<0.1) and the presence of more than 4 nodal areas affected were associated with early relapse (*P*<0.1).For patients undergoing ASCT in 1st CR male sex (p=0.05), high LDH (*P*=0.04), more than 4 nodal areas affected (P=0.01), age adjusted IPI (*P*<0.1) and FLIPI (*P*<0.1) were associated with early relpse. The poor outcome of these FL patients was independent of the use of Rituximab previously to ASCT. For patients undergoing HDT/ASCT in 2nd/3rd CR duration of first remission less than 12 months selected a subgroup of patients at high risk for early relapse (P=0.02).

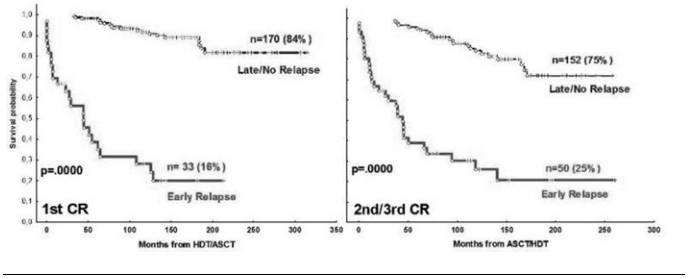

Figure 1.

Summary and Conclusions: Relapse of FL within 2 years after HDT/ASCT intensification carried out at 1st or subsequent CR is associated with really poor outcomes. Male sex, older age, high B_2 microglobuline, high LDH, the presence of more than 4 nodal areas affected and duration of first remission less than 12 months conferred a significantly high risk for early relapse. Patients undergoing HDT/ASCT at first CR relapsed early less frequently that those transplanted at subsequent CRs (P=0.02), however relapsing within 2 years of HDT/ASCT defines a unique category of patients at substantially increased risk of death, independently of having been transplanted in 1ST or in subsequent CRs. These high-risk patients need further study in directed prospective studies of FL biology and clinical trials.

P343

HEMATOPOIETIC STEM-CELL TRANSPLANTATION FOR T-CELL LARGE GRANULAR LYMPHOCYTE LEUKEMIA: A RETROSPECTIVE STUDY OF THE EUROPEAN SOCIETY FOR BLOOD AND MARROW TRANSPLANTATION (EBMT)

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Background: Large Granular Lymphocyte (LGL) leukemia is a rare lymphoproliferative disorder characterized by clonal expansion of either $CD3^+$ cytotoxic T lymphocytes or $CD3^-$NK-cells. Although usually considered as an indolent disease, most patients will require therapy based on immunosuppressive drugs such as methotrexate, cytoxan or ciclosporin. Treatment of rare aggressive forms and refractory patients remains elusive and prognosis is dramatically poor. For such patients, high-dose intensification may be proposed. However, the feasibility and efficacy of both allogeneic (allo) and autologous (auto) hematopoietic stem cell transplantation (HSCT) in LGL leukemia has not been evaluated so far and data are only limited to few sporadic case reports.
Aims: The aim of the present study was to investigate the feasibility and efficacy of allo-HSCT and auto-HSCT in T-cell LGL leukemia.
Methods: Fifteen patients treated by allo-HSCT or auto-HSCT for T-cell LGL between January 2004 and November 2011 were included in this EBMT multicenter registry-based retrospective study. Baseline information and transplantation characteristics of selected patients were downloaded. Centers were then contacted to provide written diagnostic reports in order to confirm the diagnosis of LGL and additional data. Probabilities of disease-free survival (DFS) and overall survival (OS) were calculated using the Kaplan-Meier estimate.
Results: Most patients had an advanced disease with multi-organ involvement (n=9) and were heavily pretreated with poly-chemotherapy (n=11). Median time from diagnosis to transplantation was 15 months (range: 7-211). Ten patients received an allo-HSCT at a median age of 49 years (range 29-66): at transplantation, 3 patients were in complete remission, 6 in partial response and 1 presented with refractory disease. Conditioning regimen was reduced intensity in 6 patients and peripheral blood progenitor cells were used in all but one patient. Engraftment was obtained for all patients. Grade II-IV acute GVHD was observed in 4 patients. Of the 7 patients surviving past day 100, 2 developed extensive and 1 limited chronic GVHD. The main cause of death after allo-HSCT was infection whereas relapse accounted only for a single death. Five patients were still alive after a median follow-up of 30 months (range: 19-95), translating into a 2-year DFS and OS of 50% (95%CI, 18% to 75%). Auto-HSCT was performed in 5 patients with a median age 49 years (range: 38-57): 3 of them were in complete remission and 2 in partial remission at the time of transplantation. Conditioning regimens were BEAM (carmustine, etoposide, cytarabine and melphalan) in 4 patients and TAM6 (cytarabine, melphalan, Total Body Irradiation 12 Grays) in 1 patient. Two patients relapsed at 1 and 31 months post auto-HSCT and died. One patient did not obtain a complete remission after HSCT but is still alive after a follow-up of 40 months. Two patients remain alive and disease-free 27 and 52 months post HSCT, respectively. For the entire group, 2-year DFS and OS were 43% (95%CI, 16% to 67%) and 60% (95%CI, 32% to 79%), respectively.
Summary and Conclusions: Here, we report the first cohort of patients treated by HSCT for T-cell LGL leukemia. Despite the small number of patients, HSCT appears as a potential therapeutic option in rare aggressive and relapsing forms of T-LGL leukemia.

P344

UPDATED RESULTS OF THE MAYO CLINIC RISK ADAPTED ALGORITHM FOR PERIPHERAL BLOOD STEM CELL MOBILIZATION UTILIZING G-CSF AND PLERIXAFOR

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Background: The likelihood of successful mobilization has improved with the addition of Plerixafor to G-CSF in ASCT. Due to its high cost, risk adapted algorithms were instituted to identify patients who would benefit from plerixafor.
Aims: We aimed to assess the appropriateness of our risk adapted algorithm in selecting appropriate patients to receive plerixafor.
Methods: The current algorithm was instituted in December 2009. Mobilization commences with GCSF alone; plerixafor is added if day 4 PBCD34 is <10/μL for single transplant, <20/μL for multiple transplants, day 1 yield is <1.5 million CD34/kg, or subsequent yield is <0.5 million CD34/kg. Goal of collection varied by disease and by number of intended transplants (3-5 million CD34/kg/transplant). The results of this mobilization strategy are presented here.
Results: We studied 1080 mobilizations in 1068 patients using the current protocol between June 2010 and December 2013; 548 MM (51%), 327 NHL (30%), 144 amyloidosis or light chain deposition disease (AL/LCD; 13%) and 61 Hodgkin's (6%). Overall, 55.4% of patients required plerixafor (MM 58%, NHL 61%, AL/LCD 35%, HL 46%). The majority (77%) of patients initiated plerixafor on day 4 for low PB CD34 count; 23% started beyond 4 days for suboptimal CD34 collection. There was no difference between the diagnoses in terms of the need to initiate plerixafor for low PB CD34 or for suboptimal CD34 collection. Median days of plerixafor was 2 (IQR 1-3); 42% and 22% required >2 days and >3 days, respectively. More patients with NHL (50%) needed >2 days of plerixafor *vs* MM (41%), HL (40%) and AL/LCD (23%); p =0.004. The median number of CD34 cells collected was 7.4 million/kg (IQR 5.2-9.9). Median CD34 collection was highest for MM and AL/LCD, reflecting the practice to collect for more than one transplant. Overall 9 (<1%) and 22 (2%) patients collected <2 million and <2.5 million CD34 cells/kg, respectively. More patients with NHL (4%) collected <2.5 million/kg compared with MM (1%), AL/LCD (2%) and HL (0%), p=0.02. The median number of days of apheresis was 2 (IQR 1-3) and was higher in the NHL group (p=0.002). The median CD34 collected per day was 3.6 million/ kg (IQR 2-5.8); higher in MM and AL/LCD *vs* NHL and HL (p <0.001). The median CD34 collection was very similar for those patients getting plerixafor *versus* those not requiring plerixafor (p=0.26). The median time to ANC and platelet engraftment were 14 days (IQR 13-16) and 13 days (IQR 12-15), respectively. While there was no difference in ANC engraftment, platelet engraftment was longer among patients requiring plerixafor.
Summary and Conclusions: This data confirms the effectiveness of this risk adapted algorithm in over 1000 patients over 4 years. The mobilization failure rate remains low. Approximately 45% of patients did not require plerixafor, supporting the use of this risk adapted algorithm to select appropriate patients who require plerixafor.

P345

POST-TRANSPLANT CYCLOPHOSPHAMIDE (PT-CY) IS EFFECTIVE TO PREVENT GVHD AFTER T-CELL REPLETE HAPLOIDENTICAL PERIPHERAL BLOOD STEM CELL TRANSPLANTATION (HAPLO-SCT)

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Background: PT-Cy has been developed as GVHD prophylaxis after T-cell-replete Haplo-SCT. Initially, bone marrow has been considered as the preferred stem cell source because of the known higher risk of GVHD after PBSC transplantation. However, there is no evidence that this observation is applicable after Haplo-SCT using PT-Cy. For practical reasons we early decided to use PBSC rather BM in this setting.
Aims: The objective was to evaluate the outcome of patients receiving PBSC from a related haploidentical donor, using PT-Cy as GVHD prophylaxis.
Methods: Patients receiving a Haplo-SCT between March 2012 and December 2014 from 2 centres were included with following criteria: 1) high risk haematological disease requiring Haplo-SCT in the absence of matched related and unrelated donors; 2) PBSC as graft source; 3) PT-Cy 50 mg/kg at day +4 and +3 used as part of GVHD prophylaxis. Study end points were the cumulative incidences of acute and chronic GVHD, non-relapse mortality (NRM), cumulative incidence of relapse (CIR) as well as progression free (PFS) and overall survival (OS). Additionally, we analysed the composite endpoint GVHD and relapse free survival (GRFS) for which the occurrence of relapse, death or sever chronic GVHD were considered as relevant events.
Results: We analysed 102 patients (pts) with a median age of 57 years (range: 22-73). Fifty-two (51%) and 50 pts (49%) had myeloid and lymphoid diseases, respectively. Seven pts (7%) with refractory AML received sequential RIC regimen after intensive chemotherapy. Thirty-seven pts (36%) had high or very high according to Disease risk index (DRI), while 64 pts (63%) had a HCT-CI of 3 or more. Sixteen (16%) previously relapsed after a first allogeneic transplantation. Conditioning regimens were classified as non-myeloablative (cyclophosphamide (Cy)+fludarabine (Flu)+2 Gy TBI [n=69, 68%]), reduced intensity (Cy + Flu + busulfan at 200 mg/m² total dose (Bu200) [n=4, 4%] or Bu200+Flu+ thiotepa (TT) [n=10, 10%]) and myeloablative with reduced toxicity (Bu at 400 mg/m² total dose + Flu + TT [n=12, 12%]). CSA+MMF or FK+MMF were started on day +5 for additional GVHD prophylaxis to PT-Cy in 96 (94%) and 6 (6%), respectively. All but 2 pt engrafted (2%). Median time to ANC and PLT reconstitution was 21 (14-47) and 35 (10-134) days, respectively. The median CD34+ and CD3+ infused cells were 5.1x10e6/kg (range 1.9-14.8) and 262x10e6/kg (range 59-587), respectively. Day-100 grade II-IV and III-IV acute GVHD was 31% and 8%, respectively; 2-year all grades and sever chronic GVHD was 18% and 2%, respectively. CD34+ and CD3+ cell dose did not influence the incidence of acute and chronic GVHD (median cut off). After a median follow-up of 15 months (1-31), NRM at 100 days and 2 years were 12% and 23%, respectively. Relapse occurred in a median time of 3.3 months (0.5-14) after Haplo-SCT, resulting in a 2-year CIR of 24%. PFS, OS and GRFS estimation at 2 years were 53%, 55% and 48%, respectively (Figure 1). Among the 17 AML patients who were transplanted in CR, the 2-year NRM, CIR, and OS were 27%, 15% and 58%, respectively.

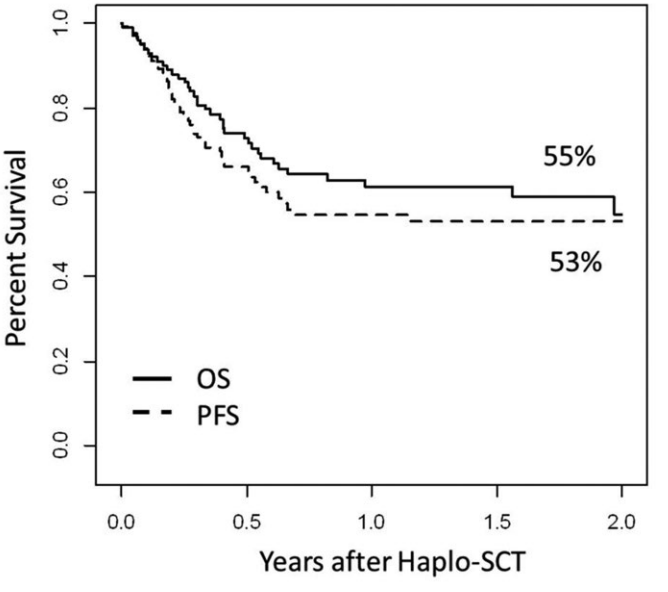

Figure 1.

Summary and Conclusions: Our results suggest the feasibility of Haplo-SCT using PBSC and PT-Cy for high risk haematological malignancies. Notably, no increased incidence of GVHD and NRM was observed comparing to bone marrow grafts. Importantly, for healthy donors, this source graft avoids risks related to the use of general anaesthetic, necessary for bone marrow harvest. With regard to the advanced pt characteristics our results are encouraging but need further evaluation with longer follow-up.

P346

COMPARABLE OUTCOMES OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION REGARDLESS OF DONOR SOURCES IN PEDIATRIC NON-MALIGNANT DISEASE

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Background: Allogeneic hematopoietic stem cell transplantation (Allo-HSCT) is a curative therapy in some non-malignant pediatric diseases including bone marrow failure, metabolic disease, immune deficiency, and so on. However, allo-HSCT is still challenging in these disease due to the risk of TRM and GVHD. Therefore, we analyzed the outcomes of allo-HSCT in pediatric non-malignant disease, and compared the outcomes according to donor types.
Aims: We performed this study to estimate transplant outcomes of pediatric non-malignant diseases, to analyze the efficacy of HSCT from alternative donors in pediatric non-malignant diseases. and to find the fators affecting transplant outcomes of pediatric non-malignant disease.
Methods: We retrospectively reviewed the medical records of 55 pediatric patients with non-malignant disease who received allo-HSCT at Asan Medical Center Children's Hospital from January 2009 to November 2014. Thirty-six patients had SAA, 7 had inherited bone marrow failure syndrome (1 Fanconi anemia, 2 CAMT, 1 CDA, 1 Pearse syndrome, 1 PRCA, 1 Kostmann syndrome),

6 had primary immune deficiency (1 IPEX, 2 WAS, 3 CGD), 5 had HLH, and 1 had Krabbe disease. To analyze the efficacy of HSCT, we investigated overall survival (OS), event-free survival (EFS), incidence of TRM, cumulative incidence (CI) of acute and chronic GVHD, incidence of graft failure (GF).
Results: Of the 55 patients, 40 were male and 15 were female. Median age at HSCT was 12.9 years (range, 0.7-21.7). Seventeen patients received HSCT from MSD (MSD-HSCT), 16 patients from URD (URD-HSCT) and 22 patients from haploidentical family donor (HFD-HSCT). Six patients (11%) experienced graft failure (GF). Of the 6 patients, 5 patients achieved engraftment after additional HSCT, and the remaining 1 survived with autologous recovery. Four patients died of TRM, and one died of disease. TRM at 1 year and 2 years for all patients were 4% and 6%, respectively. TRMs at 2 years were not different according to the donor type (0% for MSD-HSCT, 14% for URD-HSCT, 5% for HFD-HSCT; *P*=0.06). The CIs of aGVHD grade I-IV and cGVHD were 40% and 20%. However, CIs of severe aGVHD grade≥III and extensive cGVHD were just 15% and 9%. There was no differences in incidences of acute GVHD and chronic GVHD according to the donor type. At a median follow-up of 34.7 months (range, 1.6-69.3), the 3-year OS were not different according to the donor types (93% for MSD-HSCT, 87% for URD-HSCT, 95% for HFD-HSCT; *P*>0.05).
Summary and Conclusions: Given the high survival outcomes and low TRM, our study suggests that allogeneic HSCT could be safe treatment modality to the children with non-malignant disease. Especially, HSCT from alternative donor could be beneficial to patients who lack MSD. However, a larger multicenter study is needed to verify our results.

Stem cell transplantation - Clinical 3

P347

NO NEGATIVE IMPACT OF AGE ON OUTCOME OF REDUCED-INTENSITY ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR ELDERLY PATIENTS WITH ACUTE MYELOID

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Background: In recent year, reduced intensity conditioning (RIC) is developed and increasingly used in allogeneic hematopoietic stem cell transplantation (allo-HSCT) for elderly acute myeloid leukemia (AML) patients. Increasing age is a repeatedly found significant risk factor for worse prognosis after allo-HSCT among AML patients. However recent studies showed conflict results regarding the association between age and outcome in the setting of RIC.
Aims: We conducted a large-scaled, nationwide retrospective study to examine the impact of age on RIC transplantation outcomes for elderly AML patients and other prognostic factors.
Methods: Clinical data were collected from the registry database of the Japan Society for Hematopoietic Cell Transplantation. Patients with AML (excluding acute promyelocytic leukemia) aged 50 years or older who underwent RIC allo-HSCT from related or unrelated donor between January 2008 and December 2012 were extracted from the database. Patients who underwent second or more HSCT were excluded. We retrospectively analyzed the clinical impact of patient age on outcome of allo-HSCT. Patients were divided into the following four group for analysis: 50 to 54, 55 to 59, 60 to 64 and ≥65 years old. Outcomes after allo-HSCT of four age groups were compared. Overall survival (OS) was estimated by the Kaplan-Meier method and was compared using a log-rank test. Relapse and nonrelapse mortality (NRM) were considered competing risk events for each other and were compared using Gray's test. In a multivariate analysis, the Cox proportional hazard model and Fine-Gray methods were used for OS and cumulative incidence of relapse and NRM respectively, using the following variables: gender, donor source, disease status at allo-HSCT, graft *versus* host disease (GVHD) prophylaxis, donor-recipient gender mismatch, ABO mismatch, cytogenetic risk category and hematopoietic cell transplant comorbidity index (HCT-CI).
Results: Of the 757 patients, 89 patients (11.8%) were 50-54 years old, 249 patients (32.9%) were 55-59 years old, 301 patients (39.8%) were 60-64 years old and 118 patients (15.6%) were 65 years old or older. Higher proportion of patients aged 60-64 years received graft from ABO matched donor, although difference was not significant (P=0.06). There was no difference in other factors, including gender, disease status, cytogenetic risk category, HCT-CI, donor source, gender mismatch and GVHD prophylaxis. The 3-year OS (47.8%, 45.2%, 37.9% and 36.6% for patients aged 50-54, 55-59, 60-64 and ≥65, respectively, P=0.24) and NRM (24.0%, 22.8%, 29.2% and 27.6% for patients aged 50-54, 55-59, 60-64 and ≥65, respectively, P=0.49) were not significantly different among 4 age groups. In multivariate analysis, age had no significant effect on both OS and NRM after adjusting for covariates. On the other hand, a multivariate analysis showed mismatched related donor (HR 1.80; 95%CI 1.13-2.87; P=0.01) and HCT-CI ≥3 (HR 1.51; 95%CI 1.04-2.17; P=0.03) were associated with higher NRM; non-CR at allo-HSCT (HR 2.51 95%CI 2.01-3.14; P<0.001), mismatched related donor (HR 1.44; 95%CI 1.04-1.97; P=0.03) and HCT-CI ≥3 (HR 1.46; 95%CI 1.14-1.87; P=0.003) were risk factors for worse OS.
Summary and Conclusions: The current study showed increased age had no significant adverse impact on RIC allo-HSCT outcome and pre-transplantation comorbidity has more impact than age in older AML patients.

P348

THE IMPACT OF ANTITHYMOCYTE GLOBULIN ON THE OUTCOME OF UNRELATED CORD BLOOD TRANSPLANTATION FOR CHILDREN WITH HIGH-RISK OR ADVANCED HEMATOLOGICAL MALIGNANCIES

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Background: Acute graft-*versus*-host disease (GVHD) is the most important cause of death after allogeneic hematopoietic stem cell transplantation (allo-HSCT), and antithymocyte globulin (ATG) has been used in the conditioning regimens to prevent the severe GVHD in this setting. But the role and potential efficacy of ATGin patients receiving cord blood transplantation (CBT) remains controversial.
Aims: In the present study, we retrospectively compared the outcomes of routine GVHD prophylaxis (cyclosporine-based regimens) plus pre-transplantation ATG (ATG group) to routine GVHD prophylaxis alone (non-ATG group) in children with high-risk or advanced hematological malignancies receiving unrelated CBT.
Methods: The study included patients younger than 18 years at transplantation with hematological malignancies who received unrelated CBT between February 2000 and August 2013 at 8 child blood disease centers in China. Total of 207 patients enrolled in the study, and patients were divided into 2 groups: those who received ATG (n=98) and those who received no ATG in the conditioning regimens (n=109).
Results: The cumulative incidence of platelet recovery on day 100 was significantly lower in the ATG cohort as compared with the non-ATG cohort (77.3% *vs* 89.8%) (p=0.046). There was no significantly difference in the incidence of grade II–IV acute and chronic graft-*versus*-host disease (GVHD), and transplant-related mortality (TRM) between the two groups (p =0.76, 0.57, 0.46). The incidence of CMV infection was significantly higher among the ATG group patients compared with that among the non-ATG group patients (p=0.003). The 5-year cumulative incidence of relapse was significantly higher in the ATG cohort (30.7% *vs* 15.4%) (p=0.009). The overall survival (OS) in the non-ATG group was slightly higher than the ATG cohort (64.1% *vs* 52.1%, p=0.093), and the leukemia-free survival (LFS) in the non-ATG cohort was significantly higher than the ATG cohort (56.6% *vs* 37.7%, p=0.015).
Summary and Conclusions: Our study demonstrated that, for high-risk or advanced childhood hematological malignancies receiving unrelated CBT, the omission of ATG in the conditioning had a faster platelet recovery, a comparable GVHD and TRM, a significantly lower relapse risk, and an improved long-term survival compared to the inclusion of ATG in the conditioning.

P349

TCR REPERTOIRE DIVERSITY ASSESSED WITH IMMUNOSEQUENCING IS ASSOCIATED WITH PATIENT MORTALITY FOLLOWING CORD BLOOD TRANSPLANT

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Background: Immunosequencing represents a promising, fast and relatively inexpensive method to assess the reconstitution of effective cellular immunity following stem cell transplantation and has the potential to identify patients at higher risk of developing life-threatening complications and treatment-related mortality.
Aims: Diversity of the T-cell repertoire can provide a measure of immune competence. We hypothesized that restoration of TCR repertoire diversity would be associated with overall survival after UCBT.
Methods: In order to study the clinical impact of T-cell receptor (TCR) diversity in the setting of umbilical cord blood transplantation (UCBT), we retrospectively analyzed samples from 76 patients in 2 independent study cohorts at separate institutions. At Fred Hutchinson Cancer Research Center (FHCRC), we followed 34 patients with hematological malignancies who underwent myeloablation and primarily double UCBT (2 single cord). This cohort was composed of 11 pediatric and 23 adult patients (median age, 26.5yrs), primarily with acute leukemia (n=26), 50% of whom had evidence of MRD at UCBT. They received fludarabine, cytoxan or treosulfan, and total body irradiation (TBI) with cyclosporine and mycophenylate mofetil as GvHD prophylaxis. At Dana-Farber Cancer Institute (DFCI), we followed 42 adult patients (median age, 52.3 yrs), 19 with acute leukemia, 18 of whom had evidence of disease at UCBT. All patients received-reduced-intensity conditioning with fludarabine, melphalan, and anti-thymocyte globulin (ATG) followed by double UCBT with tacrolimus and sirolimus as GvHD prophylaxis. DFCI participants were selected to have predominantly cord T cell chimerism. We analyzed peripheral blood pre-transplant, and at 1, 2, 3, 6 and 12 months after UCBT, based on sample availability. We performed high-throughput sequencing of rearranged (TCR) loci to track presence and frequency of individual T cell clones in each patient across time-points, as well as to calculate estimated diversity of the TCR repertoire as a whole. We correlated our measure of TCR repertoire diversity with clinical outcome, using one-year survival as our primary endpoint.
Results: For the combined group of 63 patients with samples available 3 months after UCBT, 19 subsequently died within 1 year of transplant. Patient with treatment-related mortality had significantly lower TCR repertoire diversity at 3 months than other patients; when analyzed as 2 independent cohorts, the 29 patients at FHCRC with samples at 3 months after UCBT demonstrated significantly lower TCR repertoire diversity in TRM patients (p=0.04), as did the 34 patients with samples available 3 months after transplant at DFCI (p=0.02). In a univariate survival analysis using both cohorts, low TCR diversity at 3 months post-transplant was significantly associated with treatment-related mortality (TRM; p=0.0006 by logrank test; see Figure). This result remained significant at p=0.006 in a multivariate analysis including several other clinical variables (patient age and CR2+ at transplant were associated with TRM in the multivariate analysis; %CD3+ at 3 months and GVHD were not).

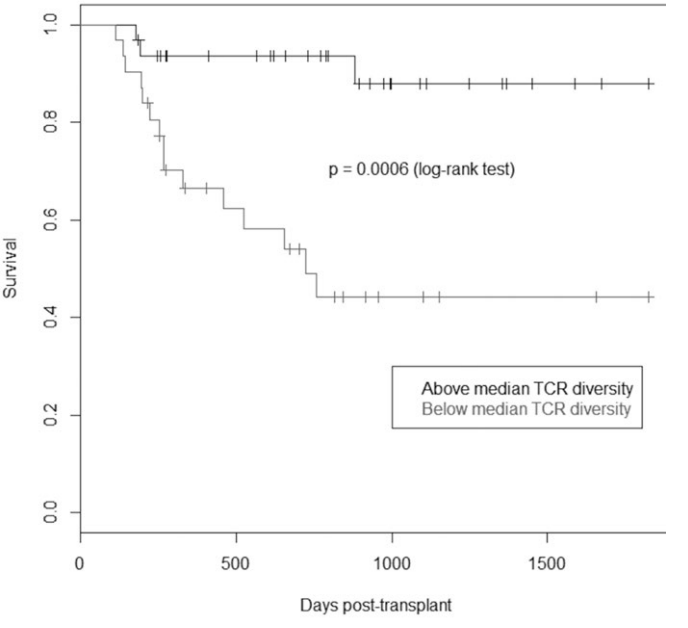

Figure 1.

Summary and Conclusions: In this study, we demonstrate that measurement of TCR repertoire diversity generated using high-throughput sequencing genes at 3 months after UCBT is significantly correlated with subsequent TRM mortality within the first year. Low T cell diversity in the peripheral blood as soon as 3 months may therefore be an early indicator of inadequate immune reconstitution and could potentially be used to tailor monitoring and therapy after UCBT in several clinical contexts.

P350

NEUROREGENERATIVE POTENTIAL OF INTRAVENOUS INFUSION OF G-CSF FOLLOWED BY MOBILIZED PERIPHERAL BLOOD MONONUCLEAR CELLS IN CHILDREN WITH CEREBRAL PALSY

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Background: Up to now most of the cells used in cell therapeutics have been mesenchymal stem cells (MSCs). However, mononuclear cells from cord blood or granulocyte-colony stimulating factor (G-CSF)-mobilized peripheral blood mononuclear cells (mPBMCs) may be an alternative option. In addition, cellular components other than purified HSCs and MSCs could play important roles in tissue regeneration.
Aims: We performed a randomized, double-blind, cross-over study to assess the neuroregenerative potential of intravenous infusion of G-CSF followed by mPBMCs in children with cerebral palsy (CP).
Methods: Children with the non-severe type of CP were enrolled in this study. G-CSF was administered for 5 days, when mPBMCs were collected by apheresis and cryopreserved. One month later (M1), recipients were randomized to mPBMCs or placebo infusion, and switched at 7 months (M7). We assessed the efficacy of treatment over 13 months after the G-CSF injection using various tools for evaluating motor and cognitive function, as well as brain magnetic resonance imaging-diffusion tensor imaging (MRI-DTI) and brain positron emission tomography (PET).
Results: Fifty-seven patients aged 4.3±1.9 (2~10) years and weighing16.6±4.9 (11.6~56.0) kg were enrolled; data for the mPBMCs collected from them were total nucleated cell counts 5.97±1.99×10^{8}/kg, CD34+ cells

3.07±2.1×10^6/kg. Forty-seven patients for whom serial data were available were included in this analysis. After randomization, functional changes noted by parents and neurodevelopmental tests showed tendencies to improve with no significant differences between the mPBMC group and the placebo group. Brain MRI-DTI revealed that fractional anisotropy (FA) in the splenium of mPBMC group (p=0.048) and the right corona radiata of placebo group (p=0.045) comparatively increased to similar extents, although increasing tendencies in FA values in most regions of interest after infusion of mPBMCs or placebo were noted without statistical significance. Additionally, in a subgroup analysis, in the diplegia children, FA values in the mPBMC group were significantly higher than in the placebo group in the left midbrain (p=0.044) and right temporal lobe (p=0.05), and lower in the right midbrain (p=0.022). In the brain PET analysis, we noted increased metabolism in 9 patients (19.1%) in the mPBMC group and in 8 patients (17.0%) in the placebo group, though the increases were not statistically significant. Interestingly, the parents noted functional improvements between the time of G-CSF infusion (M0) and the time (M1) when the children were randomized to the two groups in 27 (57.4%) of the total of 47 patients. Furthermore, there were significant differences in score changes of neurodevelopmental tests between a span of M0-M1 and M1-M7 or M7-M13 in both groups.

Summary and Conclusions: Although we did not detect any additional effect of mPBMC reinfusion, functional and anatomical changes were noted in the brains of CP children after receiving G-CSF followed by mPBMCs. Interestingly, we observed significant differences in score changes of neurodevelopmental tests between a span of M0-M1 and M1-M7 or M7-M13, which suggests that G-CSF administration on its own, without mPBMC collection and reinfusion, may be beneficial in countering neurological impairment in children with CP.

P351

PRE-ENGRAFTMENT SYNDROME AFTER UNRELATED CORD BLOOD TRANSPLANTATION: THE CLINICAL CHARACTERISTICS AND STRATIFIED INTERVENTION TREATMENT

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Background: Pre-engraftment syndrome (PES), a clinical entity of unknown pathogenesis, has been described in patients receiving umbilical cord blood transplantation(CBT). Although a uniform definition is lacking, PES is commonly defined as noninfectious fever and various other clinical findings before neutrophil engraftment, including skin rash, pulmonary infiltrates, and diarrhea, jaundice, or weight gain. However, PES remains poorly characterized, the prognosis and appropriate management of PES are also unclear.

Aims: The aim of this study was to investigate the incidence, risk factors, clinical outcomes of PES in CBT recipients and establish a prognosis assessment score system to guide the stratified intervention treatment.

Methods: Between June 2006 and April 2014, a total of 204 consecutive hematological malignancies patients underwent UCBT in Anhui Provincial Hospital were analyzed. All the patients received TBI-based(n=119) or $BuCY_2$ based(n=85) myeloablative conditioning. Cyclosporine and mycophenolate mofetil were used as GVHD prophylaxis. A retrospective analysis of 94 patients between June 2006 and March 2012 was first done to find out the poor prognostic risk factors of PES. Then we made a PES score system according to the high risk factors, and between April 2012 and April 2014, 88 patients with PES in 110 patients were given stratified intervention treatment.

Results: In all 204 patients, PES developed in 154 patients (75.5%) at a median of 7 days after UCBT. Risk factors for developing PES included TBI-based myeloablative conditioning and double units CBT. The age, sex, weight, underlying malignancy, BM status at CBT, HLA match, ABO compatibility, total infused TNC dose and total $CD34^+$ cell dose had no impact on PES incidence. Of the 94 patients in the period of June 2006 and March 2012, the cumulative incidence of grade II to grade IV acute GVHD by 100 days after CBT was higher in patients with PES than in those without PES (52.5% *versus* 20.3%, P <0.01). The cumulative incidence of neutrophil engraftment at 42d after CBT was also higher in patients with PES than in those without PES (97.5% *versus* 78.0%, P <0.05). PES occurrence did not impact chronic GVHD, treatment-related mortality, relapse, overall survival(OS) or disease-free survival(DFS). But we found that PES occurred in 7 days (<7days), over three or more clinical symptoms, poor effectiveness of MP (ineffectiveness within in one week) were the poor prognostic risk factors of PES. So we made a PES score system according to 3 high risk factors, the existence of one risk factor was defined as 1 point, and no risk factors with 0 score. We found that the higher the PES score, the lower the OS(P<0.001). Then between April 2012 and April 2014, 88 patients with PES in all 110 patients were given stratified intervention treatment, patients with 1 high risk factor were treated with MP 1 mg/kg/d, and patients with 2 or more high risk factors were treated with MP 2 mg/kg/d. If MP was invalid or the disease progressed, anti-CD25 antibody was timely used. After the stratified treatment according to the PES score system, the prognosis of severe PES(score 2+3) was significant improved by 1-year OS between the two different periods (June 2006 to March 2012 and April 2012 to April 2014) (26.4% *versus* 65.1%) (*P<0.05*).

Summary and Conclusions: PES seems to be common after CBT and may be associated with enhanced engraftment and aGVHD incidence. We have established a prognosis assessment score system according to poor prognostic risk factors of PES and for the first time put forward a new insight for PES stratified intervention treatment. Severe PES patients could be benefit from active treatment.

P352

OUTCOME AFTER RELAPSE OR PROGRESSION FOLLOWING FIRST ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN CHILDREN WITH ACUTE LEUKEMIA. A RETROSPECTIVE ANALYSIS FROM THE SFGM-TC

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Background: Hematopoietic stem cell transplantation (HSCT) has contributed to improved outcome in childhood acute leukemia (AL). However, post-HSCT relapse is associated with a dismal prognosis and its optimal treatment remains unclear.

Aims: We aimed to compare patients' related factors and treatment strategy, in case of relapse or progression postallogeneic HSCT in children with AL in a recent ten-year period.

Methods: A total of 334 children who received a first allogeneic HSCT for ALL or AML from January 2000 to December 2009 experienced a relapse or progression thereafter. They were treated in the 33 centers of the SFGM-TC, among them 286 cases were analysable. Primary endpoint was overall survival (OS) after diagnosis of relapse or progression post first HSCT whatever the treatment post relapse was.

Results: 286 patients (113 females and 173 males) with a median age of 8.9 years at transplantation (range: 0.52-17.99) were included. Diseases were 151 ALL (B-ALL, n=125; T-ALL n=29), 123 AML. At transplantation, 234 patients were in complete remission (CR) (CR1 n=122, CR2 n=94, CR3 n=18) . Donors were matched related siblings (36%), mismatched related (4%), matched unrelated (18%), mismatched unrelated (24%), unrelated without precision (16%) or syngenic (2%). Stem cell source was bone marrow, peripheral stem cells, umbilical cord blood in 65%, 12%, 23% of cases, respectively. Median time from diagnosis to first HSCT was 263 days. 91% children received myeloablative conditioning and 55% received TBI. Acute GVHD after first HSCT occurred in 157 patients (grade I n=62 grade II to IV n=94). Median delay from first HSCT to failure was 182 days. Treatments for relapse after first HSCT consisted in chemotherapy alone (n=107), chemotherapy followed by second HSCT (n=70), supportive and palliative care (n=68), combination of chemotherapy and DLI (n=28), or DLI only (n=13). With a median follow up of 1315 days (range 58;4182), median OS duration after relapse was 149 days. Median survival duration in days according to therapy was as follows [confidence interval]: DLI after chemotherapy =407 [156;658], Second allograft: 391 [264;518], chemotherapy: 174[118;239], DLI alone: 140 [10;270], Palliative care: 43 [33;53] . Cox model analysis showed the following hazard ratio, when comparing to the best line of treatment, ie chemotherapy+DLI; second allograft HR 1.41, (p=0.20), chemotherapy alone; HR 2.05, (p=.008), DLI HR 2.00 (p=0.08), palliative care HR=5,7, (p<.001). The time interval from transplantation to relapse was also associated with prognosis: when comparing to relapses occuring within the first 90 days, day 90-179, day 180-269,and ≥270 days yielded hazard ratios of respectively .549, .479 and .293 (p ≤.003). There was no impact on outcome from the following variables: gender, age at transplant, leukemia subtype,type of conditioning, TBI, GVHD occurrence after thefirst transplant,stem cell source.

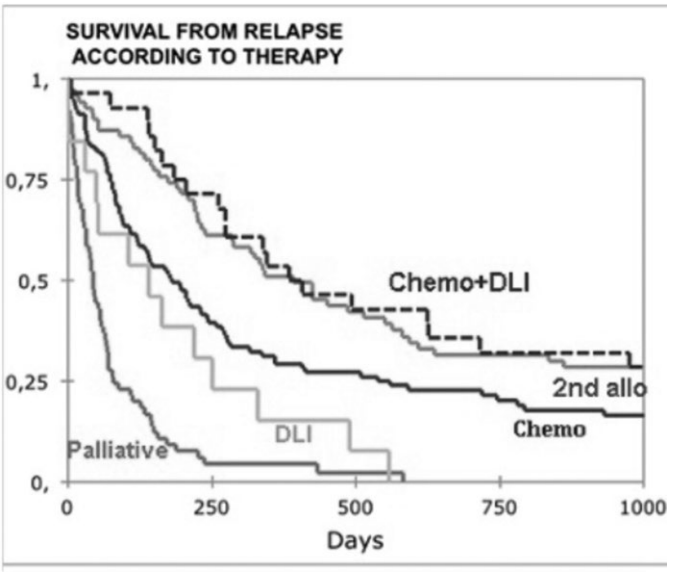

Figure 1.

Summary and Conclusions: The therapeutic choice after post transplant relapse is a sensitive decision influenced by several factors. However, this study highlights the positive role of immunotherapy: the longest survival was achieved either through a second HSCT or through a combination of chemotherapy and DLI, both yielding similar long-term results.

P353

BENDA-BEAM HIGH-DOSE THERAPY PRIOR TO AUTO-SCT IS EFFECTIVE IN RESISTANT/RELAPSED DLBCL: A PHASE II MULTICENTER STUDY

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Background: The major pitfall affecting clinical trials of high-dose therapy (HDT) followed by autologous stem cell transplant (ASCT) in lymphomas is the high heterogeneity of histological entities. As a consequence, the statistical power is reduced when we focus on a specific histological subset, and data are often not conclusive. We previously demonstrated (Visani et al, Blood 2011) the safety of a new conditioning regimen with bendamustine, etoposide, cytarabine, and melphalan (BeEAM) prior to ASCT in resistant/relapsed lymphoma patients (EUDRACTnumber2008-002736-15). The regimen showed long-lasting significant anti-lymphoma activity, with a 3-year PFS of 75%. However, that study enrolled both Hodgkin and non-Hodgkin lymphoma patients.
Aims: We designed a phase II study to evaluate the efficacy of the BeEAM conditioning in resistant/relapsed diffuse large B-cell non-Hodgkin lymphoma (DLBCL) patients.
Methods: The study was registered at European Union Drug Regulating Authorities Clinical Trials (EudraCT) N. 2011-001246-14. Until now, 57 patients (median age 54 years, range 19-69) were enrolled. At the time of writing,44 patients with resistant/relapsed diffuse large B cell non-Hodgkin lymphoma are evaluable. The primary end-point of the study is to evaluate the 1-year complete remission rate. Fixing the lowest acceptable rate as 55% and the successful rate as 70%, with a significance level alpha=0.05 and a power 1-beta =0.90, the sample size was estimated in 88 patients.
Results: Briefly, 33/44 patients had advanced stage disease (III-IV), 14 were primary refractory and 30 had relapsed after a median number of 2 lines of therapy (range: 2-3). Eleven patients had 1 or more relevant comorbidities (range: 1-5). 22 patients were in II or subsequent CR after salvage therapy, whereas 19 were in PR and 3 had progressive disease. A median number of 5.90x10^6CD34+/kg cells (range 2,8-9,42) collected from peripheral blood was reinfused to patients. All patients engrafted, with a median time to ANC>0.5x109/l of 10 days. Median times to achieve a platelet count >20x10^9/l and >50x10^9/l were 12 and 16 days respectively. Ten out of 44 patients presented a fever of unknown origin (23%), whereas 21 patients (47%) presented a clinically documented infection. All patients received G-CSF after transplant for a median time of 8 days (range: 8-13). One patient died due to an incomplete hematological recovery after transplant, producing an overall transplant related mortality of 2.7%. Thirty-eight out of 44 patients are evaluable up to now for response to treatment. 31/38 (81.5%) obtained a CR, 3/38 a PR, whereas 4/38 did not respond to therapy. After a median follow-up of 12 months after transplant (range 2-30), 4/38 patients were refractory, 7/38 relapsed, and 27/38 (71%) are still alive, in continuous CR.
Summary and Conclusions: The stringent inclusion criteria at enrollment allowed theevaluation ofthe impact of HDT with Bendamustine followed by ASCT in a highly selected population of patients with DLBCL only. Accordingly, our data preliminary provide the evidence that the Benda-BEAM regimen is safe and has promising high efficacy in resistant-relapsed aggressive diffuse large B cell lymphoma patients.
Acknowledgements: The study was supported in part by AIL Pesaro Onlus. Mundipharma Italy is grateful acknowledged for providing Bendamustine free of charge.

P354

TREATMENT OF CHRONIC GRAFT VERSUS HOST DISEASE WITH A COMBINATION OF B-CELL DEPLETION AND TYROSINE KINASE INHIBITION, PRELIMINARY RESULTS

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Background: Chronic Graft *Versus* Host Disease (cGVHD) affects about 60% of all patients receiving an allogeneic HSCT and surviving beyond day 100. Incidence of cGVHD is rising because of the widespread use of Peripheral Blood Stem Cells (PBSC) as stemcell source. Patients with cGVHD have high morbidity and mortality rates. Patients require long term use of immunosuppressive drugs, mainly corticosteroids, which lead to development of severe side effects. Therefore new therapies are urgently needed.
Aims: We tested whether the sequential therapy of the anti CD20 antibody rituximab followed by a 6 month treatment period with the tyrosine kinase inhibitor nilotinib is a good treatment strategy for patients with sclerotic cGVHD.
Methods: We treated 19 patients with a combination of 4 weekly infusions of rituximab followed by a 6 month period of treatment with nilotinib (300mg b.i.d.). Patients were evaluated monthly for 13 months and sequential blood samples and skin biopsies were analyzed. All patients gave informed consent before enrollment.
Results: 3 patients experienced severe side effects from either rituximab or nilotinib treatment and therefore were taken off study. Of the remaining 11 patients who thus far have completed the study protocol, 65% showed a response (4 patients went from NIH scoring 'severe' to 'moderate', 1 patient went from NIH scoring 'moderate' to 'no cGVHD'). There is also a significant decrease in cGVHD affected body surface area (Figure 1). Moreover, two out of four patients who suffered from severe ulcerations at the start of the study had a complete resolution of ulcers at the end of the treatment period. Patients with a (partial) response also showed a decrease in self attributed severity of cGVHD and their immunosuppressive drugs could be tapered.

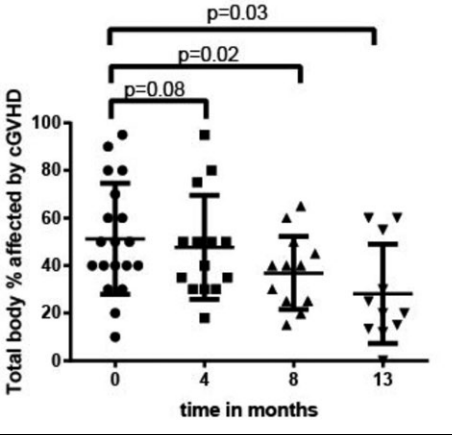

Figure 1. Percentage of total body surface area affected by chronic Graft versus Host Disease is plotted against time in months after start of the study. Each dot represents 1 patient. P-values are calculated by means of Wilcoxon matched-pairs signed rank test.

Summary and Conclusions: The sequential therapy of B-cell depletion and tyrosine kinase inhibition provides a new and interesting alternative treatment option for this difficult and heavily pretreated patient category.

P355

LOW-DOSE DECITABINE COMBINED WITH MODIFIED BUCY AS CONDITIONING REGIMENT FOLLOW BY ALLOGENEIC STEM CELL TRANSPLANTATION FOR THE TREATMENT OF ADVANCED AML/MDS PATIENTS

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Background: Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is the only curative treatment options to hematologic malignancies. However, majority of patients with refractory or resistant hematologic malignancies can not achieve remission before transplantation. It is necessary to design a safe and affective conditioning regimento reduce the tumor burden, improve theremission rate, decrease thetransplantation related mortality andimprove disease-free survivalin patients with advanced acute myloid leukemia(AML) and myelodysplastic syndrom(MDS). One of the promising drugs of epigenetics is decitabine (DAC), which has a significant effect on a variety of hematologic malignancies including MDS and advanced AML. Furthermore, decitabine can not only up-modulate the tumor-associated antigen express on surface of leukemia cells to increase graft-*versus*-leukemia (GVL) effect but also can reduce the incidence of graft-*versus*-host disease (GVHD) by increase the number of regulatory T Cells (Tregs).
Aims: Therefore, this clinical study will investigate the security and efficacy of conditioning regimen containing low-dose decitabine combined with modified BUCY regimen for advanced AML/MDS patients and explore the role of immunomodulatory activity post transplantation.
Methods: 20 cases of patients with advanced AML/MDS underwent allo-HSCT with low-dose decitabine (integral dose100mg/m^2) combined with modified BUCY conditioning regimens.
Results: 19/20 (95%) patients achieved complete remission and hematopoietic reconstitution after transplantation.The median time of neutrophil and platelet recovery were 12 (10-22) and 14.5 (12-35) days respectively. The transplantation-related mortality (TRM) rate was 0.The cumulative rate of aGVHD and cGVHD were 25.6% and 48.3%.And the cumulative rate of aGVHD for grade III and IV were 10.6%. The cumulative relapse rate was 26.7%. During a median follow-up of 246 (19-613) days,15 patients weredisease free survival.The estimated 2-year overall survival (2yr-OS) rate was 78%.The 2 year disease-free survival (2yr-DFS) rate was 62.6%. Furthermore, Therewasnosignificantdifferenceof the estimated 2-yr OS and DFS between patients with or without DNMT3A mutations or abnormalities of chromosome 7 and complex chromosomal karyotype.
Summary and Conclusions: 1.It is feasible to use conditioning regimen containing low-dose decitabine combined with modified BUCY regimen before allo-HSCT.The treatment were well tolerated and transplantation-related mortality (TRM) rate was 0. 2..The incidence of aGVHD and cGVHD were not increased in this setting. 3. 92.3% patientsachieved complete remission with salvage allo-HSCT with ralative high 2-yr rate of OS and DFS. 4.Theprognosisand survival of the patients with complex chromosomal karyotype or chromosome 7 abnormalities or DNMT3A mutation may be improved by treating with decitabine containing conditioning regimen.

P356

STUDY OF PERIPHERAL STEM CELLS MOBILIZATION AS A TREATMENT LINE FOR PEDIATRIC IDIOPATHIC DILATED CARDIOMYOPATHY

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Background: Mobilizing hematopoietic stem cells may be a promising intervention to the treatment of idiopathic dilated cardiomyopathy (IDCM) in infant and children.
Aims: Evaluating the efficacy of granulocyte-colony stimulating factor (G-CSF) as a therapeutic modality in pediatric idiopathic dilated cardiomyopathy (IDCM).
Methods: This randomized clinical trial was conducted on 15 pediatric patients with IDCM admitted at Cardiology Unit, Pediatric Department, Tanta University Hospital. They were subjected to history taking, clinical examination, total CPK, CK-MB isoenzyme, and peripheral blood CD34+ cell assessment before and at day 6 after subcutaneous G-CSF injection for 5 consecutive days. Echocardiography was done before and one month after therapy.
Results: Clinical improvement in the form of regression of patients ROSS heart failure classes. Increased percentage of CD34+ mobilized cells from the bone marrow, and significant increase in blood counts especially white blood cells 6 days after G-CSF injection. Significant improvement in echocardiographic data evaluating systolic function of the heart (Ejection fraction, Fractional shortening and systolic velocity at mitral annulus. But, with no significant correlation between increased percentage of CD34+ cells and improvement in echocardiographic parameters.

Tabella 1. Modified Ross H.F Classes before and after one month from receving G-CSF therapy.

Modified Ross H F classification	Before		After	
	No.	%	No.	%
Class I	0	0.0 %	3	20 %
Class II	**4**	**26.7%**	**5**	**33.3%**
Class III	8	53.3 %	4	26.7%
Class IV	3	20 %	2	13.3%

Tabella 2. Systolic functions of the heart among studied group before & one month after receving G-CSF therapy.

Echo finding	Before		After		P-value
	Mean	SD	Mean	SD	
FS %	16.9	±4.1	**21.88**	±5.6	**<0.001****
EF %	39.8	±6.1	**49.1**	±8	**<0.001****
S-wave (mm/sec)	0.08	±0.05	0.16	±0.1	0.05*

*: Significance, **: High significance

Summary and Conclusions: Administration of G-CSF may be beneficial in improving systolic functions of the heart in pediatric idiopathic dilated cardiomyopathy.

Hematopoiesis, stem cells and microenvironment

P357

IKAROS CONTRIBUTES TO B CELL TOLERANCE BY MODULATING MAPK ACTIVITY

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Background: B cell differentiation is a multiple process that involves the dynamic activation and silencing of hundreds of genes. The Ikaros transcription factor is a key regulator of B cell differentiation and function. The IKZF1 gene, which encodes the Ikaros transcription factor, was recently identified as a susceptibility locus for systemic lupus erythematosus (SLE), and leukocytes from SLE patients have reduced IKZF1 mRNA levels, suggesting a possible link between Ikaros loss and SLE development.
Aims: How Ikaros deficiency may contribute to SLE is presently unknown.With this study, we want to understand the biological processes influenced by Ikaros resulting in defectiveB cell tolerance associated with autoimmune disorders.
Methods: To study the function of Ikaros transcription factor as a regulator of peripheral B cell maturation, activation, and tolerance, we used hypomorphic and B cell conditional knock-out models for Ikaros.
Results: We show that mice with Ikaros null B cells spontaneously produce high levels of IgM autoantibodies associated with SLE (against chromatin/nucleosomes, double-stranded DNA, all histones), demonstrating a direct role for Ikaros in generating autoreactive B cells. Naive Ikaros null B cells are hyperreactive to B cell receptor (BCR) induced stimulation and show a diminished requirement for secondary co-stimulatory signals, with enhanced survival and increased proliferation. Unexpectedly, Ikaros deficiency induces gene expression changes in naive B cells that render them poised for activation. Freshly isolated Ikaros null B cells show defects in the MAPK signaling pathway that result in enhanced and sustained phosphorylation of p38 and ERK-1/2 upon activation. Inhibition of p38 and ERK activity rescues the hyper-reactive phenotype. Interestingly, Ikaros deficiency in B cells is not sufficient for progression to a pathological state as mutant mice do not develop clinical signs of lupus, suggesting that Ikaros deficiency functions as a trigger for disease initiation but is not sufficient for SLE progression.
Summary and Conclusions: Altogether, our results implicate Ikaros as a novel negative regulator of BCR signaling which helps to maintain B cell tolerance to self-antigens during maturation by modulating MAPK events downstream of the BCR.

P358

HIGH-THROUGHPUT SIRNA SCREENING REVEALS GATA-2 UPSTREAM TRANSCRIPTIONAL MECHANISMS IN HEMATOPOIETIC CELLS

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Background: Hematopoietic stem cells (HSCs) are capable of self-renewing and differentiating into all blood cell types. The transcription factor GATA-2 is expressed in HSCs and progenitor cells and is essential for cell proliferation, survival, and differentiation. Recently, studies of aplastic anemia, MonoMAC syndrome, and lung cancer demonstrated a mechanistic link between GATA-2 and human pathophysiology. GATA-2-dependent disease processes have been extensively studied; however, GATA-2 upstream transcriptional mechanisms remains poorly understood.
Aims: We conducted high-throughput screening analysis of siRNA libraries to identify novel factors involved in GATA-2 regulation.
Methods: Human hematopoietic cell lines, YN-1 (Endo *et al*, 1993), K562, KG1a, Jurkat, U937, and NALM6 were analyzed. GATA-2 regulatory element was cloned into luciferase plasmid (pGL4.20, Promega) and mutation within the *cis*-element was introduced using a site-directed mutagenesis kit (Agilent). For siRNA screening, we targeted 995 genes encoding transcription factors from the siPerfect transcription factor library (RNAi Co., Ltd.). YN-1 cells stably expressing *GATA-2* +9.9/1S-luciferase were transfected with each siRNA duplex by 96-well plate GenomONE-Si (Ishihara Sangyo), and luciferase activity was analyzed using a ONE-Glo Luciferase Assay System (Promega). siRNA-mediated knockdown (Dharmacon) and CRISPR/Cas9-mediated knockout (Invitrogen) were conducted with nucleofector (Lonza).
Results: GATA-2 transcription involves two different exons, a distal (1S) and proximal (1G) promoter, with the former being considered important in hematopoietic cells (Pan *et al*, 2000). We first demonstrated that GATA-2 1S transcript was most abundantly expressed in YN-1 cells. Next, using transient luciferase analysis to determine the optimal configuration for siRNA screening, we demonstrated that 1S promoter fused to a +9.9 kb intronic enhancer and involved in MonoMAC syndrome pathogenesis (Johnson *et al*, 2012), exerted the highest promoter activity. Disruption of the GATA binding motif within the +9.9 kb enhancer resulted in a significant decrease in +9.9 kb/1S luciferase activity. Next, we established a stable YN-1 cell line expressing *GATA-2* +9.9 kb/1S-luciferase and demonstrated significantly diminished luciferase activity following knockdown of BRG1, a previously reported GATA-2 regulator (Sanalkumar *et al*, 2014). Following siRNA library screening, 10% of genes with the highest luciferase activity repression after their knockdown were initially selected from each 96-well plate (n =83), which included potential GATA-2 regulators of GATA1, GFI1B, and ERG. The list of candidate genes was further refined based on their potential importance in hematopoiesis. CITED2, previously reported as a CBP/p300-dependent transcriptional co-activator involved in pathogenesis of bone marrow failure (Chen *et al.* Blood 2007; Kranc *et al.* Cell Stem Cell 2008), and EGR1, a transcription factor involved in HSC homeostasis as well as leukemogenesis (Min *et al.* 2008; Stoddart *et al.* 2014), were subsequently identified. siRNA-mediated knockdown of CITED2 and EGR1 led to significant *GATA-2* downregulation. Further, CRISPR/Cas9-mediated knockout of CITED2 resulted in significant *GATA-2* downregulation. We are currently performing a molecular analysis how CITED2 and EGR1 affect GATA-2 expression.
Summary and Conclusions: Our approach represents a powerful tool for identifying GATA-2 regulatory mechanisms and may lead to the development of novel therapeutic approaches for human diseases, including bone marrow failure syndromes.

P359

FUMARATE HYDRATASE IS AN ESSENTIAL REGULATOR OF EMBRYONIC AND ADULT HAEMATOPOIETIC STEM CELL

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Background: Lifelong adult haematopoiesis critically depends on rare multipotent haematopoietic stem cells (HSCs). HSCs reside in niches within the foetal liver (FL) during embryogenesis and the bone marrow (BM) after birth. These specific microenvironments allow HSCs to maintain a particular metabolic status that is essential to preserve their properties. Quiescent long-term HSCs rely on glycolysis but switch to oxydative phosphorylation in order to comply with the energetic demands of self-renewing or differentiation. However, the tricarboxylic acid (TCA) cycle's contribution to HSC metabolism remains poorly understood.
Aims: The aim of this study is to elucidate the role of the TCA cycle in haematopoiesis by deleting one of the key enzymes, fumarate hydratase (FH), that drives the hydration of fumarate into malate.
Methods: In order to determine the role of FH within the haematopoietic system, we opted for an *in vivo* genetic loss of function approach. We crossed floxed *Fh1* conditional mice with Vav-iCre mice to induce a constitutive haematopoiesis-specific *Fh1* deletion.
Results: We demonstrate that *Fh1* is indispensable to the haematopoietic system as its deletion is embryonic lethal. Indeed, *Fh1* deletion leads to complete foetal liver failure resulting in a severe defect in differentiation coupled with an expansion of the most primitive cell compartments. Using a different mouse model, we validated these results in the adult by reproducing the phenotype after conditional deletion (*Mx-Cre*). Metabolic analysis of haematopoietic cells lacking *Fh1* (combining LC/MS and SeaHorse approach) revealed that in addition to harbouring a compromised TCA cycle, *Fh1*-deficient cells exhibit a higher cellular concentration of fumarate. In order to discriminate which of these two metabolic changes triggers the observed phenotypes we reintroduced the cytosolic isoform of Fh1 thereby restoring intracellular fumarate concentration while maintaining the TCA cycle impairment. We demonstrate that this genetic addition is enough to rescue the embryonic lethality, the multilineage differentiation potential and the self-renewing capacity of HSCs upon transplantation.
Summary and Conclusions: With this study, we demonstrated an absolute requirement for *Fh1* for embryonic and adult haematopoiesis. From the dual function of Fh1 we established that while the mitochondrial isoform of Fh1 is largely dispensable for HSC maintenance and multilineage differentiation, efficient clearance of fumarate from the cytoplasm by the cytosolic isoform of Fh1 is fundamental.

P360

DECODING THE CENTRAL ROLE OF RCOR1 IN PROLIFERATION AND DIFFERENTIATION OF HAEMATOPOIETIC STEM AND PROGENITOR CELLS IN ZEBRAFISH

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Background: The corepressor Rcor1 was initially discovered as a novel genetic determinant of platelet number in meta-analyses of genome wide association stud-

ies (GWAS) for mean platelet volume and platelet count. More recently, it has been suggested to play a role in terminal differentiation of murine erythrocytes.
Aims: However, the mechanisms by which *rcor1* affects haematopoiesis during development and what transcription factors it mediates its effect through remain unclear.
Methods: To address this lack of knowledge, in this study, we used already validated methods of reverse genetics in zebrafish.
Results: Here we report on the novel role of Rcor1 in proliferation of haematopoietic stem and progenitor cells (HSPCs) during zebrafish embryonic development. Indeed, knock down of Rcor1 activity resulted in enhanced proliferation of HSPCs after their migration to the caudal haematopoietic tissue, which is the zebrafish equivalent to the mammalian foetal liver. This expansion of HSPCs was accompanied by a block in differentiation. Interestingly, the loss of transcription factor *gfi1aa* (*gfi1aa*$^{sa18719/sa18719}$) completely phenocopied the rcor1 defect in proliferation of HSPCs. However, analysis of *gfi1aa*$^{sa18719/sa18719}$ mutant HSPCs revealed that they had normal differentiation capacity. We further show that inhibition of Notch signalling partially rescued the ability of *rcor1*-depleted HSPCs to undergo differentiation.
Summary and Conclusions: Taken together, our data suggest that Rcor1 mediates the complex interplay between HSPC proliferation and differentiation by modulating the activity of diverse transcription factors.

P361

DUVELISIB (IPI-145) INHIBITS MALIGNANT B-CELL PROLIFERATION AND DISRUPTS SIGNALING FROM THE TUMOR MICROENVIRONMENT THROUGH MECHANISMS THAT ARE DEPENDENT ON PI3K-DELTA AND PI3K-GAMMA

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Background: In indolent non-Hodgkin lymphoma (iNHL) and chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), the neoplastic B cells rely upon support from non-neoplastic cells within their microenvironment for proliferation and survival. Support cells include T cells, myeloid-derived cells, and mesenchymal stromal cells, which provide phosphoinositide-3 kinase (PI3K)-dependent survival and growth signals for the neoplastic cells, as well as signals that maintain the tumor microenvironment (TME). Duvelisib (IPI-145) is an oral dual inhibitor of PI3K-δ and PI3K-γ in clinical development for iNHL and CLL/SLL.
Aims: To better understand the roles of the PI3K-δ and PI3K-γ isoforms in mediating signaling between the tumor and TME cells in B-cell malignancies,Infinity's potent PI3K isoform-selective compoundsthat targeteither PI3K-δ or PI3K-γ with >100-fold selectivity over other PI3K isoforms were utilized in *in vitro* experiments.
Methods: A mixture of cytokines (CD40L/IL-2/IL-10) was utilized in an assay that recapitulated TME-induced malignant B-cell proliferation.
Results: Duvelisib inhibited CD40L/IL-2/IL-10-induced proliferation of primary CLL cells with an average EC50 in the sub-nanomolar range. The use of PI3K isoform-selective inhibitory compounds revealed these proliferative signals are PI3K-δ dependent, as the PI3K-δ-selective inhibitor was more active than the PI3K-γ-selective inhibitor. While these experiments established the PI3K-δ dependence of TME-derived cytokines on CLL cell proliferation, the role of PI3K-γ in key functions such as the directed migration of non-malignant immune cells of the TME was also tested. The stromal chemokine, CXCL12, resulted in upregulation of phospho (p)-AKT in the CD3+ T cell populations in CLL patient PBMCs. Using isoform-selective inhibitors, the increase in CXCL12-induced pAKT in CD3+ T cells was found to be mediated by PI3K-γ. Chemotactic assays demonstrated reduced migration of total CLL PBMCs towards CXCL12 in the presence of the PI3K-δ and PI3K-γ inhibitor duvelisib. Flow cytometric analyses of these migrating cells revealed that duvelisib had the greatest effect on CXCL12-induced migration of T-cell population. Utilizing PI3K isoform-selective compounds, the inhibition of T-cell migration toward CXCL12 was found to be a PI3K-γ mediated process, as the PI3K-γ-selective inhibitor was more potent than the PI3K-δ-selective inhibitor in blocking T-cell migration. Myeloid-derived cells can also support CLL cell survival as components of the TME. Murine bone marrow cells were polarized *in vitro* into M2-macrophages with MCSF and IL-4. This polarization was blocked by the PI3K-g-selective inhibitor but not the PI3K-d-selective inhibitor. Finally, co-cultures of M2 macrophages with CLL cells led to extended CLL cell survival. These data show that macrophage polarization toward an M2 phenotype is dependent upon PI3K-γ and that M2-cells can support CLL cell survival.
Summary and Conclusions: T cells and myeloid cells provide a survival and proliferative advantage to malignant CLL cells within the TME. The role of PI3K-γ in the migration and polarization of these cells supports the potential for therapeutic benefit from inhibition of PI3K-γ. By inhibiting both the PI3K-δ and PI3K-γ isoforms, duvelisib is uniquely positioned to inhibit key signals important in the pathogenesis of B-cell malignancies.

P362

BONE MARROW ADIPOGENESIS BY ACTIVATING PPARΓ SIGNALING INHIBITS POSTINJURY RECOVERY OF MOUSE HEMATOPOIETIC STRESS MODEL

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Background: Adipocytes have been considered as passive tissue filler of bone marrow(BM) niche for a long time.However, recent reportsshow thatadipocyte-rich BM harbored a decreased frequency of progenitor cells. Furthermore, "fatless" mouse BM cells exhibit improved engraftment after transplantation. Our previous studies alsoreveal that hematopoietic recovery is accelerated by using PPARγ inhibitor BADGE after chemotherapy. However, it is still unclear how adipocytes affect hematopoietic recovery under stress conditions. In our study, we set out to test the hypothesis that adipocytes could affect the function and migration of hematopoietic stem cell(HSC) following hematopoietic stress.
Aims: To investigate the effect of BM adipogenesis on the function and migration ofHSC in response to stress in mice.
Methods: For induction of hematopoietic stress, C57BL/6J female mice(6-8 weeks, 18-20g, n=90) were administrated once by intraperitoneal injection with 250mg/kg 5-Fu. Rosiglitazone group animals were fed 5g chow/d containing 0.15mg/g.d rosiglitazone before 5-Fu. BADGE group animals were administered 60mg/kg/d BADGE following chemotherapy. To observe the level of BM adipogenesis, tibias were collected and detected by histopathology. PPARγ, aP2, adiponectin mRNA level was evaluated by RT-PCR. Peripheral blood cells(PB) and BM mononuclear cells(BMMNC) were counted using the hematology analyzer. The number of progenitor cell in BM were analyzed by colony-forming cell assay. Immunophenotype and cell cycle of hematopoietic stem cells were quantitatively estimated by flow cytometry.
Results: We found that BM adipogenesis could be successfully induced by rosiglitazone treatment under homeostatic condition. Rosiglitazone treatment mice exhibited normal hematopoiesis compared with control group, as indicated by normal levels of blood and BM cellularity. In response to 5-Fu induced hematopoietic stress, mice exhibited hypocellular BM with dilated sinus. Compared to control group, adipocytes number in BM of rosigolitazone group mice was increased and the expression of PPARγ, adiponectin was also significantly higher in rosiglitazone group. After treatment with 5-Fu, all groups showed similar nadirs of WBC and BMMNC. On recovery, however, WBC and BMMNC increased more slowly in rosiglitazone group mice. HGB and platelet counts didn't show similar pattern of delayed recovery after 5-Fu treatment in rosiglitazone group. As a cytotoxic agent, 5-Fu induces a multistep BM stress response by killing actively cycling cells, causing the number of BM cells to decline, followed by proliferation, differentiation and mobilization of HSPCs. We observed that rosigalitazone group mice had increased percentage of LSK compared to control groups while a decreased percentage of GMP and CLP cells at d14 after 5-Fu. Meanwhile, we found a 1.5-fold reduction of CFU-G counts in rosiglitazone group BM. However, we didn't find any difference of LSK cell counts in PB and spleen, and cell cycle of LSK cells was also not affected by treatment of rosiglitazone. These results indicated that BM adipocytes maintain the number of LSK cells in BM while impair myeloid and lymphoid differentiation, which may explain the delayed hematopoietic recovery in rosiglitazone group. Finally, we treated rosiglitazone mice with an PPARγ inhibitor BADGE for 2 weeks to check if such effects were reversed. Surprisingly, there was a twofold increase in the proportion of LSK cells in the spleen at d14. Moreover, WBC and BMMNC counts of BADGE groups were elevated compared to rosiglitazone group and the proportion of LSK of BADGE treated BM was decreased compared with rosiglitazone group at d14.
Summary and Conclusions: Our studies revealed that PPARγ induced BM adipogenesis could inhibit the differentiation of HSC while maintain them in undifferentiated state. These results may suggest a plausible mechanism for the impaired hematopoiesis in patients with fatty bone marrow and provide new evidence for benefits of PPARγ inhibitor treatment to improve hematopoietic recovery.

P363

A CROSSTALK IS MEDIATED BY THE NOTCH PATHWAY BETWEEN THE HAEMATOPIETIC STEM CELLS AND THEIR STROMAL MICROENVIRONMENT

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Background: The very well conserved Notch pathway is known to be important in many aspects of haematopoiesis. The Notch ligands are expressed in the stromal bone marrow microenvironment and interact with Notch receptors expressed on the haematopoietic stem cells and progenitors (HSCs/HSPCs) in order to maintain their immature and quiescent phenotype (Anjos Afonso *et al.*, 2013).
Aims: The cell autonomous role of the Notch pathway has been recently elu-

cidated in human HSCs/HSPCs maintenance. However little is known about the non-cell autonomous role played by the Notch pathway in the stromal microenvironment.

Methods: The long-term culture (LTC) assay has been used as a model to study the interaction mediated by the Notch pathway between the HSPCs and the MS-5 stromal cell line *in vitro* and *in vivo*. For *in vivo* studies it has beenused a technique recently established in the laboratory. MS-5 stroma and HSPCs cells were pre-seeded in gel-foam scaffolds that were, after few days, implanted in the back of mice for 8 or more weeks.

Results: In this study, for the first time, is shown that the HSPCs activate the Notch pathway in the MS-5 stromal cells through Notch2 receptor (Fig1 A). The activation of the pathway induces an increase in the expression of Notch ligands Jag1, Jag2 and Delta4 and of Notch2 receptor in the stromal cells (Fig1 B). Thus, enhancing in a positive feed-back loop the activation of the Notch pathway in the HSPCs and in the MS-5 cells (Fig1 C and D). These data suggest that a cross-talk mediated by the Notch pathway is happening between the HSPCs and the stromal cells. Interestingly, in absence of Notch activation in the stroma, the HSPCs are pushed to proliferate and differentiate loosing their LTC-IC and *in vivo* engraftment potentials after co-culture (Fig2). However, the inhibition of the cross-talk mediated by Notch inhibitors (GSIs) trigger a stronger phenotype, confirming the cell autonomous role played by Notch in HSPCs maintenance *ex vivo* (Fig3). These results have been confirmed using human primary Mesenchymal Stem Cells (MSCs).

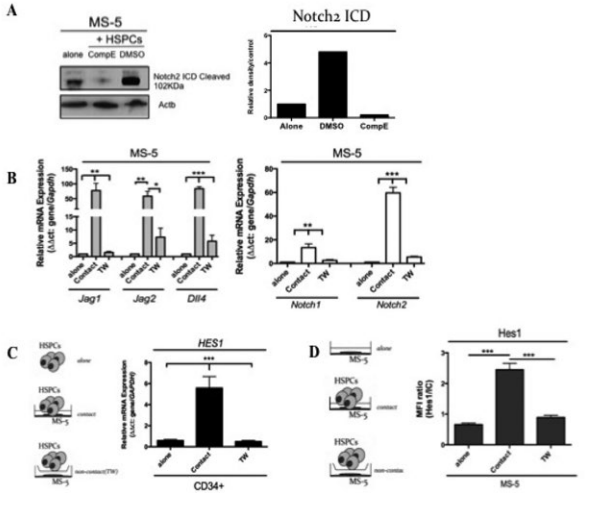

Figure 1.

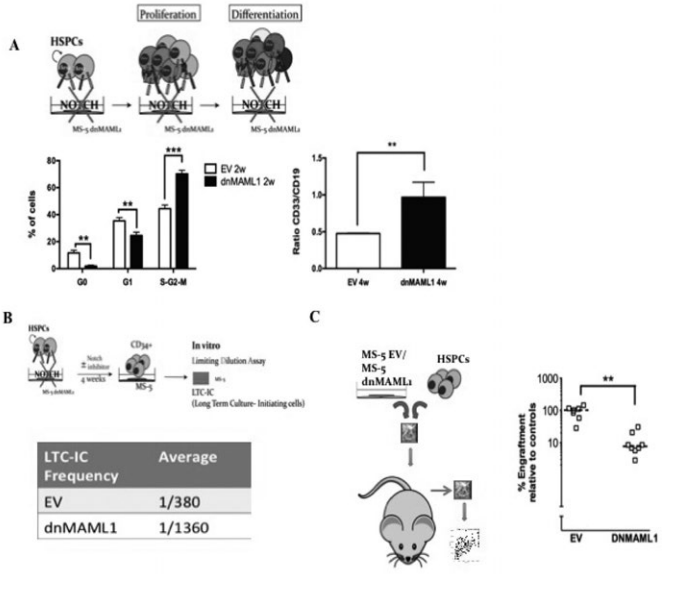

Figure 2.

Summary and Conclusions: These results show that a crosstalk mediated by the Notch pathway between the HSCs and the stromal microenvironment is fundamental to maintain the properties of the HSPCs*ex vivo*.A better understanding of the molecular mechanism involved in the crosstalk mediated by the Notch pathway between the haematopoietic cells and the stromal microenvironment would not only help in the optimization of their *in vitro* expansion for clinical purposes, but also in a better understanding of the role of Notch in haematological malignancies.

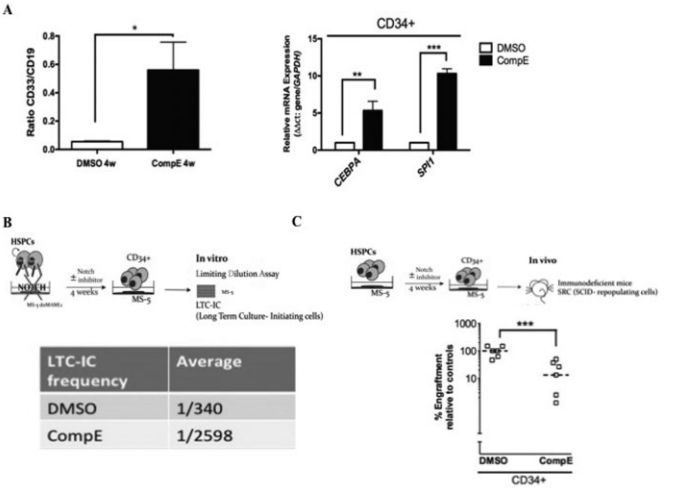

Figure 3.

P364

A CRITICAL ROLE FOR O-GLCNACYLATION IN MURINE FETAL LIVER AND ADULT HEMATOPOIESIS

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Background: Posttranslational modifications such as phosphorylation or glycosylation are crucial for regulating protein function. Among these, glycosylation of serine or threonine residues with *O*-linked N-acetylglucosamine (*O*-GlcNAcylation) by *O*-linked N-acetylglucosamine Transeferase (OGT) is particularly important, as this modification regulates various cellular processes through numerous targets including signaling molecules or transcription factors. Dysregulation of *O*-GlcNAcylation is critically linked to metabolic or degenerative diseases (*i.e.* diabetes, Alzheimer disease), tumorigenesis and aging. However, a role of *O*-GlcNAcylation in hematopoiesis still remains elusive.

Aims: To investigate the role of *O*-GlcNAcylation by *Ogt* in normal hematopoiesis.

Methods: We utilized *Ogt* conditional knockout mice (*Ogt*-cKO) to delineate the role of O-GlcNAcylation in hematopoietic system. To accomplish hematopoietic-specific or inducible deletion of *Ogt*, *Ogt*-cKO mice were crossed to Vav-Cre transgenic (Tg) or Mx-Cre Tg mice, respectively. Since *Ogt* is located on X-chromosome, $Ogt^{flox/Y}$ or $Ogt^{flox/flox}$ mice were considered to be equivalent.

Results: Since both $Ogt^{flox/flox}$ Vav-Cre^{+}and $Ogt^{flox/Y}$Vav-Cre^{+} mice were embryonic or perinatal lethal, we analyzed E14.5 fetal liver cells (FLs) to examine the effect of *Ogt* loss in FL hematopoiesis. Quantitative RT-PCR and western blot analysis showed that *Ogt*-mRNA and OGT protein were absent in $Ogt^{flox/Y}$ Vav-Cre^{+}or $Ogt^{flox/flox}$ Vav-Cre^{+} FL cells, indicating that floxed-alleles created a null mutation. Interestingly, the number of FL cells was remarkably decreased in $Ogt^{flox/Y}$ Vav-Cre^{+}or $Ogt^{flox/flox}$ Vav-Cre^{+} embryos as compared to the wild-type (WT) counterparts. In contrast, the percentage of lineage^{-}Sca-1^{+}c-kit^{+} (LSK) cells was significantly increased in $Ogt^{flox/Y}$ Vav-Cre^{+}or $Ogt^{flox/flox}$ Vav-Cre^{+} FLs. Surprisingly, these *Ogt*-deficient LSK cells completely lacked clonogenic activity in methylcellulose culture *in vitro*, and a few surviving cells did not form secondary colonies in serial replating assays. Furthermore, when 3,000 LSK cells from either $Ogt^{+/Y}$Vav-Cre^{+} or $Ogt^{flox/Y}$Vav-Cre^{+}were transplanted into lethally irradiated recipient mice along with 200,000 recipient-type whole BM cells, $Ogt^{flox/Y}$Vav-Cre^{+}donor-derived cells were scarcely detectable in peripheral blood(PB) of recipient mice at 3 weeks after transplantation, while more than 50% of PB cellswere donor-derived in mice recieving $Ogt^{+/Y}$Vav-Cre^{+}LSK cells. These data suggest that *Ogt* is critical for proliferation, differentiation and self-renewal of FL hematopoietic progenitors (HPCs).

We next examined $Ogt^{flox/Y}$Mx-Cre^{+}and $Ogt^{+/Y}$Mx-Cre^{+} mice to investigate the role of *Ogt* in adult hematopoiesis. Ogt deletion was initiated via five intraperitoneal injections of polyinosinic–polycytidylic acid (pI-pC) at dose of 400 μg/body, and then complete blood cell counts were monitored every 2 weeks. Interestingly, the number of white blood cells, red blood cells and platelets were decreased over time in *Ogt*-deleted mice. At four weeks after pI-pC injection, the percentages of hematopoietic stem cells (HSCs) (CD48^{-}LSK, CD150^{+}CD48^{-} LSK) in *Ogt*-deleted mice were significantly decreased as compared to those of WT mice.We then evaluated competitive repopulating activity of *Ogt*-deficient cells. 1,000,000 bone marrow (BM) cells from either $Ogt^{flox/Y}$ Mx-Cre^{+} or Ogt$^{+/Y}$

Mx-Cre+ mice (CD45.2+) were transplanted with the equal number of competitor cells (CD45.1+CD45.2+) into lethally irradiated CD45.1+ recipient mice. 4 weeks after transplantation, pI-pC was administered to the recipient mice to delete floxed alleles. The result showed that the percentage of *Ogt*-deleted cells was remarkably decreased at 2 weeks after pI-pC injection and it progressively decreased over time. These data suggest that *Ogt* is essential for competitive and long-term repopulating capacity of BM-HSCs.
Summary and Conclusions: The *O*-GlcNAcylation by *Ogt* plays a critical role for maintaining homeostasis of FL and BM hematopoiesis in mice.

LB365

EXPLORING THE ROLE OF KIT LIGAND AT THE ONSET OF HEMATOPOIESIS
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Background: Hematopoietic stem cells (HSCs) are generated during mammalian development from hemogenic endothelium in the major arteries through a process called endothelial-to-hematopoietic transition. The extrinsic and intrinsic factors that orchestrate this process are poorly understood. Kit ligand (KitL; also known as Stem Cell Factor/SCF) is a cytokine that plays a pivotal role in the adult bone marrow HSC niche, and it is thought to act on several cell types during embryogenesis. It is known that the absence of KitL causes a significant reduction in the fetal liver HSC pool and leads to death *in utero* with severe anaemia. However, it is currently unclear where, when and how the hematopoietic defect originates in embryos lacking KitL, and what is the precise role of this cytokine in the first steps of hematopoiesis.
Aims: Here we use a novel KitL transgenic reporter mouse line to map expression of this cytokine in the microenvironment of the Aorta-Gonad-Mesonephros (AGM) and yolk sac hematopoietic sites in the developing embryo, and investigate the effect of loss of KitL in these pre-liver sites of hematopoiesis.
Results: We show that KitL expressing cells are found in the microenvironment of all hematopoietic sites of the E8.5-E10.5 mouse embryo. Loss of KitL as assessed in *Steel* mutant (*Sl/Sl)* embryos showed smaller and less proliferative hematopoietic clusters in the dorsal aorta and in the yolk sac. Already at E10.5, homozygous *Steel* mutants show a reduction in pre-HSC numbers, yolk sac erythropoiesis, and fetal liver cellularity. At E11.5 these mutants display decreased progenitor numbers in all hematopoietic sites.
Summary and Conclusions: Our data suggest that, contrary to earlier reports, Kit signalling plays a role in early pre-liver hematopoiesis. In addition, our data identified a previously unrecognized role for KitL in the maturation and/or expansion of hematopoietic progenitors originating from the yolk sac, that could contribute to the anaemia observed later in development. Ongoing studies aim at elucidating the mechanism by which KitL exerts its role in early hematopoiesis.

Red blood cells and iron - Biology

P365

DEREGULATION OF GENES RELATED TO IRON AND MITOCHONDRIAL METABOLISM IN REFRACTORY ANEMIA WITH RING SIDEROBLASTS
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Background: The presence of *SF3B1* gene mutations is a hallmark of Myelodysplastic Syndrome with ring sideroblasts (MDS-RS) and could be related to the iron accumulation in these patients. However, the mechanisms underlying that characterize the MDS-RS are not completely understood.
Aims: In order to gain insight in the molecular basis of MDS-RS, an integrative study of the expression and mutational status of genesrelated to iron andmitochondrial metabolism was carried out.
Methods: A total of 231 low-risk MDS patients (50,3% MDS-RS, 49,7% refractory cytopenia with unilineage dysplasia patients-RCUD) and 81 controls were studied. The gene expression profile was analysed using the Human Genome Expression Array (U133 Plus 2.0) from Affymetrix. Array-based sequence capture (Roche NimbleGen) followed by next-generation sequencing (Roche GS FLX Titanium sequencing platform) was used to analyze 39 genes related to iron and mitochondrial metabolism. The genes were selected according to our previous gene expression data. Spliceosome-related genes were studied using an amplicon sequencing design in GS Junior Instrument (Roche Applied Science). In addition, Sanger sequencing was carried out.
Results: Patients with refractory anemia with ring sideroblasts (RARS) showed a differential expression of 1145 genes compared to controls. Interestingly, 38% (266 genes) of the over-expressed genes were related to iron and mitochondrial metabolism. The comparison between RARS and RCUD patients showed a set of 192 differentially expressed genes: 33% (42 genes) of the over-expressed genes were also related to iron and mitochondrial metabolism. *ALAD* and mitochondrial transporters *SLC25* (*SLC25A37* and *SLC25A38*) genes were overexpressed in RARS. Most of the MDS-RS patients (94,3%) carried mutations in any spliceosome-related gene. In addition, our analysis of the *ALAD* gene identified two polymorphisms (rs8177807 and rs2228083) in exon 6 and located 49 bases from each other. The occurrence of both polymorphisms ("variant haplotype") was more frequent in MDS-RS (12%) than in members of the other groups analyzed (5%). (p=0.07). Furthermore, an un-described variant in the exon 7 was found in the *ALAD* gene in one RARS patient with a mutation in *SF3B1*. The positively charged arginine residue (R174) was replaced by an uncharged cysteine residue. The three-dimensional structure showed that R174 residue is completely buried into the monomeric structure and the protein was predicted to be potentially damaging. The variant was not found in any of the control samples or in those analyzed from RCUD patients. An over-expressed gene-signature of 71 genes was identified between patients with *SF3B1* mutations and patients without the mutations. Interestingly, *GDF15* was overexpressed in patients showing *SF3B1* mutations. In addition, other genes such as *PPP2R5B*, *PPP1R16A* and *DDIT4L*, related to *SF3B1* and *GDF15*, were up-regulated in the mutated group. A functional analysis with this gene set showed two deregulated pathways: porphyrin biosynthesis and heme biosynthesis (p<0.001).
Summary and Conclusions: The deregulation of genes involved in iron and mitochondrial metabolism provides new insights in our knowledge of MDS-RS. Our study revealed mutations in spliceosome-related genes in almost 100% of the MDS-RS. The presence of variations identified in *ALAD* gene could have a possible role in the predisposition to disease (as a first event) as well as contributing to the pathogenesis of MDS-RS where spliceosome-related genes mutations could be the trigger cause.

P366

THE PRESENCE OF HEMOGLOBIN S IS ASSOCIATED WITH MIRNA DEREGULATION IN CD34+-DERIVED ERYTHROID CELLS
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Background: Sickle cell disease (SCD) is a recessive genetic abnormalitycaused by a point mutation of the *HBB* gene (Glu6Val) that results in theproduction of hemoglobin S (HbS), leading to chronic hemolysis andvaso-occlusion. In addition to its ability to polymerize under hypoxicconditions, HbS may give rise to increased oxidative stress caused by freeheme and iron secondary to its intrinsic instability. MicroRNAs (miRNAs) canmodulate post-transcriptional erythroid-specific regulators.

Aims: The aim of thisstudy was to investigate miRNA expression profiles and the possiblepost-transcriptional role of these molecules associated with the presence ofHbS in sickle cell trait and in compound heterozygosity for HbS and δβ-thalassaemiaSicilian type.
Methods: We obtained samples from individuals from the same family with sickle cell trait (β^S/β^A),two sickle-δβ-thalassaemia Sicilian type patients ($\beta^S/\beta^{\delta\beta}$), two δβ-thalassaemia Sicilian type heterozygotes($\beta^{\delta\beta}/\beta^A$) and normal control ($\beta^A/\beta^A$).CD34$^+$-derived erythroid cells werecultured for 13 days and used to determine the miRNA expression profile.miRNAs were hybridized using an Agilent miRNA microarray platform and theprofiles were subjected to bioinformatics data analysis using GeneSpringsoftware (v11.0). Different databases, such as miRBase (Release 21),TargetScan (Release 6.2) and microRNA.org (Release 2010) were used todetermine the predicted targets of miRNAs. The RNAhybrid tool (BiBiServ) wasused to find the minimum free energy hybridization of each miRNA and apossible target gene.
Results: Twenty five miRNAs were up-regulated in$\beta^S/\beta^{\delta\beta}$ and in β^S/β^A compared to β^A/β^A and $\beta^{\delta\beta}/\beta^A$. *In silico* analysis showedthat eight miRNAs target genes involved in cellular adhesion (*CD36, ICAM4*,and *ITGB1*), antioxidant defense mechanisms (*CAT*, catalase), and hemoglobinaffinity for oxygen (*BPGM* and *MINPP1*, 2,3-bisphosphoglycerate mutase andphosphatase, respectively). *CD36, ICAM4*, and *ITGB1* are possible targets ofthe miR-17, 20a and 20b and the latter is additionally targeted by miR-29b,29c and 30e. The *CAT* gene is also targeted by miR-30e as well as by miR-148aand 148b. *BPGM* is targeted by miRs 20a and 20b, while *MINPP1* is onlytargeted by miR-30e. We also found hemopexin (*HPX*), a major heme-bindingprotein, is a possible target of miR-26b.
Summary and Conclusions: Our data suggest that,in an *in vitro* model, the presence of HbS is associated with miRNAderegulation that may be associated to pathologic processes found *ex vivo* in reticulocytes and mature erythrocytes from SCD patients. Financial supportby FAPESP and CNPq/INCTS.

P367

ORAL IRON SUPPLEMENTS INCREASE HEPCIDING AND DECREASE ABSORPTION FROM DAILY OR TWICE DAILY DOSES: STUDIES WITH STABLE IRON ISTOPIC LABELS IN YOUNG WOMEN

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Background: Oral iron supplementation is used to treat iron deficiency, but absorption is often low and correction is variable.
Aims: Determine the duration and magnitude of the plasma hepcidin (pHep) response induced by oral iron (Fe) supplements and concomitantly measure bioavailability in healthy iron depleted young women.
Methods: After randomization of 42 subjects with plasma ferritin<20 µg/L, pHep (measured by C-ELISA), iron status and inflammation markers were monitored at regular intervals. On day 1, no supplements were given (control day). On days 2 and 3, subjects received iron supplements containing 40, 60, 80, 160 or 240 mg Fe as $FeSO_4$ as either single or two consecutive daily doses extrinsically labeled with stable iron isotopes $^{54}FeSO_4$, $^{57}FeSO_4$ or $^{58}FeSO_4$. Iron bioavailability was measured by assessing the isotopic enrichment of erythrocytic iron 14 days after administration.
Results: Both Fe dose (P<0.05) and time of day (P<0.05) were associated with increase in pHep. Compared to control days, pHep was significantly higher at 8h and 24h after administration for 60, 80, 160 and 240 mg (P<0.05) but not for 40 mg Fe. Total Fe absorption from the Fe dose on the second day of the consecutive administration compared to a single Fe dose on the first day was not significantly decreased for 40 mg, but was decreased by 37% for 80 mg, 31% for 160 mg and 45% for 240 mg (for all, P<0.01). Fractional absorption was highest from the 40 mg dose. With twice per day dosing (60 mg Fe) the afternoon dose was less bioavailable (P<0.05).
Summary and Conclusions: In Fe-depleted women, consecutive day doses of supplemental Fe at 60 mg or above increase pHep and decrease fractional Fe bioavailability, while a dose of 40 mg does not. These important new data will help guide optimal dosing regimens for Fe supplements in women.

P368

RED BLOOD CELL GLYCOLYTIC INHIBITION ALTERS INTRACELLULAR ION CONCENTRATIONS, LEADS TO DECREASED DEFORMABILITY, AND UPREGULATES EXPRESSION OF CLEARANCE SIGNALS

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Background: enzymopathies is group of metabolic disorders of the erythrocyte that leads to decreased intracellular energy production. Energy deprivation ultimately results in premature clearance from the circulation in the spleen (extravascular hemolysis). The precise mechanism by which energy-deprived cells are recognized and cleared is largely unknown. One candidate mechanism could involve disturbed ion balance due to defective function of ATP-dependent ion channels in the red cell membrane, with consequent expression of clearance signals, and loss of red blood cell deformability.
Aims: To investigate the effect of energy-depletion on ion channel function, the exposure of clearance signals on the surface of the red cell, and red blood cell deformability in a model for energy-depleted red blood cells.
Methods: Isolated erythrocytes were resuspended in HEPES buffered Ringers solution (pH=7.4) and incubated overnight at 37ºC with 1mM D-glucose and various concentrations (5-10mM) of 2-deoxy-D-glucose. 2-deoxy-D-glucose (2dDg) is a glycolytic inhibitor acting on hexokinase, thereby serving as a model for energy-depleted red blood cells. Intracellular calcium was analysed by flow cytometry with fluo-4 AM as a fluorescent calcium probe and intracellular sodium and potassium concentrations were measured by flame photometry. ATP was measured using the CellTiter-Glo Luminescent Cell Viability Assay. Deformability of erythrocytes was measured using osmotic gradient ektacytometry by LoRRca. Expression of phosphatidylserine (Annexin-V) and CD47 on the erythrocyte membrane was examined by FACS.
Results: Upon incubation with various concentrations of 2-deoxy-D-glucose (2dDg) sodium levels (39 mM *vs* control 8 mM) were increased in a dose-dependent manner. Potassium (51 mM *versus* control 82 mM) and ATP concentrations (23 nM *versus* 7 nM) were decreased following treatment with 2dDg. FACS analysis revealed increased intracellular concentration of calcium and increased expression of CD47 and phosphatidylserine after incubation with 2dDg. Osmotic gradient ektacytometry showed decreased deformability after incubation with 2dDg, reflected by a decreased EI_{max} (maximal Elongation Index, Figure). When compared with ektacytometry curves from a patient with either hexokinase or pyruvate kinase deficiency, similar curves were obtained. Importantly, osmotic gradient ektacytometry curves of erythrocytes incubated with 2dDg are comparable (Figure).

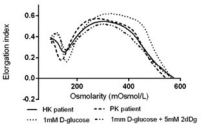

Figure 1.

Summary and Conclusions: Glycolytic inhibition alters intracellular red blood cell ion concentrations, and causes decreased deformability of erythrocytes. The decreased deformability as seen in 2dDg-incubated erythrocytes is comparable with the decreased deformability seen in patients with hexokinase and pyruvate kinase deficiency. Moreover, glycolytic inhibition is associated with upregulation of removal signals CD47 and phosphatidyl serine on the erythrocyte membrane. Loss of deformability and upregulation of removal signals in energy-deprived red blood cells may play a role in the premature removal of erythrocytes in red blood cell enzymopathies.

P369

MODIFIED ACTRIIB-MFC FUSION PROTEIN (RAP-536) INCREASES FUNCTIONAL RED BLOOD CELLS AND IMPROVES SICKLE CELL DISEASE PATHOLOGY WITH OR WITHOUT HYRDOXYUREA IN A MURINE MODEL

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Background: Sickle cell disease (SCD) is characterized by the presence of the sickle hemoglobin variant (HbS) of the b-globin gene. Under hypoxic conditions, HbS polymerizes, causing irreversibly sickled red blood cells (RBC). Manifestations of SCD include increased reticulocytes, splenomegaly, impaired blood flow due to intravascular sickling, and vaso-occlusive crises. The only approved therapy for SCD is hydroxyurea (HU), which decreases irreversibly sickled cells and painful events.. However, myelosuppression is a dose limiting toxicity and ~1/3 of patients do not respond to HU therapy, thereby highlighting the need for alternative treatment strategies
Aims: Luspatercept (ACE-536) is a modified type IIB activin receptor-Fc fusion protein[1] which acts as a ligand trap for members of the TGF-b superfamily to promote late-stage erythroid differentiation. In a murine model of b-thalassemia, RAP-536 (murine ortholog of ACE-536) corrected anemia and mitigated disease complications of b-thalassemia[2]. In this study, we evaluated RAP-536 as a monotherapy and in combination with HU in a murine model of SCD
Methods: SCD mice[3] (β^S/β^S, 6 and 12 weeks old) were dosed with RAP-536 (10 mg/kg, twice weekly SC) or TBS (VEH) control (N=4-6/group). Combination treatment with HU (100 mg/kg, i.p.) and RAP-536 (10 mg/kg SC) twice weekly for 3 months was compared with vehicle or HU monotherapy. Non-symptomatic compound heterozygote (β/β^S) littermates were used as controls (N=5/group).

Results: RAP-536 significantly decreased spleen weight (-16.4%) and serum bilirubin (-40.8%, *P<0.01*) compared to VEH, indicating decreased hemolysis. RAP-536 increased RBCs (+20%, *P<0.01*) and decreased reticulocytes (-30%, *P<0.05*). Blood smears from RAP-536 treated SCD mice displayed a 40-60% decrease in number of irreversibly sickled erythrocytes. In 6-week old SCD mice, RAP-536 treatment resulted in increased oxy-hemoglobin (+41.3%, *P=0.06*), functional oxygen saturation (+40.9%, *P=0.06*), oxygen content (+65.2%, *P=0.05*), and oxygen carrying capacity (+14%, *P<0.05*), and reduced carboxy-hemoglobin (-25.2%, *P<0.05*) compared to VEH. Histologic analysis of RAP-536 treated SCD mice showed substantial reduction in vascular congestion and damage in lung, spleen and kidneys, as well as reduced thickening of the alveolar wall compared to VEH. These data are consistent with reduced RBC sickling and qualitative improvements in SCD pathology in mice treated with RAP-536 monotherapy. We then evaluated RAP-536 in combination with HU in SCD mice. Treatment with HU alone reduced spleen weight (-20.2%, *P<0.05*), reticulocytes (-23.7%, *P<0.05*), phosphatidyl serine exposure on RBC membranes (-22.2%, *P<0.05*), and hemolysis (-48.4%, *P<0.05*) compared to VEH. The addition of RAP-536 to HU therapy further decreased splenomegaly (-50.7%, *P<0.05*), phosphatidyl serine exposure (-35.6%, *P<0.001*) and hemolysis (-55%, *P<0.01*) compared to VEH. The combination also further reduced vascular damage and congestion compared to VEH or HU monotherapy.
Summary and Conclusions: RAP-536, alone or in combination with HU, substantially improves RBC morphology and sickle cell disease pathology in SCD mice. Luspatercept (ACE-536) is currently being tested in Phase 2 clinical trials in MDS and β-thalassemia, and merits evaluation in SCD patients.

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P370

QUANTIFICATION AND VISUALIZATION OF DIETARY IRON UTILIZATION UNDER ERYTHROPOIETIC STIMULATION BY USING STABLE IRON ISOTOPE 57FE AND MASS SPECTROMETRY

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Background: Epoetin beta pegol (C.E.R.A.) is a novel, long-acting erythropoiesis-stimulating agent; once-monthly administration of C.E.R.A. effectively maintains hemoglobin levels in renal anemia patients. We previously demonstrated that C.E.R.A. promotes iron absorption through the reduction of serum hepcidin levels and subsequent up-regulation of the iron transporters, divalent metal transporter 1 (DMT1) and ferroportin. However, utilization of dietary iron following C.E.R.A. administration has not yet been fully quantitatively analyzed.
Aims: In this study, we used the stable iron isotope ^{57}Fe as a tracer of dietary iron incorporation. The primary objective was to quantify the utilization of dietary iron for erythropoiesis and its distribution into hematopoietic and iron storage tissues under C.E.R.A. stimulation by using inductively coupled plasma mass spectrometry (ICP-MS). We also attempted to visualize the incorporation of dietary iron into newly synthesized heme in the spleen by using high-resolution Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS).
Methods: To trace the incorporation of dietary iron into hematopoietic and iron storage tissues, we used a diet containing 200 ppm of the stable iron isotope ^{57}Fe instead of natural iron (^{57}Fe-diet). As a control, we used a diet containing 200 ppm of natural iron (control diet). C57BL/6NCrl mice fed the control diet were switched to the ^{57}Fe-diet immediately after being intravenously injected with 10 μg/kg of C.E.R.A. or vehicle. On Day 2, 5, 8, and 14 after administration, mice were killed by exsanguinations under anesthesia with isoflurane, and iron parameters and hematological parameters were analyzed. To quantify dietary iron incorporation, the content of hemoglobin containing ^{57}Fe in red blood cells and ^{57}Fe levels in bone marrow cells, spleen and liver were measured by ICP-MS. To visualize newly synthesized heme containing ^{57}Fe (^{57}Fe-heme), on Day5 after C.E.R.A. administration we spatially mapped ^{57}Fe-heme in the spleen by FT-ICR MS imaging.
Results: Compared with the vehicle-treated group, the C.E.R.A.-treated group showed a reduction in hepcidin levels from Day 2 to Day 8 and higher levels of hemoglobin. On Day 14, the content of hemoglobin containing ^{57}Fe in red blood cells was significantly higher in the C.E.R.A.-treated group than in the vehicle-treated group (3.6±0.7 g/dL *vs* 1.5±0.2 g/dL). ^{57}Fe levels in hematopoietic tissues (*e.g.* bone marrow cells and spleen) and iron storage tissues (liver) were also increased until Day 14 after C.E.R.A. administration. On Day 5, newly synthesized ^{57}Fe-heme in the spleen was more obviously detected in the C.E.R.A.-treated group than in the vehicle-treated group.
Summary and Conclusions: The present study focused on the effect of C.E.R.A. on dietary iron incorporation and utilization for erythropoiesis. We demonstrated that C.E.R.A. stimulated the effective use of dietary iron for erythropoiesis both in a quantitative manner with ICP-MS and in a visible manner with FT-ICR MS. Using these tools, we could trace dietary iron dynamics from incorporation to utilization for erythropoiesis or to retention in hematopoietic tissues such as bone marrow and spleen. It is well-known that abnormal iron metabolism contribute to the development of renal anemia. Therefore, our methods can not only provide useful information to understand iron metabolism under hematopoietic stimulation in basic research, but could be helpful in clinical applications.

P371

FIVE NEW CASES OF HEXOKINASE DEFICIENCY: BIOCHEMICAL AND MOLECULAR CHARACTERIZATION OF A NOVEL SPLICE SITE MUTATION AND 2 NOVEL MISSENSE MUTATIONS IN HK1

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Background: Hexokinase (HK) deficiency is a very rare cause of hereditary nonspherocytic hemolytic anemia (HNSHA). Hexokinase is one of the key regulatory enzymes of glycolysis, on which the red blood cell is totally dependent the production of ATP. Deficiency of HK disrupts cellular metabolism, which ultimately results in HNSHA. To date, 24 cases of hexokinase deficiency have been described. Only four of them have been characterized on the DNA level. We here describe five new cases and 3 novel mutations in *HK1*.
Aims: Characterize a genetic defect in patients who were found to be HK-deficient by spectrophotometrically determined red blood cell HK and pyruvate kinase (PK) enzymatic activities.
Methods: PK/HK ratio was applied to evaluate the effect of age-related increases in enzymatic activity. To confirm the diagnosis, DNA sequence analysis of *HK1* was performed by Sanger sequencing. Novel mutations were characterized by biochemical methods (K_m for glucose and Mg.ATP, pH stability and thermolability) and molecular studies (RT-PCR, quantitative RT-PCR, and Western Blot analysis on *ex vivo* cultured patient erythroblasts).
Results: DNA sequence analysis of *HK1* of 5 patients with suspected HK deficiency revealed a total of 5 different mutations in *HK1*. Three of them were novel (Table, novel mutations in bold). In *silico* analysis of mutations p.His868Tyr and p.Thr601Met by Polyphen-2 and SIFT predict that both substitutions are not tolerated. Both are located in the catalytic region, and kinetic properties are likely to be impaired upon mutation. Kinetic studies of the p.His868Tyr HK mutant showed that its affinity for ATP was indeed markedly decreased (3.2-times), whereas the affinity for glucose was slightly increased (Table). The molecular effects of the novel splice site mutation c.876-2A>G in intron 7 was studied on mRNA isolated from *ex vivo* cultured erythroblasts from the patient's father, who was heterozygous for this mutation. RT-PCR analysis showed the presence of normal as well as two aberrant mRNAs species. The aberrantly spliced transcripts lacked either exon 8 or both exons 8 and 9. Quantitative RT-PCR analysis showed that expression levels of the normally spliced mRNA variant were down-regulated 3-times compared to normal. These findings were confirmed on the protein level by Western blot analysis of HK from nucleated erythroid cells (Figure).

Tabella 1. Biochemical and molecular data of HK-deficient patients

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
HK [0.8 – 1.5]	0.64	0.58	0.71*	1.14*	0.65
PK [6.1 – 12.3]	12.3	12.6	3.25*	12.5*	12.2
PK/HK ratio [4.8 – 11.9]	19.2	21.7	9.50*	10.90*	18.7
Allele 1	-193A>G	-193A>G	c.281A>G p.Arg94Gln	**c.2602C>T p.His868Tyr**	**c.1802C>T Thr601Met**
Allele 2	**c.876-2A>G**	**c.876-2A>G**	c.281A>G p.Arg94Gln	**c.2602C>T p.His868Tyr**	Normal
Km Mg.ATP [0.99-1.59]	1.11	1.42	0.94	4.22	ND
Km glucose [0.09 - 0.13]	0.07	0.06	0.26	0.15	ND
Thermal stability	Slightly decreased	Slightly decreased	Heat labile	ND	ND
pH stability	Normal	Normal	Normal	ND	ND

*HK [1.09 – 1.65], PK[5.12 – 5.78], PK/HK [3.60 – 6.30]; *HK [0.62 – 1.26], PK [8.40 – 15.2], PK/HK [7.2 – 15.6]; ND- not determined

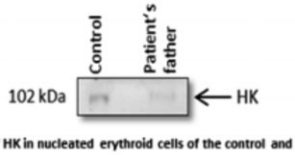

Figure: Detection of HK in nucleated erythroid cells of the control and patient's father.

Summary and Conclusions: We report 5 mutations in *HK1* in 4 unrelated families. Three of them, c.876-2A>G, Thr601Met, and p.His868Tyr have not been previously reported. The pathogenic nature of novel mutations was confirmed by molecular and biochemical studies. Our results contribute to a better

understanding of the genotype-to-phenotype correlation of HK deficiency, a very rare enzyme disorder of the red blood cell. *Supported by Ministry of Health of Czech Republic, grant NT/13587 and IGA_LF_2015_015.*

P372

CHRONIC PSYCHOLOGICAL STRESS STIMULATES MEDULLARY ERYTHROPOIESIS BY MODULATING LOCAL NITRIC OXIDE PRODUCTION

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Background: Stress elicits an integrated neuroendocrine response disturbing the homeostasis of different physiological systems. Thus, the experimental studies have shown that chronic psychological stress stimulates erythropoiesis but molecular mechanisms underlying this stress response remain to be elucidated. There is evidence that stress increases nitric oxide (NO) synthesis as well as that NO can modulate hematopoiesis.
Aims: This study was undertaken to investigate whether NO is involved in the regulation of erythropoiesis during chronic psychological stress using restraint experimental model and NO synthase (NOS) inhibitor Nw-nitro-L-arginine-methyl ester (L-NAME).
Methods: Adult male CBA mice were randomly assigned to following groups: 1) restraint group exposed to 2 h daily restraint stress during 7 consecutive days; 2) L-NAME + restraint group, treated with L-NAME (50 mg/kg, s.c.) 30 min prior to daily restraint; 3) L-NAME group, received only the daily dose of L-NAME; and 4) control group, simply handled daily. In the bone marrow, the number of erythroid progenitors *burst forming units-erythroid (*BFU-E) and colony-forming unit-erythroid (CFU-E) was determined using colony assays. The expression of neuronal NOS (nNOS) and endothelial (eNOS) in the bone marrow cells was evaluated by both Western blot and immunohistochemistry. In addition, the expression of nuclear factor kappa B (NFκB) subunit p65 was assessed by Western blot in the nuclear fraction of bone marrow cells.
Results: Chronic psychological stress significantly increased the number of BFU-E and CFU-E cells in the bone marrow. Inhibition of NOS activity by L-NAME completely abolished the effect of repeated restraint on erythroid progenitors. Similar to restraint stress, L-NAME alone induced a significant increase in the frequency of erythroid progenitor cells compared to controls. To further investigate a role of NO in regulation of erythropoiesis during chronic stress, we evaluated the expression of nNOS and eNOS in bone marrow cells. The results, obtained by Western blot analysis, demonstrated significantly decreased expression of nNOS in cytoplasmatic fraction of bone marrow cells following chronic restraint stress. Although not statistically significant, a similar trend was observed for eNOS in restrained mice. In accordance, a few nNOS- and eNOS-immunoreactive cells were detected within the bone marrow of chronically stressed animals. Furthermore, the activation of NFkappaB, known to induce the expression of NOS isoforms, was evaluated by its active nuclear component p65. Consistent with decreased expression of nNOS, the data showed that chronic psychological stress significantly attenuated the expression of p65 in the nuclear fraction of bone marrow cells.
Summary and Conclusions: Taken together, obtained results demonstrate for the first time that signal molecule NO modulates medullary erythropoiesis during chronic psychological stress. These findings are of importance because chronic psychological stress, as a part of modern style of life, may be involved in the pathogenesis of hematological malignancies.

P373

RECOMBINANT HUMAN ERYTHROPOIETIN-INDUCED ERYTHROPOIESIS REGULATES HEPCIDIN EXPRESSION OVER IRON IN A RAT MODEL

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Background: Iron has essential roles in several physiological processes, including red blood cell formation. Stimulation of erythropoiesis increases iron demand in bone marrow. Hepcidin, a small peptide produced by hepatocytes, plays a central role in regulating iron by promoting internalization and degradation of ferroportin, the only known cellular iron exporter. Several factors regulate hepcidin expression, as erythropoiesis, erythropoietin (EPO) and iron levels; however, the impact of each factor is not well established.
Aims: We aimed to study the effect of recombinant human erythropoietin (rHuEPO) therapy on erythropoiesis and on iron metabolism in a rat model.
Methods: Male Wistar rats, 12 weeks old, were divided in 5 groups: control group and rHuEPO-treated groups (100, 200, 400 and 600 IU/kg bw/week) during 3 weeks. Hematological and iron data were evaluated, as well as, the expression of several genes involving iron metabolism. Mann-Whitney test was used to evaluate differences between groups.
Results: One week rHuEpo treatment to healthy rats caused a dose-dependent increase in erythrocyte count, hemoglobin concentration, hematocrit and reticulocyte count. At the end of the protocol (3 weeks), rHuEPO-treated groups (200, 400 and 600 IU) showed higher values for these parameters, as compared to control group, except for the reticulocyte count that was reduced in the 200 IU rHuEPO-treated group. After 3 weeks of treatment, iron and ferritin levels do not differ between groups, transferrin levels reduced in the 100, 200 and 600 rHuEPO-treated groups, whereas transferrin saturation increased in the 200, 400 and 600 rHuEPO-treated rats. Liver hepcidin mRNA levels increased in the 200 IU rHuEPO-treated animals, as well as, the mRNA levels of the up-regulatory hepcidin mediators (*tfr2*, *hjv*, *bmp6*); in contrast, reduced mRNA levels of matriptase-2, an inhibitory modulator, were found. The 400 and 600 IU treated groups presented reduced levels of hecpidin mRNA and of the up-regulatory hepcidin mediators, and an increase in mRNA levels of matriptase-2. Serum EPO rat levels increased in the 200 IU rHuEPO-treated group, at the same extent we found an increase in liver EPO and EPO receptor mRNA levels. Extramedullary erythropoiesis was found in the spleen of 600 IU rHuEPO-treated group.
Summary and Conclusions: An increasing EPO stimuli leads to a rise in reticulocyte/erythrocyte production, which seems to be supported by an increase in iron absorption and mobilization, as suggested by the increase in transferrin saturation. However, the erythropoiesis stimuli triggered by the 200 IU rHuEPO dose seems to overwhelmed the bone marrow capacity, as showed by the decrease in reticulocyte production. Strengthening this hypothesis we found an increased serum EPO levels and liver mRNA expression of EPO and EPO receptor. At higher EPO stimuli, such as 600 IU rHuEPO dose, the increase in erythrocyte production is also supported by extramedullary erythropoiesis. The increase in hepcidin expression in the 200 IU rHuEPO-treated group seems to result from the reduction in erythropoiesis (an inhibitory stimuli for hepcidin production), as the transferrin saturation presents a similar value in the 600 IU rHuEPO-treated group. Actually, it seems that erythropoiesis prevails over iron regulation on hepcidin production. Supported by FCT (PTDC/SAU-TOX/114253/2009 and SFRH/BD/79875/2011) and POPH/FSE.

P374

MICRO-RNA CANDIDATES FOR REGULATION OF FETAL HEMOGLOBIN IN SICKLE CELL ANEMIA

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Background: Due to the important role of maintaining the hemoglobin in the metabolic activity, many factors are known to regulate the expression of globin genes, specifically gamma-globin genes. MicroRNAs (miRs) are endogenous regulatory elements, non-coding, small size, that act on gene regulation in post-transcriptional level. The miRs differential expression in sickle cell anemia and beta thalassemia homozygous has been studied and identified differences in miRNA expression in individuals with sickle cell anemia in relation to individuals without anemia.
Aims: We aimed to evaluate differential expression of miRNAs selected from in silico analysis and characterized as candidates for regulation of gamma-globin genes (HBG1 and HBG2) in subjects with sickle cell anemia in order to identify novel components involved in modulating levels of Hb F.
Methods: Peripheral blood samples from 20 individuals adults-10 with Hb AA and 10 with Hb SS (Bantu haplotype and HU use) were collected, regardless of gender. The quantification of the hemoglobin fractions was performed by HPLC-Ultra 2 (Trinity Biotech®). The homozygous for Hb S and the Bantu haplotype was confirmed by PCR-RFLP. MicroRNAs were selected from in silico analysis, through databases miRNApath (http://lgmb.fmrp.usp.br/mirnapath), Diana Lab (http://diana.cslab.ece.ntua.gr/microtiter /), Ferrolab (The miR ontology database) (http://ferrolab.dmi.unict.it/miro/), TargetScan (http://www.targetscan.org/), miRecords (http: // mirecords .biolead.org /) and miRBase (http://www.mirbase.org). Were considered candidates for regulation of Hb F levels the elements listed in at least three of the databases used.Reticulocytes were isolated and the microRNA extraction was performed with use of the kit miRvana miRNA Isolation Kit (Ambion®). CDNA synthesis (RT-PCR) was performed using the High Capacity cDNA Reverse Transcription Kit Kit (Applied Biosystems). For analysis of expression of microRNAs (qRT-PCR) reactions were performed by TaqMan MicroRNA Assay Detection System (Applied Biosystems). Statistical analyzes were performed with Statistica 8 software (Statsoft®), GraphPad Prism version 5 (GraphPad Software, San Diego, USA) and R version 3.1.1 Statistics (http://www.r-projet.org). We considered Statistically significance was $p < 0.05$.
Results: The in silico analysis shown 20 miRNA indicated as a candidates for regulation of Hb F. Quantitative analysis showed that 14 miRNAs showed significant differential expression: miR-449th, miR-449b, miR-34c-5p, miR-4795-3p, miR-96, miR-26b, miR-151-3p, miR-210, miR-15a, miR-16-1, miR-451, miR-144, miR-221 and miR-222. Only miR-34c-5p showed higher expression levels in the group with sickle cell anemia. Higher levels of expression of miR-221 and

miR-222 in the control group enhancing the performance of these two elements as possible negative regulators of the Hb F levels. miR-449th, miR-449b and miR-4795 were highly expressed in the control group.The miR-34c-5p, was more expressed in the group with sickle cell disease and was correlated with miR-26b (ρ=0.7). The miR-221 and miR-222 were correlated with each other (ρ=0.7) and miR-151-3p (ρ=ρ=0.72 and 0.98, respectively). Furthermore, miR-96 showed more correlations in the group with sickle cell anemia.
Summary and Conclusions: Our results shown the presence of differentially expressed miRNAs predicted the regulation of gamma-globin genes among Hb AA individuals with low levels of Hb F and individuals with sickle cell anemia with increased Hb F induced by treatment with HU. Some of these elements have validated and compared with the erythropoiesis regulation of gamma-globin genes, whereas other miRNAs have not yet been associated with these procedures.

LB375

POSSIBLE INTERPLAY OF MICRORNA AND TRANSCRIPTION FACTORS DURING IN VITRO ERYTHROID CULTURE OF CD34+ CELLS IN SICKLE CELL ANEMIA

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Background: The clinical severity of the sickle cell anemia (SCA) can be affected by a number of modifying factors including HbF levels and the coinheritance of alpha-thalassemia. Furthermore, key regulators such as microRNAs (miRs) and transcription factors (TFs) are involved in the regulatory mechanism of HbF production, however this regulatory mechanism is unclear.
Aims: To investigate miRs and TFs expression during *in vitro* erythroid differentiation in normal and SCA erythropoiesis.
Methods: Peripheral blood CD34$^+$ cells were isolated from 4 patients who presented homozygous HbS genotype (HBSS) and from 4 healthy subjects (CON). The cells were cultured in a liquid system stimulating erythropoiesis and collected on days 7, 10, and 13 to analyze miRs and the TFs expression profile. *In silico* prediction was performed by TargetScanHuman software v 6.2 to verify possible interactions between miRs and TFs.
Results: Quantitative PCR showed that two miRs were down-regulated during HBSS erythropoiesis: miR-223 on days 7 and 10, and miR 486-3p on day 10 (P<0.05). Seven miRs were up-regulated: 146a-5p, 221 and 222 on day 10, and 15a, 144-3p, 150-5p and 155-5p on day 13 (P<0.05). *NFE2*, *SP1*, *ETO2* and *FOXO3* were decreased on day 13 (P<0.05), while *LMO2* and *LDB1* were up-regulated on days 10 and 13, respectively (P<0.05). Increased levels of *TAL1* were observed on days 10 and 13 (P<0.05). *GATA1* expression did not change significantly. *In silico* analysis predicted miR interactions for the 3' untranslated region of TFs : 146a-5p–*NFE2*, miR-155-5p– *SP1*, miR-221–*FOXO3*, miR-222 – *FOXO3*, miR-486-3p–*LDB1*, and miR-486-3p–*TAL1*. Studies have shown that mice lacking the transcription factor NF-E2 present hemolytic anemia, while the downregulation of *ETO2* and *SP1* contributes to the activation of TFs involved in the *GATA1*-complex, such as *LDB1*, *TAL1* and *LMO2.* Based on our preliminary results, the upregulation of these TFs may be associated with the decreased expression of *ETO2* and *SPI1* in HBSS cultures. We also suggest that the miRs-targets 155, 15a, 486-3p and 146a-5p may be contributing to changes in the expression of these TFs. Studies demonstrated that miRs 15a and 150-5p regulate the TF *cMYB* and its downregulation leads to an increase in HbF levels. Our results of low levels of *cMYB* with concomitant increases in these miRs in HBSS cultures are in agreement with the previous studies. An association of high miR-144-3p expression with a severe anemia phenotype in SCA, oxidative damage and hemolysis of HBSS erythrocytes cells has been previously described. Additionally, up-regulation of *FOXO3* correlated to an antioxidant gene expression response, which protects erythroid cells from cellular stress. We also found the high levels of miR-144-3p in HBSS cultures and also showed a downregulation of *FOXO3,* which may be associated with upregulation of its miRs-target 221/222.
Summary and Conclusions: Our data show relevant associations between miRNAs and their possible targets during *in vitro* erythroid differentiation. These results suggest that miRNAs represent a class of molecules that may contribute to hematopoietic process and may have a role in physiopathology of SCA.

Red blood cells and iron - Clinical 1

P375

CATEGORIZATION OF CLINICAL SEVERITY IN PYRUVATE KINASE DEFICIENCY (PKD) IN AN INTERNATIONAL, OBSERVATIONAL COHORT

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Background: PKD is the most common glycolytic defect causing congenital non-spherocytic hemolytic anemia. An international, multicenter Natural History Study (NHS) has been established to collect retrospective and prospective clinical data on PKD patients (pts).
Aims: To categorize PKD clinical severity based on degree of anemia, transfusion history, and splenectomy status and to identify predictors and prevalence of complications in the severity groups.
Methods: 105 pts with PKD enrolled on the PKD NHS at 14 IRB approved sites from March 2014 to January 2015. Pts were randomly assigned to either the test set (n=75) or future validation set. For this analysis, baseline and retrospective enrollment data were included from the 75 test set pts. PKD pts were categorized into 4 distinct severity groups (Gp1-Gp4), with increasing severity of the disease: Gp1. Never regularly transfused +/-prior acute transfusions; Gp2. Regular transfusions prior to splenectomy (SPL), post-SPL baseline hemoglobin (hb) >8.7 g/dl; Gp3. Regular transfusions prior to SPL, post-SPL baseline hb ≤8.7 g/dl; and Gp4. Splenectomized and currently regularly transfused. Gp2 and 3 were regularly transfused prior to SPL and transfusion independent after SPL and are distinguishable by post-SPL baseline hb. The median hb (8.7 g/dl) from Gp2 and 3 was used to discriminate between pts who were less anemic *versus* more anemic. Ordinal logistic regression models were used to test for association between clinical characteristics and severity group.
Results: The Table shows the characteristics of Gp1-4. In Gp1, only 42% of pts were splenectomized. Younger age at diagnosis (p=0.04) was significantly associated with increased severity of disease. There were no significant differences in groups based on gender, ethnicity, or race. There was a significant trend for increasing ferritin (p=0.03) and liver iron concentration (LIC, p=0.04) with increasing PKD clinical severity, and more severe groups were more likely to have received chelation therapy (p<0.01). Some Gp1 pts developed iron overload despite the absence of a history of regular transfusions. There was also a trend for higher rates of cholecystectomy (p=0.01) with increasing clinical severity. For certain complications, there was no evidence to support an association with clinical severity, including aplastic crises (overall rate 16%), extramedullary hematopoiesis (12%), and pregnancy complications of the affected mother (55%), as well as the requirement for exchange transfusion in the newborn period (44%). The reticulocyte count was incrementally higher with increasing clinical severity in the non-regularly transfused groups from Gp1 to Gp3 (p=0.02), even after controlling for splenectomy status. As expected, multiple genotypes were found in this cohort. Pts with the common, Amish, homozygous 1436G>A mutation were found in Gp1, 2, and 3, which may indicate a contribution of other genetic or environmental factors to clinical severity.

Tabella 1. Demographyc and Clinical Features of the Classification Groups of Clinical Severity in PKD.

Severity Group Characteristics	Group 1: Never Regularly Transfused, +/-Acute Transfusion	Group 2: Regularly Transfused before SPL, Less Anemic (Hb >8.7 g/dl)	Group 3: Regularly Transfused before SPL, More Anemic (Hb ≤8.7 g/dl)	Groups 4: Currently Regularly Transfused after SPL	P-value
n[1]	24	16	19	6	
Age at enrollment (years) Median (range)	18.9 (1.7-69.9)	24.5 (3.1-44.4)	19.2 (4.5-49.2)	13.0 (7.9-27.9)	0.06[2]
Age at diagnosis (years) Median (range)	1.8 (0-60.3)	0 (0-5.4)	0.4 (0-35.1)	2.6 (0-13.8)	0.04[2]
Hb (g/dl) Median (range)	10.1 (6.4-14.2)	9.3 (8.8-10.9)	8.0 (6.2-8.6)	8.1 (6.7-9.2)	NA
Reticulocyte Count (%) Median (range)	9.6 (1.6-82.9)	30.9 (8.2-61.0)	36.2 (5.4-61.2)	21.4 (2.4-27.7)	0.02[3]
MCV (fL) Median (range)	97.2 (76.0-119.7)	109.7 (101.1-133.4)	115.3 (89.7-128.0)	96.4 (84.8-100.0)	0.01[3]
Cholecystectomy	7/24 (29%)	6/16 (38%)	14/19 (74%)	3/6 (50%)	0.01[2]
History of chelation	1/23 (4%)	3/16 (19%)	9/18 (50%)	3/6 (50%)	<0.01[2]
Ferritin (mg/ml) Median (range)	403 (48-2623) n=9	625 (327-3106) n=15	642 (324-2134) n=12	1696 (308-5630) n=6	0.03[2]
LIC (mg/g DW) (median, range)	7.5 (4.9-8.0) n=4	5.8 (2.0-10.0) n=12	8.7 (3.0-11.8) n=7	46.0 n=1	0.04[2]

[1]Non-splenectomized, regularly transfused infants (n=10) were excluded from groups, because patients were considered too young for splenectomy and too early to be categorized in Groups 2-4. Hb = hemoglobin; SPL = splenectomy; NA: Factor is associated with classification of severity
[2]P-value of testing the association of a given factor with the level of severity (groups 1-4).
[3]P-value of testing the association of a given factor with the level of severity (groups 1-3).

Summary and Conclusions: The PKD NHS represents the largest cohort of pts with PKD assembled to date. We have identified 4 distinct severity groups, based on transfusion history, degree of anemia, and splenectomy status. Complications, such as cholecystectomy and iron overload, are correlated with severity of disease. Categories of severity will be validated in the next 75 PKD pts enrolled on the PKD NHS, and prospectively followed in this cohort for at least two years. This classification may be helpful for determining monitoring and treatment practices in this rare anemia.

P376

EFFICACY AND SAFETY OF VITAMIN C AS AN ADJUVANT TO IRON CHELATION THERAPY IN YOUNG PATIENTS WITH B-THALASSEMIA MAJOR: A RANDOMIZED PROSPECTIVE TRIAL

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Background: Ascorbate deficiency is common in iron overloaded subjects and may modulate iron distribution. Animal studies showed that ascorbate replacement in the presence of desforaxamine (DFO) can markedly improve urinary iron elimination.
Aims: To investigate the effects of vitamin C as an adjuvant therapy to the three used iron chelators in moderately iron overloaded young vitamin C-deficient β-thalassemia major (β-TM) patients in relation to tissue iron overload.
Methods: This randomized prospective study was registered in the ClinicalTrials.gov (NCT02083575). Inclusion criteria were moderately iron overloaded β-TM patients who had vitamin C deficiency, without clinical symptoms of cardiac dysfunction and with serum ferritin (SF) >1000-2500 ng/mL and cardiac T2* >10 ms. Of two-hundred ninety β-TM patients screened for eligibility, 180 were enrolled. Patients were randomly assigned into 3 groups (60 patients in each group) receiving DFO, DFP or DFX. Patients in each of the 3 chelation groups were further randomly divided into 2 subgroups according to vitamin C supplementation. Thirty patients in each chelation group received morning oral vitamin C in a dose of 100 mg daily. All patients received vitamin C (group A) or no vitamin C (group B) were followed-up for one year with assessment of transfusion index, hemoglobin, iron profile, liver iron content (LIC) and cardiac magnetic resonance imaging (MRI) T2*.
Results: Baseline clinical, laboratory and radiological variables were similarly distributed among β-TM patients with and without vitamin C supplementation and also, among patients on the three used chelating agents. Transfusion index, serum iron, serum ferritin (SF), transferrin saturation (Tsat) and LIC were significantly decreased among group A β-thalassemia patients after vitamin C supplementation compared with baseline levels ($p<0.05$). Hemoglobin and vitamin C levels as well as cardiac MRI T2* were significantly increased at the end of treatment ($p<0.001$). No significant difference was found between baseline and study end as regards any of the studied variables among thalssemia patients who did not receive vitamin C therapy (group B). After vitamin C supplementation, DFO-treated patients had significantly lower transfusion index ($p=0.004$), serum iron ($p<0.001$), SF ($p<0.001$), Tsat ($p<0.001$) and LIC ($p<0.001$) while hemoglobin ($p<0.001$) and cardiac MRI T2* ($p=0.017$) were increased compared to baseline levels. Patients on DFP or DFX showed non-significant improvement in hematological variables. Cardiac MRI T2* was significantly higher among DFP-treated patients ($p=0.002$) while LIC was significantly decreased in patients receiving DFX after vitamin C supplementation ($p<0.001$). When the three thalassemia subgroups were compared post therapy, DFO-treated patients had the highest hemoglobin ($p=0.046$) and vitamin C levels ($p=0.038$) with the lowest iron ($p=0.032$), SF ($p=0.021$) and Tsat ($p=0.033$) compared with the other two subgroups. No significant difference was found between the 3 groups as regards LIC or cardiac MRI T2*. Baseline vitamin C levels were negatively correlated to SF and LIC ($p<0.001$).
Summary and Conclusions: vitamin C supplementation to patients with β-TM represents a potential therapeutic adjuvant agent increasing the efficacy of iron chelation therapy, decreasing transfusion frequency and elevating hemoglobin levels. Vitamin C in a dose of 100 mg possibly potentiates the efficacy of DFO more than DFP and DFX in reducing iron burden in the moderately iron overloaded vitamin C-deficient β-TM patients, with no adverse events.

P377

TREATMENT OF IRON DEFICIENCY ANAEMIA OF LATE PREGNANCY WITH A SINGLE INTRAVENOUS IRON POLYMALTOSE OR FERRIC CARBOXYMALTOSE VERSUS ORAL IRON SULPHATE: A PROSPECTIVE RANDOMIZED CONTROLLED STUDY (TIDAL)

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Background: Conferring the World Health Organisation (WHO) statistics, iron deficiency anaemia (IDA) is the most common nutritional deficiency disorder in the world, affecting at least two billion people, with pregnant women and young children at particularly high risk. If IDA in pregnancy is not managed properly, it may have a significant effect on mothers and babies with serious maternal and neonatal consequences. Therefore, we are proposing a pragmatic solution by employing in our trial intravenous iron that is considered an effective and safe tool in replenishing the iron stores and hence, eliminating serious consequences of IDA.
Aims: To assess the improvement in predelivery haemoglobin and iron stores after treatment with the standard care with oral iron *versus* the standard IV iron formulation "iron polymaltose" (long duration infusion) *versus* newly available IV "iron carboxymaltose" (short duration infusion) therapy in pregnant Tasmanian women, Australia.
Methods: A three arm randomised controlled trial (RCT) that aims to evaluate the treatment effects on Tasmanian pregnant women with iron deficiency anaemia, with (A) standard treatment with oral iron sulphate 325mg (elemental 105 mg) daily until delivery *versus* (B) a single dose of IV 100 mg iron polymaltose (IPM) administered over 60 minutes *versus* (C) a single dose of IV ferric carboxymaltose (FCM) administered over 15 min. We recruited 246 pregnant women after informed consentbetween September 2013 and August 2014 at our tertiary referralcentre. Median age was 28 years (range, 18-47) with a median and mean gestational age of 27 weeks. Median baseline serum ferritin level was 9 mcg/Lwith a median serum transferrin saturation of 13%.
Results: The ferritin level was significantly higher in FCM group against both Oral iron andIPM group ($p<0.001$) with a difference of 73.9 mcg/L. However, no difference was noted between the IVgroups with each IV iron showing significantly higher ferritin at 4 weeks and pre-delivery compared to oral iron.The ferritin is significant in FCM group against both oral and iron polymaltose groups ($p<0.001$) but there were no significant differences against FCM and IPM groups in terms of Hb or ferritin increment. Hb levels increased significantly at 4 weeks in FCM group compared to oral iron group ($p=0.05$) however the difference declined in pre-delivery Hb ($p=0.07$) with more apparent increase in terms of ferritin compared to the oral group at both 4 weeks and pre-delivery time points ($p<0.0001$). Cord blood ferritin was significantly higher in both IV iron groups against oral iron groups with p values of 0.05 in FCM and 0.03 in IPM without such difference in terms of cord Hb increment ($p=0.2$ and 0.8 respectively).A cost effectiveness analysis showed the total cost of administration of 1000mg of FCM is less than a similar dose of IPM and both were more economical than oral iron in terms of effectiveness.No blood transfusion was recorded in the IV iron groups, however two patients in the oral group received 2 units of blood each, however statistically not yet significant ($p=0.2$)
Summary and Conclusions: We compared two different single IV iron infusion in set of 1g *versus* 4 weeks of oral iron sulphate 325mg daily (total dose 9.1g) for the treatment of moderate IDA of pregnancy. It is of interest that IV dose compromise 1/9 of the oral dose and showed significant superior results than oral iron at 4 weeks of treatment in both ferritin and Hb levels. Even the benefits extended in terms of ferritin increment till the delivery time point ($p<0.001$), where the patients received in average a total of 27 g of oral iron *versus* 1g for the IV groups. Our data indicate thatFCM application during pregnancy is safe and leads to improved efficacy and improvement of iron stores compared to oral iron in pregnancy-related ID. There were no significant difference between FCM and IPM groups. However, FCM is considered cost effective and more-convenient to the patients. **Trial registration:** (http://www.ANZCTR.org.au/ACTRN12613000853741).

P378

HYDROXIUREA PRESCRIPTION, AVAILABILITY AND USE FOR CHILDREN WITH SICKLE CELL DISEASE IN ITALY:RESULTS OF THE ITALIAN ASSOCIATION OF PEDIATRIC HEMATOLOGY ONCOLOGY (AIEOP) MULTICENTER SURVEY

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Background: Hydroxyurea (HU) is widely recommended for treatment of children with Sickle Cell Disease (SCD) in developed countries, but information about its use comes mainly from studies performed in adults or in the United States. The National Guidelines for the Management of SCD in Childhood in Italy issued in 2012 by the Italian Association of Pediatric Hematology Oncology (AIEOP; www.aieop.org) strongly recommend HU treatment for specific disease complications; a chapter dedicated to HU therapy is included in the Guidelines. In Italy, where newborn screening is not available in most Regions, children can receive HU free of charge from Pharmacies or Hospitals located near home

upon presentation of the rare disease certificate and a HU prescription made by a physician. The only formulation available in Italy is the 500 mg capsule, not divisible. Data regarding the use of HU for children with SCD in our country are lacking.
Aims: To evaluate HU prescription, availability and use in children (<19 yrs) with SCD in Italian Pediatric Centers through a national survey.
Methods: The 22 Pediatric Hematology Oncology Units of the AIEOP were invited to participate to the survey. Eight Centers in 4 different Regions responded. Data were retrospectively collected from clinical charts in each center and included in a dedicated excel database for analysis.
Results: Out of 441 Children followed in 8 Centers, 173 (39%) were prescribed HU: 98 M, mean age at diagnosis 30 months (range 0-198 months). 72% are African, 18% European, the remaining from Middle East/South America. 94% are SS/Sβ°, 3% SC, 3% SB+/Other. Mean age at HU beginning is 90 months (range 11-221) but is higher in SC patients (120 months, range 46-165). While in 70% of the children the diagnosis of SCD is made before 3 yrs of age, less than 10% begins HU before 3 yrs. Indications for HU therapy are: 29% recurrent Vaso-occlusive crisis, 20% recurrent Acute Chest Syndrome, 16% both, 24% anemia <7 g/dl, 1% retinopathy, 1% refusal of transfusion, 9% various causes. Mean starting dose is 16 mg/kg (range 6-30), mean dose reached after 6 months is 21 mg/kg (range 10-32). 87% of the patients use the 500 mg Oncocarbide capsule, 6% a galenic prepared according to patients' weight and drug dosage pro kg, 2% the 100 mg Siklos tablets, purchased from abroad. Frequently, parents had to split the capsule or change to non-daily drug schedule (2-3 times weekly) due to the limited availability of a pediatric formulation adequate for low weights. 62% of the HU prescriptions by pediatric hematologists occurred after 2012. Surprisingly, 6 Centers (36/173 patients) use combination of HU treatment coupled with chronic transfusion (exchange or top up). Most children (>95%) achieved good hematological and clinical response after 6 months of HU treatment and 158/173 are still on HU with a mean FUP of 4 yrs (range 2 months-10 yrs); reasons for interruption were lack of clinical response with start of a transfusion program or bone marrow transplantation. No major complication have been reported.
Summary and Conclusions: This is the first survey regarding the use of HU in children with SCD in Italy, a European country with a recent increase of SCD patients. Among other results, we underline the significant number of children receiving treatment, the majority having begun HU after 2012, year of the National Guidelines publication. Some criticisms have been identified, mainly associated with no pediatric formulation available (low dose tablets or syrup), the relative high age of HU beginning, and a maximum dose lower than recommended.

P379

NOVEL SICKLE CELL DISEASE DIAGNOSTIC DEVICE DEMONSTRATES HIGH PERFORMANCE AT THE POINT OF CARE

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Background: Sickle cell disease (SCD) is one of the most common inherited blood disorders in the world[1,2]. Although newborn screening for SCD has become standard of care in many high-resource countries, many low-resource areas remain unable to diagnose patients at early age due to the expense and difficulty of current testing mechanisms[3]. Furthermore, current standard of care testing methodology (hemoglobin electrophoresis, iso-electric focusing, and high-performance liquid chromatography) take time to perform and results are often delivered to affected families (or individuals) weeks after the initial testing is performed. To improve the diagnosis of individuals worldwide with SCD, a point of care test is needed. According to the College of American Pathologists, a point of care (POC) test is designed to be used where the patient is located, does not require permanent dedicated space, and can be performed outside the physical facilities of the clinical laboratories. Having a low cost, portable POC test for SCD will a). Greatly enhance the capacity for diagnosis in rural or low-resource areas b). Reduce the time between testing and diagnosis and c). Improve the ability to deliver results, counseling, and education at the time of testing.
Test: To address this gap in technology, we have developed a novel, innovative point of care (POC) test for sickle cell disease (SCD) or sickle cell trait (SCT). This **Sickle SCAn™ test kit** is a rapid, qualitative lateral flow immunoassay kit for the identification of sickle cell disorder of hemoglobin A, S, and C. The test requires five microliters of blood added to a provided, buffered loaded Module to release hemoglobin by lysing erythrocytes. The resulting hemolyzed solution is dropped on to the sample inlet of the Sickle SCAn™ cartridge. The treated sample flows through the test cartridge in order to interact with antibody-conjugated colorimetric detector nanoparticles and travel to the capture zones. A total of four detection lines are possible including hemoglobin variants A, S, C and a control (compound heterozygotes will show all hemoglobin present, see Figure). Prior to this clinical study, an ex-vivo laboratory testing using venous blood samples was performed. Sickle SCAn™ was compared to hemoglobin electrophoresis and HPLC using guidelines. Patient samples (n=126) were collected and measured in duplicate on both systems. Sickle SCAn™ performance compared to hemoglobin electrophoresis based diagnosis demonstrated >99% specificity and sensitivity for samples of HbAA, HbAS, HbSS, HbSC, and HbAC. The Sickle SCAn™ limit of detection for hemoglobin A, S, and C was determined to be 20%, 1%, and 2%, respectively.
Aims: To assess the level of agreement between sickle cell disease genotype measured using the Sickle SCAn™ device by finger stick at the true point of care compared to known genotype of affected individuals.
Methods: IRB approval was obtained from the Medical University of South Carolina to test current SCD patients and their families using a POC device. 75 patients provided informed consent to undergo capillary sampling at the MUSC SCD clinic. Blood was obtained using a standard lancet and was collected using the capillary sampler provided in the testing kit and added to the buffered loaded module. After inverting the module x 2, the hemolyzed samples were dropped onto the inlet of the Sickle SCAn™ cartridge. Results were read within 120 seconds. Patients of all ages were included in the trial. Those who had received a blood transfusion within the last 60 days or family members whose hemoglobin genotype was not confirmed were excluded. Patients on Hydroxyurea were included in sample analysis. 43 patients met inclusion/exclusion criteria and are included in the results below.
Results: Forty-three patients with HbAA, HbAS, HbAC, HbSS and HbSC were included. Patients ranged in age from 5 weeks-72 years. The majority of individuals were 16-32 years of age. Hemoglobin of individuals tested ranged from 5.9g/dL to 14g/dL. Sample results are shown below with confirmed genotypes (table 1).

Tabella 1.

Number of Samples	Sickle SCAn™ test result	Confirmed Genotype
16	S	14 HbSS
		2 HbSB0
		1 HbSB+
7	SC	7 HbSC
12	AS	1 HbSD
		11 HbAS
3	AC	3 HbAC
5	A	5 HbAA

The Sickle SCAn™ test performed favorably with only 1 false negative result (patient with confirmed HbSD disease). In 43 subjects, this test detected the correct A, S, C presence with an overall diagnostic accuracy of 97.7%. Detailed data is shown in table 2 below.

Tabella 2.

	Sensitivity (95% CI)	Specificity (95% CI)
SCD (HbSS, HbSC, HbS +-thal, HbS 0-thal)	100% (87-100)	100% (84-100)
Sickle trait (AS	100% (74-100)	96.9% (84-99)
C trait (AC)	100% (44-100)	100% (91-100)
Normal (AA)	100% (57-100)	100% (91-100)

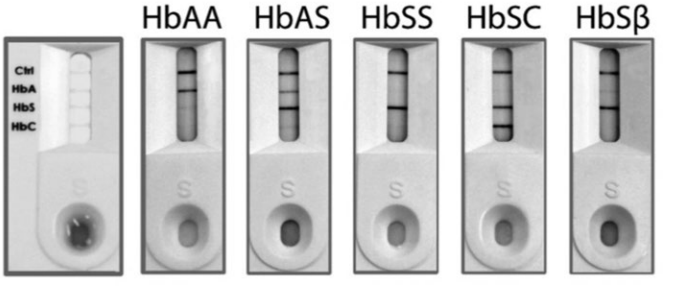

Figure 1.

Summary and Conclusions: Results indicate the Sickle SCAn™ test is highly specific and sensitive for the diagnosis of SCD at the point of care using a capillary blood samples. The Sickle SCAn™ test is especially innovative in its use of lateral-flow immunoassay technology which favors its use for large-scale screening efforts in low-resource settings. In practice, this means the test does NOT require electricity or high-level equipment. The results are easily interpreted with the naked eye with detection lines that are interpreted visually (similar to a home pregnancy test). Our goal is to make POC testing widely accessible with the same level of sensitivity and quantitation specificity as complex and expensive central lab tests. The Sickle SCAn™ test kit includes everything needed for performing SCD diagnostics outside of the laboratory setting. Further testing in children less than 6 months of age is in progress to assess the feasibility of Sickle SCAn™ test for newborn screening.

P380

TRANSCRANIAL DOPPLER IN A EUROPEAN COHORT OF SICKLE SC PEDIATRIC PATIENTS

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Background: Sickle Cell Disease (SCD) is the most frequent severe genetic disease worldwide. It's the most prevalent genetic disorder in France and the UK; its frequency is steadily rising in several European countries, including Italy and Ireland. Sickle SC disease (HbSC) is the second commonest form of SCD after sickle cell anaemia (HbSS/HbSB°) and accounts for 25–30% of cases. Neurological events are among the most frequent and disabling complications in children with SCD with an important impact on quality of life, health and educational system costs. Overt stroke, silent infarcts and psycho-cognitive impairment are reported to occur also in HbSC disease, although at a lower frequency than in HbSS. Studies suggest that the life-time risk of stroke in HbSC is 2–3%. At present, a screening program is available for stroke prevention using Transcranial Doppler (TCD) according to the Stroke Prevention Trial criteria, but only for HbSS/HbSB° patients. There is no specific evidence to guide stroke prevention in HbSC. TCD ranges of velocities in the Middle Cerebral Artery (MCA) and in the distal Internal Carothid Artery (dICA) used to stratify patients with HbSS/HbSB° in risk categories might be inappropriate for HbSC patients. The Sickle Cell Anaemia transcranial Doppler Educational Study (SCATES) is a Multicenter European Educational Study to facilitate a screening program with the purpose to achieve systematic evaluation of stroke risk in children. It has the objectives to standardize TCD application in different European Settings and make TCD a common practice in routine health care of children with SCD across United Kingdom, Ireland and Italy.
Aims: To determine mean reference values of velocities in MCA and dICA in a European prospective cohort of children with HbSC. To evaluate possible clinical and hematological risk factors of high velocities in HbSC disease.
Methods: TCD was performed at least once a year in children with HbSC disease aged 2-18 years. TCD, clinical and hematological data were prospectively collected in clinical charts and transferred in a web based dbase for statistical analysis. Descriptive statistics and regression analysis were performed.
Results: 227 HbSS and 61 HbSC were enrolled in SCATES. In HbSC patients mean MCA velocity was 95.70 cm/sec (range 54.3-132, SD 19.07) and dICA was 79.81 cm/sec (range 29-138, SD 19.29), while in SS patients velocities were much higher: MCA was 122.47 cm/sec (range 57-190, SD 24) and dICA was 107 cm/sec (range 61-176, SD 22.21). Bootstrap analysis allowed to define a mean velocity in the MCA of 98.68 cm/sec (95%CI 93.75-103.60 (N), 94.00-103.77 (P), 94.06-103.92 (BC)). There was no significant correlation between high velocities in the MCA or dICA in HbSC patients and any clinical or hematological parameters except for Diastolic Blood Pressure (p 0.030, 95%CI 0.0939-1.593). Evaluation of Magnetic Resonance Imaging (MRI) available for 15 patients did not show any correlation between stenosis and TCD velocities of the corresponding vessels.
Summary and Conclusions: This is the largest cohort of pediatric patients with HbSC disease evaluated for stroke risk using a standardized protocol, reproducible across Europe. Mean velocities are lower than the reported ones for HbSS/SB° patients and could aid in defining stroke risk categories for this group. Diastolic Blood Pressure is an important risk factor and prompts to a regular Blood Pressure monitoring and control in children with HbSC.

P381

INTRAVENOUS IRON CARBOXYMALTOSE VERSUS STANDARD CARE IN THE MANAGEMENT OF POSTOPERATIVE ANAEMIA: A PROSPECTIVE RANDOMIZED CONTROLLED TRIAL

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Background: Data from literature suggest that by having postoperative normal Haemoglobin (Hb), patients will recover significantly better after major surgery. There is growing evidence that improving the recovery period after surgery will impact on overall outcome of the surgical procedures. Despite the significant efforts in management of the preoperative, it has been found that there is a significant, although unknown, number of patients who have postoperative iron deficiency anaemia. Furthermore, there is no data regarding prevalence and management of postoperative iron deficiency anaemia and the requirement of blood transfusion in patients who undergo different surgeries.
Aims: This study is aiming to show that repleted iron stores and hence improved haemoglobin post-operatively, will improve outcomes of major surgery. Furthermore, this approach is aiming to minimise requirement for blood transfusion and the patient's stay in hospital and to lower the overall cost of procedures and to relieve the extra burden on health systems.
Methods: A prospective randomized controlled study was offered after an informed consent to adult patients who are 18 years old and above with documented Hb level at day 1 postoperatively between 70 and 120 g/L and reduced iron stores with ferritin <100 or iron saturation <20%. Patients were randomized between standard care *versus* active intervention with a single intravenous iron carboxymaltose infusion. Assessment during hospital stay and at 4 weeks was conducted to measure patients' outcomes. During the period between December 2014 and March 1st 2015, we have recruited 106 patients postoperatively after major surgery at the Launceston General hospital, a tertiary referral hospital for Northern Tasmania, Australia. Male to female ratio was 52:54 with median age of 65 years (range, 22:90). Vast majority of patients underwent major orthopaedic surgery (68), then come abdominal (18) and genitourinary surgery (12), and others (8).
Results: Mean day 1 postoperative Hb was 103 g/L with a median of 105 g/L. At four weeks assessment of Hb, there was a significant increase of mean Hb in the intervention group to 131 g/L with reduction in transfused blood units *versus* Hb of 116 g/L in the standard care group (p<0.01).Intravenous single iron carboxymaltose was commenced in average of 1000 mg as short infusion over 15 minutes and was well tolerated by the patients postoperatively.
Summary and Conclusions: Our data show the feasibility and safety of applying iron carboxymaltose in the postoperative anaemia setting. Delays in the identification of patients with preoperative anaemia may delay both, proper anaemia management and the operative procedure. We employed a pragmatic alternative or complementary approach to preoperative assessment for anaemia at day 1 postoperatively. A significant number of surgical patients who lost blood during surgery with an underlying iron deficiency could benefit from postoperative management with iron infusion. The new therapy with iron carboxymaltose was well tolerated. Further trials to assess our novel approach in treatment of the post-operative anaemia are warranted.
Trial registration: The study was approved by the Tasmanian Human Research Ethics Committee, Australia and registered in the Australian New Zealand Clinical Trials Registry (http://www.ANZCTR.org.au/ACTRN12614001261606).

P382

ORAL HIGH DOSE LIPOSOMIAL IRON SUPPORT IS SAFE, FAST, WELL TOLERATED AND COST-EFFECTIVE AS INTRAVENOUS IRON IN SIDEROPENIC ANEMIA. MULTICENTRIC RANDOMIZED STUDY

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Background: In iron deficiency anemia support with intravenous iron allows a faster anaemia correction and a faster ferritin increase than iron sulfate. Frequently iron sulfate and intravenous iron generate adverse events as hypotension, urticarioyd reactions, shock, epygastralgia, constipation or diarrhea. High doses of oral iron frequently are poorly tolerated because of adverse events.
Aims: Aim of this study is to verify if high doses of oral liposomial iron are safe, cost-effective and well tolerated as standard doses of intravenous ferrigluconate in patients with iron deficiency anemia.
Methods: We considered two group of patients (RANDOMIZED 1:1) with iron deficiency anemia without other relevant comorbidities. In group A M/F was 2/3, 7 patients had haemorragic gastritis, 3 hemorragic enteric bleeding angiodysplasia, 10 hypermenorrhaea, median level of hemoglobin (Hb) was 8 g/dl (R 7-10), median ferritin level was 10 ng/ml (R 3-20), with a normal level of CRP or ESR, and received liposomial iron 30 mg 4 tablet/day. In group B M/F was 1/3, 9 patients had haemorragic gastritis, 1 hemorragic enteric bleeding angiodysplasia, 10 hypermenorrhaea, median level of Hb was 8.5 g/dl (R 8-9.5), median ferritin level was 8 ng/ml (R 2-18), with a normal level of CRP or ESR, and received iv sodium ferrigluconate 62.5 mg iv in NS 100 ml in 3 h/day. The median treatment costs in each group were calculated considering the monthly global treatment cost for each patients in the treatment period. This provided an estimate of the costs, independent of the precise cost of the drug, but tied to the final outcome (efficacy) of the therapeutic strategy used during the observation period.
Results: In group A, 1 g Hb increase was observed after a median of 8 days (R 7-12), a target Hb level of 12 g/dl was achieved in a median time of 4 weeks (R 2-4) with a median cost of € 110/months (R 92-162), 6 (30%) patients showed adverse events (3 epigastralgia, 3 diarrhoea). In group B, 1 g Hb increase was observed after a median of 7 days (R 6-10), a target Hb level of 12 g/dl was achieved in a median time of 3.5 weeks (R 1.5-4) with a median cost of €326/months (R 250-360), 4 (20%) patients showed adverse events (2 hypotension, 2 urticaria and headhace).
Summary and Conclusions: Oral high dose liposomial iron support is safe, fast, well tolerated and cost-effective as intravenous iron in sideropenic anemia. This study needs confirmation on a larger cohort of patients.

P383

SURVIVAL AND CAUSES OF DEATH IN PATIENTS WITH ALPHA-AND BETA-THALASSAEMIA IN A DEVELOPING COUNTRY: THE FIRST REPORT FROM THAILAND

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Background: α-and β-thalassemia (thal) syndromes are the most common genetic condition found in Thailand and other Southeast Asian countries. It is expected that >40% of Thai population are carrier for thal and haemoglobinopathies resulting in >600,000 Thai people expected to have thalassemia diseases. With recent advances on thalassaemia management *i.e.* stem cell transplantation, transfusion support, iron monitoring and chelation, survival of transfusion dependent thalassaemia (TDT) in particular; β thal major have increased significantly in developed countries in the past recent years. However, there was limited data of thalassaemia survival and the causes of death in developing countries and none from Thailand. Additionally no data on survival of a less severe thalassaemia; Non Transfusion Dependent Thalassaemia (NTDT) is available.
Aims: To evaluate survival and causes of death of Thai patients with β thal major,β thal/Hb E and α thalassaemia (Hb H and Hb H/Hb CS) who received their treatment under standard health care system in Thailand.
Methods: We retrospectively analysed our hospital registry for patients who were diagnosed at our department since 1974 until December 2013. The diagnosis of thalassaemia syndromes was performed based on the standard clinical evaluation, haematology, haemoglobin and/or molecular analyses. After our diagnosis, most patients were referred back to be treated locally at their primary or secondary health care centers. All patients from our registry were verified and determined their survival through our National Health Registry database (started in 1996). Their death rate and causes of death were evaluated and compared with those of age-sex matched general Thai population during 2004-2013. Survivals were analysed by the Kaplan-Meier and compared by different syndromes, sex, decade of birth cohort (<1990, 1991-2000 and >2001) using the log-rank test. A statistically significant difference was defined at $p<0.05$.
Results: Total 4,303 thalassemia patients with the oldest patientborn in 1961 were registered in which 2,623, 317, 691 and 672 wereβ thal/Hb E,β thal major, Hb H and Hb H/CS, respectively. Sixty five percentage of these patients (n=2,570) could be identified using the National database at Ministry of Public Health, Thailand. The ratio of male: female was around 50:50 across all syndromes. The mean ages±SD of alive patients were 22.35±10.61, 17.63±7.71, 24.86±12.16 and 20.83±9.54 in β thal/Hb E (n=1,650), β thal major (n=165), Hb H (n=386) and Hb H/Hb CS (n=414), respectively. Patients withβ thal major (28 death; 17%) had the highest death rate (7.5 times over matched population controls) followed byβ thal/Hb E (146 death; 9.1%) (3.1 times compared to controls) while patients with α-thalassaemia (Hb H (6 death; 1.6%) and Hb H/Hb CS (9 death; 2.2%) who were all NTDT had the same mortality as general population. The majority of β thal major died from anemia and very few due to heart failure suggesting most patients received inadequate transfusion and only 20% of patients could reach their forth decade of life (**Figure 1A**). Up to 50% ofβ thal/Hb E patients that around 70% of them were NTDT phenotype could live to the age of 50 (**Figure 1A**). Infection was the major cause of death inβ thal/Hb E possibly related to splenectomy that widely practiced for such condition in Thailand. The majority of Hb H patients died from accidents and other natural causes not related to underlying disease similar to general population. Increasing survival was observed, although not statistically significant, inβ thal major patients who born in recent birth cohorts (**Figure 1B**) suggesting an improvement of thalassaemia care in general. There was no difference on survival by different sex for all thalassemia syndromes.

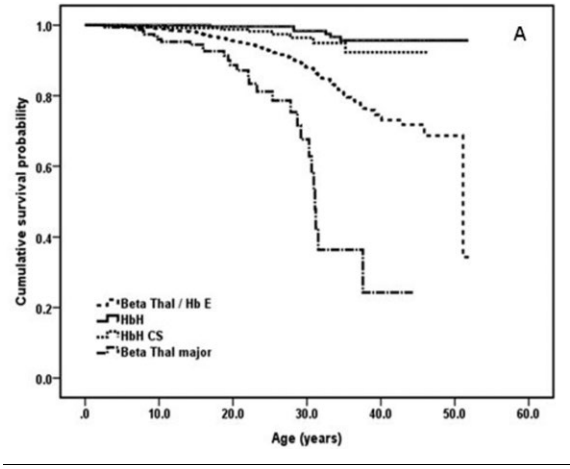

Figure 1. A) Kaplan-Meier srvival curves for all types of thalassaemia syndromes in Thailand; and B) by birth cohort in β thal major.

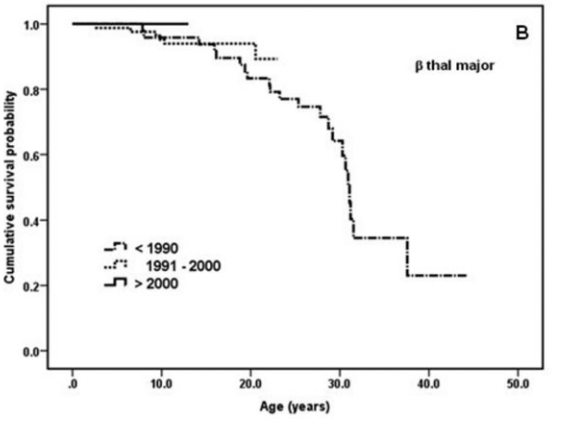

Figure 1. A) Kaplan-Meier srvival curves for all types of thalassaemia syndromes in Thailand; and B) by birth cohort in β thal major.

Summary and Conclusions: For the first time, our data suggest that survival and causes of mortality were different among thalassaemia syndromes; α-thalassaemia had a comparable survival to general population while the survival of β-thalassaemia was remarkably poor compared to those of developed countries. This suggests that management of severe thalassaemias in a developing country remains suboptimal and an improvement on standard of care is urgently required to enhance a long –term survival of these highly prevalent conditions.

P384

LONG-TERM FOLLOW-UP ON YOUNG PEDIATRIC PATIENTS TREATED WITH DEFERASIROX

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Background: The oral iron chelator, deferasirox (DFX), is approved for the treatment of transfusional hemosiderosis in both adult and pediatric patients. Safety data from 52 pediatric patients, aged 2–<6 years, who received DFX for 1 year, in registration studies were consistent with other age groups. Majority of adverse events (AEs) were transient in duration and of mild to moderate in severity. In order to collect long-term data on the safety and efficacy of DFX in young children, a 5-year observational multinational registry study was initiated.
Aims: To evaluate the long-term safety and efficacy of DFX in an unselected population of children aged 2–<6 years at enrollment and with transfusional hemosiderosis.
Methods: Pediatric patients (2–<6 years of age) receiving DFX according to local prescribing information were enrolled and followed for up to 5 years. The primary objective was to evaluate the safety profile of DFX, specifically, renal and hepatic function. Growth and sexual development, and long-term efficacy measured by serum ferritin, were also assessed. This interim analyses reports data for up to 61 months since study initiation.
Results: Of 268 evaluable patients, 99 completed, 107 (mean age, 3.3±1.2 years) patients had ≥4.5 years of clinical data, 126 discontinued, and 43 were ongoing at time of data cut off. Diagnoses included: β-thalassemia (n=177, 66.0%), sickle cell disease (n=52, 19.4%), Diamond–Blackfan Anemia (n=12, 4.5%), and other anemias (n=27, 10.1%). Most common reasons (>5%) for study discontinuation were "others" (20.1%), lost to follow up (7.5%), and protocol deviation (5.2%), while 11 (4.1%) patients discontinued due to AEs. There was one fatal event due to graft *versus* host disease. Mean±SD of DFX exposure was 43.1±21.5 months and average±SD actual dose was 25.9±6.6 mg/kg/day. Median serum ferritin decreased from 1708 to 1050 ng/mL after 61 months. Most common AEs related to DFX for the overall population and for those completing ≥4.5 are presented in Table 1. Serious AEs (SAEs) regardless of relationship to study drug were seen in 86 (32.1%) patients, most common (≥5%) being pyrexia and pneumonia in 24 (9%) and 15 (5.6%) patients, respectively. Of 107 patients, 31 (29.0%) had SAEs. Of these 3 SAEs in 2 (1.9%) patients were suspected to be related to study-drug, including accidental overdose, increase in aspartate aminotransferase (AST) and increase in alanineaminotransferase (ALT). At data cut off, 227 (85%) patients had serum creatinine (SrCr) ≤upper limit normal (ULN); 7 (2.6%) patients had SrCr increases >33% and >ULN on 2 consecutive measurements. In patients completing 4.5 years, 2 (1.9%) patients had SrCr increases >33% compared to baseline

and >ULN on 2 consecutive measurements. These cases were resolved without dose adjustments. Increases in ALT >5xULN and 10xULN were observed in 6 (2.2%) and 4 (1.5%) patients, respectively. In patients completing ≥4.5 years, elevated ALT >5xULN on 2 consecutive post-baseline assessments was seen in 1 (0.9%) patient and resulted in drug interruption. Elevated ALT >10xULN on 2 consecutive post-baseline assessments was observed in 2 (1.9%) patients and drug was not interrupted.

Tabella 1. Most common investigator-assessed drug-related AEs (≥4 patients, from the overall population).

Most common investigator-assessed drug-related AEs (≥4 patients) by preferred term*		
AEs, n%	Overall Population (Safety Set) (N=267)*	Patients (Safety Set) (N=107)
Patients with investigator assessed drug-related AE(s), n (%)	102 (38.2)	43 (40.2)
ALT increased	37 (13.9)	12 (11.2)
AST increased	26 (9.7)	6 (5.6)
Transaminases increased	13 (4.9)	5 (4.7)
Rash	13 (4.9)	5 (4.7)
Vomiting	12 (4.5)	6 (5.6)
Blood creatinine increased	10 (3.7)	3 (2.8)
Abdominal pain	8 (3.0)	3 (2.8)
Liver function test abnormal	8 (3.0)	8 (7.5)
Diarrhea	5 (1.9)	3 (2.8)
Proteinuria	5 (1.9)	1 (0.9)
Hepatocellular injury	4 (1.5)	1 (0.9)
Blood bilirubin increased	4 (1.5)	1 (0.9)
Hepatic enzyme increased	4 (1.5)	1 (0.9)
Hematuria	4 (1.5)	2 (1.9)

AEs, adverse events; ALT, alanine aminotransferase; AST, aspartate aminotransferase.
*Safety set defined as, all patients who received at least one dose of deferasirox over the analyzed period and had at least one post-baseline safety assessment

Summary and Conclusions: The results show a favorable safety profile of DFX with long-term treatment in pediatric patients of 2–<6 years age. Reported increases in ALT parameters above 5 times ULN were consistent with previous studies. Conversely, increases in SrCr were lower than those observed in previous studies. These results continue to support that DFX is effective in decreasing serum ferritin levels in the pediatric population.

Infectious diseases, supportive care 1

P385

COMPARISON OF MICAFUNGIN AND LIPOSOMAL AMPHOTERICIN B FOR EMPIRICAL ANTIFUNGAL THERAPY IN FEBRILE NEUTROPENIC PATIENTS WITH HEMATOLOGICAL MALIGNANCIES: A RANDOMIZED CONTROLLED TRIAL

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Background: Invasive fungal infections (IFIs) incur significant morbidity and mortality among neutropenic patients after chemotherapy. The risk for IFIs is related to the intensity and duration of neutropenia, and varies from 2 to 40%. Mortality rates associated with documented IFIs are considerable, reportedly ranging from 30% to 60%. Empirical antifungal therapy is the standard care for neutropenic patients with hematological malignancies (HEM) who remain febrile despite broad-spectrum antibacterial treatment. Several antifungal agents including voriconazole (VRCZ) or liposomal amphotericin B (L-AMB) have been studied as empirical therapies for febrile neutropenia (FN). However, limited data are available concerning the efficacy of micafungin (MCFG) in febrile neutropenic patients with HEM.
Aims: We conducted a randomized, cooperative group, open-label trial comparing MCFG (150 mg once daily) with L-AMB (2.5 mg/kg once daily) as first-line empirical antifungal treatment for FN patients with persistent fever of HEM.
Methods: Eighty hospitalized FN patients with persistent fever of HEM (AML 56, ALL 8, MDS-AML 7, MDS (RAEB) 3, NHL 4, MM 2 cases) were randomized to each drug group (MCFG, 40; L-AMB, 40). The study drug was started to administer as empiric antifungal therapy at least for 5 days without severe drug toxicity, and the efficacy and safety were evaluated.The efficacy end point was a favorable overall response, as determined by a five-component end point according to the criteria of Walsh et al (N Engl J Med 2004; 351: 1391).
Results: At the time of enrolment, there were no significant differences in the demographics or baseline characteristics between the two groups. The mean treatment duration for MCFG and L-AMB was 16 and 15 days, respectively. There were no significant differences in clinical efficacy between the two treatments (MCFG group, L-AMB group), evaluated based on: (1) successful treatment of baseline fungal infection (no evaluation), (2) absence of breakthrough fungal infection (90.0% *vs* 92.5%, p=0.692), (3) survival for ≥7 days after study completion (87.5% *vs* 85.0%, p=0.745), (4) premature study discontinuation due to poor efficacy (22.5% *vs* 20.0%, p=0.784), and (5) resolution of fever during neutropenia (55.0% *vs* 52.5%, p=0.822). However, discontinuation due to drug-related adverse events occurred less frequently in the MCFG group (12.5% *vs* 35.0%, p =0.018). In safety evaluation, total adverse events were less often in the MCFG group than in the L-AMB group (25.0% *vs* 47.5%, P=0.036).
Summary and Conclusions: MCFG was as effective as L-AMB, and better tolerated than L-AMB as empirical antifungal therapy in FN patients with HEM.

P386

CAN THERAPEUTIC DRUG MONITORING OPTIMISE EXPOSURE OF PIPERACILLIN IN FEBRILE NEUTROPENIC PATIENTS WITH HAEMATOLOGICAL MALIGNANCIES? A RANDOMISED CONTROLLED TRIAL

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Background: Conventional antibiotic doses may recurrently fail to offer adequate exposure in febrile neutropenic patients due to disease-related pharmacokinetic (PK) changes. Therapeutic drug monitoring (TDM) guided dose optimisation is recommended to improve antibiotic exposure although this has not been described for piperacillin in febrile neutropenic patients.
Aims: To describe piperacillin exposure in febrile neutropenia patients and determine whether TDM can be used to increase the achievement of piperacillin pharmacokinetic/pharmacodynamic (PK/PD) targets at PK 'steady state'
Methods: In a two-arm prospective randomised controlled study (Australian New Zealand Registry, ACTRN12615000086561), patients were subjected to TDM for three consecutive days. Dose was adjusted in the intervention group to achieve a free drug concentration above the minimum inhibitory concentration for 100% of the dose interval (100% $fT_{>MIC}$) which was also the primary outcome measure. The secondary PK/PD target was 50% $fT_{>MIC}$. Duration of fever and days to the recovery from neutropenia were recorded.
Results: Thirty-two patients were enrolled. Initially, patients received 4.5g piperacillin-tazobactam eight or six hourly along with gentamicin co-therapy in

30/32 (94%) patients. At the first TDM, 7/32 patients (21%) achieved 100% $fT_{>MIC}$ and 12/32 patients (38%) achieved 50% $fT_{>MIC}$. Following dose adjustment, 69% of the intervention *versus* 19% of the control patients (p=0.012) attained 100% $fT_{>MIC}$, and 94 % of intervention *versus* 31% of control patients (p=0.001) achieved 50% $fT_{>MIC}$. After the third TDM, the proportion of patients attaining 100% $fT_{>MIC}$ improved from a baseline 19% to 73% in the intervention group while it declined from 25% to 7% in the control group. No difference was noted in the duration of fever and days to recovery from neutropenia.
Summary and Conclusions: Conventional doses of piperacillin-tazobactam may not offer adequate piperacillin exposure in febrile neutropenic patients. TDM provides useful feedback of dosing adequacy to guide dose optimisation.

P387

PREDICTORS OF MORTALITY IN 100 PATIENTS WITH NEUTROPENIC ENTEROCOLITIS AFTER CYTOTOXIC AGENTS FOR ACUTE MYELOID LEUKEMIA

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Background: Neutropenic enteroclitis (NEC) is a life-threatening complication of intensive chemotherapy regimen in patients with acute myeloid leukemia (AML) and the optimal treatment of patients with NEC remains controversial.
Aims: This retrospective study analyzed the predictors of mortality for NEC occurring in AML patients undergone to intensive chemotherapy, in order to evaluate the optimal management and the risk factors.
Methods: From January 2002 to December 2012, among 100 patients developing NEC, 30 received chemotherapy protocols based on standard doses of cytarabine (CY) (100 mg/m^2 daily), 19 intermediate doses (1000 mg/m^2 daily) and 51 high doses (6000 mg/m^2 daily). Empirical treatment regimen was defined as the initial antibiotic therapy of neutropenic fever while post-US treatment regimen was the antibiotics used at the time of ultrasonographic (US) diagnosis of NEC. The outcome measured was death within 30 days of the NEC onset. Survivor and nonsurvivor groups were compared.
Results: The overall mortality rates at 30 days after the diagnosis of NEC was 23%. We observed no significant differences in the clinical, ultrasonographic and microbiological characteristics of NEC episodes between survivors and unsurvivors. Patients in the latter group were more likely to have received cytotoxic agent schedule based on high-dose CY (p<0.001). Front-line tigecycline (TYG)-sparing antibiotic regimen (p=0.006) was associated to a higher mortality. Among the 37 patients, whose post-US antibiotic regimens were classified as 2-drug combinations, 13 (35%) died whereas 10 of the 63 patients (16%) who received 3-drug combination regimens died (p=0.02). Mortality rates within the post-US antibiotic combination regimen sub-groups were: 14% (1/7) for those treated with TYG plus piperacillin-tazobactam; 20% (1/5) for those treated with TYG plus meropenem; 33% (2/6) for those treated with meropenem plus daptomycin; 43% (3/7) for those treated with meropenem plus amikacin; 50% (6/12) for those treated with piperacillin-tazobactam plus amikacin; 0% (0/17) for those treated with TYG, meropenem and daptomycin; 14% (2/14) for those treated with meropenem, amikacin and teicoplanin; 22% (4/18) for those treated with ceftazidime, amikacin and teicoplanin; and 29% (4/14) for those treated with piperacillin-tazobactam, amikacin and teicoplanin. The only multidrug regimen of the post-US phase that was significantly more common in the survivor group (p=0.036) was the combination of TYG, meropenem and daptomycin. Overall, the 30-day survival distributions were also significantly different in patients treated with regimens including TYG *vs* regimens sparing TYG in the empirical, post-US and/or post-antibiogram phases of treatment [21/23 patients (91%) *vs* 56/77 (73%) patients, respectively; p=0.01]. Logistic regression analysis indentified cytotoxic schedule based on high-dose CY for hematological remission induction (p<0.0001) and 2-antibiotics combination (p<0.0001) as independent predictors of 30-day mortality, whereas front-line TYG-including regimens (p<0.0001) and post-US combination therapy with TYG, meropenem and daptomycin (p=0.013) were associated with a lower risk of mortality.
Summary and Conclusions: Our study represents the largest cohort of patients with NEC analyzed to evaluate the impact of several factors on mortality. High-CY doses are associated with higher incidence and mortality for NEC in AML patients. Prompt administration of TYG in patients with neutropenic fever as empirical therapy, preceding US diagnosis of NEC, seems to be effective. As for post-US antibiotic therapy, treatment with 3 drugs and overall TYG including regimen seems to improve survival for NEC.

P388

THE ASSOCIATION OF COMBINED ANTIBODY DEFICIENCY AND CHEMOTHERAPY REPRESENTS A HIGH-RISK FACTOR OF NON-NEUTROPENI MAJOR INFECTION IN CLL

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Background: Chronic lymphocytic leukemia (CLL), the most common leukemia in western is still an incurable disease and infections are the major cause of morbidity and mortality. Susceptibility to infections in CLL patients can be an intrinsic characteristic of the disease or due to chemo-immunotherapy.
Aims: In this study we will identify a clinical subset of patients characterized by a very high risk of major infectins, in which Ig replacement therapy (IgRT) should be appropriate.
Methods: We retrospectively analyzed clinical and biochemical data of 706 patients referred to the Hematology and Clinical Immunology Unit of Padua University Hospital. In this series, we identify patients with at least one episode of major infections (MI), defined as those events requiring inpatient management and/or intravenous antibiotic treatment; we excluded chemotherapy related neutropenic MI. FISH analysis (n=450), CD38 expression (n=529), ZAP70 (n=502), IGHV (n=473), TP53, NOTCH1, BIRC3, SF3B1 (176 genes tested) mutational status, were evaluated at diagnosis or before starting treatment. Staging and Ig levels were collected at the time of the MI; for patients who didn't complain any MI we recorded the last available data. We used ROC curve analysis, Mann-Whiney, Fisher exact or Chi-square, Log-rank test, Kaplan-Meier method and Cox model when appropriated. Time to MI (TTMI) was calculated from the date of initial presentation to the MI event or last known follow-up (censored).
Results: 79 patients had 98 MI events, 67 were pneumonia (5 pulmonary aspergillosis), 27 sepsis, 2 meningitides, 1 uveitis and 1 endocarditis. Ig levels were significantly lower in patients with a history of MI with respect to patients who did not experience any serious infections (p<0.0001). Some clinical and biological markers were more common in patients with a history of MI (del 17p [p<0.0001], unmutated IGHV [p<0.0001], CD38$^+$ [p=0.0205], Rai stage III-IV [p<0.0001] and needed treatment [p<0.0001]) than patients without any MI event. No differences were found for ZAP70 expression, TP53, NOTCH1, BIRC3, SF3B1 gene mutations. Using ROC analysis we identified the best Ig isotype cut-offs to identify patients at high risk to development a MI event *i.e.* 7.44g/L for IgG, 0.79g/L for IgA and 0.21g/L for IgM. In the univariate analysis, pretreated patients and subjects with combined antibody deficiency (CAD, *i.e.* low levels of IgG and IgA or IgM) had a shorter TTMI with respect to patients who did not need a specific CLL therapy (239 months *vs* not reached, p<0.0001) or those with only IgG deficiency or Ig within normal range (239 months *vs* 270 *vs* not reached, p<0.0001), respectively. By multivariate analysis the HR in treated patients and those presenting a CAD were 2.98 and 3.10 respectively (p<0.0001 in both cases). We evaluated the importance of the presence of a combination of these two markers using a unique model. Median TTMI was significantly shorter in patients who had both a history of treatment and a CAD than in subjects presenting only one of this prognostic marker (i.e treatment history or CAD presence) (217 months *vs* 258 *vs* not reached, p<0.0001). These data were also confirmed in multivariate analysis.
Summary and Conclusions: We, herein, demonstrated that previously treated patients with CAD carried the highest risk of contracting a major infection during their clinical history. Furthermore, we described the half of incidence of MI (0.044 *vs* 0.019 MI/people-year) in a small cohort of patients with such clinical phenotype after IgRT.

P389

EMERGING RESISTANT BACTERIA STRAINS IN ACUTE LEUKAEMIA PATIENTS: IS THERE STILL A ROLE FOR FLUOROQUINOLONE PROPHYLAXIS? RESULTS OF A PROSPECTIVE STUDY BY THE RETE EMATOLOGICA LOMBARDA (REL)

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Background: Multiresistant (multiR) bacteria are emerging pathogens in haematologic cancer patients (pts). The antibiotic pressure, including fluoroquinolones (Fq) prophylaxis, may be one of the factors responsible for this phenomenon.
Aims: In order to better define the very recent epidemiology and outcome of bloodstream infections (BSI) in a real-life setting, we planned a prospective study collecting all consecutive febrile/infectious episodes occurring in acute leukaemia (AL) pts admitted to 9 haematological institutions participating to REL.
Methods: From Dec-12 to Dec-14, all febrile/infectious episodes were record-

ed. The following data concerning BSI were analysed: age, gender, type/phase of leukaemia, neutropenia, Fq prophylaxis or other antibiotic treatment, presence of central venous catheter (CVC), concomitant pulmonary infiltrates, antibiotic resistance.
Results: In 293 AL pts (M/F 174/119; median age 55y, range 18-80; AML/ALL 238/55), 433 BSI were diagnosed. In 246 (56.8%), BSI occurred in pts on Fq prophylaxis and in 72 (16.6%) in pts already receiving systemic antibiotic therapy. In 94 (21.7%) pneumonia was also present. Gram-positive cocci (GPC) were isolated in 194/433 BSI (44.8%), Gram-negative rods (GNR) in 164 (38.3%), polymicrobial (PMB) aetiology in 68 (15.7%) and fungi (F) in 5 (1.1%). Coagulase-negative staphylococci (CoNS) were the most frequent GPC (130/433, 30%); *S. aureus*, *S. viridans* and enterococci were observed in 10 (2.3%), 24 (4.6%) and 52 (12%) cases, respectively. Methicillin-resistant strains accounted for 76.4% of all staphylococci and vancomycin-resistant for 1.9% of enterococci. Fq resistance was detected in 50.2% of all GPC. GPC aetiology was associated with CVC-related BSI (OR 1.7, CI 1.1-2.6, p=0.009), and was typical of pts not in complete remission (OR 0.7, CI 0.4-0.9, p=0.04). Considering GNR, Enterobacteria were isolated in 155 BSI (35.8%) and *P. aeruginosa* in 37 (8.3%). GNR occurred more frequently during consolidation cycles (OR 1.8, CI 1.2-2.8, p=0.003). Frequency of Enterobacteria BSI was also higher during consolidation cycles in comparison with other AL phases (OR 2.5, CI 1.7-3.9, p<0,0001) and neutropenia was a risk factor (OR 2.2, CI 1.1-4.5, p=0.04). Fq resistance was observed in 88/155 (56.8%) Enterobacteria; in 84.1% of cases it was detected during Fq prophylaxis. ESBL+ strains, which accounted for 21.3% of Enterobacteria, were also associated with previous antibiotic exposure, including Fq prophylaxis (OR 4.05, CI 1.3-12.4, p=0.01). Carbapenemase producing (CP) strains occurred in 9% of Enterobacteria. Among *P. aeruginosa* strains, 21.6% were multiR. Thirty-day mortality was 8.5% (37/433); it was lower, although not significantly so, for GPC (6.7%) in comparison with GNR (9.1%) and PMB BSI (13.2%). CP Enterobacteria or multiR *P. aeruginosa* BSI but not ESBL+ strains were independent predictors of death (30d mortality: 30.8%; OR 5.7, CI 1.9-16.8, p=0.002). Furthermore, having relapsed/resistant AL (18.3%; OR 4.7, CI 2.1-10.4, p=0.0002) and the presence of concomitant pulmonary infiltrates (26.6%; OR 7.5, CI 3.4-16.1, p<0.001) significantly correlated with the risk of death.
Summary and Conclusions: The proportion of multiR GNR is becoming a major problem in our real-life prospective study and negatively impacts on prognosis of AL pts, as well as a concomitant diagnosis of pneumonia during BSI. Fq prophylaxis is not protective against multiR pathogens and it is associated to Fq resistance and to ESBL+ strains. The role of Fq prophylaxis in AL leukaemia patients should be reconsidered.

P390

PATIENTS WITH HEMATOLOGICAL MALIGNANCIES AND ACUTE RESPIRATORY FAILURE FROM UNDETERMINED ETIOLOGY ARE AT HIGHEST RISK OF DYING: A GRRR-OH STUDY

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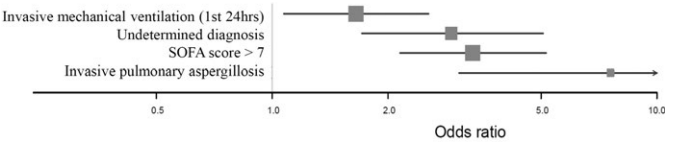
Background: Acute respiratory failure (ARF) is the most frequent complication in patients with hematological malignancies (HM), occurring in 20% to 50%, and is associated with high morbidity and mortality. ARF etiologies are numerous, and despite extensive diagnostic investigations, some patients remain with undetermined ARF etiology.
Aims: We sought to appraise the relation between an undetermined ARF etiology and mortality in a large cohort of critically ill patients with HM, and secondary, the yield of the bronchoalveolar lavage (BAL) in this population as compared with non-invasive tests.
Methods: A post-hoc analysis from a prospective multicenter study of 1011 critically ill hematological patients performed by the French and Belgian research network on critical respiratory diseases in patients with malignancies (Groupe de recherche en réanimation respiratoire en onco-hématologie, GRRR-OH) was conducted. All patients with ARF were included in the statistical analysis. ARF etiology was sought by noninvasive diagnostic tests or bronchoscopy and BAL. Relationship between ARF etiology and hospital mortality was assessed using a multivariable model adjusting for confounders.
Results: Seven hundred and one patients with respiratory symptoms were reviewed and 604 patients with ARF were included. There were mainly males (61%) with a median age of 60 [50-70]. The most prevalent HM were acute myeloid leukemia (AML, 28%) and non-Hodgkin lymphoma (NHL, 27%). Twenty three percent of the patients were in complete or partial remission. One hundred and seven patients (17.7%) were allogeneic hematopoietic stem cell transplantation recipients. Twenty percent of the population had a performans status of more than 1, 47% had a SOFA score over 7 and 30% were neutropenic. At ICU admission, the median respiratory rate was 32 [26-38]/min and two-thirds of the patients had 2 or more quadrants involved on chest radiography. Two hundred and fifty patients (41.4%) needed invasive mechanical ventilation at day 1. Patients were classified into four etiological categories: infectious etiologies (44.4%), non-infectious diagnoses (32.6%), opportunistic infection (10.1%) and undetermined (12.9%), with corresponding mortality rates of 39.2%, 35%, 55.7% and 60%, respectively. Overall hospital mortality was 42.2%. One hundred and fifty five patients had BAL (25,6%), with no impact on hospital mortality or rate of undetermined diagnosis as compared with non-invasive tests (16.8% *vs* 11.8% respectively, p=0.10). By multivariate analysis, factors associated with hospital mortality were invasive pulmonary aspergillosis (OR =7.57 (95% CI 3.06-21.62); p<0.005), invasive mechanical ventilation in the first 24 hours (OR =1.65 (95% CI 1.07-2.55); p=0.02), a SOFA score >7 (OR =3.32 (95% CI 2.15-5.15); p<0.005) and an undetermined ARF etiology (OR =2.92 (95% CI 1.71-5.07); p<0.005) (Figure).

Tabella 1. Multivariate analysis of risk factors for hospital mortality.



Summary and Conclusions: In patients with hematological malignancies and ARF, up to 13% remain with undetermined ARF etiology despite comprehensive diagnostic workup. Undetermined ARF etiology is independently associated with hospital mortality. Studies to better guide second line diagnostic strategies are warranted.

P391

APREPITANT PREVENTS NAUSEA AND VOMITING AFTER A HIGH-EMETOGENIC DOSE OF CYCLOPHOSPHAMIDE FOR PBSC HARVESTING: A PHASE III, DOUBLE-BLINDED, RANDOMIZED, PLACEBO-CONTROLLED TRIAL

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Background: Peripheral blood haematopoietic stem cells for autologous transplantation are often mobilized by means of highly emetogenic, intermediate doses of cyclophosphamide which are administered in an out-patient regimen. As prophylaxis of chemotherapy induced nausea and vomiting (CINV) Aprepitant, a 5-HT3 receptor antagonist, is recommended in combination with dexamethasone and a 5-hydroxytryptamine 3 (5-HT_3) receptor antagonist. Since aprepitant moderately inhibits CYP3A4, concomitant administration with cyclophosphamide might decrease cyclophosphamide clearance, thus impairing efficacy of autologous stem cell mobilization.
Aims: This study was designed to determine whether the aprepitant-palonosetron-dexamethasone regimen was better than palonosetron-dexamethasone therapy in preventiing CINV and to assess its impact upon stem cell harvesting and toxicity.
Methods: This single centre, randomized, double-blinded, placebo-controlled phase III trial was conducted in patients who received a highly emetogenic cyclophosphamide IV chemotherapy (3 g/m^2) for autologous PBSC harvesting. Efficacy of retching and vomiting, rescue medication, severity of nausea and overall quality of and safety data were obtained from the patient's daily diary (days 1-5) which reported episodes life. The Functional Living Index-Emesis questionnaire was completed on days 1 (before starting chemotherapy) and 6 (after chemotherapy). All side effects were recorded daily.
Results: A total of 122 patients were enrolled and randomized. The analysis was performed according to the intention-to-treat principle. Primary endpoint: When the 2 groups were compared, the number of patients with no emetic episodes and no rescue medication in the first 120 hours post-chemotherapy, was significantly lower in the aprepitant group (*p*.0114). Secondary endpoints: Acute (*p*.0272) and delayed (*p*.0039) complete response rate, complete control rate (*p*<.0001), number of emetic events (*p*.0003) and the impact of nausea and vomiting on daily life (*p*<.0001) were also significantly better in the aprepitant group. The adverse event rate and stem cell harvest (*p*.821) were not significantly different.
Summary and Conclusions: The aprepitant regimen more effectively prevented CINV and weakened its impact on daily life without impairing the efficacy of autologous PBSC mobilization and harvesting in patients treated with highly emetogenic, intermediate doses of cyclophosphamide.

P392

A PROSPECTIVE STUDY ON BED-SIDE ULTRASOUND IN NEUTROPOENIC ENTEROCOLITIS (NEC): 76 NEC OUT OF 1680 NEUTROPENIC EPISODES. EARLY ULTRASUOND DIAGNOSIS REDUCES MORTALITY

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Background: Neutropenic enterocolitis (NEC) is a life threatening complication of leukemic and solid tumors patients (pts) treated with chemotherapy (CHT) with mortality rate up to 50%. It's a clinical syndrome in neutropenic patients (pts) characterized by abdominal pain (AP), fever (F) and diarrhoea (D). Ultrasound (US) was used to evaluate bowel-wall thickening (BWT), and >4 mm is considered diagnostic of NEC. Perforation occurs in 5%>10% of cases. Early diagnosis is crucial to start conservative medical management (CMM) which appears the optimal strategy for most cases.
Aims: Aims:to evaluate prospectively if Bed-side-US(BUS) can detect early signs of NEC and guide a prompt treatment (CMM or surgical) in order to reduce mortality.
Methods: In the last 7 years all pts admitted in Our Hematology/BMT Unit wards at University of Pisa (Italy), undergoing chemotherapy (CHT), autologous or allogeneic transplant (AutoTx,AlloTx) were enrolled. Abdominal US was performed, baseline before treatment, and as only one symptom (or a combination) appeared within 12h from onset: F and/or D and/or AP in CHT-related neutropenic pts.
Results: Out of 1680 neutropenic pts 76 episodes were identified (4.7%). Seven pts had 2 separate episodes of NEC. Disease diagnosis were HD (N=10), ALL (N=8), AML(N=21),MM (N=9) and NHL (N=28). Treatment received was intensive CHT (N=35), AutoTx (N=37) and AlloTx (N=4). At time of diagnosis (Dx) symptoms were: F+AP+D 48%, F+D 4%, F+AP 1%, AP+D 34%,D 3%,AP 9%. F alone were never present at diagnosis of NEC. At Dx, F was absent in 35/76 NEC episodes (46%) and in 17/76 F never developed and 18/76 had delayed onset of F (from 10h to 72 h) from NEC Dx. There is a trend but not a statistical (St) significant difference in mortality among the 3 F groups (P=0.09). As control group (CG) we considered pts with CHT related mucositis and pts restaged with US during neutropenia in absence of symptoms. A total of N=509 pts were randomly chosen in the CG. None of them had BWT. Overall 11 pts died (14.5%) without a St difference between 1 or 2 episodes (P=0.309) of NEC. Treatment was CMM in 92% of pts, and was promptly started as BUS diagnosis was made. Mortality in pts treated with CMM was 11.5%. Six pts underwent surgery, guided by US features, during neutropenia, and 50% are alive. Median BWT was 8.6 mm in surviving pts (range 4.2-30mm) and 11mm in deceased (range 9.3-15mm). Authors have suggested BWT to be prognostic of outcome; in our study pts with >10mm had 60% survival. Median time to response from beginning of CMM was 24h and the first sign of was a decrease in AP, while median time to death was 26h (range 10.5-72h). The likelihood of NEC Dx in a discriminant St model (Bayes theoreme) for pts with BWT and AP=98.8%, AP+D=99.9%, AP+D+F=100%, AP+F=99.9%, D+F=5%.
Summary and Conclusions: BUS allowed to detect early signs of NEC and to start prompt treatment in this life threatening complication, which was CMM in 92% with a 88.5% survival rate. With BUS pts do not live the isolation room. Early diagnosis and intervention allowed to reduce mortality. US guided surgical intervention with 50% survival rate. Images of BUS and CT were superimposable. Fever is not a condition sine qua non for NEC diagnosis. A prompt BUS in neutropenic patients as just one symptom presents allows to make early diagnosis of this life threatening complication and guide prompt treatment (conservative or surgical) reducing mortality.

Transfusion medicine

P393

STREAMLINING THE MANAGEMENT OF BLOOD GROUP O RH NEGATIVE BLOOD STOCK: A SINGLE CENTRE IRISH EXPERIENCE

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Background: Group O Rh D negative blood is an important resource as it is can be transfused to patients of all blood types. According to most recent figures from the Irish Blood Transfusion Service, 8% of the general population are blood group O Rh D negative, but 14% of all blood used in clinical practice is group O Rh D negative. Inappropriate usage (use of O Rh D negative blood for non O Rh D negative patients on an elective rather than emergent basis that is greater than 3 days from expiry of the red cell concentrate (RCC)) and use of short dated group O Rh D negative blood (RCC less than 3 days to expiry) frequently occurs. This should be minimised given both the importance of this blood group and its scarcity. However, the use of short-dated O Rh D negative blood is acceptable to avoid wastage when all reasonable efforts have been made to give it to O Rh D negative patients.
Aims: To develop a system to minimise inappropriate use of group O Rh D negative blood within the UL Hospitals.
Methods: In 2011, a strategy was adopted within the UL Hospital group in Ireland to streamline stock management of group O Rh D negative blood supply. The UL Hospital group is made up of the main university hospital (the hub) and 5 other clinical sites. The Munster Regional Transfusion Centre, a branch of the Irish Blood Transfusion Service (IBTS), supplies blood to the UL Hospitals (ULH). Currently, ULH maintains 16 units of emergency O Rh D negative blood in the 5 satellite off site hospitals. Prior to 2011, these were supplied via the hub. In 2011, a mathematical model developed by the Mathematics Applications Consortium for Science and Industry (MACSI) in the University of Limerick determined that replenishing the oldest emergency stock in any of the six hospital sites (the hub plus 5 satellite sites) from the source as opposed to from the hub and moving the oldest stock in the satellite sites to the hub would improve O Rh D negative stock management and reduce inappropriate use. Stochastic simulation was used to prove that the mathematical model would work in real time. Use of group O Rh D negative blood was then audited before (2009), immediately after (2011) and 3 years after (2014) implementation of this model.
Results: The breakdown of usage of our group O Rh D negative red cell concentrate was audited, paying particular attention to inappropriate usage and use of short dated group O Rh D negative blood. The initial audit (2009) showed that the rate of inappropriate use was 22% and the rate of transfusion of short dated O Rh D negative units to non-group O Rh D negative patients was 21%. Following implementation of the model (2011), the rate of inappropriate use and short dated use had markedly improved at 8% and 6%, respectively. A repeat audit performed 3 years later (2014) showed that the rate of inappropriate use was 0% and rate of short dated use was 13.7%.
Summary and Conclusions: Our audit shows that the rate of inappropriate use of group O negative Rh D negative blood has reduced to 0 with our current group O Rh D negative blood stock management system. The use of short dated group O Rh D negative blood did increase from 6% to 13.7%. This remains well below the pre 2011 figure of 21% and illustrates the improved management implemented. In conclusion, this audit shows an effective blood stock management system for O Rh D negative blood that minimises wastage and is likely to be directly applicable to other hub and spoke model hospital networks. Future efforts may include requisition of newer blood to the hub to prevent short dating.

P394

CELL SALVAGE DURING CARDIAC SURGERY MAY DECREASE RED BLOOD CELL TRANSFUSION: A SYSTEMATIC REVIEW AND META-ANALYSIS

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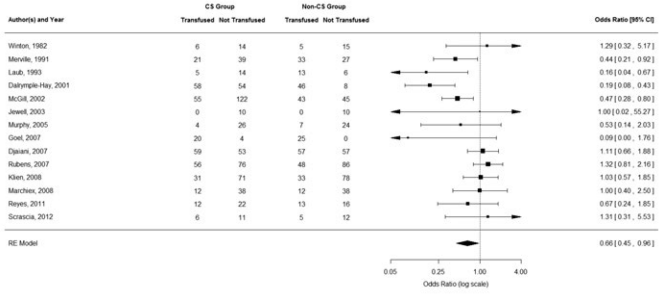
Background: Red blood cell transfusion in cardiac surgery is known to be associated with increased morbidity and mortality. The use of the cell salvage (CS) as a blood conservative strategy in cardiac surgery has been under debate with conflicting results from different studies.
Aims: We examined the current evidence behind the use of the CS by performing a systematic review and meta-analysis of existing randomized clinical trials that assessed the impact of the CS use on red blood cell (RBC) transfusion.

Methods: We searched MEDLINE, CENTRAL, American Society of Hematology and bibliographies of relevant studies (all searched July 2014) for randomized clinical trials comparing CS *versus* non-CS strategies in cardiac surgeries. We performed a meta-analysis using random effects model (DerSimonian-Laird) to estimate the pooled odds ratio and used I^2 statistic to assess the heterogeneity.
Results: Total of 587 citations were retrieved, out of which 14 trials were eligible for inclusion with a total of 1661 patients (899 in the CS group and 762 in the non-CS group). The vast majority of patients had coronary artery bypass graft surgery. All trials used shed blood for CS. In the CS group, 345 patients (38%) received RBC transfusion compared to 340 patients (45%) in the non-CS group. The odds of receiving blood transfusion were lower in the CS group (odds ratio of 0.66, 95% confidence interval [CI]: 0.45-0.96; Figure). Nevertheless, there was substantial heterogeneity between the trails in the outcome [I^2 of 60% (95% CI: 19-87%) which was statistically significant (P<0.01).

Tabella 1.



Summary and Conclusions: Available evidence suggests that the use of CS during cardiac surgery as a blood conservative strategy may decrease RBC transfusion with a reasonable effect size. However, these findings need to be taken with caution, given the substantial between-study heterogeneity in the outcome. We therefore recommend a large randomized controlled clinical trial to confirm these findings.

P395

IMMUNOGLOBULIN PROPHYLAXIS AGAINST HUMAN T CELL LYMPHOTROPIC VIRUS TYPE I PREVENTS INFECTION IN A HUMANIZED MOUSE MODEL

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Background: Adult T-cell leukemia (ATL) is a malignant disease caused by infection with human T-lymphotropic virus type 1 (HTLV-1). Only about 2–5% of HTLV-1-infected patients develop ATL, but prevention of HTLV-1 infection is the most effective strategy to eradicate ATL. After the introduction of HTLV-1 antibody screening of blood donations by the Japanese Red Cross, detection of HTLV-1 infections caused by transfusion has ceased. However, HTLV-1 infection continues to spread via breastfeeding and sexual intercourse. In Kyushu, an endemic area of Japan, pregnant HTLV-1 carriers are recommended against breastfeeding, and this policy has successfully reduced the number of HTLV-1 vertical infections. Despite this recommendation, 3–5% of HTLV-1 infections are still due to the virus passing from mother to child, and the number of HTLV-1 infections in non-endemic areas is increasing. Thus, an effective anti-viral agent is still needed to inhibit HTLV-1 infection. At present, there is no effective vaccine or anti-viral agent for HTLV-1, although it has recently been shown that anti-HTLV-1 antibody is effective in preventing mother to infant infection in a rabbit model of HTLV-1.
Aims: In this study, we aimed to develop an effective HTLV-1 immunoglobin isolated from HTLV-1 carriers that were screened at the Japanese Red Cross and to test whether HTLV-IG can prevent HTLV-1 infection in a humanized mouse model.
Methods: First, we developed and standardized effective screening methods to evaluate and characterize the anti-viral effect of HTLV-1 positive plasma. To monitor the infectivity and the ability to form syncytia we used MT-2, a HTLV-1-harboring T-lymphoblastoid line that can infect normal cells. Cocultures of 10^3 MT2 and 10^5 Jurkat cells treated with mitomycin C (MMC; 50 µg/mL) were successfully used to evaluate HTLV-1 infectivity.
Results: We found that HTLV-1 positive plasma, which was isolated from an HTLV-1 carrier who had a proviral load over 4, can effectively inhibit both HTLV-1 infection and syncytia formation. We purified HTLV-IG from the HTLV-1 positive plasma and evaluated its effect in a humanized mouse model, NOG (NOD.Cg-Prkdcscid Il2rgtm1Sug/Jic). Mice were treated with HTLV-IG for 5 days before HTLV-1 infection. HTLV-1 infection of 6-week-old NOG mice was carried out by injection with MMC+ MT-2 (10^5) 3 days after the mice received a transfer of human peripheral blood mononuclear cells (PBMC; 10^7). At day 11 post-infection, more than 80% of human CD45+ cells were found in the PBMC from the abdominal cavity and spleen. In those tissues at day 11 post-infection, HTLV-1 infection was observed in the untreated infected mice, but not in the HTLV-IG-treated mice. HTLV-1-infected cells, CD3+/CD4+/CCR4+/CD25+, were detected in the spleen, liver, and lung in the control MT-2-treated mice, but no HTLV-1-infected cells were observed in these tissues in the HTLV-IG-treated mice, even though their T cells (CD3+/CD4+ and CD8+) were normally colonized. These data suggest that HTLV-IG inhibits both HTLV-1 infection and the infiltration of HTLV-1-infected cells to the target tissues in a humanized mouse model.
Summary and Conclusions: We developed a screening method for evaluating the anti-viral effect of HTLV-IG. Our results show that HTLV-IG effectively inhibits both HTLV-1 infection and the infiltration of HTLV-1-infected cells into various types of tissues in a humanized mouse model. Our model is suitable for evaluating and confirming the anti-viral effect of HTLV-IG.

P396

ENDOGENOUS THROMBIN POTENTIAL FOLLOWING HEMOSTATIC THERAPY WITH 4-FACTOR PROTHROMBIN COMPLEX CONCENTRATE: A 7-DAY OBSERVATIONAL STUDY OF TRAUMA PATIENTS

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Background: Purified prothrombin complex concentrate (PCC) is increasingly used as hemostatic therapy for trauma-induced coagulopathy (TIC).
Aims: However, the impact of PCC administration on coagulation status among patients with TIC has not been adequately investigated.
Methods: In this observational, descriptive study, data relating to thrombin generation were obtained from plasma samples gathered prospectively from trauma patients upon emergency room (ER) admission and over the following 7 days. Standard coagulation tests, including measurement of antithrombin (AT) and fibrinogen, were performed. Three groups were investigated: patients receiving no coagulation therapy (NCT group), patients receiving fibrinogen concentrate only (FC group), and patients treated with PCC and fibrinogen concentrate (FC-PCC group).
Results: The study population (77 patients) was predominantly male (84.4 %); mean age was 40 +/-15 years and mean injury severity score was 25.6 +/-12.7. There were no significant differences between the three study groups in thrombin-related parameters upon ER admission. Endogenous thrombin potential (ETP) was significantly higher in the FC-PCC group compared with the NCT group on days 1 to 4 and the FC group on days 1 to 3. AT levels were significantly lower in the FC-PCC group from admission until day 3 (*versus* FC group) or day 4 (*versus* NCT group). Fibrinogen increased over time, with no significant between-group differences after ER admission. Despite ETP being higher, prothrombin time and activated partial thromboplastin time were significantly prolonged in the FC-PCC group from admission until day 3 to 4.
Summary and Conclusions: Treatment with PCC increased ETP for several days, and patients receiving PCC therapy had low AT concentrations. These findings imply a potential pro-thrombotic state not reflected by standard coagulation tests. This is probably important given the postoperative acute phase increase in fibrinogen levels, although studies with clinical endpoints are needed to ascertain the implications for patient outcomes. We recommend careful use of PCC among trauma patients, with monitoring and potentially supplementation of AT.

P397

HIGH LEVEL OF UNDERSTANDING FOR BLOOD TRANSFUSIONS IN PATIENTS CAN BE ACHIEVED BY EXPERT STAFF FROM THE TRANSFUSION UNIT

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Background: Enough understanding for blood transfusion is essential for patients treated with a blood transfusion. The American Association of Blood Banks (AABB) and other societies concerned with blood transfusions have recommended the minimum acceptable requirements for obtaining informed consent (IC). Specifically, the doctor should give each patient a sufficient amount of information on the procedure as well as the opportunity to ask questions. We have recently initiated a new service in which expert staff from the transfusion unit provide patients with basic information on the blood transfusion procedure. This new system has drastically improved patient understanding of blood transfusion.
Aims: The difference in the level of understanding for each basic fact related to blood transfusion was assessed between two groups of patients that were, or were not, provided with information from staff of the transfusion unit.
Methods: Expert staff from the transfusion unit started to provide patients with basic information on blood transfusion before further disease-specific explanation by the primary doctor (transfusion group; n =253). The effectiveness of this approach was assessed by comparison with a group of patients that were furnished with information by the doctors only (doctor group; n=74). We carried

out a questionnaire survey of patients to analyze the depth of understanding of blood transfusion from July to October 2013 and October to December 2014. The level of understanding in patients before blood transfusion was compared between the two groups. Statistical analyses were performed by the Chi-square test using the SAS analysis suite (Tokyo, Japan). Probability values less than 0.05 were considered statistically significant.
Results: The level of understanding for each issue was as follows. For precautions during blood transfusion, 88% of patients answered "Completely" or "Mostly" in the transfusion group, but only 26% in the doctor group. For requirement of tests for post-transfusion-transmitted infections, 90% answered "Completely" or "Mostly" in the transfusion group, but only 33% in the doctor group. For the scheme of blood transfusion, 90% answered "Completely" or "Mostly" in the transfusion group, while 36% in the doctor group. For agreement for signing a consent form, 94% answered "Completely" or "Mostly" in the transfusion group, but only 50% in the doctor group. For a sense of ease in receiving a blood transfusion, 86% answered "Completely" or "Mostly" in the transfusion group, but 45% in the doctor group. Patients were also asked for ten risks associated with blood transfusion after being given the explanation. The results are summarized in the figure, and the representative results were shown as follows. In the transfusion group, a good understanding was obtained in 94% of patients for "Allergenic reaction", 90% for "Fever", 85% for "Hepatitis virus" and 74% for "HIV". By contrast, in the doctor group, a good understanding was obtained in 49% of patients for "Allergenic reaction", 30% for "Fever", 42% for "Hepatitis virus" and 39% for "HIV. Notably, TRALI, TACO and GVHD were understood by only about 10% of patients in the doctor group. There were significantly higher degrees of understanding in the transfusion group (P <0.001). For the other issues, the degree of understanding was significantly higher in the transfusion group than in the doctor group (P <0.001). The significances were more prominent than our previous smaller study.

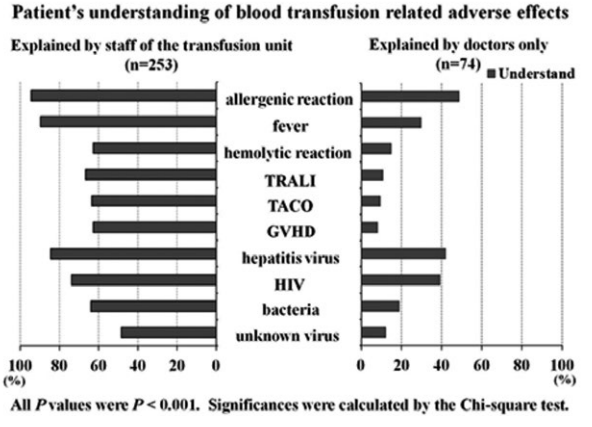

Figure 1.

Summary and Conclusions: Providing patients with information on blood transfusion by expert staff from the transfusion unit prior to further explanation by the primary doctors was found to be useful. All of the issues related to blood transfusion were significantly better understood by the patient after input from the transfusion unit staff. Intervention by the transfusion unit in this field will be useful for the process of obtaining IC and achieving high-quality transfusion therapy.

P398

INTRAVENOUS HIGH-DOSE IRON SUPPLEMENTATION FOR BLOOD DONORS WITH IRON DEFICIENCY

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Background: Iron deficiency (ID) is one of the most common nutritional deficiencies worldwide. It is highly prevalent in premenopausal women and also frequently found in industrial countries. About 23% of the population participates in blood donation programs. Blood donations often contribute to ID, but for donor clearance, only a capillary hemoglobin (Hb) threshold is required. However, Hb does not reliably predict iron stores. ID is associated with restless legs syndrome, cognitive and physical symptoms and an increased risk for preterm birth. Currently, only in anemic donors, iron supplementation is routinely recommended. Oral iron substitution is often associated with significant gastrointestinal side effects and poor compliance.
Aims: In our ongoing study, we compare the effect of a single intravenous high-dose iron carboxymaltose preparation (1000mg) for blood donors with iron deficiency (IV) with the effect of a corresponding dose (10g) of oral iron over 10 weeks (PO).
Methods: In our randomized, controlled clinical trial we include male and female blood donors (whole blood and platelet apheresis) who fulfill the criteria of a predonation hemoglobin value of ≤13.5g/dl and a ferritin value of ≤30ng/ml (target sample size 160 in total). Stratified by gender, participants are randomized with a web-based randomization tool to IV or PO in a 1:1 ratio. 12 weeks after the first visit hemoglobin and ferritin values of both groups are determined.
Results: Out of 306 donors with Hb ≤13.5g/dl, 187 (61%, 166 female, 21 male) had a ferritin value of ≤30ng/ml and of these, 112 had a ferritin ≤15ng/ml.
To date, 32 participants (27 female, 5 male) have completed the trial. Hb and ferritin levels after treatment are shown in the Table. No serious adverse events occurred. Before iron treatment, but after donation, mean Hb and ferritin levels were 11.5±0.7g/dl and 6.5±3.9ng/ml.

Tabella 1. Hb and ferritin values (mean±SD) 12 weeks after iron supplementation

	IV, n=16	PO, n=16	P value
Hb (g/dl)	13.5±0.5	13.5±0.5	n.s.
Ferritin (ng/ml)	116.1±70.7	27.6±15.5	<0.01

In the IV group, 2/16 participants (12.5%) still had ferritin values <30ng/ml after iron supplementation (both of whom donated blood after their first visit) compared to 8/16 (50%) participants of the PO group.
Summary and Conclusions: Iron deficiency is common in blood donors, especially in females. Both IV and PO iron supplementation improves iron stores but IV iron is more effective. The diagnosis and treatment of this common condition could be useful both for donors and transfusion medicine services.

P399

MORTALITY REDUCTION WITH A MASSIVE TRANSFUSION PROTOCOL IN A NON-TRAUMA UNIVERSITY MEDICAL CENTER

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Background: Management of massive bleeding requires rapid hemostatic control and timely blood product support. Our center has developed a non-trauma massive transfusion protocol (MTP) that includes blood product supply and use of adjuvant pro-coagulant agents.
Aims: We aimed to evaluate the impact of a MTP on patient outcome.
Methods: Retrospective-observational study in patients treated with our MTP between January 2006-December 2012, and an historical cohort (year 2005) as control. MTP consisted in a goal-directed transfusion strategy with early use of adjuvant procoagulant medications (fibrinogen, aFVII, prothrombin complex concentrates or antifibrinolytics). Primary endpoints were 24-h and 30-day mortality. Secondary endpoints were: 1) Fresh frozen plasma/Red blood cells (FFP:RBC) and platelet/RBC transfusion ratios, 2) Time to first FFP unit, and 3) Pro-coagulant agents frequency of use. A multivariate logistic regression model was used to establish associations with 30-day mortality.
Results: 324 patients were included (67% male, median age 61yr). Analysis was divided into two periods (2006-2009 and 2010-2012). RBC use remained stable, with a progressive increase in FFP and pro-coagulant agents use after MTP implementation. A statistically significant increase in FFP:RBC ratio was observed after the MTP (p=0.03), without differences in PLT:RBC ratio. Time to first FFP showed a tendency to reduction (p=0.08). A significant reduction in 30-day mortality was seen (25% in 2006-2009; 20% in 2010-2012; 38% in 2005, p=0.037), with unchanged 24-h mortality. Multivariate logistic regression model showed an independent effect of MTP on 30-day mortality.

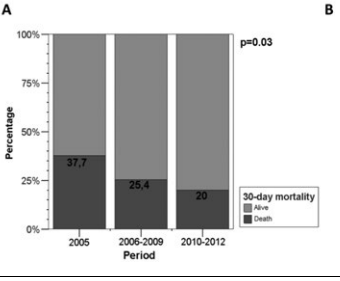

Figure 1. (A) 30-day Mortality reduction after massive bleeding protocol implemetation. (B) Results of the multivariate logistic regression model for 30-day mortality.

Summary and Conclusions: An independent association between the non-trauma MTP implementation and 30-day mortality has been found, with a parallel improvement in transfusion ratios. MTP might be valuable for non-trauma patients, and may have an effect on survival not entirely dependent on the improved transfusion ratios.

Platelet disorders

P400

THE TAIL MUTATIONS OF MYH9 INHIBIT THE DISASSEMBLY OF NON-MUSCLE MYOSIN IIA

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Background: MYH9 disorders include autosomal dominant macrothrombocytopenias with leukocyte inclusion bodies. These disorders were caused by heterozygous mutations in MYH9, the gene encodes non-muscle myosin heavy chain-IIA. However, It has yet remained to be clarified how these mutations make an impact on the molecular property of myosin. In order to address this issue, we need to examine the whole myosin hexamer, consisting of a pair of heavy chains, essential light chains and regulatory light chains, because it has been known that interaction between the tail and the head-neck domain is critical for regulation of non-muscle myosin filament formation. We have overcome difficulties and successfully produced the recombinant non-muscle myosin hexamer as a whole molecule.
Aims: To clarify the effects of the tail mutations on the molecular property of non-muscle myosin IIA.
Methods: We used Baculovirus expression system, and successfully produced functional recombinant myosin hexamer, and analyzed the Mg^{2+}-ATPsase activities and ability of filament formation, wild type and two representative tail mutants, namely p.Asp1424His and p.Glu1841Lys.As *in vivo* analysis, we overexpressed GFP-tagged MYH9, wild type and tail mutants in NIH3T3 cells, and examined the localization.
Results: The ATPase activities of these mutants were essentially the same as the wild type, indicating that these tail mutations don't influence the motor activity of non-muscle myosin IIA. Non-muscle myosin forms large head to head aggregation in the absence of ATP. However, both mutants failed to disassemble to monomers upon the addition of ATP in contrast to the wild type. Additionally electron microscopic analysis also revealed that they impaired the ATP-induced disassembly of myosin filaments, and produced large aggregates. For further investigation on the single molecule, we studied at reduced protein concentration using rotary shadowing. In the presence of ATP, the wild type myosin monomers predominantly presented a compact shape with two folds in the tail. On the other hand, two tail mutants predominantly formed an extended conformation. Additionally the p.Glu1841Lys mutant proned to stick to each other even at low protein concentration in the presence of ATP. Next we overexpressed GFP-tagged MYH9, wild type and two tail mutants in NIH3T3 cells. Wild type MYH9 diffusely localized in cytoplasm. In contrast, both tail mutants accumulated to the myosin filaments, where the regulatory light chains were phosphorylated, suggesting that these mutants were unable to disassemble once they formed filaments.
Summary and Conclusions: These tail mutations hampered the ATP-induced formation of folded conformation thus stabilizing the myosin aggregates. They disturbed the normal assembly-disassembly dynamics of myosin filaments, thus inhibiting dynamic actomyosin cytoskeletal rearrangements. These effects may cause the clinical features of MYH9 disorders to some extent.

P401

PLATELET PROGENITORS AFTER LEAVING BONE MARROW

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Background: *in vitro* observations have indicated that preplatelets, which are anucleate discoid particles that can reversibly into proplatelet fragments, are one of platelet progenitos from cultured megakaryocytes (MKs) (Thon JN, *et al.* JBC 2010). Recently, we identified that bone marrow (BM) MKs form and release two types of platelet progenitors, using intra-vital imaging and ultrastructural analysis of mice BM (Kowata S, *et al.* Thrombosis and Haemostasis 2014). However, *in vivo*, how the platelet progenitors translate into individual platelets after leaving BM remains poorly understood.
Aims: To elucidate whether platelet progenitors have an ability of conversion into individual platelets *in vivo* and *in vitro*.
Methods: We isolated platelet progenitors from whole blood of green fluorescence protein (EGFP) transgenic mice by the centrifugation (100 g, 15 min) after the intra-peritoneal injection of anti-platelet antiserum (APS). Mature platelets were isolated from whole blood of GFP mice by the centrrifugation (100 g, 15 min). *in vivo*: The freshly enriched progenitor isolates or mature platelets, both of which were positive for EGFP, were transfused into wild type mice. The peripheral blood was obtained and analyzed by flow cytometry continually. The EGFP positive platelets in platelet gate were counted. *in vitro*: The freshly enriched progenitor isolates were cultured in IMDM medium at 37°C. The continual change of their characteristics in the shape and size were assessed using fluorescent microscopy with high resolution. Time lapse images of the processes were also captured.
Results: During an acute thrombocytopenic period after the APS injection, platelet progenitors increased remarkably in whole blood from vena cava. The length and size of platelet progenitors was extremely varied (Figure 1). The maximum length of platelet progenitor was more than 200 μm. These data were confirmed by our previous study of intra-vital imaging of BM MKs (Thrombosis and Haemostasis 2014). *in vivo*: Flow cytometric analysis showed that there was a time-dependent increase in EGFP platelet number in the gate of mature platelet, after the injection of EGFP positive platelet progenitors. The control was carried out using EGFP positive platelets instead of platelet progenitors. (0 h: 100%, 6h: 111±10.4%, 12h: 105±6.3% n=3, control 0 h: 100%, 6h: 86±8.8%, 12h: 73±5.3% n=3, Data were shown as % of the number of EGFP positive platelets at 0 h). These data suggest that the infused platelet progenitors convert into mature platelets *in vivo* as time passed. *in vitro*: The platelet morphogenesis from platelet progenitors was observed as highly dynamic process, shown as follows. (1) A long proplatelet made several formation cleavage furrows, where it snapped, making short proplatelets. Then, they snapped to become the platelet-sized particle. (2) Oval proplatelet, which was several times bigger in diameter than mature platelet, extended to short proplatelet. Then it snapped to become the platelet-sized particle.

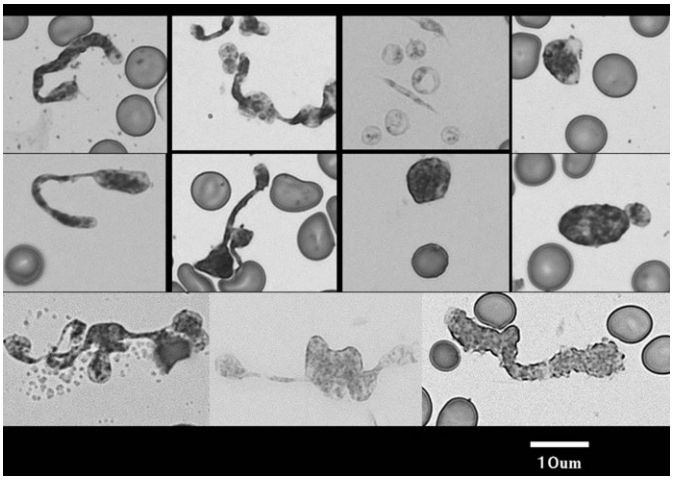

Figure 1.

Summary and Conclusions: These data indicated that, after leaving BM sinusoid, these platelet progenitors convert into individual platelets in the blood stream.

P402

HELICOBACTER PYLORI INFECTION INFLUENCES THE SEVERITY AND TREATMENT RESPONSE IN CHRONIC HEPATITIS B COMPENSATORY CIRRHOTIC PATIENTS WITH THROMBOCYTOPAENIA

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Background: Thrombocytopaenia is a frequent complication of liver cirrhosis, and ITP is closely related with *H. pylori* infection. Several studies illuminate the associations between *H. pylori* and viral hepatitis, but most of the studies focus on hepatitis C and *H. Pylori* infection.There is no information availableon the association between *H. pylori* infection and hepatitis B compensatory cirrhosis with thrombocytopaenia.T. Umemura *et al.* reported that *H. pylori* seropositivity potentially contributes to thrombocytopaenia in patients with HCV infection. Therefore, we wanted to determine whether *H. pylori* infection plays a role in compensatory cirrhosis in chronic hepatitis B patients with thrombocytopaenia.
Aims: The aim of this projectis to explore the role that *H. pylori* infection plays in chronic hepatitis B compensatory cirrhotic patients with thrombocytopaenia.
Methods: A total of 255 patients were enrolled in the study. All of the patients were received nucleoside analogs (NA) therapy and were screened for *H. pylori* infection. Patients were divided into three groups according to the different therapy administered and the state of *H. pylori* infection: patients without *H. pylori* infection and receiving NA therapy alone (N=146); patients with *H. pylori* infection and receiving NA therapy alone (N=48); patients with *H. pylori* infection and receiving *H. pylori* eradication treatment combined with NA therapy (N=61). Clinical laboratory parameters and platelet response to the treatment were compared between three groups.
Results: In hepatitis B patients experiencing compensatory liver cirrhosis who were infected with *H. pylori,* platelets counts were significantly reduced compared with non-infected patients (31 *vs* 60×10^9/L, respectively, P<0.01).For

long-term follow-up, the platelet count was significantly increased in HBV/*H. pylori* co-infection patients who received NA combined *H. pylori* eradication treatment compared with the other two groups who received NA alone (P<0.01). The titre of anti-*H. pylori* IgG was significantly correlated with thrombocytopaenia severity and inversely correlated with platelet count. *H. pylori* infection is an independent risk factor of thrombocytopaenia (P<0.01).
Summary and Conclusions: In conclusion, we are the first to report that thrombocytopaenia is significantly correlated with *H. pylori* infection in compensatory liver cirrhosis of chronic hepatitis B patients. The platelet response after 2 years follow-upis markedly increased in HBV/*H. pylori* co-infection patients who received NA combined *H. pylori* eradication treatment. *H. pylori* infection is an independent risk factor of thrombocytopaenia. Therefore, *H. pylori* may partly contribute to thrombocytopaenia in HBV patients. NA combined with *H. pylori* eradication treatment may prove beneficial in chronic hepatitis B compensatory cirrhotic patients infected with *H. pylori.*

P403

ELEVATED PLATELET AGGREGATION AND ACTIVATION ARE ASSOCIATED WITH PROLONGED ISOLATED THROMBOCYTOPAENIA VIA REDUCED B2-GPI AFTER ALLO-HAEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Prolonged isolated thrombocytopaenia (PT) remains a serious complication after allogeneic haematopoietic stem cell transplantation (allo-HSCT). Recent studies have demonstrated that several risk factors, including the stem cell source, disease status, graft-*versus*-host disease (GVHD), and cytomegalovirus (CMV) infection, are proposed to be associated with PT after allo-HSCT. PT is an independent risk factor for a poor prognosis after allo-HSCT. The pathogenesis of PT after allo-HSCT remains unknown. Our previous studies demonstrated that recurrence and engraftfailure are strongly associated with PT; increased platelet turnover from impaired platelets and increased platelet consumption have been demonstrated. β2-GPI is a major target for antibodies in Apl, and β2-GPI antibodies contribute to decreased platelets in APS patients. Hulstein*et al.* revealed that β2-GPI inhibits the vWF-dependent functions of platelet adhesion and aggregation. In present study, the function of β2-GPI was investigated in PT after allo-HSCT. Minimal data are available on β2-GPI involvement in platelet activation and aggregation in this setting.
Aims: The aim of this study was to explore whether decreased β2-GPI levels contribute to the enhanced of platelet activation and aggregation in PT after allo-HSCT.
Methods: A total of 56 consecutive patients with PT and 60 control patients after allo-HSCT were enrolled at our centre. β2-GPI Ag and anti-β2-GPI antibody levels were measured by ELISA, and vWF Ag and vWF activity levels were detected using an automated coagulation analyser. Platelet aggregation and CD62p and PAC-1 expression were confirmed using a Chrono-log aggregation analyser and flowcytometry.
Results: The β2-GPI antigen in PT was significantly decreased compared with control (164.2±12μg/ml *vs* 234.2±16μg/ml; P<0.01), and the β2-GPI antigen was negatively correlated with platelet aggregation(P<0.01). The aggregation ratio in PT was significantly increased compared with control(39±7.5% *vs* 23±8.5%; p=0.0317). A significant difference was noted after 90 days of allo-HSCT (P<0.01). Additionally, anti-β2-GPI IgG in PT was markedly increased (1.78±0.46*vs* 0.94±0.39 U/ml; P<0.01). No significant differences were noted between the two groups regarding vWF antigen levels, and vWF activity was increased in PT (133.06±30.50% *vs* 102.17±25.90%; P<0.01). β2-GPI antigen levels and vWF activity were negatively correlated (P<0.01). Platelet aggregation was induced by ADP in combination with various concentrations of β2-GPI. The aggregation ratio gradually decreased in PTwith increasing β2-GPI. CD61, CD62p and PAC-1 expression in platelets was higher in the patients with PT after allo-HSCT(33.6±11.6%*vs* 8.5±3.5%,p=0.000; 42.4±7.6% *vs* 6.8±2.2%, p=0.000; 20.6±7.9% *vs*6.1±2.0%, p=0.000; respectively).
Summary and Conclusions: β2-GPI antigen is obviously decreased in patients with PT and is closely related to increased platelet activation and aggregation. β2-GPI significant inhibit platelet aggregation in patients with PT, suggesting that it may be involved in the pathogenesis of PT after allo-HSCT.

P404

OUTCOMES OF 65 PREGNANCIES IN 34 WOMEN WITH 5 DIFFERENT FORMS OF INHERITED PLATELET FUNCTION DISORDERS ENROLLED IN A RETROSPECTIVE AND MULTICENTRIC STUDY

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Background: Pregnancy and delivery represent hemostatic challenges to women with inherited platelet function disorders (IPFD). Information on outcomes and management of IPFD in pregnancy is scarce, and the limited data available in literature, mainly deriving from case reports or small case series, suggest that major bleeding is very common.
Aims: To improve knowledge on this matter we performed a retrospective, multicentric study aimed at systematically collecting and analyzing pregnancy outcomes in a series of women with IPFD. The study was promoted by the *SWG on Thrombocytopenias and platelet function disorders* of the EHA.
Methods: Twelve centers around the world took part in the study, and 34 women with 5 different forms of IPFD confirmed by specific diagnostic criteria were enrolled, obtaining data on a total of 65 pregnancy and 48 newborns. Data on spontaneous bleeding tendency, way of delivery, hemostatic prophylaxis, bleeding at delivery and its management, as well as newborns' status, were recorded.
Results: Spontaneous bleeding diathesis did not worsen during gestation, and no severe bleeding episodes were reported during this period. Miscarriage and preterm birth incidence was similar to that observed in the normal population. Prophylactic platelet transfusions were given in 30% of cases and 73% of deliveries were vaginal. Severe bleeding events requiring blood transfusion were observed in 12.5% of deliveries, in all cases occurring in women with Glanzmann thrombasthenia (GT). Of note, 50% of deliveries in women with GT needed therapeutic blood transfusions, while no women with delta storage pool disease (delta-SPD), Hermansky-Pudlak syndrome, P2Y12 defect or defect of thromboxane A2 receptor required this treatment. However, mild bleeding was reported also in some women with the latter diseases. More than half of women with GT who received prophylactic platelet transfusions experienced severe hemorrhages at delivery, suggesting that platelet transfusion alone may be inadequate and better preventive treatments are required in this condition. No women died of delivery-related causes or received hysterectomy to stop bleeding. Neither the 9 neonates who inherited the mothers' illness nor the unaffected infants experienced bleeding at birth. Statistical analysis found significant association between excessive bleeding at delivery requiring transfusion and a history of grade 3 or 4 (OR 23.4) of WHO bleeding scale. The risk of bleeding was also significantly increased in women with GT compared with delta-SPD (OR 41.6). No significant correlations were found between severe bleeding requiring blood transfusion at delivery and the way of delivery, the age of the mother, severe bleeding at previous surgical interventions and the status of primipara; no significant correlation was found with prophylactic platelet transfusion as well.
Summary and Conclusions: The risk of bleeding at delivery in women with IPFD varies according to diagnosis and previous spontaneous bleeding diathesis. Prophylactic platelet transfusions in women with GT do not ensure that childbirth occurs without major hemorrhages in the mother, this indicating the need for better regimens of preparation to delivery in this condition.

P405

PREDICTION OF SEVERE ADAMTS13 DEFICIENCY IN PATIENTS WITH THROMBOTIC MICROANGIOPATHIES

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Background: Thrombotic microangiopathies (TMA) are a rare and heterogeneous group of diseases that include Thrombotic Thrombocytopenic Purpura (TTP), Hemolytic Uremic Syndrome (HUS) and other TMAs secondary to several conditions (ie, cancer and hematopoietic stem cell transplant). TTP is characterized by an extreme ADAMTS13 deficiency. Plasma exchange therapy and immunosuppression are the current treatment for TTP but are ineffective for most other forms of TMA. Assays exploring ADAMTS13 activity are limited to only some specialized reference laboratories. We performed an analysis of our TMA registry to identify clinical features which may allow a rapid and reliable prediction of an acquired ADAMTS13 deficiency.
Aims: The primary goal was to identify cut-offs for variables that would prove

useful in excluding the possibility of TTP, in order to facilitate a more suitable therapy based on the etiology of the TMA.
Methods: The Registry included 242 patients with TMA. All the statistical analyses were performed on a final cohort of 174 adults (including secondary TMAs) in which all the data for subsequent analyses were available. Secondary TMAs were included in the study because it provides a real-world setting for the analysis. A subset of variables was considered from a clinical standpoint as potential predictors of low ADAMTS13 activity. Patients with severe acquired (<5% of normal activity) ADAMTS13 deficiency (n=55) were compared to patients with detectable (≥5%) ADAMTS13 activity (n=119). Then, for the statistically significant variables (age, platelet count and creatinine), we generated receiver operator characteristics (ROC) curves and compared area under the curve (AUC) values to find cut-offs helping in the prediction of a severe ADAMTS13 deficiency (thresholds of 68 years old for age, 2.0 mg/dL for creatinine and 44×10^9/L for platelet count). Finally, we conduct multivariate logistic analysis to test if a model with combined variables improves the prediction capability of the variables taken alone. A fourth variable (absence of Hematopoietic Stem Cell Transplant (HSCT) in the patient) performed well in the multivariate analysis and was included in the final model.
Results: The combined model (table 1) had an AUC of 0.925, thus improving the prediction capability of the individual variables. The percentage of correct predictions of the model was 89,7%. Patients with severe ADAMTS13 deficiency had platelets ≤44×10^9/L, creatinine ≤2,0 mg/dL, age ≤68 years old and did not undergo HSCT (Odds Ratio 64,0; p <0,001). If all the four criteria are met, the probability of TTP is 82,5%. If any of the conditions is not met, the probability of TTP drops to 6,8%.

Tabella 1.

Variables included	Condition in severe ADAMTS13 deficiency	AUC	% of correct predictions	Specificity	Sensibility	Negative Predictive Value	Positive Predictive Value
Creatinine	≤2.0 mg/dL	0,925	89,7% 8 false - 10 false+	91.6%	85.5%	93.2%	82.5%
Platelet	≤44×10^9/L						
Age	≤68 years						
HSCT	Absent						

Summary and Conclusions: TTP share features of atypical HUS and secondary TMAs. Our results may enable the rapid exclusion (if any of the conditions is not met) and accurate diagnosis of TTP (if all the four criteria are met) using readily available laboratory data and this could help to choose the suitable therapy for different pathophysiological groups. Three out of 8 false negatives were TTP relapses that were misclassified due to a high platelet count. This could be explained because the patients were already diagnosed and advised that they have to be promptly hospitalized in a relapse. Within the 10 false positives, 30% of the cases were shown to be associated to gastric or breast tumors days after ADAMTS13 testing. Therefore, if ADAMTS13 test results normal in a patient who met all the four conditions, a diagnosis of TMA secondary to cancer should be suspected.

P406

PDGF-BB PROTECTS BONE MARROW MESENCHYMAL STEM CELLS AGAINST APOPTOSIS AND SENESCENCE THROUGH THE P53/P21 PATHWAY IN PATIENTS WITH IMMUNE THROMBOCYTOPAENIA

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Background: Immune thrombocytopaenia (ITP) is characterized by platelet destruction and megakaryocyte dysfunction resulting from autoantibodies induced by the polarization of Th1 and deficient regulatory T cells. Mesenchymal stem cells (MSCs) display not only stem cell multipotency but also robust immunosuppressive properties. However, MSCs from ITP patients do not exhibit conventional proliferative abilities and thus exhibit defects in immunoregulation, suggesting that MSCs impairment might be a mechanism involved in ITP. In addition, reversal of the defects in MSCs may represent an attractive therapeutic alternative. Platelet-derived growth factor (PDGF) improves growth and survival in various cell types. Moreover, PDGF promotes MSCs proliferation. However, the effects of PDGF on MSCs in patients with ITP have not been fully characterised.
Aims: The aim of this study was to investigate the molecular pathways underlying the increased apoptosis and senescence and to analyse the*in vitro*effectsofPDGFon MSC from patients with ITP
Methods: Bone marrow MSCs were derived from primary ITP patients and healthy donors. Gene expression profilechanges in MSCs from ITP patients and controls wereinvestigated by DNA microarray analysis. RT-PCR and Western blot were employed for molecular expression analyses. Cellular senescence was detected using the CCK-8 assay, cell cycle analysis and senescence-associated β-galactosidase (SAb-gal) assay. Apoptosis was assessed by nuclear staining and flow cytometry. Mitochondrial membrane potential (MMP) was investigated by JC-1 staining.
Results: Microarray analysis revealed that the expression of p53, p21, and the apoptosis regulators Caspase-3,-9, and-8 were increased in ITP MSCs compared with controls. ITP MSCs expanded more slowly and appeared larger in size and flattened. ITP MSCs exhibited increased senescence as demonstrated by decreased proliferative capacity and increased SAb-gal positive cells and G0/G1 cells. The increased apoptosis rate was detected in ITP MSCs compared with normal controls. Molecular changes involving the intrinsic apoptosis pathway were assessed. A decreased Bcl-2/Bax ratio and MMP levels were observed, which leads to cytochromeC release from mitochondria and consequently activates Caspase-9 and Caspase-3 in ITP MSCs. Moreover, Fas and FasL expression and Caspase-8 activation, which are associated with the extrinsic apoptosis pathway, were up-regulated in ITP MSCs, suggesting that both the intrinsic and extrinsic pathways account for enhanced apoptosis. p53 and p21 mRNA and protein expression were up-regulated in ITP MSCs, whereas the inhibition of p53 with PFT-αmarkedly inhibits apoptosis and senescence. PDGF-BB treatment significantly decreased p53 and p21 expression and increased surviving expression in. In addition, the apoptotic rate and number of senescent cells in ITP MSCs were reduced after PDGF-BB treatment.
Summary and Conclusions: Increased apoptosis and senescenceare noted in ITP MSCs. Both the intrinsic and extrinsic pathways participate in excessive apoptotic induction. For the first time, we demonstrate that PDGF-BB protects MSCs against apoptosis and senescence in patients with ITP. This protective effect of PDGF-BB is likely mediated via the p53/p21 pathway, thus potentially providing a new therapeutic alternative for immune thrombocytopaenia.

P407

EFFICACY OF PROPHYLACTIC SINGLE DONOR PLATELETS (SDP) TRANSFUSION IS RELATED TO THE TIME OF STORAGE AND NOT TO THE ABO COMPATIBILITY IN CHILDREN WITH MALIGNANCY

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Background: The prophylactic transfusion of apheresis platelets remains the gold standard in order to avoid spontaneous bleeding in children undergoing intensive chemotherapy protocols. Due to inventory constrains, several times transfusion services are issuing ABO mismatched platelets.
Aims: To provide descriptive data based on our centre's experience with regard to platelet increments post prophylactic transfusions in children with malignancy and investigate for factors that could be related to the effectiveness of SDP transfusion.
Methods: We retrospectively analysed data from a 3 years period (2011-2014) on the prophylactic SDP use on 48 children (25 boys/23 girls) with a median age of 7(2.5-14)years diagnosed with haematologic or solid malignancies (29 ALL, 3 AML, 4 NHL, 2 Wilms' tumor, 4 Ewing sarcoma, 3 myeloblastoma, 2 neuroblastoma and 1 rhabdomyosarcoma). Transfusion efficacy was assessed based on the corrected count increment (CCI) that takes into consideration the difference in platelet counts pre and post transfusion together with the number of platelets transfused as well as the patients' body surface area. Response was evaluated at 24 hours post transfusion and any CCI <$5x10^3$ was considered inadequate. Patient related (age, gender, disease, ABO/D, previous exposure to SDP or random platelets, status at last follow up) as well as product related (duration of storage, ABO/D, apheresis machine) factors were recorded and included in the analysis.
Results: In total 228 transfusions, meeting the inclusion criteria were analysed. In 146 (64%), patients received ABO identical SDPs whereas in 48 (21%) and 34 (15%) children received SDPs with major or minor ABO mismatch respectively. The mean value of CCI was 17.9 (0-66.4)x$10^3$and transfusion refractoriness was noticed in 35 SDPs administration (15%). The median SDP age was 2.59 (0.5-5) days and each child received 3(1-21) prophylactic transfusions. Previous exposure to SDP was recorded in 207 transfusions (136 with major mismatch) whereas random platelets transfusion had preceded the current transfusion in 189 cases (37 with major mismatch). In a univariate analysis a strong negative correlation was found between the time of SDP storage and the transfusion efficacy (p<0.002). In case of refractoriness (CCI <$5x10^3$) the mean product age was 3.35 days, significantly longer compared to that of the efficacious transfusions (2.47 days) (p<0.003). At the contrary the ABO compatibility between the patient and the transfused SDP did not influence the CCI (18.5, 16.7 and 17 x10^3forABO identical and with major and minor mismatch respectively). When CCI was analysed by the patients' ABO blood type significant differences were found between groups with patients on group B showing the modest response (CCI 12.8 x10^3).
Summary and Conclusions: Based on our findings, prophylactic transfusion of fresh SDPs (<48 hours) leads to bigger platelet increments while transfusion of older SDP is related to refractoriness. Interestingly enough, opposite to literature, we found no correlation between the ABO compatibility status and the SDP transfusion efficacy. This could partly be explained by the strong immunosuppression that our study population suffers due to both the underlying disease as well as the chemotherapy received. In summary for children with malignancy, should a prophylactic platelet transfusion is needed the first choice should be to provide fresh SDP ideally of the same ABO, without though firm restrictions on the use of other ABO blood types if the first are not available.

Procoagulant states

P408

IMPACT OF HIGH ON-TREATMENT PLATELET REACTIVITY ON 5-YEAR MORTALITY IN PATIENTS AFTER MYOCARDIAL INFARCTION

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Background: High on-treatment platelet reactivity (HTPR) is expected to be a negative prognostic factor in patients with coronary artery disease. However, long-term results in patients with acute myocardial infarction are lacking.
Aims: The aim of the study was to assess the relationship between HTPR and five-year mortality in patients with acute myocardial infarction.
Methods: We performed a prospective cohort study of 198 patients with acute myocardial infarction. In these patients, the response to aspirin and clopidogrel was assessed by impedance aggregometry. According to their response to antiplatelet treatment, the patients were divided into groups with adequate response, dual poor responsiveness (DPR), poor responsiveness to aspirin (PRA) and poor responsiveness to clopidogrel (PRC). After five years, the myocardial infarction recurrence and overall mortality were assessed.
Results: Five-year mortality was significantly higher in all groups of patients with HTPR compared with patients with sufficient response to antiplatelet treatment: in PRA patients 38.1 % *vs* 19.2 %, p <0.01, in PRC patients 45.2 % *vs* 17.3 %, p<0.001 and in DPR patients 50.0 % *vs* 19.9 %, p<0.05. Risk of repeated myocardial infarction was also increased (HR 4.0, 95 % CI 1.25-11.5, p<0.05 for DPR, HR 4.37, 95 % CI 1.51-12.77, p<0.01 for PRA, HR 3.25, 95 % CI 1.11-9.36, p<0.05 for PRC). In a multivariable analysis, HTPR and left ventricle systolic dysfunction were proven to be independent predictors of mortality.

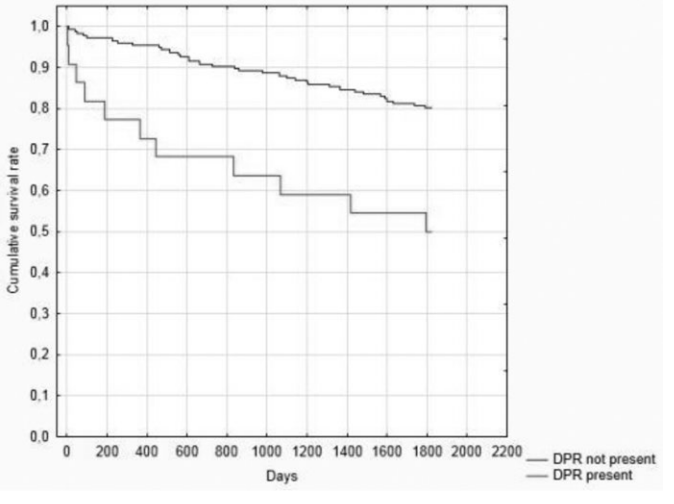

Figure 1.

Summary and Conclusions: PRA, PRA and DPR are independent predictors of increased five-year mortality and risk of repeated myocardial infarction.

P409

NEUTROPHILS CONTRIBUTE TO THE GENERATION OF PROCOAGULANT PLATELETS BY A MYELOPEROXIDASE INDEPENDENT MECHANISM

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Background: The procoagulant subset of highly activated platelets has been associated with increased pathological thrombotic states such as ischemic stroke and sepsis. Increased generation of reactive oxygen species has been implicated in procoagulant platelet formation, thus we postulated a role for activated neutrophils, and in particular neutrophil myeloperoxidase (MPO), in procoagulant platelet associated occlusive thrombosis. We have shown a novel marker for procoagulant platelets, trivalent arsenical GSAO, identifies procoagulant, necrotic platelets in a whole blood flow cytometry assay and enables direct visualization of fibrin supporting platelets in occlusive thrombi after 8% $FeCl_3$ injury.
Aims: We aimed to use GSAO to study the role of activated neutrophils and MPO in generation of procoagulant platelets *in vitro* and *in vivo*.
Methods: Thrombus formation in the 8% $FeCl_3$ model of thrombosis in the murine cremasteric arterial circulation was visualized using high speed confocal intravital microscopy in presence of GSAO and fluorochrome-conjugated anti-fibrin, and anti-CD42b antibodies. 1A8 anti-Ly6G antibody administered via intraperitoneal injection was used to achieve neutrophil depletion, which was confirmed by flow cytometry after 48 hours. Thrombus formation was induced by 8% ferric chloride.
Results: Compared with controls, neutrophil–depleted C57Bl6 mice demonstrated decreased GSAO+ platelets, decreased occlusive thrombi and reduction in fibrin formation indicating a role for neutrophils in formation of procoagulant platelets *in vivo*. We examined whether the mechanism for this neutrophil effect involved MPO and downstream oxidants. However, ex-vivo addition of hypochlorous acid to washed human platelets did not induce formation of procoagulant platelets. In murine models, there was no difference in generation of procoagulant platelets at rest or after agonist stimulation in MPO-/-mice *versus* wild type C57Bl6 controls. There was also no difference in stimulus-induced procoagulant subset when mice were exposed to increased circulating MPO via 30 day continuous intravenous infusion of human MPO using a small animal pump compared with saline control mice.
Summary and Conclusions: Thus, neutrophils are important for the generation of procoagulant, fibrin supporting platelets *in vivo*. However, the mechanism is not via MPO.

P410

RELEVANT ROLE OF VON WILLEBRAND FACTOR IN EXPERIMENTAL SEPSIS BY CECAL LIGATION AND PUNCTURE IN MOUSE

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Background: Sepsis is a serious inflammatory response syndrome, in which systemic activation of both inflammatory and coagulation pathways are provoked by severe microbial infection. In addition to the known hemostatic functions, von Willebrand factor (VWF) is assumed to participate in a cross-talk between inflammation and thrombosis. However, little is known about the detailed mechanisms or relevant role of VWF functions in inflammation.
Aims: We therefore studied the physiologic relevance of VWF-dependent inflammatory responses in a mouse model of experimental sepsis by cecal ligation and puncture (CLP).
Methods: The mouse CLP was performed according to the established standard protocol (Rittirsch D, *et al*., Nat Protoc, 2009). Briefly, mouse cecum was ligated distal to the ileocecal valve under laparotomy, punctured with 18 gauge needle and gently pressed until a small drop of stool appeared. The cecum was returned to the peritoneal cavity and 200 mL of saline was injected into the cavity to avoid dehydration before body wall and skin incision were closed with a 4-0 Sofsilk. We compared20 wild-type (WT) and 20 VWF-gene deleted (knock-out; KO) mice (from The Jackson Laboratory, Bar Harbor, ME), all of which were 10-12 weeks of age, healthy and fertile. Excess blood loss was not observed in all (WT or KO) mice during the CLP operation.
Results: Kaplan-Meier analysis revealed the significantly (p <0.05) lower survival rate of KO mice than that of WT (KO; 20.0% *vs* WT; 60.0% at the Day 7 of CLP). The impaired survival rate of KO mice was restored by the bolus administration of human VWF (n=22, 2.5 U/mouse) to an extent comparable to that of WT. Peripheral blood analysis at 24 hours after CLP showed the severe leukocytopenia and neutrophil decrease in KO mice, as compared to WT. In addition, formation of walled-off abscess was confirmed at the peri-cecal space in all the WT and KO mice alive even at the Day 7 of CLP, while such focal abscess formation was not found in mice died before the Day 3 of CLP.
Summary and Conclusions: Our results altogether indicate that VWF could play a role on the recruitment or accumulation of neutrophils for microbial killing at the local inflammatory focus. VWF-mediated platelet aggregate formation in peripheral capillary vessels then could shut down the local microcirculation, thereby blocking systemic microbial expansion as a crucial biological defense mechanism.

P411

A NOVEL ASSAY DEMONSTRATES PROCOAGULANT PLATELETS ARE INCREASED IN PATIENT UNDERGOING CORONARY ANGIOGRAPHY WITH DIFFERENTIAL EFFECTS BY ANTI-PLATELET AND ANTI-COAGULANT THERAPY

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Background: Procoagulant platelets are a subset of activated platelets that support thrombin generation. Excess procoagulant platelets are involved in pathological thrombosis. Study of procoagulant platelets is hampered by the lack of specific and sensitive marker. Recently, we have developed a novel assay for procoagulant platelets using a cytoplasmic necrosis marker, GSAO that identifies necrotic/procoagulant platelets in whole blood flow cytometric assay.
Aims: We postulated that platelets from patients with coronary artery disease (CAD) have heightened propensity to stimulus-induced procoagulant phenotype, which may be modified by antiplatelet agents (APA).
Methods: Following an informed consent, 216 blood samples from 60 patients were collected during coronary angiography from radial/femoral and coronary arteries. Thrombin (2 U/mL) or a combination of thrombin (2 U/mL) and collagen (10 µg/mL) were used to induce procoagulant platelet in diluted re-calcified whole blood. Previously, we established a normal response profile to thrombin and thrombin/collagen stimulation in healthy controls. Linear mixed effects models were used to explore the effect of antiplatelet agents and the presence of angiographically-confirmed CAD on platelet procoagulant propensity.
Results: Compared to healthy controls, study participants undergoing coronary artery angiography had higher proportion of procoagulant platelets at baseline (4.1% *vs* 0.4%, P<0.0001) and in response to thrombin/collagen (31.7% *vs* 13.3%, P<0.0001). Controls and patients without CAD showed a marked synergistic increase in procoagulant platelet generation with dual agonist (thrombin/collagen) stimulation compared to either agonist alone. In contrast, patients with angiogram-confirmed CAD reached maximal procoagulant potential with thrombin stimulation alone. Aspirin alone had no effect on stimulus-induced procoagulant platelet subset, however patients on dual antiplatelet therapy (DAPT) demonstrated reduced thrombin/collagen-induced procoagulant platelets compared to no APA (OR 0.61, P=0.0295). Patients receiving intravenous unfractionated heparin showed marked reduction in procoagulant platelets regardless of the stimulus: thrombin (OR 0.32, P<0.0001) thrombin/collagen (OR 0.38, P=0.0001).
Summary and Conclusions: Compared to healthy volunteers, platelets in patients undergoing coronary angiography showed increased procoagulant potential, which was favorably modified by DAPT but not aspirin alone. Novel cytoplasmic necrosis marker, GSAO has potential as a biomarker in coronary disease.

P412

SUITABILITY OF THE CAPE BABOON IN HUMAN-TARGETED ANTI-PLATELET STUDIES

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Background: Animal models play an integral part in the drug development process and are among other things used to test the pre-clinical safety and efficacy of anti-platelet agents. Non-human primates are considered to be the most suitable animal model for anti-platelet studies. However, limited data exist on the molecular make-up of some commonly used non-human primate species, such as the Cape baboon (*Papio ursinus*). For this reason, concerns are raised regarding the translatability of results obtained with these models to humans.
Aims: The aim of our study was to compare four commonly targeted platelet receptors ($P2Y_{12}$, GPIIb/IIIa, GPVI, and GPIbα) between the Cape baboon and human.
Methods: We performed light transmission aggregometry (LTA), surface-receptor quantification, and Sanger DNA sequencing on baboon platelets and compared it with normal human results.
Results: Baboon ADP-, arachidonic acid-, and collagen-induced platelet aggregation results were significantly different compared to normal human results, even with increased agonist concentrations. However, the differences in collagen-induced aggregation results were not clinically relevant because all except one result (at 8-µg/ml) fell within the normal human reference range. At double the highest human concentration for ristocetin (2.5-mg/ml) baboon platelets gave statistically similar results.
Baboon quantification results showed a 37%, 27% and 25.5% increase in GPIIb, GPIIIa, and GPIbα, respectively. GPVI quantification failed due to non-reactive monoclonal antibodies. P2Y12 quantification was not possible, as no commercial monoclonal antibodies were available at the time of the study.
The P2Y12 protein sequences were 98.8% similar. It differed by four amino acids, none of which have been described as functionally essential. The GPVI protein sequence showed 95% similarity. It included a 14 amino acid difference and a three amino acid deletion. One change was in a region where an amino acid change has been implicated in reduced collagen-induced platelet aggregation in humans. Two differences were directly adjacent to a collagen-binding amino acid. The deletion was within the signaling area of GPVI. Exon 28 of GPIIb failed to sequence. The GPIIb protein sequence for exon 1-27 was 98.2% similar and for exons 29-30 there was 98.3% similarity. There was an 18 amino acid difference. Only one conservative amino acid change was in the ligand-binding region. The GPIIIa protein sequence was 99.6% similar, with three amino acid changes. Again, only one conservative substitution was in the ligand-binding region. 54 amino acid changes were found in GPIbα. The protein sequences of the signal peptide, vWF-binding-, PEST/macroglycoprotein-, transmembrane-and cytoplasmic domains showed 93.8%, 89.4%, 57.9%, 90.5% and 95.0% similarity, respectively. 246 bases in the PEST/macroglycoprotein domain of GPIbα failed to sequence. However, they were in a notoriously variable region. However, no radical amino acid substitutions were found at functionally vital sites.
Summary and Conclusions: Sequentially and functionally baboon P2Y12, GPIIb/IIIa, and GPIbα is comparable to humans. Non-reactive antibodies and changes in critical amino acids caused the baboon GPVI to be not comparable to humans. For this reason, the Cape baboon is deemed a suitable animal model for the evaluation of human-targeted anti-platelet agents directed against P2Y12, GPIIb/IIIa, and GPIbα, but not against GPVI.

P413

CHARACTERIZATION OF PROCOAGULANT PLATELETS IN WHOLE BLOOD USING A NOVEL CELL DEATH MARKER

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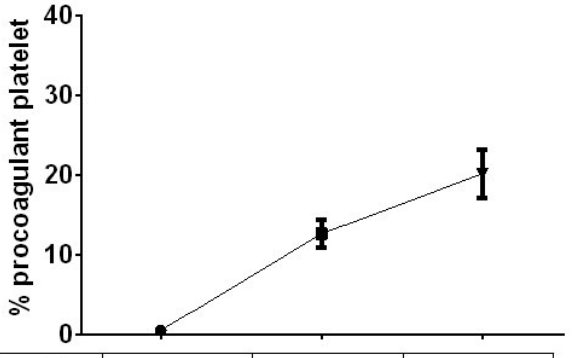
Background: Procoagulant platelets are implicated in the pathogenesis of arterial thrombosis disorders including coronary events, ischaemic stroke and sepsis outcomes. However, our understanding of the contribution this platelet subset has been hampered by the lack of a suitable marker for clinical studies. We have previously reported that a novel cell death marker, GSAO, identifies the procoagulant platelet fraction in assays using washed human platelets.
Aims: Here we present a flow cytometry-based, non-lyse assay for detection of procoagulant platelets in whole blood, which reduces volume of blood required, improves throughput, and enables the interactions between platelets and leukocytes within a physiological matrix.
Methods: Following informed consent, blood was collected from healthy volunteers.15 µL of citrated blood was diluted in 96-well plate wells with a buffer (HBSS, pH 7.35) containing GPRP (2.5 mM), $CaCl_2$ (2.5 mM)±various agonist(s) to 50 µL. Incubation at room temperature was stopped after 10 min by adding 150 µL of HBSS. 20 µL aliquots were stained for 15 min at room temperature with GSAO AF647 (2 µM), CD62P PE (KO2.3), CD45 BUV395 (HI30), and CD41a BV510 (HIP8) in 100 µL final volume. Fixation with 2 volumes of PamFix for 5 min was followed by a single wash step with 10 volumes of HBSS with 0.35% HSA (HBSS/HSA). Resuspended cells were analysed on an LSRFortessa flow cytometer. The lowest FSC threshold was used to acquire 10,000 platelets defined as $CD41a^+/CD45^-$.
Results: At baseline, <1% of platelets showed the procoagulant phenotype. Thrombin (EC_{50} 0.8 U/mL) consistently induced largest PP subset (12.6%±5.1%) whereas collagen (EC_{50} 37 µg/mL) was significantly less potent (1.5%±0.4%). Compared to collagen, GPVI agonist, CRP induced somewhat stronger procoagulant response. However, the maximal procoagulant platelet response (15.8%±7.1%) was induced by a synergistic combination of submaximal doses of thrombin and collagen (Figure 1). The thrombin-induced response was replicated fully by PAR1 agonist TFLLR-NH_2 (EC_{50} 10.2 µM), and only partially by PAR4 stimulation with AYPGKF-NH_2 (EC_{50} 66.9 µM) indicating that the thrombin effect is PAR1 mediated. Stimulation with ADP up to 200 µM failed to induce any increase in PP subset consistent with the concept that the procoagulant response is induced by strong agonists.



Thrombin (2 U/mL)	-	+	+
Collagen (10 µg/mL)	-	-	+

Figure 1.

Summary and Conclusions: The whole blood procoagulant platelet assay makes it feasible to investigate the pathological roles of procoagulant platelets in in mouse models of disease and to explore the potential of GSAO as a biomarker of disease in large scale clinical cohorts including cardiovascular and sepsis settings.

P414

NO DIRECT ASSOCIATION BETWEEN ON-TREATMENT PLATELET REACTIVITY AND BLEEDING EVENTS FOLLOWING CORONARY INTERVENTION AND DUAL ANTIPLATELET THERAPY: A POST HOC ANALYSIS OF THE PRASFIT-ACS STUDY

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Background: Few studies have examined the relationship between the pharmacodynamics of antiplatelet drugs and the risk of clinically significant bleeding following percutaneous coronary intervention for treating acute coronary syndrome (ACS).
Aims: We examined the associations between the pharmacodynamics of prasugrel and clopidogrel and the incidence of bleeding events in the acute and chronic phases after PCI.
Methods: We performed a post hoc analysis of the PRASFIT-ACS (PRASugrel compared with clopidogrel For Japanese patIenTs with ACS undergoing PCI) study of patients in whom platelet reactivity was determined as P2Y12 reaction units (PRU; VerifyNow® P2Y12 assay) or vasodilator-stimulated phosphoprotein-platelet reactivity index (VASP-PRI). Japanese patients were randomized to prasugrel (loading/maintenance dose: 20mg/3.75 mg/day) or clopidogrel (300mg/75mg/day), both in combination with aspirin, for 24–48 weeks. The bleeding outcome was a composite of TIMI major, minor, and clinically relevant bleeding.
Results: Overall, 66/685 (9.6%) and 65/678 (9.6%) of prasugrel-and clopidogrel-treated patients, respectively, experienced major, minor, or clinically relevant bleeding. PRU and VASP-PRI at 5–12 h or in steady state conditions (at 4 weeks) were not associated with the risk of bleeding in the acute (to day 3) or chronic (from day 4 to 14 days after treatment discontinuation) phases of treatment, respectively. The incidence of bleeding was not increased in patients with very low on treatment platelet reactivity (defined as PRU <85 or VASP-PRI <16).
Summary and Conclusions: No direct association observed in the pharmacodynamics of prasugrel and clopidogrel with the risk of bleeding in this cohort of Japanese ACS patients following PCI.

P415

CYSTATHIONINE BETA SYNTHASE (CBS) GENE 844INS68 POLYMORPHISM IN SICKLE CELL DISEASE PATIENTS: FREQUENCY OF VASO-OCCLUSIVE CRISIS

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Background: Sickle cell disease is (SCD) is a chronic hereditary hemolytic anemia characterized by a hypercoagulable and inflammatory state which can lead to vaso-occlusive episodes. Increased serum homocysteine level is an independent risk factor for thromboembolism and cardiovascular disease and is therefore of interest in sickle cell disease. The 844ins68 polymorphism, occurring on the Cystathionine Beta Synthase (CBS) gene which controls the CBS enzyme activity, is accompanied by hyperhomocysteinemia. This mutation, in homozygous or heterozygous state, lowers CBS enzyme activity and is thus considered an independent risk factor for artery occlusion.
Aims: To determine the frequency of the 844ins68 CBS gene polymorphism and its contribution to hyperhomocysteinemia and vaso-occlusive episodes in sickle cell anemia and sickle beta thalassemia patients.
Methods: Nineteen sickle cell anemia, 32 sickle beta-thalassemia and 2 sickle trait subjects together with 42 age and sex matched healthy controls were included after approval of the institutional ethical committee. All patients/guardians signed an informed consent. Fasting serum homocysteine level was measured using immunonephlometry. The CBS 844ins68 polymorphism was detected using conventional PCR.
Results: A significant increase in fasting serum homocysteine level (p-value <0.001) was found in subjects with either thehomozygous or heterozygous variant compared to wild type subjects. A significant increase in the frequency of vaso-occlusive crisis (VOC) was found in SCD patients exhibiting these variants (p-value =0.05). Positive correlation was found between fasting serum homocysteine level and frequency of VOC (r=0.36, p-value=0.009).
Summary and Conclusions: The 844ins68 polymorphism of the CBS gene is a risk factor for VOC and hyperhomocysteinemia in sickle cell disease.

Quality of life, palliative care, ethics and health economics

P416

THE DANISH LYMPHOMA REGISTRY HAS A HIGH COVERAGE AND HIGH DATA QUALITY

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Background: The Danish Lymphoma Registry, LYFO, prospectively includes all lymphoma patients that are diagnosed at Danish hematology departments. It was founded in 1982, and became nationwide from January 2000. It contains detailed information on more than 20,000 patients and is characterized by a very high coverage (>90%); however, the validity of LYFO has not previously been assessed in detail.
Aims: The purpose of this study is to validate data quality and coverage of LYFO.
Methods: A random sample of 3% (N=364) was made from all the patients in LYFO. In addition, four subtypes of lymphomas were validated in detail; CNS lymphomas (N=370), Diffuse Large B-Cell Lymphomas (N=159), Peripheral T-Cell Lymphomas N=141), and Hodgkin Lymphomas (N=672). For all patients date of diagnosis, histological subtype, ECOG performance status and Ann Arbor stage were validated. For each subgroup additional variables were included, *i.e.* treatment, relapse and lab test results. A total of 1,706 patients from the period 2000-2012 were included. The positive predictive values (PPV*s*) of selected variables were calculated for each subgroup and for the entire cohort of patients. Information from medical records was used as reference standard. Data coverage was tested by merging of LYFO with the Danish Cancer Registry (DCR) for the period 2000-2011.
Results: Table 1 shows the joined cohort validation (N=1706). PPV's were above 90% both in the joined cohort and for each subgroup, except for SR and Albumin, which only were validated for Hodgkin Lymphomas. DCR and LYFO contained information on 13,100 lymphoma patients; 11,350 patients appeared in both registries, 12,084 in LYFO and 12,366 in DCR, giving a coverage of 93.9% for LYFO, and 91.8% for DCR. For the 1,016 patients not registered in LYFO, 469 patients were never referred to a hematology department. For 357 patients without clinical lymphoma diagnosis, 105 patients had an unconfirmed pathology diagnosis and 82 patients were diagnosed by autopsy. Registration was missing in the remaining 190 patients. For the 734 patients only registered in LYFO, 56% had indolent lymphoma. Overall survival (OS) was significantly better for patients not registered in DCR, whereas patients not included in LYFO had significantly worse OS. For 10,452 of the 11,350 patients registered in both LYFO and DCR the date of diagnosis +/- one month was consistent (92.1%), in 581 patients the difference was 1-6 months, in 75 patients 6-12 months, and finally in 242 patients the difference exceeded one year.

Tabella 1. Validation for the joined cohort, N=1706

Variable	Number of correctly coded records/number of relevant records reviewed	PPV (%) (95% CI)
Histological subtype	1678/1701	98.7 (98.0;99.1)
Date of diagnosis (±14 days)	1605/1699	94.5 (93.3;95.5)
Ann Arbor Stage (1-2/3-4)	1588/1701	93.4 (92.1;94.5)
Performance status (0-1/2-4)	996/1022	97.5 (96.3;98.3)
LDH above upper limit	948/972	97.5 (96.4;98.4)
Extranodale involvement(0-1/>1)	346/363	95.3(92.6;97.3)
Planned treatment	354/363	97.5 (95.4;98.9)
Chemotherapy	879/885	99.3 (98.5;99.8)
Immunotherapy	799/815	98.0 (96.8;98.9)
Radiotherapy	831/858	96.9 (95.5;97.9)
Relapse	531/550	96.6 (94.7;97.1)
Albumin	521/599	87.0 (84.0;89.6)
SR	411/531	77.4(73,6;80.9)

Summary and Conclusions: The information registered in LYFO is characterized by high validity with PPV's of 77-100% and a high coverage of more than 93%. The fact that only patients in contact with specialized hematology departments are registered in LYFO explains the majority of the discrepancy between LYFO and DCR, since only 190 patients referred to a hematology department, were not registered. For patients missing in DCR it is a plausible explanation that the majority had indolent lymphomas with sparse inpatient contacts. This is supported by the fact that the median OS was dismal for patients registered in DCR only. In conclusion, LYFO is a unique nationwide clinical database characterized by high validity, good coverage and prospective data entry. Therefore LYFO is a valuable resource for future lymphoma research.

P417

QUALITY OF LIFE IN RELAPSED/REFRACTORY MANTLE CELL LYMPHOMA PATIENTS TREATED WITH LENALIDOMIDE *VS* INVESTIGATOR'S CHOICE: MCL-002 (SPRINT) TRIAL

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Background: Measurement of health-related quality of life (QoL) is especially important in patients with relapsed/refractory (R/R) mantle cell lymphoma (MCL), as they generally receive multiple lines of therapy over the course of their disease. The European multicenter MCL-002 (SPRINT) phase II study was the first randomized study of lenalidomide *vs* investigator's choice (IC) in R/R MCL patients with ≤3 relapses on prior therapy and who were ineligible for intensified treatment or stem cell transplantation.
Aims: Examine health-related QoL in R/R MCL patients receiving lenalidomide *vs* IC.
Methods: Patients were randomized 2:1 to receive lenalidomide (25 mg/day on days 1-21 of each 28-day cycle until progressive disease or intolerability *vs* single-agent IC (chlorambucil, cytarabine, fludarabine, gemcitabine, or rituximab). As a planned secondary study endpoint, QoL was measured using the EORTC QLQ-C30 at baseline, after cycles 2, 4, 6, and 8, and at treatment discontinuation. EORTC QLQ-C30 included 5 functional domains, 9 symptom scales, and 1 global health status/QoL scale. Changes from baseline QoL score at each visit for the primary (global health status/QoL) and secondary domains (physical function and fatigue) and their 95% confidence intervals were calculated. A mixed model was employed to analyze differences in mean domain/scale scores between the treatment arms to account for repeated measurements of QoL during follow-up visits. Patients with ≥10 percentage point change (clinically meaningful) for each domain/scale at one or more visits were compared between the two groups using the Chi-square test.
Results: 254 patients (lenalidomide n=170, IC n=84) were enrolled in the trial. QoL data completion declined from 93% at screening to 51% at treatment discontinuation during the course of the study, with higher non-compliance rates typically seen among IC than among lenalidomide patients. QoL was maintained (non-deterioration: no worsening >10 points) with lenalidomide from baseline through last treatment cycle for evaluated primary and secondary QoL domains. Patients treated with lenalidomide reported similar QoL *vs* IC single agents across all domain/scale scores and at each follow-up visit. A trend towards higher rates of clinically meaningful improvement in QoL was observed in lenalidomide treated patients across most function and symptom domains/scales at one or more follow-up visits. Statistically significant QoL differences (≥10%) comparing lenalidomide *vs* IC treatment arms were identified for physical function (24% *vs* 8%, *P*=0.003) and pain (29% *vs* 18%; *P*=0.047).
Summary and Conclusions: Patients with R/R MCL maintained their QoL while receiving lenalidomide despite a longer duration of treatment compared with single agent IC therapy. In addition, patients receiving lenalidomide experienced higher rates of clinically meaningful improvement in QoL for physical function and pain.

P418

QUALITY OF LIFE AND SYMPTOM PROFILE IN LYMPHOMA PATIENTS IN COMPLETE REMISSION AFTER AUTOLOGOUS HAEMATOPOIETIC STEM CELL TRANSPLANTATION (AHSCT)

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Background: It is now clearly established that cure or control of the underlying malignancy is not necessarily accompanied by full restoration of health or quality of life (QoL) in long-term survivors. Self-reported QoL and symptom assessment may be a useful tool to identify patient benefits and risks of AHSCT at long-term follow-up.
Aims: We aimed to examine QoL in lymphoma patients in complete remission at long-term follow-up after AHSCT as compared with population norms and to evaluate symptom profile in this patient population.
Methods: A total of 80 patients with lymphomas (non-Hodgkin lymphomas-36, Hodgkin lymphoma-44) in complete remission after AHSCT with the follow-up of at least 12 months were enrolled in the study. Mean of follow-up period was 18 months. Mean age-34.6 yrs (SD=9.9); male/female 38/42. QoL was assessed using generic questionnaire SF-36; for symptom assessment MDASI was used. To compare patient population with normative data the sample from population norm (PN) data base adjusted to age and gender (n=72) was used. For comparisons t-test for independent samples or Mann-Whitney test was used. Distribution of patients according to the grades of QoL impairment was analyzed: no QoL impairment (Integral QoL index similar to the one in normative population sample), mild (25% decrease from a PN), moderate (25-50% decrease), severe (50-75% decrease) and critical (>75% decrease).
Results: QoL parameters in lymphoma survivors were similar to the ones in normative population for the majority of scales: physical functioning-85.4 *vs* 85.3, role-physical functioning-74.0 *vs* 77.0, bodily pain-80.4 *vs* 80.0, general health-63.9 *vs* 64.0, vitality-68.9 *vs* 65.3, social functioning-86.8 *vs* 86.0, role-emotional functioning-85.6 *vs* 87.0, mental health-74.0 *vs* 70.0. No statistically significant differences between patient group and normative population were obtained. In the most cases patients experienced mild (ratings-1–4) symptoms; 22 % of patients experienced at least one moderate-to-severe (ratings ³5) symptom. QoL parameters were significantly lower ($p<0.001$) in the patient group with moderate-to-severe symptoms as compared with the ones who did not have moderate-to-severe symptoms. The majority of patients (n=58; 73.4%) had no QoL impairment; 7 patients (8.8%) exhibited mild QoL impairment, 7 patients (11.4%)-moderate QoL impairment; and 5 patients (6.4%)-severe QoL impairment. There were no patients with critical QoL impairment. The common symptoms in lymphoma survivors were sleep disturbance (70%), fatigue (67%), memory loss (55%), shortness of breath (52%), numbness (49%), and sadness (39%). Severity of fatigue and sleep disturbance was 1.1 and 1.4 in patients with no QoL impairment, and 5.1 and 4.5 in the group with different grades of QoL impairment, respectively.
Summary and Conclusions: In conclusion, lymphoma patients in complete remission at the mean follow-up of 18 months after ASHT had QoL parameters similar to normative data. The vast majority of patients exhibited either no or mild to moderate QoL impairment. The most common symptoms in lymphoma survivors were sleep disturbance, fatigue, memory loss, and shortness of breath. In the majority of cases they were of mild severity. The subgroup of patients with moderate-to-severe symptoms was identified; these patients experienced significant QoL impartment. Clinical self-reported symptom assessment in lymphoma patients in complete remission after ASHCT provides an important opportunity to promote proactive management of transplant-related side effects and maintain or improve their QoL.

P419

GERMAN PATIENT AND PHYSICIAN PREFERENCES FOR RELAPSED/REFRACTORY MANTLE CELL LYMPHOMA TREATMENT OUTCOMES: A DISCRETE CHOICE EXPERIMENT

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Background: Despite the availability of a wide number of treatments for relapsed/refractory (r/r) mantle cell lymphoma (MCL), no standard of care has emerged. There are no studies evaluating preferences for treatment outcomes for r/r MCL.
Aims: This study was a discrete choice experiment (DCE) designed to elicit preferences for r/r MCL treatment outcomes among patients and physicians experienced in treating MCL in Germany.
Methods: 6 treatment attributes with 3 levels each were identified through lit-

erature review and qualitative interviews with 6 hematologists and 5 r/r MCL patients; overall survival (OS; Levels: 3,2,1 years), progression-free survival (PFS; 2,1,0.5 years;), fatigue (no to mild, mild relieved by rest, mild not relived by rest), nausea (no, mild, moderate), risk of serious infection (low, moderate, high), treatment administration (oral pill daily at home, monthly infusion at hospital, weekly infusion at hospital). The mean relative attribute importance was assessed in a DCE using a Hierarchical Bayes model. Patients were asked to base choices on their own preferences. Physicians were asked to make their choices as if they were patients. 19 r/r MCL patients and 50 physicians completed the online DCE survey.
Results: The analysis of mean relative attribute importance revealed that OS (38%), treatment administration (17%) and risk of serious infection (16%) were the most important treatment choice attributes to r/r MCL patients, followed by PFS (11%), fatigue (10%) and nausea (8%). The most important attributes to physicians were OS (44%), risk of serious infection (16%) and PFS (15%), followed by fatigue (11%), treatment administration (8%) and nausea (7%).
Summary and Conclusions: OS is the most important treatment attribute to both German r/r MCL patients and treating physicians, however, the relative importance of the other attributes differ between patients and physicians. Our results suggest the importance of mode of administration to patients may be underestimated by physicians. The results may be used to guide what treatment endpoints to include in clinical trials for the purpose of designing trials that reflect the needs of the patients.

P420

COST EFFECTIVENESS OF BORTEZOMIB, RITUXIMAB, CYCLOPHOSPHAMIDE, DOXORUBICIN AND PREDNISONE FOR THE FIRST-LINE TREATMENT OF MANTLE CELL LYMPHOMA PATIENTS NOT ELIGIBLE FOR STEM CELL TRANSPLANTATION

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Background: Mantle cell lymphoma (MCL) is a rare type of non-Hodgkin's lymphoma, incorporating the worst aspects of aggressive and indolent lymphoma subtypes; it progresses quickly and is typically incurable. In the UK, patients unsuitable for haematopoietic stem cell transplantation (HSCT) primarily receive rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone (R-CHOP). Although most patients initially respond to this therapy, the durability of these responses is limited, and the majority of patients relapse within 2 years. The LYM-3002 trial demonstrated that the use of bortezomib, rituximab, cyclophosphamide, doxorubicin and prednisone (VR-CAP) close to doubled progression-free survival (PFS) relative to R-CHOP (24.7 *vs* 14.4 months; HR=0.63, p<0.001). Duration of overall response for VR-CAP was more than double that of R-CHOP (median of 36.5 *vs* 15.1 months), resulting in an increase in treatment free interval of almost 20 months *vs* R-CHOP (median of 40.6 *vs* 20.5 months).
Aims: To assess the cost effectiveness of VR-CAP compared with R-CHOP as first-line treatment for patients with MCL unsuitable for HSCT, from the perspective of the UK National Health Service (NHS).
Methods: A cost-effectiveness model was constructed based upon line of treatment, progression status and survival. The model has a 20-year time horizon, extrapolating LYM-3002 clinical trial data using parametric models that were fit to PFS and overall survival (OS) Kaplan–Meier curves. The patient population was assumed to be the same as in the LYM-3002 trial with demographic data taken from US, Canadian and Western European patients. Utilities were derived from EQ-5D data from the clinical trial, supplemented with data from published literature for long-term health status. Costs were included for drugs, administration, adverse events and medical resources such as blood transfusions and haematologist visits. Drug dosing and concomitant medications, adverse events, blood transfusions and second-line treatment information were taken from the LYM-3002 trial data. Other resource use, such as blood tests and haematologist visits, was based upon UK clinician advice. Probabilistic and structural sensitivity analyses were conducted to assess the uncertainty of the results.
Results: Total lifetime costs in the VR-CAP arm were £45,185 compared to £27,460 in the R-CHOP arm. Treatment with VR-CAP resulted in an increase in life years (7.67) compared to R-CHOP (6.66). The same is seen for quality-adjusted life years (QALYs), 4.14 and 3.35 for VR-CAP and R-CHOP, respectively. Thus the additional cost associated with VR-CAP is partially offset by additional benefit; resulting in an incremental cost-effectiveness ratio of £22,227. In probabilistic sensitivity analysis, there was an 83% chance that VR-CAP was cost effective at the standard UK threshold of £30,000 per QALY gained. The model was most sensitive to assumptions regarding the extrapolation of PFS and OS and utility associated with post-progression from second-line treatment.

Tabella 1.

Model results	VR-CAP	R-CHOP	Difference
Clinical outcomes			
Median PFS, months	26.5	15.0	11.5
Median predicted OS, months	75.0	62.6	12.4
Mean predicted life years (over a patient lifetime)	7.67	6.66	1.01
QALYs	4.14	3.35	0.80
Cost outcomes			
Medication and administration	£27,195	£9,290	£17,905
Monitoring	£11,715	£10,741	£974
Next line of treatment	£6,275	£7,429	-£1,155
Total	£45,185	£27,460	£17,725
ICER – Cost/life year	£17,631		
ICER – Cost/QALY	£22,227		

Key: ICER, incremental cost-effectiveness ratio; OS, overall survival; PFS, progression-free survival; QALYs, quality-adjusted life years; R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone; VR-CAP, bortezomib, rituximab, cyclophosphamide, doxorubicin and prednisone.

Summary and Conclusions: Based upon data from the LYM-3002 trial, VR-CAP is a cost-effective treatment for previously untreated patients with MCL who are unsuitable for HSCT in the UK.

P421

COST COMPARISON OF HEMATOPOIETIC STEM CELL TRANSPLANTATION AND CONVENTIONAL THERAPY FOR PATIENTS WITH THALASSEMIA MAJOR IN CHINA

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Background: The conventional therapy (CT) for b-thalassemia major (TM) consists of regular transfusion and iron-chelating therapy for lifetime. However, the high cost of CT can lead to poor compliance. Hematopoietic stem cell transplantation (HSCT) provides an alternative option for TM patients. To our knowledge, recent data of China has not been available in the literature on cost comparison between HSCT and CT, leading to debate on selection of treatment for patients with TM.
Aims: To compare the lifetime undiscounted mean cost (UMC) of HSCT with UMC of CT in the TM patients and to investigate the relationship of the clinical features to cost outcomes of HSCT.
Methods: We estimated UMC of 93 TM-HSCTs in 2011 with a median age of 6 years (range, 3-16) and a median follow-up time of 3.6 years (range, 3.1-4.1). The relationship between the UMC of HSCT and patient characteristics were analyzed. The UMC of 93 TM-HSCTs was compared with UMC of CT based on total 1526 TM patients. Age was used as matching variant, and the mean cost of each age was calculated, then cumulative cost was further adjusted for age in patients.
Results: UMC of TM-HSCT was CNY (Chinese Yuan) 235,254/USD 37,664 (95% confidence interval (CI) CNY208,081-262,719/USD 33,293-42,035) with mean hospital stay of 60.5 days (95% CI 49-71). With the addition of the UMC of CNY 345,317/USD55,251 for 20 years of follow-up, total undiscounted lifetime (55 years) cost of TM-HSCT were CNY 465,975/USD 74,602. However, the corresponding costs of patients undergoing CT were CNY 2,101,488/ USD336,238 and CNY 7,489,519/ USD1,198,323 respectively. Patient characteristics were helpless to predict the costs. HLA-mismatched transplants increased significantly UMC of HSCT than matched transplants (USD 35,818 *vs* USD 52,771; p<0.001). The development of GVHD were associated with higher costs (USD 63,933 *vs* USD 44,547; p =0.001).

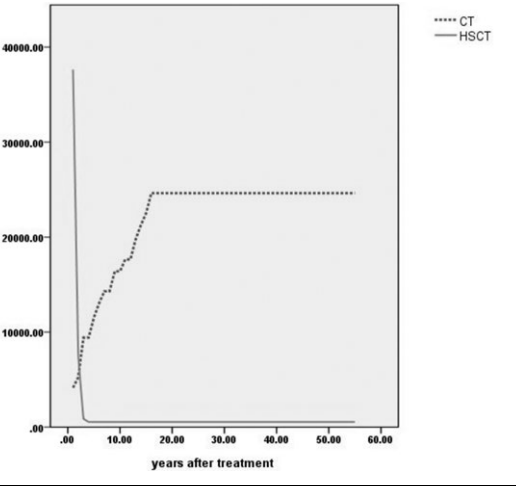

Figure 1.

Summary and Conclusions: Cost comparison of HSCT and CT suggests that HSCT is an efficient and high cost-effective treatment for TM patients. Mismatched donor transplant increases the cost of HSCT.

P422

CROSS-CULTURAL DEVELOPMENT OF FOUR EORTC QUESTIONNAIRES TO ASSESS QUALITY OF LIFE IN PATIENTS WITH HODGKIN LYMPHOMA, NON-HODGKIN LYMPHOMA AND CHRONIC LYMPHOCYTIC LEUKAEMIA

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Background: Advances in the management of patients with lymphoma or chronic lymphocytic leukaemia (CLL) have changed the survival outcomes dramatically the last two decades. The number of new agents for these patients is increasing and quality of life (QoL) is becoming a primary objective. Unfortunately, questionnaires to assess disease-specific QoL among patients with Hodgkin lymphoma (HL), high-grade non-Hodgkin lymphoma (HG-NHL), low-grade non-Hodgkin lymphoma (LG-NHL) and CLL are lacking.
Aims: This study describes the combined development of four disease-specific QoL questionnaires for patients with HL, HG-NHL, LG-NHL and CLL to supplement the European Organization for Research and Treatment of Cancer (EORTC)-QLQ C30 core cancer questionnaire.
Methods: Questionnaire development was conducted according to guidelines from the EORTC Quality of Life Group. Phase I comprised generation of QoL issues relevant to patients. Phase II included operationalization and assessment of item relevance. In phase III, items were pretested in a cross-cultural sample representing all four malignancies to assess issues such as comprehensibility and intrusiveness of items. Data were analysed per malignancy.
Results: Seventy-five QoL issues were identified through focus groups and systematic literature searches. Semi-structured interviews with 80 health-care professionals and with 245 patients (75 HL, 66 HG-NHL, 41 LG-NHL and 63 CLL) resulted in a provisional module of 39 items representing items relevant for all or at least one of the four malignancies. In phase III this was further tested in 67 HL, 117 HG-NHL, 67 LG-NHL and 86 CLL patients with different phases of disease and treatment from five European countries. Results from the interviews, clinical experiences and statistical analyses resulted in a questionnaire with 27 items for HL (EORTC QLQ-HL27), 29 items for HG-NHL (EORTC QLQ-NHL-HG29), 20 items for LG-NHL (EORTC QLQ-NHL-LG20) and 17 items for CLL (EORTC QLQ-CLL17). The items are conceptualized in several multi-item scales: symptom burden, physical condition/fatigue, worries/fears health and functioning, emotional impact (not in the CLL module), neuropathy (only in the HG-NHL module).
Summary and Conclusions: This study provides four modules for use in clinical trials and observational research in conjunction with the EORTC QLQ-C30 for assessment of QoL for patients with HL, HG-NHL, LG-NHL and CLL. Phase IV of the questionnaire development will start in 2015 and comprises international field testing of the four questionnaires, *i.e.* EORTC QLQ-HL27, QLQ-NHL-HG29, QLQ-NHL-LG20 and QLQ-CLL17.

P423

INCREASED TREATMENT COST ASSOCIATED WITH MANTLE CELL LYMPHOMA DISEASE PROGRESSION

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Background: While recent advances in the treatment of mantle cell lymphoma (MCL) have increased remission rates and time to relapse, the cost of treatment has not been well described.
Aims: This study evaluated the cost of care across the first, second, and third treatment lines (1L/2L/3L) in patients with MCL.
Methods: MCL patients were identified in the IMS PharMetrics Plus database containing de-identified health plan claims on >100 million U.S. lives. All patients had ≥2 MCL claims (ICD-9-CM: 200.4X) at least 30 days apart between 2008 and 2012, no MCL claim 6 months prior to the initial claim (index), and continuous enrollment in the health plans for ≥3 months post-index. Monthly costs were calculated from the beginning of each line of therapy and up to 24 months post-index. Patients receiving autologous stem cell transplant (ASCT) during 1L treatment were analyzed separately. The 1L was defined as a MCL treatment with all agents administered within 90 days of the index and a new line was determined when restarting therapy following a 90 day gap in a previous line, or addition of a new agent >90 days from the start of the previous line.
Results: A total of 872 patients (mean age 63 years, 72.0% male) were selected, of whom 172 (19.7%) received ASCT, 334 (38.3%) and 119 (13.6%) were followed to the end of 2L and 3L, respectively. For patients who relapsed/were refractory while on 1L treatment, the median TTNT was 350 days for pts after ASCT (n=54) and 422 days for non-ASCT pts from initiation of 1L treatment to the beginning of 2L (n=280). For patients receiving ASCT, the mean monthly cost between diagnosis and the start of 2L treatment was $12,565 with the highest mean monthly cost occurring between months 4 and 6 ($43,703). Pharmacy, inpatient, and outpatient costs accounted for approximately 26%, 47%, and 27%, respectively. For non-ASCT patients, the mean monthly cost following diagnosis to the next line treatment was $5,964, with the highest mean monthly cost occurring in the first 3 months ($24,363). For these patients, pharmacy, inpatient, and outpatient costs accounted for approximately 43%, 27%, and 30%, respectively. For all patients, monthly 1L costs stabilized at about 16 months to approximately $4,000. Upon initiating 2L and 3L therapy, monthly costs rose to $21,660 and $22,718 over the first three months, respectively, then followed by a pattern of reduction similar to what was seen in 1L treatment. For 2L and 3L therapy, pharmacy costs made up approximately 45% of all costs, with inpatient and outpatient costs each accounting for 25%>30% of remaining costs. Inpatient costs rose to 47% of total costs during months 4-6 after the initiation of 3L therapy.
Summary and Conclusions: Treatment costs for MCL were the highest during the first 3 months of treatment in each line of therapy then declined and stabilized to about 25%>30% of the initial costs. This pattern suggests that prolongation of time to progression may yield economic as well as humanistic benefits. Patients receiving ASCT incurred more than twice the expense compared with those not receiving ASCT during 1L therapy.

P424

A MODEL-BASED ASSESSMENT OF THE BENEFITS AND RISKS OF PONATINIB VERSUS BOSUTINIB IN THIRD-LINE TREATMENT OF CHRONIC-PHASE CHRONIC MYELOID LEUKEMIA (CP-CML)

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Background: Ponatinib is a potent oral 3rd-generation tyrosine kinase inhibitor (TKI) with evidence of better efficacy than 2nd-geneation TKIs in 3rd-line treatment of CML (Lipton 2014), but with potentially higher risk of vascular occlusive adverse events (AEs). Bosutinib, a 2nd-generation TKI, differs in both efficacy and characteristic AEs.
Aims: A model was developed to quantify the benefit-risk difference between ponatinib and bosutinib for CP-CML patients after failure of 2 previous TKIs, in terms of survival and quality-adjusted survival.
Methods: The Excel-based model uses estimates of treatment response, in terms of complete cytogenic response (CCyR) at 12 months, and AEs to quantify the overall benefit-risk difference with ponatinib *vs* bosutinib using common metrics: outcomes of survival and quality-adjusted survival are measured in life-years (LYs) and quality-adjusted life-years (QALYs) accrued over patients' remaining lifetimes. Quality of life (QoL) is quantified using health-state utilities, where each health state is assigned a value from 0 (death) to 1 (perfect health). A decision tree captures the risk of transient AEs, assumed to occur immediately upon start of treatment that may cause death or short-term decrements to QoL, but if not fatal, have no long-term consequences. Chronic AEs occur either immediately or over time and have both short-and long-term consequences. AEs of interest are grade 3 or 4 affecting survival and/or QoL that differed between treatments in clinical trials, including cardiovascular, hematologic, and gastrointestinal AEs, as well as blindness and amputation. In an ensuing Markov model, patients are assigned to health states based on treatment response and their most serious previous chronic AE. In each 3-month Markov cycle, patients are at risk of incident chronic AEs and of dying from prior or incident chronic AEs, CML, or age-specific other causes. Each cycle, patients accrue LYs, and QALYs reflecting response status, less any QoL loss for their most serious chronic AEs. In the base case, the model applies an evidence-based difference in utility between responders and non-responders, but assumes no survival advantage for responders. Treatment response was estimated from a recent synthesis of CML studies, estimating 60% response for ponatinib and 22% for bosutinib. Rates of AEs were derived from published literature and prescribing information. Utility decrements for responders/non-responders and AEs, and mortality for AEs were estimated from published literature. Extensive sensitivity and scenario analyses were conducted.
Results: In the base case assuming response confers no survival advantage, patients receiving ponatinib *vs* bosutinib accrue 0.43 fewer LY (9.5 *vs* 9.9, respectively) of survival. When LY are adjusted for QoL, ponatinib provides an

additional 0.24 QALYs *vs* bosutinib (Figure). In scenario analyses where response is assumed to confer a 5% survival advantage, patients on ponatinib are estimated to survive an additional 0.37 years (10.8 *vs* 10.4 LY, respectively) *vs* bosutinib and accrue 7.9 *vs* 7.0 QALYs.

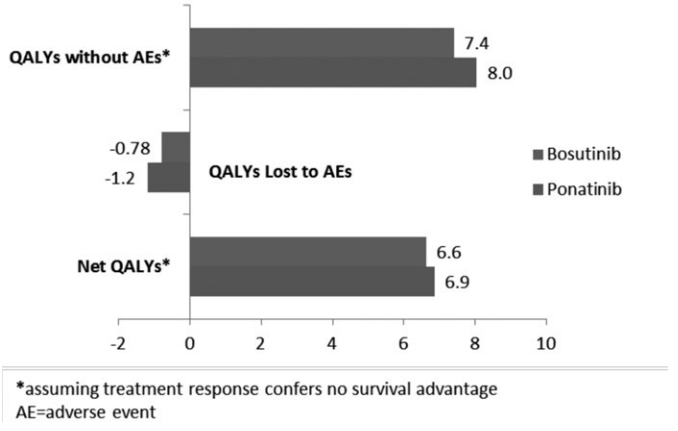

Figure 1. Estimated lifetime quality-adjusted life-years (QALYs) for 3rd-line CP-CML patients treated with ponatinib versus bosutinib.

Summary and Conclusions: Given the assumptions and limitation of this analysis, we found that in 3rd-line CP-CML patients, the positive effect of ponatinib' s higher efficacy on quality of life more than offsets losses due to AEs, even if no survival advantage for treatment responders was assumed. This analysis highlights the need to consider the negative effect of non-response as well as AEs on patients' quality of life and survival when evaluating alternative treatments in CML.

P425

A COST-UTILITY ANALYSIS OF DEFERIPRONE COMPARED TO DESFERRIOXAMINE AND DEFERASIROX FOR THE TREATMENT OF CHRONIC IRON OVERLOAD IN PEOPLE WITH THALASSAEMIA: AN ITALIAN ADAPTATION OF THE UK MODEL

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Background: In thalassemia major (TM) three chelators are available to treat chronic iron overload due to blood transfusions: subcutaneous desferrioxamine (DFO), oral deferiprone (DFP) and oral deferasirox (DFX).

Aims: This study evaluated the relative cost effectiveness of the three chelators in monotherapy.

Tabella 1.

	Rx cost p.a.(€)	Administration costs p.a. (€)	Monitoring costs p.a. (€)	AE costs p.a. (€)	Total costs (€)	QALYs
Scenario 1: iron chelators impact cardiac morbidity and mortality. Time horizon: 5 years.						
DFP	24,401	0	1,439	0	25,840	3.921
DFX	151,546	0	1,378	0	152,924	3.825
DFO	78,683	16,735	1,106	0	96,524	3.242
Scenario 2: iron chelators impact only cardiac mortality. Time horizon: 5 years.						
DFP	24,401	0	1,439	0	25,840	3.925
DFX	151,546	0	1,378	0	152,924	3.851
DFO	78,683	16,735	1,106	0	96,524	3.263
Scenario 3: iron chelators impact only cardiac morbidity. Time horizon: 5 years.						
DFP	24,401	0	1,439	0	25,840	3.921
DFX	151,546	0	1,378	0	152,924	3.899
DFO	78,683	16,735	1,106	0	96,524	3.304
Scenario 4: iron chelators are all equivalent. Time horizon: 1 year. No discount.						
DFP	5,222	0	308	0	5,530	0.840
DFX	33,054	0	307	0	33,360	0.840
DFO	17,162	3,650	241	0	21,053	0.712

Methods: Patients enrolled in the MIOT (Myocardial Iron Overload in Thalassemia) project and who received monotherapy with a single iron chelator for at least 18 consecutive months were evaluated for assessment. The cost-effectiveness model was adapted from the previously published UK model (Bentley A *et al.* Pharmacoeconomics 2013;31:807–22). Based on literature data, it was assumed that all three iron chelators had comparable efficacy in controlling serum ferritin and liver iron concentration, wherea 4 different efficacy-based scenarios were explored in respect to cardiac morbidity and mortality using Markov-type models. Treatment costs reflect the Italian market: Incremental costs and quality-adjusted life-years (QALYs) were calculated for each iron chelator, with cost effectiveness expressed as incremental cost per QALY.
Results: Within the MIOT project, none of the adverse events (AE) considered in the UK model (neutropenia, agranulocytosis, Franconi syndrome, hepatitis) were detected for the 193 TM patients who had been received the same chelator for at least 18 months and were considered as reference group. The table shows costs and QALYs in the different modelled scenarios. For the first three scenarios a 5-year period was considered per patient while for the last scenario a 1-year time horizon was used and the discount rate of 3.5% was not applied. DFP was the dominant strategy in all scenarios, providing greater QALY at a lower cost.
Summary and Conclusions: The results of this analysis indicate that, from an Italian perspective, DFP is the most cost-effective treatment available for managing chronic iron overload in β-thalassaemia patients. Use of DFP in these patients could therefore result in substantial cost savings.

SIMULTANEOUS SESSIONS

Multiple myeloma: Clinical studies 2

S426

RESULTS FROM TWO PHASE 3 STUDIES OF POST-TRANSPLANT BORTEZOMIB (BTZ) CONSOLIDATION *VS* OBSERVATION (OBS) IN PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA (NDMM)

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Background: HDT followed by ASCT remains the gold standard for treatment of NDMM patients who are able to tolerate the procedure. Following ASCT, consolidation with novel agents for a fixed time period (or number of cycles) can improve outcomes. However, there are currently few published data on BTZ consolidation therapy, although a study by the Nordic Myeloma Study Group has reported results in BTZ-naïve patients (Mellqvist *et al.* Blood 2013).
Aims: The results from two large randomized controlled phase 3 studies were combined to investigate BTZ consolidation or OBS in patients with NDMM both with and without prior BTZ treatment.
Methods: MMY3012 (NCT00416273; 222 patients aged ≤60 yrs) and MMY3013 (NCT00416208; 158 patients aged 61-75 yrs) recruited adults with NDMM who underwent induction therapy followed by ASCT. Patients were randomized 1:1 to receive BTZ consolidation (1.6 mg/m² IV days 1, 8, 15, 22; 4x35-day cycles) or OBS, 60-120 days after ASCT. The primary endpoint was progression-free survival (PFS) from the start of induction; secondary endpoints included response rate, overall survival (OS), and safety. Factors affecting PFS were also assessed by post-hoc multivariate analysis. Responses were assessed per EBMT response criteria, with VGPR as an additional category. Adverse events (AEs) were graded per NCI-CTCAE v3.0.
Results: In 371 randomized patients, median age was 59 yrs (35-76); 62% were male, 14%/84% were Durie-Salmon stage II/III. Of the 278 patients assessed for cytogenetics, 37% were classified as high-risk (32% del13q, 10% t(4;14), 6% del17p). Overall, 50% of patients had received prior BTZ therapy; the most common induction regimen was VCD (40%). Others included dexamethasone/idarubicin (14%), dexamethasone (13%), VAD (9%), adriamycin/dexamethasone (6%) and VD (6%). In the overall study population, patients who received BTZ-based induction showed a trend towards improved PFS (HR 1.24; 95% CI: 0.97, 1.59; p=0.084) by unadjusted Cox regression analysis. Outcomes for patients receiving BTZ consolidation or OBS only are shown in the Table 1 (median follow-up 50 months from start of induction). PFS was significantly improved by approximately 6 months, but there was no improvement in OS. In a post-hoc exploratory multivariate analysis, BTZ consolidation remained a predictor for PFS (HR 0.69; 95% CI: 0.51, 0.93; p=0.016). There appeared to be an increased risk of progression in patients with high-risk cytogenetics (HR 1.45; 95% CI: 1.05, 1.99; p=0.025) or those with <VGPR at baseline (HR 1.44; 95% CI: 1.05, 1.98; p=0.024). Age >60 had no effect on PFS (HR 1.24; 95% CI: 0.87, 1.77; p=0.240); neither did the use of non-BTZ induction (HR 1.17; 95% CI: 0.83, 1.66; p=0.375).

Table 1.

	BTZ (N=186)	OBS (N=185)	HR (95% CI)	P-value
Best response ≥VGPR before consolidation, n (%)	102 (55)	109 (59)		
Best response ≥VGPR after consolidation, n/N* (%)	109/177 (62)	86/180 (48)		
Median PFS, months†	33.6	27.8	0.70 (0.55, 0.90)	0.0058
Best response <VGPR‡	33.3	24.5	0.58 (0.39, 0.88)	0.0089
Best response ≥VGPR‡	33.4	29.3	0.81 (0.59, 1.31)	0.218
BTZ-naïve‡	33.3	29.0	0.74 (0.53, 1.05)	0.090
BTZ-pretreated‡	33.6	27.8	0.77 (0.54, 1.10)	0.152
High-risk cytogenetics‡	30.6	24.2	0.66 (0.41, 1.05)	0.074
Standard cytogenetics‡	34.3	30.3	0.77 (0.47, 1.27)	0.297
Median OS, months†	NR	NR	0.94 (0.64, 1.39)	0.75
Any-grade (grade ≥3) TEAE, %	95.2 (30.6)	94.1 (24.3)		
Most frequent any-grade (grade ≥3) TEAE, %				
Diarrhea	42.5 (8.6)	8.1 (0.5)		
Nausea	33.3 (4.8)	4.9 (0)		
Vomiting	27.4 (3.2)	3.2 (0)		
Fatigue	24.2 (0.5)	9.2 (1.1)		
Discontinuations due to AE, %	15.1	0		
SAE, %	10.8	16.8		
Deaths, %	25.3	30.3		

NR, not reached
*Response-evaluable patients
†Cox proportional hazards regression model
‡Univariate Cox regression

Summary and Conclusions: These data indicated that a fixed period (4 cycles) of BTZ consolidation was beneficial in NDMM patients with or without prior BTZ exposure. A higher proportion of patients achieved ≥VGPR after BTZ consolidation than OBS. PFS was significantly improved, but there was no improvement in OS at data cutoff, possibly related to limited follow-up and the use of effective salvage options. Subgroups that seemed to benefit from BTZ consolidation were patients with <VGPR and those with high-risk cytogenetics; age and BTZ pretreatment did not affect PFS. The BTZ dosing regimen was generally well-tolerated.

S427

EFFECT OF CARFILZOMIB, LENALIDOMIDE, AND DEXAMETHASONE *VS* LENALIDOMIDE AND DEXAMETHASONE IN PATIENTS WITH RELAPSED MULTIPLE MYELOMA BY LINE OF THERAPY: INTERIM RESULTS FROM THE PHASE 3 ASPIRE STUDY

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Background: Previously reported results from ASPIRE (NCT01080391; N=792 patients) showed that carfilzomib, lenalidomide, and dexamethasone (KRd) significantly improved progression-free survival (PFS) *vs* lenalidomide and dexamethasone (Rd) in patients with relapsed multiple myeloma, with a favorable benefit-risk profile (Stewart *et al. N Engl J Med* 2015;372:142-52).
Aims: A secondary analysis of efficacy and safety results from patients treated with KRd or Rd after first relapse (1 prior line of therapy) *vs* ≥2 prior lines of therapy in the ASPIRE study is presented.
Methods: Adults with relapsed multiple myeloma who received 1-3 prior lines were eligible. Patients were randomized (1:1) to KRd or Rd. All patients received lenalidomide (25 mg) on days 1-21 and dexamethasone (40 mg) on days 1, 8, 15, and 22 of a 28-day cycle. Patients in the KRd arm received carfilzomib as a 10-min infusion on days 1, 2, 8, 9, 15, and 16 during cycles 1-12 (20 mg/m^2 [days 1 and 2 of cycle 1]; 27 mg/m^2 thereafter). Carfilzomib was omitted on days 8 and 9 during cycles 13-18 and was not administered beyond 18 cycles. All patients provided informed consent.
Results: Median PFS for patients receiving 1 prior line (n=341) was 29.6 months (95% confidence interval [CI]: 23.2-33.5) for KRd *vs* 17.6 months (95% CI: 15.0-22.2) for Rd (hazard ratio [HR]: 0.694; *P*=.0083). Median PFS for patients receiving ≥2 prior lines (n=451) was 25.8 months (95% CI: 22.2-31.0) for KRd *vs* 16.7 months (95% CI: 13.9-22.0) for Rd (HR: 0.688; *P*=.0017). Best overall responses in patients who received 1 *vs* ≥2 prior lines of therapy are presented in the Table 1. Overall response rates (partial response or better) were 87.0% (KRd) *vs* 70.1% (Rd) in patients with 1 prior line and 87.3% (KRd) *vs* 64.4% (Rd) in patients with ≥2 prior lines. In patients with 1 prior line, 33.7% (KRd) *vs* 7.0% (Rd) achieved a complete response (CR) or better, including 12.5% (KRd) and 3.2% (Rd) who achieved a stringent complete response (sCR). In patients with ≥2 prior lines, 30.2% (KRd) *vs* 10.9% (Rd) achieved a CR or better, including 15.6% (KRd) and 5.0% (Rd) who achieved a sCR. Adverse events (AEs) grade ≥3 were reported in 85.7% (KRd) and 79.9% (Rd) of patients who received 1 prior line of therapy and 81.9% (KRd) and 81.3% (Rd) of patients who received ≥2 prior lines. AEs of interest (grade ≥3; grouped terms) included dyspnea (1 prior line: 2.7% [KRd] and 2.6% [Rd]; ≥2 prior lines: 3.3% [KRd] and 1.7% [Rd]); cardiac failure (1 prior line: 3.3% [KRd] and 1.9% [Rd]; ≥2 prior lines: 4.3% [KRd] and 1.7% [Rd]); ischemic heart disease (1 prior line: 4.9% [KRd] and 1.3% [Rd]; ≥2 prior lines: 1.9% [KRd] and 2.6% [Rd]); hypertension (preferred term; 1 prior line: 3.8% [KRd] and 0.6% [Rd]; ≥2 prior lines: 4.8% [KRd] and 2.6% [Rd]); and acute renal failure (1 prior line: 3.3% [KRd] and 3.2% [Rd]; ≥2 prior lines: 3.3% [KRd] and 3.0% [Rd]).

Table 1. Best overall responses by prior line of therapy (1 *vs* ≥2).

	1 Prior Line of Therapy		≥2 Prior Lines of Therapy	
	KRd (n=184)	Rd (n=157)	KRd (n=212)	Rd (n=239)
Best Overall Response, n (%)[a]				
Stringent Complete Response	23 (12.5)	5 (3.2)	33 (15.6)	12 (5.0)
Complete Response	39 (21.2)	6 (3.8)	31 (14.6)	14 (5.9)
Very Good Partial Response	78 (42.4)	57 (36.3)	73 (34.4)	66 (27.6)
Partial Response	20 (10.9)	42 (26.8)	48 (22.6)	62 (25.9)
Overall Response Rate (ORR), n (%)[a]	160 (87.0)	110 (70.1)	185 (87.3)	154 (64.4)
95% Confidence Interval of ORR[b]	(81.2–91.5)	(62.2–77.1)	(82.0–91.4)	(58.0–70.5)

[a]Determined by Independent Review Committee according to IMWG-URC. Patients evaluated for ORR had a best overall response of partial response or better.
[b]Clopper–Pearson interval.

Summary and Conclusions: The use of KRd led to a 1-year improvement in median PFS *vs* Rd after first relapse, and a 9-month improvement in median PFS *vs* Rd in patients with ≥2 prior lines of therapy, with similar HRs. KRd had a favorable benefit-risk profile compared with Rd after 1 and ≥2 prior lines of therapy in patients with relapsed multiple myeloma.

S428

THE QUADRUPLET COMBINATION OF CARFILZOMIB, CYCLOPHOSPHAMIDE, LENALIDOMIDE, AND DEXAMETHASONE IS SAFE AND WELL TOLERATED AS INDUCTION THERAPY FOR NEWLY DIAGNOSED, TRANSPLANT ELIGIBLE, MYELOMA PATIENTS

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Background: Triplet regimens containing an immunomodulatory agent (IMiD), proteasome inhibitor (PI) or both, are standard induction therapy for myeloma (MM) patients. Attempts to improve responses with quadruplets containing bortezomib and lenalidomide led to increased toxicity without improving outcomes. New generation PIs such as carfilzomib, with less off-target activity and better toxicity profiles are now available and recent data from the ASPIRE trial demonstrate that the triplet carfilzomib, lenalidomide, dexamethasone is safe and effective at relapse. Therefore, it is important to define whether a quadruplet combining carfilzomib and lenalidomide at induction can improve outcomes whilst minimising additional toxicity. The UK NCRI Myeloma XI trial is the first large, phase III study aiming to answer this question with the addition of the quadruplet carfilzomib, cyclophosphamide, lenalidomide and dexamethasone (KCRD) experimental arm to the transplant eligible (TE) pathway. Overall the trial seeks to establish the optimum induction and maintenance approaches for all newly diagnosed MM (NDMM) patients with almost 4000 recruited to date.
Aims: To assess the safety and tolerability of KCRD in TE, NDMM.
Methods: In 2013, the TE pathway of the NCRI Myeloma XI trial was amended to include an assessment of the quadruplet carfilzomib (20/36mg/m^2 IV D1-2,8-9,15-16. 20mg/m^2 only cycle 1 D1-2), cyclophosphamide (500mg PO D1,8), lenalidomide (25mg PO D1-21), dexamethasone (40mg PO D1-4,8-9,15-16). This up-front quadruplet is being compared to the sequential strategy of triplet IMiD combinations (with thalidomide or lenalidomide) followed by additional PI triplet therapy (with bortezomib) for those with a suboptimal response (<VGPR) prior to ASCT. Treatment is given to max. response/unacceptable toxicity before proceeding to ASCT, followed by a maintenance randomisation. Here we report a safety analysis for the KCRD regimen.
Results: Following informed consent 257 patients have commenced KCRD treatment to date. KCRD is well tolerated with patients completing a mean of 4.2 cycles (±1.17), median 4 (range 1-7) prior to transplant. Dose modifications have been required in 63% of KCRD patients (55% with modifications to carfilzomib, 38% to lenalidomide). Data for study drug-related toxicity in patients who have completed at least one cycle of KCRD show a low incidence of grade 2-4 neuropathy (sensory 1.2%, motor 4.4%), all grade infusion reactions (4.4%) and thrombo-embolism (5%). Grade 3-4 haem toxicities were: 14.5% neutropenia, 6.9% thrombocytopenia, 7.5% anaemia. There were few reported cases of cardiac or renal failure so we looked for ARs suggestive of these previously reported side effects, finding grade 3-4 dyspnoea in only 1.3%. Serious adverse events suspected to be due to trial medications have occurred in 41% of patients receiving KCRD with the majority due to infections or cytopenias. Overall the toxicity profile is similar to that reported previously for patients receiving the CRD triplet, despite the addition of carfilzomib.
Summary and Conclusions: These results suggest that KCRD, an outpatient delivered 4-drug regimen combining second generation IMiD and PI drugs, is well-tolerated and safe in TE NDMM patients. With no significant additional toxicity over 3-drug combinations and a favourable profile when compared to other 4-drug regimens, KCRD represents a highly promising induction option. Longer follow-up is awaited to assess the efficacy and tolerability compared to a sequential IMiD/PI strategy.

S429

EFFECT OF AGE ON EFFICACY AND SAFETY OUTCOMES IN PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA RECEIVING LENALIDOMIDE AND LOW-DOSE DEXAMETHASONE (RD): THE FIRST TRIAL

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Background: Combination therapy with melphalan-prednisone-thalidomide (MPT) is considered a standard treatment (Tx) option for patients (pts) with

newly diagnosed multiple myeloma (NDMM) who are ineligible for stem cell transplant (SCT; Facon, *Lancet Oncol*, 2007; Fayers, *Blood*, 2011; NCCN Guidelines, Multiple Myeloma, V3.2015). The pivotal, randomized, international, multicenter phase 3 FIRST trial demonstrated that first-line use of lenalidomide continuous plus low-dose dexamethasone (Rd continuous) improved progression-free survival (PFS) compared with MPT (HR=0.72; *P* <0.001). An overall survival (OS) analysis also showed improvement, with a 22% reduction in risk of death with Rd continuous *vs* MPT (HR=0.78; *P*=0.02) (Benboubker, *N Engl J Med,* 2014).

Aims: To determine PFS, OS, and DOR outcomes stratified by age of patients with NDMM in the FIRST trial.

Methods: NDMM pts ineligible for SCT were randomized 1:1:1 to Tx with Rd continuous (28-day cycles) until disease progression (n=535); 18 cycles (72 wks) of Rd (Rd18; n=541); or 12 cycles (72 wks) of MPT (n=547). Starting doses were reduced in pts aged >75 *vs* those aged ≤75 yrs: dexamethasone (20 *vs* 40 mg), melphalan (0.20 *vs* 0.25 mg/kg), and thalidomide (100 *vs* 200 mg). The primary endpoint was PFS. Secondary endpoints included OS, overall response rate (ORR), time to response, duration of response (DOR), time to Tx failure, time to second anti-myeloma Tx, health-related quality of life, and safety.

Results: This analysis based on the final PFS analysis using a data cutoff of May 24, 2013, with a median follow-up of 37 mos. The proportion of pts aged ≤75 yrs and >75 yrs was 65% (n=1056) and 35% (n=567), respectively. Pt characteristics, including rate of adverse cytogenetics, were well balanced across all Tx arms. In pts ≤75 yrs, ISS stage III disease was detected in 37% of those treated with Rd continuous or Rd18 and 36% of pts treated with MPT. In pts >75 yrs, these rates were 47% with Rd continuous or Rd18 and 50% with MPT. Severe renal impairment (CrCl <30 mL/min) was observed in 6%, 8%, and 8% of pts ≤75 yrs *vs* 13%, 11%, and 15% of pts >75 yrs (Rd continuous, Rd18, and MPT, respectively). PFS and OS outcomes favored Rd continuous over MPT in both age groups. Median PFS was 27.4 mos in Rd continuous pts *vs* 21.8 mos in MPT pts aged ≤75 yrs (HR=0.68; *P* <0.001); HR for pts aged >75 yrs was 0.81 (*P*=0.11; Table 1). PFS for Rd continuous *vs* Rd18 pts was also increased in both age groups (HR=0.68; *P* <0.001 and HR=0.75; *P*=0.03, respectively). OS showed an improved trend for Rd continuous *vs* MPT in pts aged ≤75 yrs (HR=0.77; *P*=0.06) and >75 yrs (HR=0.80; *P*=0.16). ORR was consistently higher with Rd continuous *vs* MPT in pts aged ≤75 yrs (77% *vs* 66%) and >75 yrs (71% *vs* 55%). Median DOR with Rd continuous was longer *vs* MPT in pts aged ≤75 yrs (40 *vs* 22 mos) and pts >75 yrs (31 *vs* 24 mos). Hematologic and non-hematologic adverse events (AEs) were as expected for Rd and MPT, with no relevant differences in Grade 3/4 toxicity between pts ≤75 yrs and >75 yrs. Tx discontinuation due to AEs was comparable across Tx and age groups.

Table 1. PFS, OS, and response.

	Aged ≤ 75 Yrs			Aged > 75 Yrs		
ITT population	Rd continuous (n = 349)	Rd18 (n = 348)	MPT (n = 359)	Rd continuous (n = 186)	Rd18 (n = 193)	MPT (n = 188)
Median PFS, mos	27.4	21.3	21.8	21.2	19.4	19.2
3-yr PFS, %	46	25	23	35	19	22
PFS, HR (95% CI); *P* value						
Rd continuous vs MPT	0.68 (0.56-0.83); *P* < 0.001			0.81 (0.62-1.05); *P* = 0.11		
Rd continuous vs Rd18	0.68 (0.55-0.83); *P* < 0.001			0.75 (0.58-0.98); *P* = 0.03		
3-yr OS, %	74	70	67	63	58	54
OS, HR (95% CI); P-value						
Rd continuous vs MPT	0.77 (0.59-1.01); *P* = 0.06			0.80 (0.59-1.09); *P* = 0.16		
Rd continuous vs Rd18	0.88 (0.67-1.16); *P* = 0.36			0.94 (0.69-1.29) ; *P* = 0.70		
Response rate (≥ PR), %	77	77	66	71	66	55
Median duration of response (≥ PR), mos	40	23	22	31	20	24

HR, hazard ratio; ITT, intent to treat; MPT, melphalan-prednisone-thalidomide; OS, overall survival; PFS, progression-free survival; PR, partial response; Rd, lenalidomide-dexamethasone; Rd18, 18 cycles of Rd.

Summary and Conclusions: Regardless of age (≤75 *vs* >75 yrs), Rd continuous extended PFS with an OS benefit *vs* MPT in NDMM pts. Rd continuous was generally well tolerated in both age groups. DOR was improved with Rd continuous *vs* MPT and Rd18, irrespective of age. Rd continuous represents a new standard of care for pts in the first-line setting independent of age.

S430

PHASE 2 STUDY OF DARATUMUMAB MONOTHERAPY IN PATIENTS WITH ≥3 LINES OF PRIOR THERAPY OR DOUBLE REFRACTORY MULTIPLE MYELOMA: 54767414MMY2002 (SIRIUS)

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Background: Novel agents including proteasome inhibitors (PI) and immunomodulatory agents (IMiDs) have provided improved treatment outcomes for patients with multiple myeloma (MM). The majority of patients with MM still unfortunately relapse, with very limited options after PI and IMiD-based treatment. Daratumumab (DARA) is a human anti-CD38 IgG1κ mAb and has previously shown single agent activity and good tolerability in relapsed/refractory MM (Lokhorst HM *et al.* ASCO 2014).

Aims: This ongoing phase 2 study (NCT01985126) evaluated DARA monotherapy in the FDA breakthrough therapy designation population: MM patients with ≥3 prior lines of therapy including a PI and an IMiD, or double refractory to a PI and IMiD. Preliminary results are reported.

Methods: MMY2002 is a 2-part, open-label, international, multicenter study. In part 1 stage 1, 34 patients were randomized to DARA 8 mg/kg (n=18) q4w or 16 mg/kg (n=16) qw for 8 weeks, q2w for 16 weeks, then q4w in a Simon-2-stage design to determine the most effective dose. Subsequently, 90 additional patients were enrolled in the 16 mg/kg DARA group. The primary endpoint was overall response rate (ORR) by independent review (IRC).

Results: Data for the 16 mg/kg DARA group are presented (n=106). The median time from diagnosis was 4.8 years and patients received a median of 5 prior treatment lines. A total of 80% of patients received prior autologous stem cell transplants. Seventy five percent of patients were ISS stage 2 or higher. Ninety-six percent of patients were refractory to their last line of therapy and 95% were refractory to their last PI and IMiD. The proportions of patients refractory to other to agents included pomalidomide (63%), carfilzomib (48%) and alkylating agents (78%). Adverse events (AE; ≥20%) included fatigue (39.6%), anemia (33.0%), nausea (29.2%), thrombocytopenia (25.5%), back pain (22.6%), neutropenia (22.6%), cough (20.8%). Infusion-related reactions (IRR, 42.5%) were mainly grade 1/2 during the first infusion with 4.7% of patients with grade 3 IRRs. No grade 4 IRRs were reported and no patients discontinued the study due to IRRs. Five patients (4.7%) discontinued treatment due to AEs and none of these AEs were assessed by the investigator to be DARA-related. The ORR (IRC assessed) was 29.2%, with 3 stringent complete responses, 10 very good partial responses, and 18 partial responses. The ORR was consistent across clinically relevant subgroups. The median duration of response was 7.4 months. The median time to progression was 3.7 months. Median overall survival (OS) has not been reached and the estimated 1-year OS rate is 65%. After a median follow up of 9.4 months 14/31 (45.2%) of responders remain on therapy.

Summary and Conclusions: In a heavily pre-treated MM population (95% refractory to last PI and IMiD), DARA at 16 mg/kg showed meaningful durable single agent activity, with deep responses and a favorable safety profile.

CLL: Novel agents

S431

VENETOCLAX (ABT-199/GDC-0199) COMBINED WITH RITUXIMAB INDUCES DEEP RESPONSES IN PATIENTS WITH RELAPSED/REFRACTORY CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Venetoclax is a selective, orally bioavailable BCL-2 inhibitor. Single agent venetoclax induces responses in ~80% of pts with relapsed/refractory chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma (SLL). Pts were enrolled in a phase 1b, open-label, dose-escalation, multicenter study of venetoclax plus rituximab (R), to determine safety, PK and efficacy. We have previously reported the recommended phase 2 dose for venetoclax in this combination to be 400 mg/day.
Aims: The goal of this analysis was to determine the response rate and minimal residual disease status and to update the safety profile for the combination.
Methods: Eligible pts began treatment with 20 or 50 mg venetoclax daily, increasing weekly to final cohort doses of 200-600 mg. After the weekly lead-in phase, monthly R was added (at 375 mg/m^2 and then 500 mg/m^2) for 6 doses. Responses were assessed using iwCLL criteria, including CT scan and bone marrow (BM) biopsy at the end of combination therapy (7 months). MRD was assessed in local laboratories using ≥4 color flow cytometry on cells in BM and blood (minimum sensitivity of 0.01%).

Table 1.

Best Response		
	All pts n=49	del17p n=9
ORR, n (%)	41 (84)	7 (78)
CR/CRi	20[a] (41)	3[b] (33)
PR	20 (41)	4 (44)
nodular PR (nPR)	1 (2)	0 (0)
PR unconfirmed[c]	4 (8)	1 (11)
Stable disease (SD)	1 (2)	0 (0)
Progressive disease (PD)	2 (4)	0 (0)
Discontinued prior to assessment[d]	1 (2)	1 (11)

[a]6/20 CRi;
[b]2/3 CRi;
[c]1 awaiting next scan to confirm PR, 1 withdrew consent, 1 withdrew due to neuropathy; 1 had PD shortly after PR;
[d]Fatal TLS event; No other fatal TLS events occurred after protocol modification

BM MRD Status, n (%)			
	MRD-negative	MRD-positive	Not evaluable
CR/CRi	13	5	2
PR/nPR	10	10	1
Other	1[e]	1[f]	6[g]
Total	24/49 (49)	16/49 (33)	9/49 (18)

[e]PR unconfirmed;
[f]SD;
[g]No sample

Results: Accrual is complete with 49 pts (48 CLL/1 SLL) of median age 68 (50-88) years enrolled in 5 dose escalation cohorts (n=41) and a safety expansion cohort at 400 mg/day (n=8). The median number of prior regimens was 2 (1-5). Forty-five (92%) had prior R; 14/49 (29%) were R-refractory. Twenty-eight (57%) had prior fludarabine (F); 9/49 (30%) were F-refractory. Of 46 pts with available data, 9 (20%) had del17p; 19/27 (70%) with available data had unmutated IGVH. Eleven pts have discontinued the study: 6 due to PD, 2 due to AEs (neuropathy, TLS), and 3 withdrew consent. The median time on study as of Jan 21, 2015 was 13 (0.03-28) months. The overall response rate (ORR) was 84% (41/49), with 20 (41%) achieving either a complete response (CR) or CR with incomplete marrow recovery (CRi). MRD was evaluable in 40 pts. MRD-negativity in BM was achieved in 13/20 (65%) pts with CR/CRi and in 24/49 (49%) pts overall. Responses are summarized in the Table 1. Only 3/41 (7%) responders have progressed (at 5, 8 and 12 months). Six pts stopped daily venetoclax after achieving CR/CRi. These pts continue in follow up and all have maintained a response after a median time of 12 months (0-21) off venetoclax. Treatment-emergent AEs in >25% were neutropenia (53%), diarrhea and nausea (each 47%), upper respiratory tract infection (41%), pyrexia (37%) and fatigue, headache and cough (each 33%). Grade 3/4 AEs in >10% were neutropenia (51%), thrombocytopenia (16%) and anemia (14%). There was 1 treatment-emergent AE that led to death (TLS) and there were 2 deaths after PD.
Summary and Conclusions: The combination of venetoclax and R has a tolerable safety profile and induces deep and durable responses, with 41% of pts achieving CR/CRi and 49% of pts achieving MRD negativity in BM. A phase 3 trial comparing venetoclax and R *versus* bendamustine and R in pts with previously treated CLL is underway.

S432

TGR-1202, A NOVEL ONCE DAILY PI3K-DELTA INHIBITOR, DEMONSTRATES CLINICAL ACTIVITY WITH A FAVORABLE SAFETY PROFILE, LACKING HEPATOTOXICITY, IN PATIENTS WITH CLL AND B-CELL LYMPHOMA

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Background: TGR-1202 is a novel, next generation PI3Kδ inhibitor which lacks the hepatotoxicity associated with other PI3Kδ inhibitors and is active in patients (pts) with advanced heme malignancies (ASH 2014).
Aims: Herein we present updated safety and efficacy results from a Ph I study of TGR-1202 in pts with rel/ref CLL and B-cell lymphoma.
Methods: TGR-1202 administered orally once-daily following a 3+3 dose escalation design. Eligible pts have rel/ref B-cell non-Hodgkin lymphoma (NHL), chronic lymphocytic leukemia (CLL), or other B-cell malignancy and an ECOG PS ≤2. Endpoints: safety, PK/PD, and efficacy.
Results: As of Feb 2015, 58 pts evaluable for safety including CLL, FL, Hodgkin's (HL), DLBCL, MCL, and MZL. Median age 63 yo (range: 22-85), 72% male, ECOG 0/1/2: 19/38/1, median prior Tx: 3 (range: 1-14), 48% refractory to prior Tx. Safety: The only Gr≥3 AE in ≥10% of pts was neutropenia (10%). AEs (all grades, all causality) in >20% of pts were limited to diarrhea (34%), fatigue (31%), nausea (29%), and cough (26%). All diarrhea events Gr1/2, except one Gr3 event occurring in a pt in Cyc 1, which persisted for 2 days and resolved without dose interruption. Notably, in contrast to similar agents, no drug-related hepatotoxicity or colitis has been observed to date (ave time on study 7 Cyc). 2 episodes of Gr3 fatigue at 1800 mg of a new micronized formulation met the criteria for DLT. Expansion cohorts are open at 800 mg (CLL) and 1200 mg (NHL). Efficacy: A strong exposure-response relationship has been observed. Of 14 evaluable CLL pts, 13 (93%) achieved a nodal PR (median nodal ↓ of 76%), of which 7 (50%) achieved a PR per Hallek 2008 criteria. Responses have been limited in pts with DLBCL and HL. Of the 12 evaluable FL pts, 8 (67%) remain on study progression-free (range 7-99+ weeks), with 3 achieving a PR, notably being the 3 pts exhibiting the highest TGR-1202 plasma concentrations.
Summary and Conclusions: TGR-1202 is well tolerated in pts with rel/ref heme malignancies with no reported hepatotoxicity or colitis (41% of pts on study 6+ Cyc) and promising activity in CLL and NHL. Enrollment continues in expansion cohorts.

S433

Abstract withdrawn

S434

EARLY CLINICAL ACTIVITY AND PHARMACODYNAMIC EFFECTS OF DUVELISIB, A PI3K-DELTA, GAMMA INHIBITOR, IN PATIENTS WITH TREATMENT-NAÏVE CLL

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Background: Signaling via PI3K-δ and PI3K-γ has distinct and complementary effects on malignant B-cells and nonmalignant immune cells in chronic lymphocytic leukemia (CLL). Duvelisib, an oral dual inhibitor of PI3K-δ,γ, has

shown clinical activity in a phase 1 study, IPI-145-02 (O'Brien, ASH 2014; Flinn, ASH 2014; Horwitz, ASH 2014).
Aims: The activity of duvelisib monotherapy in patients with treatment-naïve CLL from this study are reported here.
Methods: Following dose escalation, an expansion cohort of treatment-naïve CLL patients was enrolled (n=18). Response was based on iwCLL (2008) criteria. Safety included AEs and laboratory assessments. Pharmacodynamic assessments included peripheral blood (PB) flow cytometry for phospho-S473 AKT (pAKT) and Ki67, and measurement of serum chemokines and cytokines. Numbers of PB T-cell subsets were also monitored.
Results: As of Oct 2014, 18 treatment-naïve CLL pts received duvelisib 25 mg BID. The best ORR per iwCLL was 82% (PRs in 14/17 evaluable patients) with a median time on treatment of 53 weeks (range 8-69). Ten patients remain on treatment, while 8 discontinued, including 6 patients due to AEs. AEs overall were mostly Grade 1 or 2. The most common ≥Grade 3 AEs were neutropenia (7/18) and ALT/AST increase (3/18). Inhibition of pAKT in CLL cells was rapid following a single dose and sustained through Cycle 2 Day 1 (C2D1). A reduction in the Ki67 proliferative fraction in both CLL and T-cells was also observed. Duvelisib resulted in reductions in the median serum levels of CCL3, CCL4, CCL17, CCL22, CXCL10, CXCL13, IL-10, IL-12p40, MMP-9, IL-16, and TNFα to ≤50% of baseline at C1D8 and/or C2D1 ($p < 0.01$).
Summary and Conclusions: Duvelisib 25 mg BID shows clinical activity in treatment-naïve CLL pts. The inhibition of pAKT and Ki67 in CLL cells suggests duvelisib inhibits the PI3K pathway and suppresses malignant cell proliferation in treatment-naive CLL patients. In addition, the effect of duvelisib on serum chemokines, cytokines, and T-cell proliferation suggests that modulation of the tumor microenvironment may contribute to the observed early clinical activity of duvelisib in patients with treatment-naïve CLL. These data support the further development of duvelisib in treatment-naïve CLL, including combinations with other targeted therapies.

S435

ADHERENCE AND DOSE INTENSITY FOLLOWING ADMINISTRATION OF THE IBRUTINIB 420 MG DOSE IN PATIENTS WITH PREVIOUSLY TREATED CLL

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Background: Ibrutinib is a first-in-class, once-daily, oral, covalent inhibitor of Bruton's tyrosine kinase (BTK). Pharmacokinetic analyses have shown that ibrutinib is rapidly absorbed, extensively distributed, and rapidly eliminated from systemic circulation after oral administration (Advani, JCO 2013, Poggesi, AACR 2014). In a study of BTK occupancy in peripheral blood, ibrutinib achieved complete or near complete occupancy of the BTK active site (median >90%) at 4 hours which was maintained at 24 hours following administration of the 420 mg once-daily dose (O'Brien, Lancet Oncology 2013). PK/PD analysis of BTK engagement at different dose levels predicted that at the lower doses of 140 or 280 mg fewer patients would attain complete BTK occupancy (Poggesi, AACR 2014).
Aims: To evaluate the effect of the ibrutinib 420 mg once-daily dose adherence on independent review committee-assessed progression-free survival (PFS) in patients with previously treated CLL from the phase 3 RESONATE trial.
Methods: Dose intensity was defined as the proportion of actually administered *vs* planned doses of ibrutinib 420 mg. Dose intensity was also defined in the first 8 weeks to compare statistically with post-week 8 PFS. Steady-state AUC/C_{max} was estimated per NONMEM modeling using 2 timepoint samples (weeks 1 and 4). Missed doses had to be consecutive. Mean duration of missed doses was based on the sum of all consecutive days of missed dose for each patient.
Results: Patients treated with ibrutinib (n=195) had a mean dose intensity of 95% (median 100%) with 8.3 months of treatment. Of all patients with dose reductions (4.1%), 3.6% had 1 dose reduction and 0.5% had 2 dose reductions due to adverse events. Only diarrhea led to dose reduction in >1 patient in the ibrutinib arm (n=3). The majority of dose interruptions were restarted at 420 mg as per protocol. There were fewer PFS events in patients not missing ibrutinib doses (n=136) *vs* those missing (n=59) ibrutinib doses for ≥8 consecutive days (13% *vs* 31%, respectively), with median PFS of NR *vs* 11 months, respectively. The mean duration of missed doses was 26 days for patients missing ≥8 days. For patients missing ≥2-7 days consecutively (n=33), the mean duration of missed doses was 6 days; 103/195 patients did not miss >1 day. Patients with higher dose intensity experienced longer PFS compared to those with lower dose intensity (median NR *vs* 11 months). This trend was confirmed (HR=0.4 [*P*=0.0127]) using an adjusted mean dose intensity of 96% in the first 8 weeks and post week-8 PFS. In patients with del17p CLL (n=63), p53 mutation (n=61), or del11q CLL (n=63), those with higher dose intensity also had a lower rate of progression or death events compared to those with lower dose intensity (18% *vs* 42%, 9% *vs* 29%, 14% *vs* 16%, respectively). Dose reduction due to diarrhea, infection, and arthralgia occurred in patients with del17p (n=2), p53 mutation (n=3), but not del11q CLL. No difference was seen in median PFS with lower *vs* higher ibrutinib exposure (AUC or C_{max}) in the 179 patients receiving ibrutinib 420 mg with PK assessment at weeks 1 and 4. Patients receiving ibrutinib without concomitant CYP3A inhibitors had similar outcomes.
Summary and Conclusions: Improvement in PFS is associated with a higher mean dose intensity of ibrutinib, with patients missing more than 1 week of treatment experiencing more PFS events. This improvement in PFS is seen regardless of del17p, del11q, or p53 mutation status. These results support sustained adherence to the once-daily ibrutinib 420 mg dose in patients with previously treated CLL.

Translational studies in ALL

S436

TYROSINE KINASE FUSION GENES IN PEDIATRIC BCR-ABL1-LIKE ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Pediatric B-cell precursor acute lymphoblastic leukemia (BCP-ALL) with the *BCR-ABL1* fusion gene forms a small high-risk patient group with a poor prognosis. Approximately 15% of BCP-ALL shows gene expression similar to *BCR-ABL1*-positive disease and a similar unfavorable prognosis. This group shows a high frequency of B-cell development gene aberrations, especially *IKZF1* deletions and tyrosine kinase activating lesions (Den Boer *et al.* Lancet Oncol 2009; Mullighan *et al.* N Engl J Med 2009; Roberts *et al.* Cancer Cell 2012, N Engl J Med 2014; Van der Veer *et al.* Blood 2013).
Aims: To detect the clinical value (frequency, prognosis and drugability) of tyrosine kinase fusions in childhood B-cell precursor ALL.
Methods: This study comprised 204 children with BCP-ALL in 3 Dutch trials (DCOG ALL-8, 9, 10) and 2 German trials (COALL 06-97, 07-03) including 92 previously described *BCR-ABL1*-like cases identified by hierarchical clustering and 112 non-*BCR-ABL1*-like B-other cases. Characterization included RT-PCR and FISH to detect fusions involving *ABL1*, *PDGFRB*, *JAK2* and *CSF1R*, copy number analysis, and Sanger sequencing of *JAK1* exons 13, 14 and *JAK2* exons 16, 20, 21.
Results: We identified 12 tyrosine kinase activating fusion genes among 73 *BCR-ABL1*-like cases tested (16%) and none among 87 B-other cases. Four *EBF1-PDGFRB*, two *PAX5-JAK2*, *ZMIZ1-ABL1*, and *SSBP1-CSF1R* fusions were confirmed by RT-PCR. Four cases showed split FISH and/or breaks on DNA copy number arrays for *PDGFRB*, *ABL1*, or *JAK2* with unknown fusion partners. *IKZF1* deletions occurred more frequently in tyrosine kinase fusion cases compared with B-other (55% *vs* 32%), and were enriched for rare, *i.e.* other than exon 4-7 or full deletion, variants (46% *vs* 18%). *JAK2* mutations (mainly in exon 16) were present in 7/70 B-other and 2/82 *BCR-ABL1*-like cases. *JAK2* mutations co-occurred with high *CRLF2* expression and had no prognostic impact. The cumulative incidence of relapse (CIR) in the *BCR-ABL1*-like group with tyrosine kinase fusions (5-yr CIR 25%±13%) was comparable with the remaining *BCR-ABL1*-like group (5-yr CIR 33%±5%), and worse than the B-other group (5-yr CIR 18%±4%; overall Gray p-value 0.06). Of the 12 tyrosine kinase fusion cases, 4 were late responders who only achieved remission after day 33 of induction therapy. Of 8 patients receiving a prednisone window before start of induction therapy, 3 showed a poor prednisone response on day 8. Day 15 punctures showed M2 or M3 marrows in 7/9 cases. Minimal residual disease PCR at the end of induction was high (TP1 and TP2 $\geq 10^{-3}$) in 5 and intermediate (TP1 $\geq 10^{-3}$, TP2 $< 10^{-3}$) in 2/8 cases. Leukemic cells from 3 *EBF1-PDGFRB* patients were sensitive to imatinib in *ex vivo* cultures, compared with lack of cytotoxic response in 4 *EBF1-PDGFRB*-negative samples, 2 of which even showed growth on imatinib. Combination of imatinib with prednisolone resulted in further growth inhibition in *ex vivo* cultures from 2/3 *EBF1-PDGFRB* patients.
Summary and Conclusions: Tyrosine kinase fusion genes were found in 16% of DCOG/COALL *BCR-ABL1*-like cases, representing ~3% of total BCP-ALL. *JAK2* mutations were infrequent and not associated with *BCR-ABL1*-like in our cohort. Tyrosine kinase fusion cases were characterized by poor initial response to treatment, had an unfavorable clinical outcome compared to remaining non-*BCR-ABL1*-like B-other ALL cases but had a comparable unfavorable outcome to *BCR-ABL1*-like ALL without tyrosine kinase fusions. Imatinib worked additive to prednisolone in *EBF1-PDGFRB* patient cells, indicating that this inhibitor may be clinically used in combination with at least prednisone.

S437

THE PREDICTIVE STRENGTH OF NEXT GENERATION SEQUENCING MINIMAL RESIDUAL DISEASE DETECTION FOR RELAPSE COMPARED WITH CURRENT METHODS IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Minimal residual disease (MRD) monitoring using antigen receptor-based quantitative PCR (qPCR) became a gold standard in the management of acute lymphoblastic leukemia (ALL), despite being technically and financially demanding. MRD detection based on next generation sequencing (NGS) of antigen receptor genes rearrangements allows for a highly specific and sensitive detection of MRD without the need for labourious optimization of patient-specific assays.
Aims: To establish MRD detection by NGS of immunoglobulin heavy chain (*IGH*) rearrangements and compare MRD levels at BFM-protocols stratification timepoints with qPCR and flow cytometry (FC).
Methods: The libraries for sequencing were prepared from 450ng of diagnostic bone marrow DNA and 50ng of polyclonal DNA. Two-round PCR was used for library preparation: in the 1st round the *IGH* rearrangements were amplified with Biomed-2 FR3 primers. In the 2nd round of PCR sequencing adaptors and barcodes were attached. Libraries were sequenced on Ion Torrent PGM/Ion Proton sequencers. For detection of reads containing the clonal sequence from diagnosis we used our own bioinformatics algorithm.
Results: We sequenced 213 samples from 63 patients with childhood ALL treated according to the AIEOP-BFM-ALL 2000 protocol and 14 more patients with relapse from previous and current frontline treatment protocols with the median coverage 719,904 reads per sample. Eighty-four (39.4%) samples were negative by both methods. Sixteen (7.5%) samples were positive by NGS and negative by qPCR, and 16 (7.5%) samples were positive by qPCR and negative by NGS. This caused a shift in risk group stratification in 30% of patients, mainly between standard risk and intermediate risk group patients. The overall correlation of both methods was good (R^2=0.71). NGS approach detected significantly higher MRD than qPCR at day 33 in patients who later relapsed (p=0.001). NGS-MRD positivity at day 33 seems to provide a more accurate prediction of relapse than qPCR-MRD positivity (Figure 1A). At day 78, the predictive value of NGS was comparable to qPCR (Figure 1B). Combined day 33 and 78 MRD used for defining of SR, IR and HR (+SER) groups on BFM trials gave again similar results as qPCR (Figure 1C). Similarly to FC, low NGS MRD defined a group with excellent prognosis at day 15.

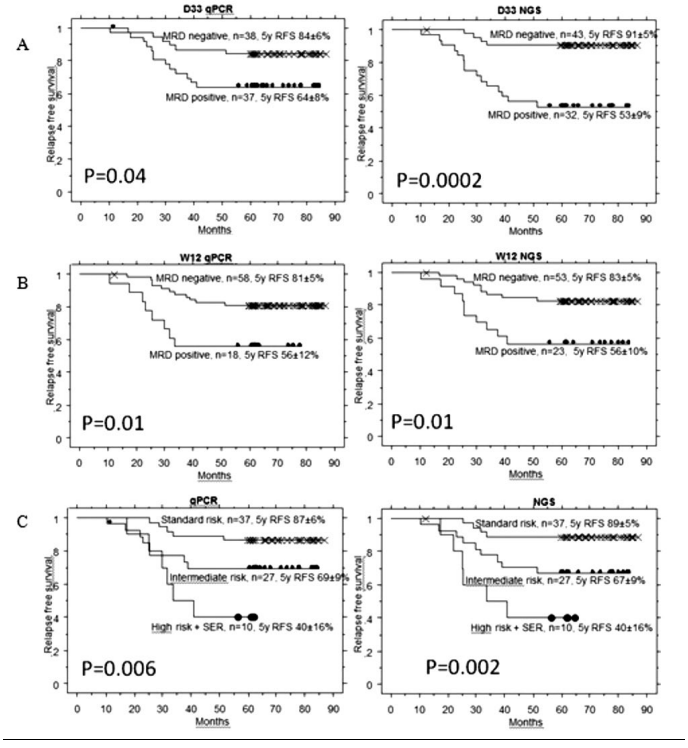

Figure 1.

Summary and Conclusions: NGS will speed up the process of MRD detection and provide results at early time points, which is currently not possible due to the long time needed for qPCR preparations. The correlation of NGS and qPCR was good with the majority of the differences below the reproducible sensitivity of methods, which caused shifts mainly between SR and IR groups. We showed that day 33 NGS MRD levels were higher than qPCR MRD levels in patients who subsequently relapsed, which was reflected in a slightly better prediction of relapse based on d33 NGS. The outcome of patients stratified into risk groups by combined d33/d78 MRD was similar for NGS and qPCR, despite the 30% of patients being differently stratified by NGS. At present, the main drawback of the Ig/TCR-exploring NGS methods is the lack of standardization both in the experimental setting and in data analysis. Therefore, the European network "EuroClonality NGS Consortium",

has been formed to optimize and standardize NGS workflow..Before NGS based MRD can safely replace qPCR for treatment guiding in ALL, robust results on comparability of both methods by standardized methodology should be validated within prospective clinical trials.
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S438

HIGH THROUGHPUT SEQUENCING AS A MEASURE OF EARLY RESPONSE TO THERAPY IN CHILDHOOD ALL

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Background: Early response to induction chemotherapy has been demonstrated to be one of if not the most significant prognostic factors in the outcome of children with acute lymphoblastic leukemia. Multiparametric flow cytometry (mpFC) has been the routinely used methodology in the US for the determination of this response. New high throughput sequencing (HTS) technologies of rearranged immune receptor (TCR and Ig) genes have raised the possibility of a more accurate, sensitive, and standardizable approach to determination of early response to therapy in ALL patients.
Aims: In this study, we investigated whether the Adaptive Biotechnologies immunoSEQ assay of IgH (immunoglobulin heavy chain (VDJ/DJ) and TCRG (T-cell receptor gamma) would be able to quantify residual disease at the end of induction therapy for children with ALL and be of prognostic value with regard to outcome (relapse free survival and overall survival) in these patients.
Methods: This study involved a total of 432 patients enrolled on COG clinical trials AALL0331 and AALL0232 for whom mpFC measurement of residual disease was <0.1% and for whom outcome data are available.
MpFC was performed at the University of Washington and Johns Hopkins as part of the evaluation for MRD. Genomic DNA was extracted from frozen bone marrow specimens collected at diagnosis and at day 29 post the start of induction therapy. High throughput sequencing of CDR3 regions of IGH and TCRG was performed on all samples. Diagnostic and d29 matched samples from a given patient were sequenced and dominant (>5%) clonal CDR3 sequences from diagnosis were searched for in the corresponding d29 sample. An exact 100 base pair match was required for identification of the sequence as residual disease in the follow-up sample. Both the presence and the frequency of the MRD clone relative to the total IGH repertoire and total nucleated cell population were determined.
Results: The assays defined the dominant clonal sequences in >90% of the patients. Approximately 60% of this subgroup was found to have residual disease present at d29. Clones from some of the patients demonstrated a single "trackable" sequence while clones from other patients demonstrated multiple trackable sequences either within or between the two immune receptor loci being assessed. Approximately, 50% of the residual disease detected by HTS was beneath the level of sensitivity of mpFC and therefore previously read out as MRD negative. Correlations between mpFC and HTS and independent correlations of each of these two methodologies with outcome will be presented.
Summary and Conclusions: This is the largest patient cohort studied to date for which mpFC, HTS, and outcome data are all available. This study allows an informative assessment of the capability of HTS to determine early response to induction chemotherapy and the relevance of that determination to patient outcome.

S439

GENOMIC DNA BREAKPOINTS IN MLL AND TRANSLOCATION PARTNER GENES IN A LARGE COHORT OF INFANTS WITH ACUTE LEUKEMIA

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Background: Acute leukemia (AL) in infants is characterized by high incidence of *MLL* gene rearrangements.
Aims: To evaluate the relation between genomic DNA breakpoints in *MLL* and translocation partner genes (TPGs) and clinical parameters of infant AL.
Methods: 87 infants (32 boys (37%) and 55 girls (63%), median age 4.9 mo) with *MLL*-rearranged acute lymphoblastic leukemia (ALL) (n=63), acute myeloid leukemia (AML) (n=22) and mixed phenotype acute leukemia (MPAL) (n=2) were included in the current study. Genomic DNA breakpoint detection in *MLL* gene and translocation partner genes (TPGs) was performed by long-distance inverse PCR (LDI-PCR). Exon-intron numbering of *MLL* gene was done according to I. Nilson *et al.*, 1996.
Results: Majority of ALL cases was characterized by presence of *MLL-AF4* fusion gene (FG) (n=35;55%), less frequently *MLL-MLLT1* (n=12;22%), *MLL-MLLT3* (n=8;13%) and others were found (Table 1). The most common breakpoint location within *MLL* gene in ALL patients was intron 11, detected in 31 cases (49%), less frequently breakpoints in intron 10 (n=13;21%) and intron 9 (n=9;14%) were found. The highest variability of *MLL* breakpoints was found in *MLL-AF4*-positive patients: only 15 of 35 (43%) had breakpoints in intron 11. The most stable pattern of *MLL* genomic DNA breakpoints was observed in *MLL-MLLT1*-positive patients: 9 of 14 (64%) had breakpoints in intron 11. In AML patients the most prevalent FG was *MLL-MLLT3* (n=8;36%). The remaining ones are listed in Table 1. The most frequent breakpoint location was intron 9 (n=10;45%), less often they were found in intron 10 (n=5;23%) and 11 (n=4;18%). The most stable pattern was revealed for *MLL-MLLT10* FG: *MLL* breakpoints in 4 of 5 (80%) cases were found in intron 9 (Table 1). In TPGs the most frequent breakpoint locations were as follows: in *AF4*-intron 3 (n=25;69%) and intron 4 (n=7;19%); in *MLLT1*-non-coding region between *ACER1* and *MLLT1* (n=9;60%) and intron 1 (n=5;33%); in *MLLT3*-intron 5 (n=13;81%); in *MLLT10*-intron 8 and intron 9 (n=2;33% each); in *EPS15*-intron 1 (n=3;75%). The pattern of breakpoints locations in TPGs was similar in ALL, AML and MPAL cases. Distribution of DNA breakpoints in *MLL* gene was similar in boys and girls and did not depend on type of TPG. ALL patients who had breakpoints in intron 11 were significantly younger (median 3.0 mo, range 0.03-11.6) than all others (median 5.6 mo, range 0.7-11.9) (p=0.025) and than patients with *MLL* breakpoints in intron 9 (median 6.6 mo, range 3.1-11.9) (p=0.017). For AML cases we did not find any relation between age and breakpoints locations. We estimated prognostic significance of *MLL* breakpoint locations in 46 cases of infant ALL homogenously treated by MLL-Baby protocol. 5-year EFS was significantly lower in patients with breakpoint in intron 11 (n=29) in comparison to patients with breakpoints localized from intron 7 to exon 11 (n=17) (0.16±0.07 *vs* 0.38±0.14 p=0.035). While cumulative incidence of relapse was remarkably higher in the first group of patients (0.80±0.33 *vs* 0.56±0.20 p=0.020). Median follow-up time was 36 months. Although in Cox regression model including breakpoint position together with age, immunophenotype, initial WBC count, initial CNS involvement, type of *MLL* rearrangement, absolute blast number at day 8 of dexamethasone profase, minimal residual disease (MRD) at time point 4 (TP4) of MLL-Baby protocol, the only significant covariate was the presence of MRD at TP4 (HR 3.160, 95% CI 1.159-17.815, p=0.021).

Table 1.

Translocation partner genes	*MLL* gene									
	Intron 7	Exon 8	Intron 8	Intron 9	Exon 10	Intron 10	Exon 11	Intron 11	Exon 12	Intron 12
Acute lymphoblastic leukemia *(n=63)*										
AF4 (n=35)	1	1		8	1	4	4	15		1
MLLT1 (n=14)						5		9		
MLLT3 (n=8)					1	1		5	1	
EPS15 (n=4)						2		2		
MLLT10 (n=1)				1						
AFF3 (n=1)						1				
Total ALL	**1**	**1**	**—**	**9**	**2**	**13**	**4**	**31**	**1**	**1**
Acute myeloid leukemia *(n=22)*										
AF4 (n=1)								1		
MLLT3 (n=8)				3		1		3	1	
MLLT10 (n=5)			1	4						
MLLT11 (n=2)				2						
MYO1F (n=2)						2				
ELL (n=1)						1				
ABI1 (n=1)				1						
SEPT6 (n=1)						1				
SEPT9 (n=1)			1							
Total AML	**—**	**—**	**2**	**10**	**—**	**5**	**—**	**4**	**1**	**—**
Mixed phenotype acute leukemia *(n=2)*										
MLLT1 (n=1)								1		
MLLT10 (n=1)								1		
Total MPAL	—		—	—	—	—	—	**2**	—	—
Totally	***1***	***1***	***2***	***19***	***2***	***18***	***4***	***37***	***2***	***1***

Summary and Conclusions: Our data provide additional information of molecular genetic features of *MLL*-rearranged infant AL.

S440

CLINICAL ACTIVITY OF ERY001 (ERYTHROCYTE ENCAPSULATED L-ASPARAGINASE) IN COMBINATION WITH COOPRALL REGIMEN IN PHASE 3 RANDOMIZED TRIAL IN PATIENTS WITH RELAPSED ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Asparaginase is a cornerstone in the treatment of ALL, but its utility is limited by toxicities including hypersensitivity. Clinical allergy is associated with inactivation of asparaginase by antibodies (A-Abs), which can also neutralize asparaginase without any clinical signs of hypersensitivity (silent inactivation). ERY001 improves pharmacokinetics, tolerability and maintain circulating asparaginase (ASPA) activity due to the protective barrier of the erythrocyte membrane.
Aims: The study aimed at evaluating the efficacy and safety of ERYASP
Methods: This open, randomized international Phase 3 study enrolled pts with relapsed ALL. The co-primary endpoints were the duration of ASPA activity >100IU/L and the incidence of ASPA hypersensitivity during induction. Key secondary endpoints were complete remission (CR), minimal residual disease (MRD), event free survival (EFS) and overall survival (OS).The study was powered to detect 3-fold difference in the incidence of allergic reactions between treatments. Pts (n=80), aged 1-55 years were randomized to ERY001 (150 IU/kg, n=26 or L-ASP (10,000 IU/m², n=28), or to ERY001-exp (prior allergy, n=26).
Results: In the non-allergic pts, ERY001 significantly reduced the incidence of ASPA hypersensitivity (0% *vs* 43%; p<0.001). ASPA activity >100 IU/l was 21±5 *vs* 9±8 days in ERY001 and L-ASP, respectively (p<0.001). The CR rate: ERY001 (65%, 95% CI: [51.6:89.8]) *vs* L-ASP (39%, 95% CI: [23.3:63.1]; p=0.026). Allograft was successfully performed in 65% of ERY001 *vs* 46% of L-ASP. The proportion of patients who achieved MRD<10^{-3} in F1-F2/VANDA was 35% and 25% in ERY001, and L-ASP arms, respectively. At 12 mo, EFS rate was 65% and 49% in ERY001 and L-ASP arm, respectively. Treatment with ERY001 was well tolerated.
Summary and Conclusions: ERY001 provides an alternative option for patients with relapsed ALL, which is well tolerated and efficacious.

Stem cell transplantation: Clinical 2

S441

DROPLET DIGITAL PCR FOR DNMT3A AND IDH1/2 MUTATIONS TO IMPROVE EARLY DETECTION OF ACUTE MYELOID LEUKEMIA RELAPSE AFTER ALLOGENEIC HSCT

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Background: Despite the considerable improvement documented over the last two decades in the outcome of allogeneic Hematopoietic Stem Cell Transplantation (allo-HSCT) for Acute Myeloid Leukemia (AML), disease relapse still represents a major unsolved issue, and efforts are aimed to anticipate relapse detection and treatment to the Minimal Residual Disease (MRD) stage. Recent studies demonstrated that mutations in the DNMT3A and IDH1/2 genes occur very early during the step-wise process of leukemogenesis, possibly representing disease founder mutations and thus optimal markers for MRD tracking.
Aims: To investigate the clinical utility of ultra-sensitive tracking of DNMT3A, IDH1 and IDH2 mutations as post-transplantation MRD monitoring technique.
Methods: By conventional Sanger sequencing, we screened for 6 mutations of interest (DNMT3A R882H and R882C, IDH1 R132C and R132H, IDH2 R140Q and R172K) 86 AML diagnosis samples from patients who underwent allo-HSCT. 113 bone marrow samples collected longitudinally over time from the 23 patients who carried at least one of the mutations were analyzed by ultra-sensitive droplet digital PCR (ddPCR) assays. As controls, we tested bone marrow samples collected at diagnosis from 11 patients typing negative for the mutations, and peripheral blood samples from 19 healthy individuals. ddPCR assays were performed using the Bio-Rad QX100 system: each sample was tested in duplicates, employing 25 ng of genomic DNA in each reaction well. Samples with a mutant-to-wild-type ratio above 0.1% were considered positive. ddPCR results were compared to those obtained testing the same samples by quantitative PCR (qPCR) assessment of the WT1 gene transcript and of hematopoietic chimerism.
Results: All the samples which resulted positive by conventional sequencing were confirmed by ddPCR, and in all of them the population carrying the mutant allele, quantified by ddPCR, consistently exceeded the morphological count of leukemic blasts, suggesting the presence of the mutation also in apparently normal bone marrow hematopoietic cells. All the samples tested at post-transplantation relapse remained positive for the mutations present at diagnosis, except for one case, originally carrying both DNMT3A and IDH2 mutations and typing negative for the latter at relapse. This observation might argue against the putative role of IDH mutations as leukemia-founder events, and suggests that, when present, DNMT3A could represent a more reliable MRD marker. When post-transplantation remission samples were tested, 55/60 (92%) of those harvested from 7 patients who remained long-term leukemia-free (median follow-up after allo-HSCT: 24 months) resulted negative for the mutations of interest. 4/5 of the samples which resulted positive belonged to the early time-points of a single patient who progressively decreased the mutation burden until becoming negative. 6/7 patients who relapsed presented at least one sample harvested during apparent disease remission which resulted positive by ddPCR, anticipating hematological relapse of at least one month. Of notice, only 3 of those relapsed patients had displayed WT1 transcript overexpression and only one had displayed host chimerism above the 1% threshold.
Summary and Conclusions: Although the small number of patients included in this preliminary analysis warrants for caution, ddPCR for DNMT3A and IDH1/2 mutations appears extremely promising, displaying very high specificity and sensitivity in relapse prediction, and comparing favorably with current qPCR-based post-transplantation monitoring techniques.

S442

KIR2DS4 AND ITS VARIANT KIR1D ARE ASSOCIATED WITH AGVHD, CMV AND OS AFTER SIBING RELATED HLA MATCHED TRANSPLANTATION FOR PATIENTS WHOSE DONORS BELONG TO KIR GENE HAPLOTYPE A

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Background: Hematopoietic stem cell transplantation (HSCT) outcomes of donor and recipient KIR genotypes have been extensively studies. However, the association of KIR2DS4 and its variant KIR1D with outcomes in patients who transplanted from a donor for KIR haplotye A after sibing related HLA matched transplantation has not been explored.
Aims: To the association of KIR2DS4 and its variant KIR1D with outcomes in patients who transplanted from a donor for KIR haplotye A after sibing related HLA matched transplantation.

Methods: To study this, 165 patients transplanted from KIR gene haplotype A were genotyped and divided into three groups: 2DS4+/1D- (two intact KIR2DS4 alleles), 2DS4+/1D+ (heterozygous), 1D+/1D+ (homozygous for the deletion variant KIR1D). No difference of the recovery of neutrophils and platelets was observed in the three groups.
Results: The cumulative incidences of Grade III-IV acute GVHD were 28.94%, 14.11% and 44.44%, respectively, in the 2DS4+/1D-, 2DS4+/1D+ and 1D+/1D+ groups within day+100 (*P*=0.0159). Multivariate analysis identified 1D+/1D+ was an independent risk factor for aGVHD (HR=4.221, 95%CI 1.470-12.124, P=0.007). In contrast, the cumulative incidences of chronic GVHD, 3-year cumulative relapse, and treatment-related mortality displayed no significant difference. The rate of CMV reactivation were 46.96%, 20.16% and 53.25% in the 2DS4+/1D-, 2DS4+/1D+ and 1D+/1D+ groups (P=0.0017). Multivariate analysis identified 2DS4+/1D+ was an independent protective factor for CMV reactivation (HR=0.268, 95%CI 0.125-0.574, P=0.001). Although the overall survival (OS) showed no difference in the first year, the OS of 2DS4+/1D- group showed a significant superiority over the other groups after one year (P=0.0361). In the disease stage of advanced patients, the 3-year probability of DFS were 51.06%, 34.01% and 0% in the 2DS4+/1D-, 2DS4+/1D+ and 1D+/1D+ group (P=0.0314).
Summary and Conclusions: Collectively, our data suggest that the KIR 2DS4/1D allelic variance is associated with the outcome of sibling related HLA matched HSCT, and donor subclassification of KIR 2DS4/1D alleles should be consider in this setting.

S443

HUMAN ATRIAL NATRIURETIC PEPTIDE (HANP) THERAPY IN THE EARLY PHASE AFTER ALLOGENEIC STEM CELL TRANSPLANT (ALLO-SCT) PREVENTS DEVELOPMENT OF CHRONIC KIDNEY DISEASE (CKD): RESULTS FROM A PHASE 2 STUDY

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Background: Reducing late complications in allo-SCT recipients is an urgent issue in the field of transplant. One of the most serious complications is renal impairment, which reportedly develops in around 20% of long-term survivors. hANP is commercially available in Japan, and preserves postoperative renal function in patients undergoing cardiovascular surgery. These findings inspired us to investigate the renoprotective effects of hANP administration in allo-SCT recipients.
Aims: The primary objective was to determine the safety, toxicity, and efficacy of hANP administration in the early phase after allo-SCT.
Methods: A prospective phase II study of hANP therapy for preventing renal impairment in allo-SCT recipients was conducted with the permission of the institutional review board. Eligible subjects underwent allo-SCT for the first time between 2009 and 2013 without any restrictions on underlying disease, donor source, or conditioning regimen. After obtaining written informed consent, hANP administration was started the day before transplant at a dose of 0.025 μg/kg/min. This dose was adjusted according to blood pressure and urine volume. Results were compared between the study group and control group who had undergone allo-SCT between 2006 and 2013. We selected a control cohort at the rate of one to three, using an optimal matching method for the following nine factors: age; sex; underlying disease; disease status; conditioning intensity; conditioning with or without total body irradiation (TBI); human leukocyte antigen disparity; donor type; and estimated glomerular filtration rate (eGFR) at baseline.
Results: Eighteen patients (9 men, 9 women; median age, 45 years; range, 18-64 years) were enrolled in this study. Underlying diseases were acute leukemia (n=6), myelodysplastic syndrome (n=6), and others (n=6). Eight, seven, and three patients received transplants from related donors, unrelated donors, and cord blood, respectively, and most patients were conditioned with TBI-containing myeloablative regimens. Median duration of hANP administration was 16 days (range, 3-46 days). Two patients experienced grade 2 hypotension attributable to hANP administration, but both cases resolved immediately upon discontinuation of hANP administration. In addition, hANP administration was discontinued in two patients, due to grade 4 bacterial meningitis and grade 4 sepsis, respectively. For matched-pair analysis, 54 patients were selected, and no significant differences in clinical characteristics were identified between the study and control groups. Although mean eGFR kept declining after transplant in both groups, hANP administration maintained eGFR at a significantly higher level until 30 days after transplant (p <0.001). Incidences of acute kidney injury within 30 days after transplant did not differ significantly between groups, but that within 90 days was significantly lower in the study group (11.1%) than in the control group (40.7%; p=0.023). The incidence of CKD by 1 year after transplant was significantly reduced in the study group (0%) compared to the control group (42.9%; p=0.005). Two-year cumulative incidence of non-relapse mortality and 2-year overall survival rates were similar between groups.
Summary and Conclusions: Our findings demonstrated that hANP administration in the early phase after allo-SCT offers excellent renoprotective effects, resulting in a significant reduction in CKD development, without any serious adverse events in allo-SCT recipients. These findings should be confirmed in a prospective, randomized manner in the near future.

S444

ALLOGENEIC TRANSPLANTATION FOR MYELOFIBROSIS: HIGH DOSE INTENSITY AND EARLY DISEASE STATE ARE FAVORABLE PROGNOSTIC FACTORS.

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Background: The Best conditioning regimen, *i.e.* one that is both safe and efficacious for treatment of patients with myelofibrosis (MF) is not known. After observing a higher relapse rate in the initial cohort receiving a reduced intensity regimen, we increased the intensity of the conditioning regimen for subsequent patients, hypothesizing that increased dose intensity delivered with pharmacokinetic- (PK-) dose guidance will reduce the relapse rate without increasing non-relapse mortality (NRM), and thereby improving overall outcome.
Aims: To report final results of a prospective phase II clinical trial of Fludarabine (Flu) and busulfan (bu) conditioning in MF patients with a median follow-up of 5 years.
Methods: Patients with advanced MF were eligible if they had adequate organ function and at least 9/10 HLA matched related or unrelated donor. All 46 patients received fludarabine 40 mg/m^{2}x4 (day -5 to -2), and in the first 15 (bu low group) IV busulfan 130 mg/m^2/dayx2 was given after the Flu dose on days -3,and -2). Of the remaining 31 (bu high group), 27 received IV busulfan dose to a target daily systemic exposure, AUC, of 4000 μmol-minx4, given on day -5 to -2, (total course AUC of 16,000 μmol-min) and 4 patients received a fixed dose of 100 mg/m^2/dayx4 (days -5 to -2), each Bu dose was preceded by the day's Flu dose.
Results: 23 males and 23 females with a median age of 58 years (27-74) had intermediate (27 Int-2, 1 Int-1) or high-risk (18) disease as per Dynamic international prognostic scoring system plus (DIPSS plus) criteria. Donors were matched sibs (19), matched unrelated (23), or mismatched unrelated (4).All patients engrafted with a median time to neutrophil engraftment of 13 (0-27) days and a median time to platelet engraftment of 24 (0-268) days. The Cumulative incidence (CI) of grade II-IV, grade III, and IV acute GVHD, and Chronic GVHD were 22%, 7%, and 40%, respectively. With a median follow up of surviving patients of 5.1 years (range 1-8.3 years), 3 year overall survival (OS), event-free survival (EFS), cumulative incidence (CI) of non-relapse mortality (NRM), and CI of relapse were 69%, 48%, 13%, and 39%, respectively. Multivariate Cox regression analysis showed that Bu-high dose (HR 0.44; P=0.07) was associated with lower relapse rate. Bu-high dose (HR 0.5; P=0.09), DIPSS plus high (HR 2.69; P=0.02) and Age (HR 1.05; P=0.08) were predictors of EFS (Figure 1). DIPSS plus high (HR 5.99; P=0.001) and Age (HR 1.07; P=0.03) were adverse predictors of OS.

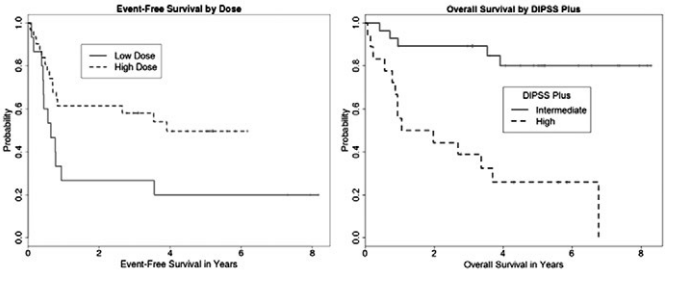

Figure 1.

Summary and Conclusions: Allogeneic transplantation results in long-term survival in patients with myelofibrosis with better outcome seen in earlier phase of the disease. PK guided myeloablative busulfan (AUC 16,000 μmol.min) appears promising in reducing relapse rate without increasing non-relapse mortality.

S445

UPDATED RESULTS OF A MULTICENTER PHASE I/IB STUDY OF CTLA4 BLOCKADE WITH IPILIMUMAB FOR RELAPSED HEMATOLOGIC MALIGNANCIES AFTER ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION

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Background: Patients with hematologic malignancies (HM) who relapse after alloHCT often have a dampened graft *versus* tumor (GVT) effect and limited

treatment options. Immune checkpoint modulation with CTLA4 blockade could be a novel pharmacologic strategy to augment GVT to restore the anti-tumor activity of the graft.

Aims: This is an ongoing multicenter phase I/Ib study of the CTLA4 blocking antibody ipilimumab to treat patients (pts) with HM of any histology who relapse after alloHCT. The primary aims are to determine the MTD and evaluate safety. Secondary aims include assessments of efficacy and changes in immune cell phenotype.

Methods: After informed consent was obtained, ipilimumab was given off-label at 3 mg/kg or 10 mg/kg IV every 3 weeks for 4 cycles of induction, followed by maintenance dosing every 12 weeks up to 1 year. Disease-specific response criteria were assessed at the mid-point (7 weeks), end of induction (13 weeks), and throughout maintenance. Immunophenotyping was performed by 8-color flow cytometry and analyzed by FACSDiva.

Results: Twenty-seven pts have received treatment to date. In phase I, 6 pts were treated at 3 mg/kg and 7 pts were treated at 10 mg/kg. An MTD was not reached, and 14 pts subsequently enrolled in the phase Ib expansion cohort at 10 mg/kg. The median number of prior therapies excluding transplant was 3 (range 2-11), and 19/27 (70.4%) of pts had received prior therapy for post transplant relapse. Histologies included AML (n=11), cHL (n=7), NHL (n=4), and MDS (n=2), and 1 pt each had MM, MPN, and ALL. The median age at enrollment was 58 yrs. (range 22-75). Immune-related adverse events (irAEs) were observed in 4 pts, including pneumonitis (n=1 gr2, n=2 gr4), diarrhea (n=2 gr 1), ITP (n=1 gr2), and colitis (n=1 gr 3), and were reversible with steroids, with 3 pts able to resume ipilimumab. Four DLTs leading to discontinuation have been observed, including cGVHD (n=2, both liver, gr 3), aGVHD (n=1, gut, gr 2), and TRM (n=1) due to presumed sepsis in the context of severe irAEs. Seventeen patients discontinued due to progressive disease, and 6 patients remain on active treatment. In an interim efficacy analysis, while none of the 5 evaluable pts treated at 3 mg/kg responded, 8 of the 20 (40.0%) evaluable pts treated at 10 mg/kg had anti-tumor activity. Six pts achieved formal response by disease-specific criteria, including a cHL patient with a PR with dramatic reduction in nodal and extranodal disease and a marrow CR at 7 weeks (see Figure 1), a MM pt with a PR with near resolution of a lung plasmacytoma, and four pts with AML who achieved CR, including two with leukemia cutis and one with myeloid sarcoma. The median follow-up time among survivors is 5.5 mo., and 6 mo. OS is currently 66%. Immunophenotyping studies revealed that while the absolute numbers of both T_{reg} and T_{conv} cells increased slightly after ipilimumab, the ratio of T_{reg}/T_{conv} decreased (range 24% to 41% decrease).

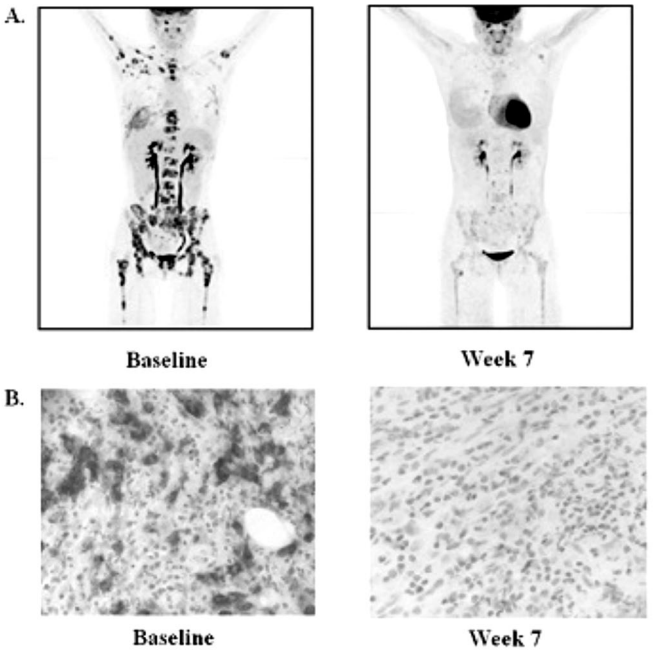

Figure 1. Baseline and week 7 re-staging studies in a classical Hodgkin lymphoma patient after 2 doses of ipilimumab at 10 mg/kg. A. PET/CT scan and B. bone marrow (CD30 stain).

Summary and Conclusions: While some toxicity including irAEs and GVHD was seen with ipilimumab 10 mg/kg in pts with relapsed HM after alloHCT, substantial anti-tumor activity was also observed in pts with both lymphoid and myeloid malignanices, including highly chemorefractory diseases such as leukemia cutis and myeloid sarcoma. The T_{reg}/T_{conv} cell ratio decreased with treatment, consistent with enhancement of GVT. Immune checkpoint modulation with CTLA4 blockade may be a promising new therapeutic approach for post alloHCT relapse, and is worthy of exploration in future studies.

MPN: Prognosis and treatment

S446

RISK FACTORS FOR THROMBOHEMORRHAGIC AND TRANSFORMATION EVENTS IN 3649 HIGH-RISK PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA: RESULTS FROM THE PROSPECTIVE LONG-TERM OBSERVATIONAL EXELS STUDY

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Background: The observational EXELS study (NCT00567502) is the largest prospective cohort of high-risk patients (pts) with essential thrombocythemia (ET) reported to date.

Aims: Study objectives included safety, pregnancy outcomes, and efficacy (measured by incidence of thrombohemorrhagic events and platelet reduction) of anagrelide (ANA) compared with other cytoreductive therapies (CRTs). The multivariate (MV) analysis aimed to identify risk factors for thrombohemorrhagic/transformation events.

Methods: Eligible pts were enrolled across 13 European countries between 2005 and 2009. Data were collected every 6 mo for 5 yrs. Event rates are presented as number of pts per 100 pt-yrs exposure and by treatment at registration. A Cox proportional hazards model was used for the MV analysis to predict events based on certain risk factors. All pts provided informed consent. This study was sponsored by Shire Pharmaceutical Development Ltd.

Results: 3649 pts were categorized according to treatment at registration: ANA (n=804), ANA+other CRT (n=141), other CRT (n=2666), and no CRT (n=38). More than 80% of pts received either hydroxycarbamide (HC) or ANA. Median age was lower in the ANA (55.5 yrs) *vs* other CRT group (70.0 yrs). Thrombohemorrhagic and malignancy event rates are displayed in Table 1. Median platelet counts in the ANA *vs* other CRT group were 443 *vs* 428x10^9/L, respectively, at baseline, and 402 *vs* 430x10^9/L, respectively, at the time of major thrombotic event. Median white blood cell (WBC) counts in the ANA *vs* other CRT group were 8.8 *vs* 6.0x10^9/L, respectively, at baseline, and 9.0 *vs* 6.2x10^9/L, respectively, at the time of major thrombotic event. 64 pts transformed to myelofibrosis (MF) and 31 to acute leukemia (AL). In pts who had only ever received either ANA or HC, the rate of transformation to MF was higher in the ANA *vs* HC group (0.78 *vs* 0.17), whereas transformation to AL was higher in the HC *vs* ANA group (0.22 *vs* 0). Non-hematological malignancy event rate was higher in the other CRT *vs* ANA group. MV analysis identified both a history of thrombohemorrhagic events and age ≥65 yrs at baseline as risk factors for predicting thrombohemorrhagic (hazard ratio [HR] 1.91 and 1.67), major thrombotic (HR 2.05 and 2.14), arterial thrombotic (HR 1.81 and 1.90), venous thrombotic (HR 3.70 and 3.27), and transformation to AL/myelodysplasia (MDS) events (HR 2.17 and 3.36). Presence of baseline cardiovascular risk factors increased the risk of major thrombotic (HR 1.38) and arterial thrombotic events (HR 1.65), while baseline hypertension was a risk factor for thrombohemorrhagic events (HR 1.33). ET diagnostic criteria (WHO *vs* PVSG) and aspirin at registration were not identified as risk factors for thrombohemorrhagic events. Time since diagnosis (5-<10 yrs; ≥10 yrs) and baseline platelet count increase of 100 units above normal (≤450x10^9/L) were both identified as risk factors for transformation to MF (HR 3.38; 4.38 and 5.34).

Table 1.

Treatment at registration	ANA N=804		Other CRT N=2666		ANA + other CRT N=141	
Event	Pts (events) n	Event rate	Pts (events) n	Event rate	Pts (events) n	Event rate
Total thrombohemorrhagic events	65 (81)	2.47	226 (278)	2.41	5 (6)	1.37
Major thrombotic events	43 (52)	1.62	194 (231)	2.06	4 (5)	1.09
Arterial thrombotic events	39 (48)	1.47	147 (175)	1.55	4 (5)	1.09
Venous thrombotic events	4 (4)	0.15	51 (56)	0.53	0	0
Major hemorrhagic events	24 (29)	0.89	42 (47)	0.43	1 (1)	0.27
Transformation to:						
Myelofibrosis	28 (28)	1.04	29 (29)	0.30	7 (7)	1.91
Acute leukemia	2 (2)	0.07	27 (27)	0.28	2 (2)	0.53
Myelodysplasia	0	0	12 (12)	0.12	0	0
Non-hematological malignancy	12 (13)	0.44	123 (140)	1.29	2 (2)	0.54

ANA, anagrelide; CRT, cytoreductive therapy; Pts, patients

Summary and Conclusions: In this large prospective study population, MV analysis identified the following baseline risk factors for thrombohemorrhagic events: history of thrombohemorrhagic events, age ≥65 yrs, cardiovascular risk factors, and hypertension. In contrast, within each treatment group, the baseline platelet and WBC counts do not appear to be significantly different at the time of major thrombotic events. A history of thrombohemorrhagic events, age ≥65 yrs, time since diagnosis, and platelet count increase of 100 units above normal were identified as baseline risk factors for transformation to AL/MDS/MF.

S447

RUXOLITINIB *VERSUS* BEST AVAILABLE THERAPY IN PATIENTS WITH POLYCYTHEMIA VERA: 80-WEEK FOLLOW-UP FROM THE RESPONSE TRIAL

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Background: RESPONSE, an ongoing, multicenter, open-label, phase 3 trial, compared the efficacy and safety of ruxolitinib (Rux) with best available therapy (BAT) in patients with polycythemia vera who are resistant to or intolerant of hydroxyurea. Primary analysis results - at 48 weeks from last patient first treatment (LPFT) - have been published (Vannucchi. *NEJM* 2015;372(5):426-435).
Aims: To perform a second preplanned analysis assessing the long-term efficacy and safety of Rux 80 weeks after LPFT.
Methods: Patients ≥18 years of age, who were resistant to or intolerant of hydroxyurea per modified ELN criteria, with splenomegaly, and phlebotomy requirement to control hematocrit (Hct) were eligible. Patients were randomized 1:1 to receive open-label Rux 10 mg BID or BAT; the latter was selected based on investigator's choice. Patients randomized to BAT could cross over to Rux from week 32. The primary response was a composite of (1) achieving a ≥35% reduction from baseline in spleen volume by imaging at week 32 and (2) Hct control without phlebotomy through week 32 (defined as no phlebotomy eligibility between weeks 8 to 32 with no more than 1 phlebotomy eligibility from randomization to week 8). Durability of the primary response, Hct control, spleen volume reduction, complete hematologic remission (CHR), as well as long-term safety, were evaluated.
Results: Overall, 222 patients were randomized (Rux, 110; BAT, 112). At the week 48 analysis, 93 (84.5%) patients randomized to Rux (Rux arm) were still receiving treatment (median exposure, 81 weeks); at the data cutoff for the week 80 anaysis, 91 (82.7%) patients in the Rux arm were still receiving treatment (median exposure, 111 weeks). No patients remained on BAT at the data cutoff for the week 80 analysis, compared with 3 patients at the week 48 analysis. The primary endpoint was achieved in 23 (20.9%) patients in the Rux arm *vs* 1 (0.9%) patient in the BAT arm (P <0.0001). Among the responders in the Rux arm, only 1 patient lost this response (Figure 1). Overall, 60.0% of Rux patients *vs* 19.6% of BAT patients achieved Hct control without phlebotomy through week 32; patients achieving Hct control in the Rux arm had an 89% probability of maintaining this response for 80 weeks from the time of initial response. Of the 98 patients on Rux at week 32, 89.8% did not have a phlebotomy between weeks 32 and 80. At week 32, 38.2% *vs* 0.9% of patients in the Rux *vs* BAT arm achieved a ≥35% reduction in spleen volume; all Rux patients maintained their response. At week 32, CHR was achieved in 23.6% of Rux patients and 8.9% of BAT patients, with Rux responders having a 69% probability of maintaining CHR for 80 weeks. Among patients who discontinued, 5 of 10 in the Rux arm with available data for the Pruritus Symptom Impact Scale at their end-of-study visit rated their pruritus as "very much improved." Nonhematologic adverse events were mainly grade 1 or 2; the most common in the Rux arm included headache (21.8% at the week 80 analysis [ie, entire follow-up] *vs* 20.9% at the week 48 analysis), diarrhea (20.0% *vs* 19.1%), pruritus (20.0% *vs* 17.3%), and fatigue (17.3% *vs* 17.3%). Grade 3 or 4 anemia and thrombocytopenia in the Rux arm did not increase from the week 48 analysis, occurring in 1.8% and 5.5% of patients, respectively. Treatment discontinuation due to adverse events remained low in the Rux arm (4.5%).

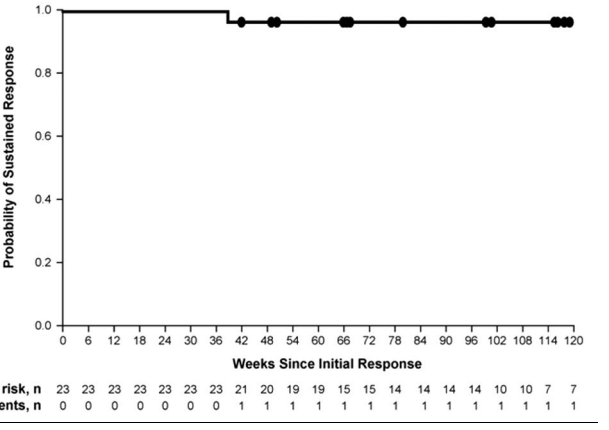

Figure 1. Durability of primary response with rux.

Summary and Conclusions: In the RESPONSE study, Rux benefit was durable and treatment remained generally well tolerated, with 82.7% still receiving Rux at a median exposure of 111 weeks.

S448

RUXOLITINIB (RUX) IN COMBINATION WITH 5-AZACYTIDINE (AZA) AS THERAPY FOR PATIENTS (PTS) WITH MYELOFIBROSIS (MF)

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Background: AZA is a DNA methyltransferase (DNMT) inhibitor with a modest response rate (20-25%) and duration (4 months) in MF. Ruxolitinib and azacytidine may target distinct clinical and pathological manifestations of myelofibrosis.
Aims: To determine the efficacy and safety of the combination in pts with MF.
Methods: A sequential approach with single-agent RUX 15 mg orally twice daily (if platelets 100-200) or 20 mg twice daily (if platelets >200) continuously in 28-day cycles for the first 3 months followed by the addition of AZA 25 mg/m2 on days 1-5 of each 28-day cycle starting cycle 4 was adopted. The AZA dosage could be gradually increased to a maximum of 75 mg/m2. Pts would be treated on study for 15 months followed by continuation of the combination off-study at the discretion of the treating physician.
Results: 35 pts were enrolled between March 2013 and October 2014. 22 (63%) had received a median of 2 (1-3) prior therapies for MF. 31 pts remain alive after a med follow-up of 15.4 (3.4-22.8) months. Study is ongoing. 28 pts have been on the study for at least 6 months and are evaluable for response. International Working Group for Myelofibrosis Research and Treatment 2013 (IWG-MRT) objective responses were noted in 23 (82%), including PR in 1, CI for spleen and total symptom score (TSS) in 5 (18), CI for TSS and hemoglobin in 2 (7), CI for TSS only in 9 (32), and CI for spleen only in 6 (21). Responses occurred in 14 of 18 (78%) previously treated patients and 9 of 10 (90%) untreated pts (P=0.42). Median time to all responses was 1.0 month (0.8-7.5 months). Median time to CI in spleen size was 1.9 months (0.9-6.6), to CI TSS was 1.0 months (0.8-7.5), and CI Hb was 2.1 months (1.1-8.9). A reduction in the baseline $JAK2^{V617F}$ allele burden was noted in 11 of 13 serially evaluable responders with 2 of the responders demonstrating a >50% reduction in allele burden. Serial evaluation of bone marrow fibrosis revealed a documented reduction in EUMNET fibrosis score in 6 of 22 (27%) evaluable responders, after a median of 8 months (5-13) on therapy. Only 5 of the 35 (14%) pts have not required a dose interruption/dose adjustment. The med time to first dose interruption/dose adjustment was 28 days (6-634). Among the 30 pts who needed a dose interruption/adjustment, 27 needed it within 3 months of initiation of the combination. The first change was a dose reduction in 17 pts and a dose interruption in 5 pts. AZA was the agent interrupted in 4 of these pts: 1 never started AZA, 1 didn't resume AZA and 2 resumed AZA at same dose. The reasons for the first dose interruption were thrombocytopenia (n=2), neutropenia (n=1), knee replacement (n=1), and pneumonia (n=1). The first change was a dose increase in 8 pts. AZA was the first drug to be increased in 1 and RUX in 7. The reasons for dose increase were leukocytosis (n=3), progressive splenomegaly (n=1), thrombocytosis (n=2), and suboptimal response (n=3). At the time of submission, 8 patients completed the predefined 15 months on the study, and 20 pts remain on study. Reasons for discontinuation in the remaining 7 pts included AML transformation (n=2), toxicity (n=2), lack of response (n=1), death (n=1), and patient preference (n=1). 4 pts experienced grade 3/4 non-hematological

toxicity including fatigue (n=2), nausea (n=1), pneumonia (n=1), respectively (Table 1).

Table 1. Baseline characteristic (N=35).

Characteristic	N (%) / [range]
Med age, years	66 (47-87)
Male	20 (57)
Prior treatment	22 (63)
ECOG PS < 1	34 (97%)
Diagnosis	
Post-essential thrombocytosis MF	8 (23)
Post-polycythemia MF	8 (23)
Primary MF	19 (54)
Splenomegaly	31 (89)
Med WBC x 10^9/L	13.3 (2.2-60.6)
Med hemoglobin g/dL	10,1 (6.8-16.2)
Med platelets x 10^9/L	259 (126-835)
Med LDH value	1865 (452-4907)
Peripheral blood blasts ≥ 1%	22 (63)
EUMNET fibrosis grade	
MF-1	4 (11)
MF-2	15 (43)
MF-3	16 (46)
JAK2 +	23 (66)
Med JAK2 allele burden	71 (4-95)
Karyotype	
Diploid	21 (60)
Abnormal	13 (37)
Insufficient metaphases	1 (3)

Summary and Conclusions: Administration of RUX with AZA was feasible and resulted in improved response rate. The sequential addition of AZA was well tolerated with a lower incidence of early discontinuation than what has been seen with other ruxolitinib combination therapies. A sequential rather than concomitant approach may be considered when administering ruxolitinib combinations.

S449

EXCELLENT LONG-TERM PROGNOSIS OF IMATINIB-TREATED FIP1L1-PDGFRA POSITIVE EOSINOPHILIA-ASSOCIATED MYELOPROLIFERATIVE NEOPLASM IN CHRONIC OR BLAST PHASE

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Background: FIP1L1-PDGFRA (FP) is the most common fusion gene in eosinophilia-associated myeloproliferative neoplasms (MPN-eo) and is exquisitely sensitive to treatment with imatinib. The vast majority of FP positive (FP+) patients (pts) are diagnosed in chronic phase (CP) but rarely also in myeloid or lymphoid blast phase (BP).
Aims: To evaluate the long-term prognosis of FP+ pts in CP or BP on imatinib.
Methods: Here we present the long-term follow-up of 62 imatinib-treated FP+ pts in CP (n=50) or BP (n=12). Median age was 49 years (range 19-70) with a striking male predominance (61/62, 98%). All pts were treated with imatinib (100-400mg/d) for a median of 79 months (range 2-140).
Results: In CP, complete molecular remission (CMR) was observed after 6, 12 and 24 months in 26/48 (54%), 35/42 (83%) and 38/39 (97%) of pts, respectively, as investigated by qualitative nested RT-PCR. The median duration of CMR was 73 months (range 3-129). Imatinib was reduced to 2-3x100mg/week in 15 pts for a median of 28 months (range 1-96) and no relapse has yet been observed. Imatinib was stopped in 4 pts with 3 pts remaining in CMR for 2, 19 and 20 months, respectively. One patient relapsed after 12 months but rapidly achieved second CMR after rechallenge with imatinib. In BP, imatinib was used as monotherapy (n=7) or after intensive chemotherapy (n=5). All pts achieved durable CMR (median 95 months, range 66-132). Overall, imatinib was well tolerated and no grade III-IV toxicities were observed. At the time of analysis, 3 pts had died (CP, n=1; BP, n=2) but no death was disease- or treatment-related. Two pts developed a secondary resistance, 7 and 10 months after start of imatinib. A T674I point mutation in the ATP-binding domain of FIP1L1-PDGFRA was identified in one patient while the mechanism of resistance in the second patient remained unknown. Because treatment with nilotinib and/or sorafenib did not result in significant remissions, both pts received an allogeneic stem cell transplantation (SCT) 5 and 8 months, respectively, after detection of imatinib-resistance. Both pts achieved a rapid complete hematologic remission. One patient has been in stable CMR for 48 months while the second patient died 18 months after SCT in ongoing CMR.
Summary and Conclusions: Treatment with imatinib in FP+ MPN-eo is associated with an excellent long-term prognosis, even if pts are initially diagnosed in myeloid or lymphoid BP. Complete remissions are rapidly achieved, are durable and can be maintained with low-dose imatinib. Discontinuation of imatinib may be feasible in some cases. Primary resistance has not yet been reported and secondary resistance seems to be very rare. If it occurs, the efficacy of second-generation tyrosine kinase inhibitors seems to be poor. Eligible pts should therefore be offered an allogeneic SCT.

S450

OUTCOMES OF ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION (HCT) IN PATIENTS WITH MYELOFIBROSIS (MF) EXPOSED TO JAK1/2 INHIBITORS

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Background: JAK1/2 inhibitors (JAK#) have the potential to effectively control MF-related symptoms, and improve the clinical status prior to HCT. However, the clinical experience is limited, and data are conflicting. (Robin *et al.*, Blood, 2013, ASH abstract 306; Stübig *et al.*, Leukemia, 2014). Additionally, the relationship between the response to JAK #, and outcomes of HCT is not clear.
Aims: To report outcomes of HCT in MF patients exposed to JAK#
Methods: In this multi-institutional retrospective study, we evaluated the outcomes of 93 MF patients (pts.) who had exposure to JAK# prior to HCT. A working definition of response to JAK# was established inspired by International Working Group-Myeloproliferative Neoplasms Research and Treatment (IWG-MRT) criteria. (Table1). The primary end point was overall survival (OS) from the date of HCT.

Table 1.

1. Gp-A: Clinical improvement: ≥50% reduction in palpable spleen length for spleen palpable by ≥10 cm, or complete resolution of splenomegaly for spleen <10 cm).
2. Gp-B: Stable disease: spleen response not meeting the criteria of clinical improvement.
3. Gp-C: New onset anemia requiring transfusions, or increase in blast to 10-19%.
4. Gp-D: Progressive disease: Loss of spleen response or progression of splenomegaly.
5. Gp-E: Progressive disease: Leukemic transformation (blast ≥20%).

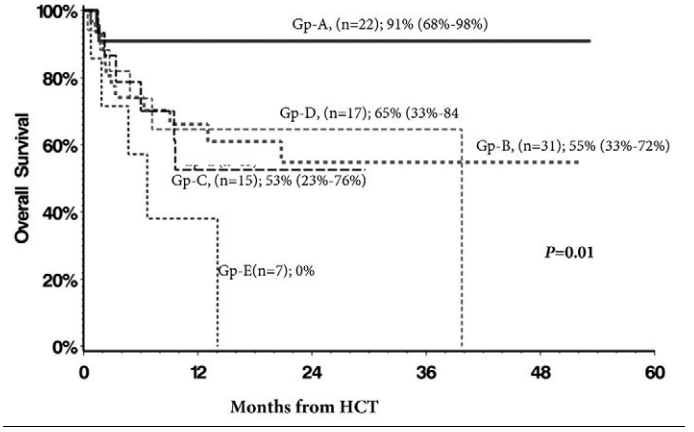

Figure 1. Probabilities of survival at 2 years and (95% CI).

Results: Median age at HCT was 59 years (range 32-72 years). Diagnosis was primary-MF (n=53), post polycythemia-MF (n=20), or post essential thrombocythemia-MF (n=20). DIPSS-plus score at the time of HCT was low in 2 (2%), INT-1 in 19 (22%), INT-2 in 47 (52%), high-risk in 22 (24%), and not available in 3 pts. Conditioning was full intensity in 42 (45%), and reduced intensity in 51 (55%). Donors were matched sibling in 36 (39%), matched unrelated in 47 (51%), and mismatched or haplo-identical in 10 (11%). JAK# used were ruxolitinib (n=84), momelitinib (n=6), or others (n=3). Sixty-five (70%) pts used JAK# leading to HCT, and stopped 0-16 days prior to conditioning regimen; 23 (25%) pts had discontinued the medication at least 4 weeks prior to HCT due to progression or intolerance, and in 5 pts this information was not available. Among pts who used JAK# leading to HCT, "withdrawal symptoms" were reported in 10 (15%), and were more common in pts who stopped JAK inhibitors ≥6 days prior to conditioning regimen compared to those who stopped

within 0-6 days (26% *vs* 11%, *p*=0.06). Withdrawal symptoms were non-severe in nature except in one pt. this resulted in rebound splenomegaly and pulmonary infiltrates necessitating splenectomy and delaying the HCT for 3 months. Primary graft failure was reported in 3 pts (3%). The cumulative incidence of grade ≥2, and ≥3 acute GVHD at 100 days were 44% and 16% respectively. Cumulative incidence of chronic GVHD at 2-years was 53%. Median follow-up of survivors were (2-53) months, and during this period, 12 (13%) pts had relapse/progression and 31 (33%) died. Probability of 2-year OS of whole cohort was 62% (95% CI, 50%>73%). Patient in group-A (n=22) had significantly superior survival *p*=0.01 (Figure 1) Other factors with significant effect on survival in univariate analysis were DIPSS-plus score, donor type, and performance status at HCT. In a limited multivariate analysis, response to JAK# was the strongest independent factor for survival (*p*=0.004). DIPSS-plus high-risk category (p=0.04), and mismatched/haplo donor groups (p=0.04) were other independent factors for survival.

Summary and Conclusions: Our data suggest that use of JAK# prior to HCT does not have adverse effects on early transplant outcomes. Continuation of JAK# until HCT appears to reduce the risk of "withdrawal symptoms". The HCT outcomes in patients responding to JAK# are particularly encouraging.

Molecular markers in AML

S451

DNMT3A MUTATIONS IN ACUTE MYELOID LEUKEMIA (AML): MONITORING OF MINIMAL RESIDUAL DISEASE (MRD). A STUDY OF THE AML STUDY GROUP (AMLSG)

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Background: *DNMT3A* is frequently mutated in AML and associated with a poor overall (OS) and relapse-free survival (RFS). The presence of *DNMT3A* mutations (*DNMT3A*^{mut}) in early preleukemic stem cells and in apparently healthy elderly highlight its pathogenic role as a founder mutation and as predisposing event in leukemia. Within our clinical AMLSG trials we addressed the question if MRD monitoring in *DNMT3A*^{mut} patients (pts) has clinical impact and delivers further information for risk-adapted therapy and clonal hematopoiesis.

Aims: To monitor MRD for the most common *DNMT3A*^{mut} (*DNMT3A*^{mut}-R882H, n=111 and -R882C, n=48) in a large cohort of AML pts entered on three AMLSG treatment trials [AML HD98A (n=14; NCT00146120), AMLSG 07-04 (n=87; NCT00151242), AMLSG 09-09 (n=58; NCT00893399)].

Methods: *DNMT3A*^{mut} MRD monitoring was performed using a cDNA-based RQ-PCR-assay by TaqMan technology with a sensitivity between 10^{-3} and 10^{-4}. MRD levels have been reported as normalized values of *DNMT3A*^{mut} transcripts per 10^4 *ABL1* transcripts (*DNMT3A*^{mut}/10^4 *ABL1*).

Results: In total, 1,168 samples [bone marrow (BM), n=615; peripheral blood (PB), n=553] from 159 *DNMT3A*^{mut} pts were analysed [diagnosis, n=256; during therapy, n=719; follow-up, n=193]. Median BM *DNMT3A*^{mut} transcript level (TL) at the time of diagnosis was 12690 (range, 0-54280); TL were not associated with known clinical characteristics or mutations in *NPM1* or *FLT3;* there was no impact on OS and RFS. *DNMT3A*^{mut} TL during therapy were significantly higher in BM after induction I, consolidation I and II (p=0.01; p=0.0003; p=0.01) than in PB. After double induction therapy (DI) there was no difference in the median of TL between 102 pts in complete remission (CR) and 12 pts not in CR (12694 and 11309, respectively). There was no prognostic impact of BM *DNMT3A*^{mut} TL as log 10 transformed continuous variable during therapy with regard to death and relapse. Strikingly, the greatest TL reduction was seen after induction I (one log) whereas subsequent therapies did not significantly influence TL. Next, we evaluated the impact of *DNMT3A*^{mut} MRD monitoring for the clinical endpoints OS, cumulative incidence of relapse (CIR) and remission duration (RD) after DI and after end of therapy (ET). After DI and ET, only 7/75 and 4/63 BM samples became MRD negative. At these two time-points MRD positivity did not significantly impact OS (p=0.89; p=0.73), CIR (p=0.74; p=0.29) and RD (p=0.61; p=0.30). Then we investigated the MRD *DNMT3A*^{mut} log10-reduction (compared to level at diagnosis) using the median as a cut-off after DI and ET. Again, there was no significant correlation for pts with a higher TL reduction compared with a lower TL reduction for OS and RD after DI and ET (p=0.87; p=0.38; p=0.67; p=0.57, respectively). Finally, we evaluated the BM *DNMT3A*^{mut} TL as 4 increasing equally sized intervals according to the quartiles of the distribution. There was no prognostic impact after DI on OS and RD (p=0.24; p=0.85) and ET (p=0.38; p=0.44). To increase the number of samples, we also performed the analyses for BM and PB samples, but again, there was no significant impact on prognosis.

Summary and Conclusions: In our study neither the absolute BM *DNMT3A*^{mut} TL at the time of diagnosis nor the reduction of TL or the increasing quartiles after DI and ET showed prognostic impact. In contrast to AML with *NPM1* mutation or associated gene fusions, most pts had persistent *DNMT3A*^{mut} TL supporting the role of persistent clonal hematopoiesis. Serial investigation of *DNMT3A*^{mut} and its concurrent mutations will provide further insights into the clonal evolution of AML.

S452

LEUKAEMIA-ASSOCIATED SOMATIC MUTATIONS DRIVE DISTINCT PATTERNS OF AGE-RELATED CLONAL HAEMOPOIESIS

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Background: Clonal haemopoiesis driven by leukaemia-associated gene mutations can occur without evidence of a blood disorder.
Aims: To investigate the incidence, target genes and age distribution of age-related clonal haemopoiesis (ARCH).
Methods: We performed targeted ultra-deep re-sequencing for hotspot mutations at 15 gene loci recurrently mutated in myeloid malignancies in the blood DNA of 4219 individuals including a large number of elderly people.To do this we developed and validated a robust methodology, employing barcoded multiplex PCR of mutational hotspots followed by next-generation sequencing (MiSeq) and bioinformatic analysis. This reliably detected mutation-associated circulating blood cell clones with a variant allele fraction (VAF) ≥0.008 (0.8%).
Results: Using only the hotspots studied, we identified clonal haemopoiesis in 0.8% of individuals under 60, rising to 19.5% of those ≥90 years; predicting that clonal haemopoiesis is much more prevalent overall than was previously realized. Indeed, using our findings as a basis for projection, we estimate the overall prevalence of age related clonal haemopoiesis (ARCH) to be >70% in those older than 90 years. *DNMT3A*-R882 mutations were most common and, although their incidence increased with age, were found in individuals as young as 25 years. By contrast mutations affecting spliceosome genes *SF3B1* and *SRSF2*, closely associated with the myelodysplastic syndromes, were only identified in those aged >70 years, with several individuals harboring more than one such mutation.This indicates that spliceosome gene mutations drive clonal expansion under selection pressures operating in the ageing haemopoietic system and explains the high incidence of clonal disorders associated with these mutations in advanced old age. Finally, despite using a very sensitive method and a mutation-calling script written specifically for this purpose, no samples with NPM1 mutations of VAF ≥0.008 were identified.

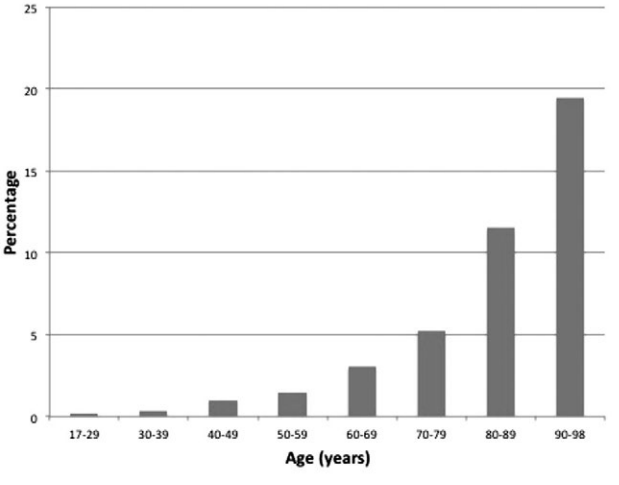

Figure 1. The prevalence of clonal haemopoiesis driven by only 9 hotspot mutations by age.

Summary and Conclusions: Our results demonstrate that the incidence of clonal haemopoiesis is much higher than suggested by exome sequencing studies, that splicesome gene mutations drive clonal outgrowth primarily in the context of an aging haematopoietic compartment, and that NPM1 mutations do not drive ARCH, indicating that their acquisition is closely associated with frank leukemia.

S453

JAM-C: A NEW LEUKEMIC STEM CELL BIOMARKER IN ACUTE MYELOID LEUKEMIA

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Background: Acute myeloid leukemia (AML) is a clonal hematologic malignancy arising from a rare population of leukemic stem cells (LSCs) that initiate and propagate the disease. LSCs are enriched within the CD34+CD38low/- compartment expressing the IL-3 receptor α-chain, CD123. In contrast, normal hematopoietic stem cells do not express CD123. Several studies have recently shown that a fraction of LSCs, residing in particular niches of the bone marrow, resists to chemotherapy and is associated with poor clinical outcome.
Aims: We were interested to explore cell-adhesion and migratory properties of LSC aiming at identification of new cell-surface marker able to define a Leukemic Initiating Cell population within the LSC compartment.
Methods: AML patient samples were analyzed by flow cytometry to characterize protein expression profile in the LSC compartment and then engrafted in NOD scid gamma chain deficient (NSG) mice. Clonogenic and cobblestone-area forming cell (CAFC) assays *in vitro* and gene-expression profiling were performed to characterize *stemness* and regenerative *potential* of defined LSCs subsets.
Results: We confirmed in a large cohort of 34 AML *de novo* patients results showing correlation between mouse engraftment and poor AML disease prognosis. Engrafting patients had a significant poorer overall survival as compared to non-engrafting. Searching for molecular factors that could affect AML engraftment, we found that the junctional adhesion molecule-C (JAM-C) was expressed by a fraction of LSCs. JAM-C is an adhesion molecule that we have previously shown to be expressed by mouse and human hematopoietic stem cells. Functionally, JAM-C interaction with JAM-B expressed on stromal cells was involved in HSC retention in the bone marrow. Here, we showed that percentages of JAM-C expressing cells in CD34+CD38low/-CD123+ leukemic cells at diagnosis were significantly increased in engrafting AML patient samples as compared to non-engrafting samples. Since engraftment was correlated to survival, we then tested if the frequencies of circulating CD34+CD38low/-CD123+JAM-C+ cells could provide prognostic information. 60 AML *de novo* patient samples at diagnostic were analyzed. We found that high frequencies of JAM-C positive cells in the LSC compartment were correlated to poor overall survival and significant reduced leukemia-free survival. Along this line, the study of 10 paired AML samples at diagnosis and relapse revealed a significant increase of JAM-C positive cells in the LSC compartment in all patient samples at relapse as compared to diagnosis. Functional properties of these cells were then tested. Clonogenic and CAFC assays showed that JAM-C+ LSCs grow into colonies whereas JAM-C- cells do not. Moreover, JAM-C+ LSCs established leukemia *in vivo* whereas JAM-C- cells didn't engraft in mice. Finally we showed that JAM-C+ cells are predominantly in G0 phase and have a more immature gene expression profile compared to JAM-C- cells.
Summary and Conclusions: All together, these data indicate that JAM-C is a unique marker for primitive leukemic stem cells and strongly suggest that JAM-C expression defines a Leukemic Initiating Cell population within the LSC compartment. Given the increased expression of this adhesion molecule by LSCs during relapse, we propose that targeting JAM-C should be a promising adjuvant therapeutic strategy to inhibit retention of leukemic stem cells within bone marrow stromal niches.

S454

UTILIZING EXOSOMES AS INNOVATIVE MICRORNA BIOMARKERS AND TARGETED DRUG-DELIVERY VEHICLES IN ACUTE MYELOID LEUKEMIA

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Background: Exosomes are microvesicles that play important roles in intercellular communications in both normal and tumor cells via their cargoes, which include microRNA (miRNA) and proteins. miRNAs play critical regulatory roles in hematopoiesis, and their abnormal expression is correlated with several hematological malignancies, including acute myeloid leukemia (AML). Exosome-derived miRNAs and proteins are increasingly recognized for their prognostic biomarker potential. Exosomes are being evaluated for their potential as novel drug-delivery vehicles due to their endogenous nature and ability to carry small molecule drugs.
Aims: We aim to characterize HSC-derived exosomes, investigate the biomarker potential of exosomes derived from AML patient samples (by examining their proteomic and miRNA profiles) and utilize exosomes as innovative drug delivery vehicles with the ability to eliminate leukemic stem cells in a targeted manner.
Methods: Secreted exosomes from murine bone marrow HSCs were isolated from conditioned medium and visualized using confocal microscopy. We isolated exosomes and performed miRNA profiling, using qPCR, and LC-MS/MS proteomic analysis to characterize the constituents. We also isolated exosomes from the CD34+ cells of three AML patient samples and profiled 372 of the

most abundantly expressed miRs in these cells compared to normal CD34+ cells. We analyzed the proteome of these exosomes as well. In order to assess the utility of exosomes as drug carriers, exosomes from bone marrow-derived OP9 stromal cells were transfected with Daunorubicin (1ug/ul). Normal CD34+ cells and patient-derived AML samples (from n=2 patients) were treated with varying doses of the drug-loaded exosomes. Drug-loaded exosomes uptake was tracked with a Texas Red siRNA. After 24 hours, cells were screened for apoptosis. To test the feasibility of targeted exosomes, OP9 cells were exposed to AML patient samples for 48 hours. The patient cells were then removed and, 24 hours later, "trained" stromal cell-derived exosomes were isolated from the media and transfected with Daunorubicin. These patient-specific, exosomes were plated with both the corresponding patient's CD34+ cells as well as normal CD34 cells (Figure 1A). After 24 hours, apoptosis was measured.

Results: miRNA profiling of murine bone marrow showed *miR-21a*, *miR-92a* and *miR-25* were most abundant in exosomes. Proteomic LC-MS/MS analysis revealed presence of exosome-associated novel proteins such as Syntenin-1. Syntenin-1, which is known to bind IL-5R and promote myelopoiesis, was present in significantly higher levels in HSCs compared to myeloid progenitors, implying a functional role for exosome derived Syntenin-1. miRNA profiling in AML samples revealed distinct signature profiles. Importantly, exosome derived miRs such as -1290, -375, -205 and -21—that are known prognostic markers in cancers such as prostate, ovarian and hepatocellular carcinoma—were significantly upregulated in all the three exosome-derived AML samples. The drug-loaded exosomes were successful in inducing significant apoptosis in two patient samples tested. These drug-loaded exosomes also induced cell death in CD34+ normal cells when compared to control exosomes. However, the patient-trained exosomes specifically eliminated 92% of CD34+ patient cells, while causing significantly less cell death (44%) of normal CD34+ cells exposed to drug-loaded, patient-trained exosomes (Figure 1B).

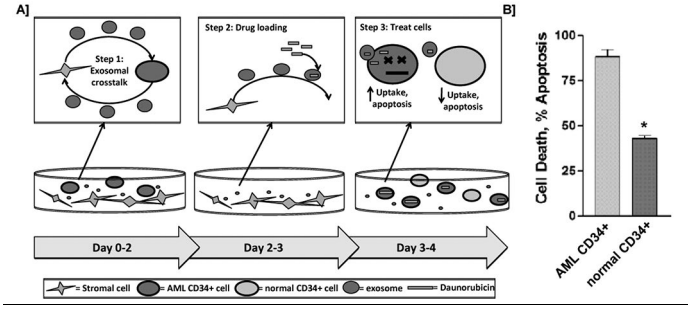

Figure 1. Trained loaded exosomes specifically target leukemic cells. A] Schematic diagram illustrating the experimental design for the generation of the trained loaded exosomes (TLE). B] Cell death analyses of the AML patient CD34+ cells compared to normal CD34+ cells upon exposure to TLE are graphen (mean +/- SEM, *p<0.05).

Summary and Conclusions: Taken together, our data predict important functional roles for exosome-derived Syntenin-1 in regulating lineage specific hematopoietic differentiations. Furthermore, for the first time, we have identified highly upregulated select exosome-derived miRs from AML patient samples whose prognostic value has been recently reported for other cancers, making these miRs promising candidates for AML biomarkers as well. Finally, stromal cell-derived, drug-loaded exosomes are not only able to induce apoptosis in AML patient samples, but they can effectively be trained by leukemic cells to favor uptake resulting in targeted elimination of leukemic over normal CD34+ cells.

S455

MICRORNA EXPRESSION BASED OUTCOME PREDICTION IN AML-CROSS-PLATFORM INTEGRATIVE ANALYSES LEAD THE WAY TO NOVEL INSIGHTS

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Background: Recent advances in omics technologies allowed for affordable generation of large scale molecular data including microRNA expression profiling. These data are expected to serve as an invaluable source for molecular based classification and prognostication of genetically heterogeneous diseases such as acute myeloid leukemia (AML). Besides, the integration of various layers of omics data may serve for the generation of novel testable hypotheses regarding disease pathogenesis.

Aims: Here, we aimed to develop a novel microRNA expression based prognostic score using data from two different platforms-microarrays and RNA-Seq data from adult patients with de novo AML and to investigate the underlying biological pathways.

Methods: We used microRNA microarray expression data from 91 adult patients with de novo AML enrolled into the AMLSG treatment protocol AML HD98A (ClinicalTrials.gov Identifier: NCT00146120) (training dataset). Another group of 177 AML patients with microRNA expression profiling data (RNA-Seq) from the Cancer Genome Atlas (TCGA) served as a validation set. A scoring system was built using the Robust Likelihood-Based Survival Modeling with Microarray Data method. The selected model included 7 microRNAs (miR-100, miR-132, miR-185, miR-186, miR-302a, miR-330, and miR-422a). A total continuous score was calculated for each patient sample using the Cox regression coefficients obtained for the training dataset multiplied by the expression levels in each sample and summed afterwards. Discrete scores (low *vs* high) were defined using Receiver Operating Characteristics (ROC) curve analysis. In addition, bioinformatic analysis of the GEP, RNA-Seq and DNA methylation data from the TCGA was performed.

Results: For both the training and validation dataset the discrete scores were significant prognostic factors in univariate analysis (p=0.001 and p=0.002, respectively). The continuous score, which performed almost identically, was also a significant factor in multivariate analysis (p<0.001 and p=0.022, respectively). In an analysis restricted to cytogenetically normal (CN) AML the discrete score was a significant adverse prognostic factor in the training dataset based on the log-rank test (p=0.045), and it appeared as independent prognostic factor in the validation subset of younger CN-AML patients in a multivariate model including age, gender, *FLT3*-ITD and *NPM1* mutational status (p=0.001). Next, we performed a network analysis of the 7 microRNAs and their known and putative targets and found an enrichment with nucleic acids binding proteins. Using the TCGA GEP data we identified 850 probe sets that were differentially expressed between Low and High Score patients at the level of p<0.01. GO analysis showed that "General transcription regulation" was the most significantly overrepresented pathway. Similarly, when Gene Set Enrichment Analysis (GSEA) was used for the subset of CN-AML <61 years the 7 top scoring gene signatures were associated with RNA metabolism and processing. The top scoring gene set was the "RNA SPLICING" signature. The differential exon usage (DEU) analysis between Low and High Score patients showed a total of 7500 differentially expressed tags at a level of significance of 0.05. Finally, we obtained the TCGA DNA methylation data and found a total of 1218 CpG sites differentially methylated between the Low and High Score patients and hierarchical clustering showed a very good correlation with the miRNA score.

Summary and Conclusions: We demonstrated the feasibility to integrate microRNA expression data from different platforms (microarray and RNA-Seq data) for building prognostic scores in AML. Moreover, the integrated analysis of omics data from microRNA expression score-defined subgroups provided further evidence for the potential biological relevance of these subgroups and a role of the RNA splicing machinery deregulation in the pathogenesis of AML.

Oncogenic mechanisms and novel targets in non-Hodgkin's lymphoma

S456

THE LANDSCAPE OF SOMATIC ALTERATIONS IN ADULT T-CELL LEUKEMIA/LYMPHOMA

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Background: Adult T-cell leukemia/lymphoma (ATL) is one of the most aggressive peripheral T-cell malignancies, which is etiologically associated with human T-cell leukemia virus type 1 (HTLV-1) infection. Although HTLV-1 can effectively immortalize infected T cells, there is a long latency period of 30~50 years before the onset of ATL, suggesting that HTLV-1 infection alone may be insufficient for the development of ATL, but additional genetic lesions that accumulate during the later life are essential. However, such somatic alterations underlying the pathogenesis of ATL remain elusive.
Aims: The aim of this study is to elucidate the landscape of genetic alterations in ATL.
Methods: We performed an integrated molecular study, in which whole-genome/exome and transcriptome sequencing were performed together with array-based methylation and genomic copy number analysis among a cohort of 81 paired ATL samples, followed by extensive validation using targeted capture sequencing of detected mutations in 370 follow-up samples.
Results: Compared with other hematologic malignancies, ATL cells harbored higher numbers of sequence mutations, copy number alterations, and structural variants, suggesting the presence of global genomic instability in ATL. In addition to previously reported mutational targets in ATL (*TP53, FAS,* and *CCR4)* and known targets frequently mutated in other lymphoid malignancies (*CARD11*, *GATA3*, *IRF4*, *POT1, CD58, STAT3* and *RHOA*), we identified a variety of recurrent mutations affecting previously unknown mutational targets, many of which are involved in development, activation and migration of T-cells, immune surveillance, transcriptional regulation, and histone modification. Molecular and functional analysis using human cell lines showed that some of these novel mutations actually enhance T-cell function, validating their biological significance in ATL. A comparison of mutations among disease subtypes revealed several subtype-specific mutations, including *TP53* and *IRF4* mutations in acute and lymphoma types, and *STAT3* mutation in chronic and smoldering types, suggesting that there is a difference in the underlying oncogenic mechanisms between indolent and aggressive forms. Moreover, several mutations, including those in *TP53*, *IRF4* and *CCR4*, independently predicted worse clinical outcomes. ATL cells showed a distinct pattern of copy number changes and genomic rearrangements. Interestingly, their gene targets showed a significant overlap to mutational targets. Intriguingly, somatic focal deletions involving the 14q31.1 locus were observed in all the cases examined by whole-genome sequencing and therefore are thought to characterize ATL genomes, although their gene targets remained to be identified. Conspicuously, pathway analysis demonstrated that multiple genes involved in the Tax interactome were systematically altered in ATL, although Tax itself underwent gene silencing in most cases. These data suggested that ATL cells can escape from immune surveillance by silencing immunogenic Tax expression, while developing alternative oncogenic mechanisms through acquiring somatic mutations or copy number alterations in the Tax-related pathway.
Summary and Conclusions: Our findings suggest that the deregulation of T-cell functionalities caused by genetic alterations, especially those related with HTLV-1 Tax oncoprotein, are pivotal to ATL pathogenesis, and provide a novel clue to contrive new diagnostics and therapeutics to combat this intractable disease.

S457

NPM-ALK MIMICS THYMIC PRE-T CELL RECEPTOR (TCR) EXPANSION BUT REQUIRES TRANSIENT TCR EXPRESSION FOR THYMIC EGRESS AND PERIPHERAL ANAPLASTIC LARGE CELL LYMPHOMA DEVELOPMENT

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Background: Anaplastic Large Cell Lymphoma (ALCL) is a peripheral T cell lymphoma of children and adults which is characterized by aberrant ALK expression and signaling, usually driven by the *NPM* promoter resulting from the t(2;5)(p23;q35). Although ALK+ ALCL cells commonly express T cell activation (CD30) and cytotoxic (Granzyme, perforin, TIA1) markers and have clonal TCR gene rearrangements, the TCR/CD3 signaling complex is strikingly absent. It is also not clear at what stage of T lymphoid development lymphomagenesis initiates, but since NPM expression is ubiquitous it is possible that this may occur before thymic egress.
Aims: We undertook a combined human and murine approach to evaluate the place of TCR rearrangement in ALCL development and the timing of NPM-ALK driven lymphoma onset.
Methods: Human ALCL were analyzed by multiplex TCR PCR from DNA (d, g and b loci) or cDNA (a locus), with Sanger sequencing and TCRa/d Agilent CGHa analysis for cases with available material. We used the murine model of T cell lymphomagenesis in which NPM-ALK is expressed from the CD4 promoter and following back-crosses to the RAG deficient and/or OTI TCR transgenic lines assessed tumor development and phenotype.
Results: Of 57 human, ALK+ ALCL (48 pediatric, 9 adult) with at least 40% tumor involvement, cases with predominantly in-frame TCRa and TCRb rearrangements were the most common category (56%), followed by 19% with predominantly in-frame TCRg and TCRd rearrangements and 14% with no clonal TCR rearrangements. More surprisingly, 6 cases (11%) demonstrated clonal out-of-frame TCRg but in-frame TCRa rearrangements in the absence of apparent clonal TCRB rearrangement, suggesting that NPM-ALK may in some way replace TCRb mediated signaling. In keeping with this, CD4 driven NPM-ALK rag competent transgenic mice (CD4NA/rag2$^{+/+}$) demonstrated a block in thymic T lymphoid development at the Double Negative (DN)3 stage, which was not observed in the CD4NArag2$^{-/-}$ mice, which developed cortical thymic tumors composed of CD4/8 DP and CD4 SP cells, but no peripheral tumors. Crossing these mice with the ovalbumin (ova) TCR transgene allowed thymic egress (in the absence of ova) and the development of peripheral ALCL, pathologically similar to those observed in humans. Intriguingly, this only occurred in a RAG competent context and in the absence of ova and was associated with loss of the TCR transgene, since CD4NA/OTImice exposed to ova and CD4NA/rag2$^{-/-}$ mice only developed non-hematopoietic hepatic or gastro-intestinal sarcomas, although TCR transgene expressing T cells were seen in the peripheryin the former.
Summary and Conclusions: These data demonstrate that NPM-ALK (and ALK signaling) allows replacement of the thymic proliferative process of TCR beta-selection, mediated by the pre-TCR, and development of thymic tumors, but that TCR expression is required for thymic egress and development of peripheral lymphoma resembling human ALCL. However, the latter is dependenton loss of TCR signaling, suggesting that simultaneous NPM-ALK and TCR activities are not permissive of ALCL development and/or survival. In this context, TCR signaling (but not unstimulated TCR expression) appears to act as a tumor suppressor. These data also represent the first murine model resembling human ALCL and provide proof of concept of an explanation of the pathogenic role for the long recognized "missing TCR" in peripheral T cell lymphomas, which may have implications beyond ALCL.

S458

IDENTIFICATION OF DIFFERENTIALLY METHYLATED DISTANT ENHANCERS RELATED TO ABERRANT EXPRESSION OF SOX11 IN MANTLE CELL LYMPHOMA

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Background: SOX11 is an SRY-related HMG-box transcription factor (TF) family member aberrantly expressed in the majority of mantle cell lymphoma (MCL) cases. The transcriptional program regulated by SOX11 and its potential oncogenic mechanisms in MCL pathogenesis have recently started to be elucidated. However, the mechanisms underlying its aberrant expression in MCL remain unclear. No mutations, genetic aberrations or direct correlations with differential DNA methylation at the promoter region related to its expression have been found in MCL. As it is becoming increasingly clear that remote enhancers may regulate gene expression, we hypothesized that distant regulatory regions might be involved in regulating aberrant SOX11 expression in MCL.
Aims: In this study, we aimed to identify and characterize distal regulatory elements associated with aberrant SOX11 expression in MCL.
Methods: We analyzed the 3-dimensional structure of the *SOX11* genomic region and its chromatin architecture by combining 4C- and ChIP-sequencing in Z-138 and JVM-2, a SOX11-positive and a SOX11-negative MCL cell line, respectively. Additionally, we analyzed the DNA methylation status of distant enhancers that potentially regulate SOX11 expression by bisulfite pyrosequencing in normal naive B cells, SOX11-positive and SOX11-negative primary MCL cases. Finally, we performed TF motif enrichment analysis to identify TFs that potentially regulate aberrant SOX11 expression in MCL.
Results: By 4C-sequencing in the SOX11-positive cell line Z-138, we identified two genomic regions that show high contact frequencies with the *SOX11* locus in 3-dimensional space. These regions were located between 500KB and 1MB from the *SOX11* locus. In Z-138, these regions were enriched for histone marks H3K4me1 and H3K27ac, indicative of enhancer activity. In the SOX11-negative MCL cell line JVM-2, neither high contact frequencies with the *SOX11* locus, nor enrichment of H3K4me1 and H3K27ac were observed at these loci. Next, we investigated whether enhancer activity and DNA methylation levels are inversely associated at the identified distant regulatory regions, as described for other loci. We observed that DNA methylation levels in these regions were significantly lower in SOX11-positive (n=12, average methylation levels 13-21%) than in SOX11-negative primary MCL cases (n=10, average methylation levels 51-85%) (*P-value* <0.01). Furthermore, in normal naive B cells these regions were highly methylated (n=4, average methylation levels 76-92%). These data support our 4C-seq findings in MCL cell lines and suggest that these regions harbour active enhancers only in SOX11-positive primary MCL cases. Next, we investigated the binding of 161 TFs (as identified by the ENCODE project) at the *SOX11* locus and its potential distant enhancers. In total we identified binding sites of 13 TFs bound both to the *SOX11* locus and to one or both potential distant enhancers. None of these were significantly higher expressed in SOX11-positive compared to SOX11-negative primary MCL cases. Surprisingly, one of the distant enhancer regions is bound by many TFs involved in B-cell development. However, it remains to be elucidated how these B-cell related TFs play a role in SOX11 expression in MCL, as in normal B cells SOX11 is not expressed.
Summary and Conclusions: Altogether, our data suggest a model in which SOX11 expression in MCL is associated with looping of the *SOX11* locus to two newly-identified distant enhancer regions. Further experiments, however, are necessary to elucidate the mechanisms underlying their aberrant activation in SOX11-positive MCL cases.

S459

BCL6 AND EZH2 COOPERATE TO ASSEMBLE NON-CANONICAL PRC1-BCOR COMPLEX TO MEDIATE GERMINAL CENTER FORMATION AND PROMOTE LYMPHOMAGENESIS BY REPRESSING BIVALENT CHROMATIN DOMAINS

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Background: The majority of B cell lymphomas arise from germinal center (GC) B cells, which form part of the humoral immune response to T cell-dependent antigen stimulation. Understanding how B cells impose and maintain the GC phenotype may thus provide important clues to explain the pathogenesis of GC B cell-derived lymphomas and inform the design of rational therapeutic strategies for patients with diffuse large B cell lymphomas (DLBCLs) and follicular lymphomas (FLs). Upon activation, GC B cells upregulate EZH2, a histone methyltransferase that forms part of Polycomb Repressive Complex 2 (PRC2). EZH2 is targeted by gain-of-function somatic mutations that enhance its ability to trimethylate histone 3 lysine 27 in DLBCLs and FLs. We previously found that EZH2 mediates the GC phenotype through *de novo* formation of bivalently marked chromatin domains (characterized by overlapping H3K27me3 repressive mark with the H3K4me3 activation mark) at the promoters of target genes involved in cell cycle regulation and in GC exit, to transiently suppress GC B cell differentiation. Mutant EZH2 reinforced these effects through enhanced silencing of these bivalent genes. EZH2 mutation induced GC hyperplasia in mice, and DLBCL patients with mutant EZH2 displayed more profound repression of the GC bivalent genes, suggesting that mutant EZH2 contributes to human lymphomagenesis through repression of bivalent chromatin domains.
This scenario is reminiscent of the role of the transcriptional repressor BCL6, which is also required for GC formation and survival of DLBCL cells. Notably BCL6 represses its targets by associating with BCoR, which forms a variant of Polycomb Repressive Complex 1 (PRC1).
Aims: We hypothesized that EZH2, BCL6, and PRC1-BCoR cooperate to mediate the GC B cell phenotype and when aberrantly active may cooperate to form GC-derived B cell lymphomas.
Methods and Results: Using transgenic animal models we found that EZH2, BCL6, and BCoR are mutually dependent to induce formation of GCs. ChIP-seq studies revealed that EZH2 bivalent promoter genes significantly overlap with BCL6 targets only when is associated with BCoR in primary human GC B cells and lymphoma cells (hypergeometric test, p=2.9e-20). Treatment of DLBCL cells with EZH2 or BCL6-BCoR inhibitors or siRNA partially derepressed these genes, indicating that these factors cooperate and are required to mediate full repression of these crucial bivalent loci. Accordingly, ChIP assays in DLBCL cells treated with EZH2 inhibitor showed impaired binding of PRC2 complex proteins (EZH2, EED and SUZ12) and PRC1-BCoR complex proteins (BCoR, RNF2 and KDM2B) to bivalent promoters. This was accompanied with corresponding loss of H3K27me3 and H2AK119ub1, the histone repressive marks catalyzed by PRC2 and PRC1, respectively. Similar results were obtained when cells were exposed to a small molecule that disrupts BCL6 recruitment of BCoR.
Conditional expression of mutant EZH2 in mice accelerated lymphomagenesis driven by BCL6. Accordingly, the combination of EZH2 inhibitor with BCL6-BCoR inhibitor, suppressed DLBCLs proliferation and tumor growth in xenografted mice more effectively than either agent alone. The combination also yielded further killing of primary human DLBCL cells growth.
Summary and Conclusions: We identified the first epigenetic mechanism of lymphomagenesis involving aberrant repression of GC-specific bivalent domains by PRC2 and BCL6 in cooperation with PRC1-BCoR complex, as well as a rational epigenetic-based and molecular targeted therapeutic approach with the potential to eradicate lymphomas without harming normal tissues.

S460

A HYPOPHOSPHORYLATED AUTOANTIGEN IS THE FIRST MOLECULARLY DEFINED RISK FACTOR AND A DOMINANT ANTIGENIC TARGET/STIMULUS OF THE B-CELL RECEPTOR FROM ABC-TYPE DLBCL

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Background: Chronic antigenic stimulation may has been hypothesized to play an important role in the pathogenesis of malignant lymphomas. We have previously shown that a hyperglycosylated version of neurabin/SAMD14, a protein strongly expressed in the CNS, is the antigenic target of the B-cell receptor (BCR) of 2/3 of all primary CNS lymphomas, but antigenic targets of peripheral DLBCL-BCRs have not been defined to date.
Aims: To identify the antigenic targets/stimuli of BCR derived from peripheral DLBCL.
Methods: BCRs were expressed as recombinant Fabs based on corresponding pairs of functional variable region heavy and light chain genes, which had been amplified from isolated genomic DNA of snap-frozen lymphoma specimens and DLBCL-derived cell lines. The purified BCR-Fabs were checked for binding to proteins expressed on macroarrays of human cDNA expression libraries.
Results: The BCR from 10 DLBCL cell lines (5 of the germinal center type and 5 of the activated B-cell type) were tested on the protein macroarray. None of the GC-type BCR reacted with any of the proteins expressed on the protein macroarrays, but the BCR from 3/5 (60%) of the ABC-derived cell lines reacted with ARS2 (arsenite resistance protein 2), a conserved mammalian protein which is important for microRNA biogenesis. Isoelectric focusing and phosphatase treatment of ARS2 derived from ABC cell lines with a BCR specific for ARS2 revealed that ARS2 was hypophosphorylated (hypo-ARS2) in the respective cell lines. Analysis of peripheral blood lymphocytes from patients with DLBCL of unknown cell of origin and healthy controls revealed that 5/100 patients (5%), but only 1/200 controls (0.5%) were carriers of hypo-ARS2, resulting in a 10x increased risk for healthy carriers of hypo-

ARS2 to develop DLBCL. All patients with BCRs targeting ARS2 had polyclonal antibodies against ARS2 in their serum.
Summary and Conclusions: Hypo-ARS2 is the first molecular defined risk factor for DLBCL identified to date. The increased risk for healthy carriers of this posttranslational modification to develop DLBCL supports the hypothesis of chronic antigenic stimulation as an important factor in the pathogenesis of DLBCL of the ABC subtype and indicates that posttranslationally modified autoantigens are a frequent target and stimulus for ABC-DLBCL-BCR. That antibodies against ARS2 are found in the respective patients suggests that the hypo-ASR reactive DLBCL evolves from a polyclonal B-cell response against this autoantigen. Investigations into the mechanism underlying the hypophosphorylation of ARS2 are underway and therapeutic options will be discussed. *Supported by Wilhelm-Sander-Stiftung.*

Novel actors in chronic myeloid leukemia biology

S461

SECOND GENERATION TYROSINE KINASE INHIBITORS SUPPRESS THE CIP2A/C-MYC/E2F1 PATHWAY

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Background: Impairment of PP2A activity by its negative regulators SET and cancerous inhibitor of PP2A (CIP2A) plays an important role in the pathogenesis and progression of chronic myeloid leukaemia (CML). CIP2A is associated with increased proliferation in several human malignancies and its over-expression can cause cellular transformation. High levels are an adverse prognostic indicator in many malignancies. In CML a high CIP2A protein level at diagnosis in imatinib treated patients may be a biomarker for subsequent blast crisis. However, its role in CML patients receiving second generation tyrosine kinase inhibitors (2G TKIs) is not known.
Aims: Our aim was to investigate if the superior clinical outcome seen in patients treated with 2G TKIs could be explained by modulation of the CIP2A pathway.
Methods: CIP2A, PP2A, PP2A^{Y307}, c-Myc and E2F1 proteins were assessed by FACS. CIP2A, c-Myc and E2F1 were depleted in K562 and CD34+ cells using siRNA.
Results: In mononuclear cells taken at diagnosis from 20 chronic phase CML patients, those with high CIP2A levels significantly decreased following dasatinib or nilotinib treatment (p=0.007 and p=0.001 respectively) and this was accompanied by a significant decrease in c-Myc, (p=0.002). Y307 phosphorylated (inactive) PP2A also decreased in dasatinib (p=0.03) and nilotinib (p=0.04) treated cells, thus increasing PP2A activity, but imatinib had no significant effect. These differences in TKI effects are not mediated by differential suppression of BCR-ABL activity (as assessed using the pCrKL assay). E2F1 transcriptionally regulates both c-Myc and CIP2A and has previously been shown to be over-expressed in CML. E2F1 is high in patients with high diagnostic CIP2A protein levels compared to those with low CIP2A protein levels (p=0.04). Following 1 month of clinical 2G TKI treatment, E2F1 protein levels significantly decreased (p=0.01) and this was accompanied by a decrease in mRNA levels of its transcriptional target *CIP2A*. In sharp contrast, imatinib did not suppress E2F1, and thus *CIP2A* mRNA levels remain unchanged. Our data demonstrate that E2F1 is stabilised in CML as a result of PP2A inactivation. CIP2A acts to inhibit PP2A leading to stabilisation of E2F1, creating a positive feedback loop generating constant transcription and stabilisation of both CIP2A and c-Myc (Figure 1). The positive feedback loop was interrogated by sequentially depleting CIP2A, c-Myc and E2F1 in K562 and CD34+ CML cells. Depleting CIP2A in CD34+ CML cells reactivates PP2A, resulting in the dephosphorylation of E2F1, and thus a decrease in E2F1 and c-Myc. This may occur by two mechanisms; firstly the decrease in CIP2A level means that CIP2A can no longer stabilise c-Myc, and secondly the reduction in E2F1 removes its drive to transcribe c-Myc. In CD34+ cells, c-Myc knockdown resulted in a decrease in CIP2A (p=0.04) and E2F1 (p=0.04). This is consistent with our model, whereby decreased CIP2A will reactivate PP2A, leading to the dephosphorylation of E2F1 and therefore a decrease in E2F1. Finally E2F1 was depleted in CD34+ cells. This resulted in a decrease in CIP2A (p=0.002) and c-Myc.

A suggested model for the CIP2A/c-Myc/E2F1 pathway. Arrows denote activation, blunt ended arrows indicated inhibition. Phosphorylated E2F1 can transcribe both CIP2A and c-Myc, which stabilise each other and cause PP2A inhibition. Inactive PP2A cannot dephosphorylate E2F1 and thus E2F1 remains active, creating a positive feedback loop.

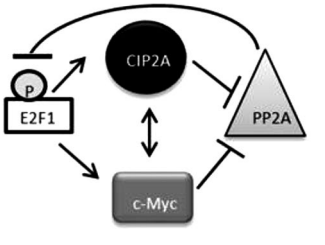

Figure 1. CIP2A/c-Myc/E2F1 pathway.

Summary and Conclusions: In summary, we have shown that a positive feedback loop occurs between CIP2A, E2F1 and c-Myc which inhibits PP2A activity and that only the 2G TKIs and not imatinib are able to supress it. Our study highlights a role for E2F1 in the CIP2A pathway, although it cannot be assumed that E2F1 is the only transcription factor regulating CIP2A and others may influence this dynamic process. Thus, CIP2A remains an attractive therapeutic target since high levels are only found in malignant cells.

S462

MYC-MAX HETERODYMER POSITIVELY REGULATES BCR AND BCR/ABL EXPRESSION

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Background: Chronic Myeloid Leukemia(CML) is caused by the BCR/ABL fusion gene. Both the presence and the levels of BCR/ABL expression seems critical for CML progression from Chronic phase(CP) to Blast crisis(BC). At the molecular level BC is a heterogeneous disease. Regardless of the additionally secondary changes, one common feature during the evolution from CP to BC is a marked increase in BCR/ABL expression. After the oncogenic translocation, the BCR/ABL gene is under the transcriptional control of BCR promoter but the molecular mechanisms involved in the regulation of oncogene expression are mostly unknown.
Aims: The aim of this study is the identification of molecular mechanisms responsible for BCR promoter regulation and BCR/ABL expression.
Methods: To identify the transcription factors directly acting on BCR promoter, we studied in-silico a region of 1443bp upstream of the human BCR gene coding sequence(Gene ID:613). Chromatin Immunoprecipitation was used to confirm in-silico data. The role of transcription factors on BCR and BCR/ABL expression have been determined through transfection of the K562 cell line with selected expression vectors and through specific silencing in K562, LAMA-84 and KCL-22 cells. mRNA and protein expression levels of BCR and BCR-ABL were detected by quantitative PCR(RT-qPCR) and western blot. BCR reporter activity was also analyzed by reporter luciferase assay in the 293T cell line. The role of the identified transcription factors in CML progression was investigated with in-vitro experiments.
Results: These data demonstrate that MYC-MAX heterocomplex binds to the BCR promoter at four different binding sites(PBS1-4), leading to up-regulation of BCR and BCR/ABL at both transcriptional and protein levels in the K562 cells. We found that both MYC and MYC-MAX overexpression significantly upregulate BCR/ABL compared to control cells, as assessed by RT-qPCR (MYC_BCR/ABL fold induction: 2.27±0.48,p=0.01; MYC-MAX_BCR/ABL fold induction: 2.55±0.34,p=0.002) and western blot. Accordingly, silencing of MYC(shMYC) expression in various BCR/ABL+ cell lines causes significant down-regulation of BCR and BCR/ABL compared to the negative controls(BCR fold change: KCL-22shMYC 0.49±0.07, p=0.013; LAMA-84shMYC 0.07±0.03, p=0.0003. BCR/ABL fold change: KCL-22shMYC 0.58±0.02, p=0.0007; LAMA-84shMYC 0.38±0.006, p<0.0001). In addition, MYC silencing in these cell lines leads to decreased proliferation and induction of cell death as assessed by cell cycle analysis and PARP-1 cleavage. In order to assess the effect of MYC-MAX on BCR promoter, we tested the activity of the luciferase reporter assay in presence of MYC expression and in a lentiviral MYC silencing model, showing that MYC silencing significantly decreases BCR promoter activity in all of the constructs analysed(p<0.0001). Notably, deletion of PBS1 and PBS2 and/or deletion of PBS3 and PBS4, dramatically decreases the promoter strength, therefore confirming the critical role of these region in controlling BCR promoter activity. Interestingly, the deletion of PBS3 and PBS4 induces a greater down-modulation of luciferase activity compared to PBS1 and PBS2 deletion (BCR-promoter: 6.11±0.11; PBS3-4deleted: 1.86±0.14; PBS1-2deleted: 3.92±0.07).
Summary and Conclusions: This is the first description of a new pathway which places BCR and BCR/ABL under the transcriptional control of the MYC-MAX heterodimer. Since MYC is frequently over-expressed in BC, this phenomenon could play a critical role in BCR/ABL up-regulation and blast aggressiveness acquired during CML evolution.

S463

CORRECTION OF MUTATIONS OF THE EPIGENETIC MODIFIER ASXL1 IN MYELOID LEUKEMIA CELLS BY CRISPR/CAS9 GENOME EDITING

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Background: The CRISPR/Cas9 genome editing system has recently emerged as a powerful tool for genome engineering, and has been used for gene correction in cultured cells from patients with monogenic hereditary defects. We reasoned that this technology could be employed to correct acquired gene mutations in human cancer cells. Recurrent somatic mutations of the epigenetic modifier *ASXL1* are common in myeloid malignancies, including myelodysplastic syndromes and chronic myeloid leukemia (CML). Loss-of-function *ASXL1* mutations are strongly associated with a poor prognosis in these disorders. We studied the CML line KBM5 which lacks wild-type ASXL1 protein expression, due to a homozygous nonsense mutation (G710X) in the *ASXL1* gene.
Aims: We used CRISPR/Cas9-mediated homology directed repair (HDR) to correct the *ASXL1* mutation in KBM5 cells and performed functional studies to determine whether the wild-type function of ASXL1 was restored.
Methods: We used three synthetic guide RNAs (sgRNAs) targeting the genomic region overlapping the *ASXL1* mutation and a single-stranded oligonucleotide as a repair template. We sequenced 1,027 colonies expanded from single FACS-sorted KBM5 cells to determine successful HDR-mediated correction of *ASXL1* mutation. Each sgRNA generated heterozygous precise correction (yield 0.46-2%). Importantly, homozygous precise correction was observed for two sgRNAs (yield 1.13-1.63%). The frequency of correction obtained is consistent with previous studies using the CRISPR/Cas9 system for precise genome editing through HDR.
Results: Western blot analysis showed restoration of full-length ASXL1 protein expression in heterozygous and homozygous corrected KBM5 clones. ASXL1 plays an important role in Polycomb Repressive Complex 2 (PRC2) recruitment to specific target loci, including the HOXA cluster. Loss of ASXL1 is associated with increased expression of *HOXA* genes. We observed significant downregulation of *HOXA5, 6, 7, 9, 10* and *13* in *ASXL1* mutation-corrected KBM5 clones. The PRC2 plays a critical role in the deposition of H3K27me3 histone repressive marks, and *ASXL1* mutations are associated with H3K27me3 loss in myeloid cells. Western blots on purified histones showed a marked increase in global H3K27me3 levels in *ASXL1* mutation-corrected KBM5 clones. ASXL1 forms a protein complex *in vitro* with the chromatin deubiquitinase BAP1. This interaction is reduced in cell lines carrying *ASXL1* mutations. Immunoprecipitation studies showed that the interaction between ASXL1 and BAP1 was restored in *ASXL1* mutation-corrected KBM5 clones. Moreover, we observed growth suppression and a significant increase in the expression of the myeloid marker CD15 in *ASXL1* mutation-corrected cells. No sequence alterations were detected in any of the off-target sites examined in all *ASXL1* mutation-corrected clones identified, suggesting high specificity of CRISPR/Cas9 system in our experiments. Preliminary *in vivo* data show that mice xenografted with *ASXL1* mutation-corrected KBM5 clones have a longer survival compared to mice xenografted with uncorrected KBM5 cells (p=0.046) (Figure 1). A larger cohort is in progress.

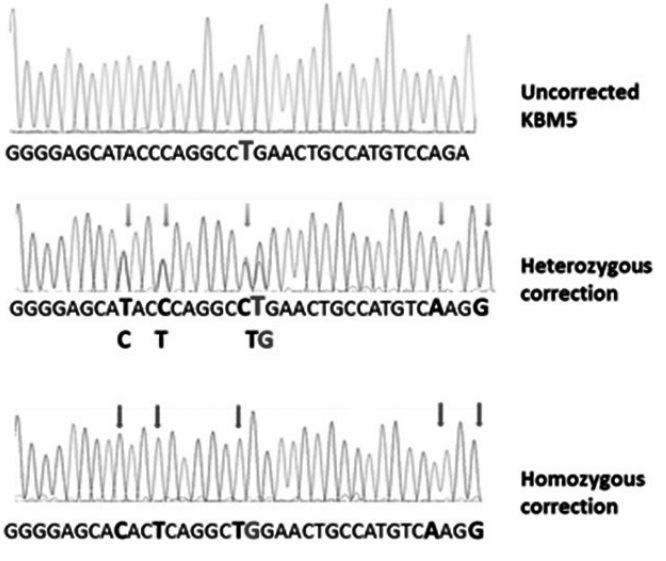

Figure 1. Evaluation of *ASX1* mutation correction in KBM5 cells using Sanger sequencing. The arrows in the middle and bottom panel indicate silent nucleotides changes that were introduced in the single-stranded oligonucleotide (repair template) sequence to avoid undesired Cas9 activity in mutation-corrected cells.

Summary and Conclusions: In summary, we have corrected a specific *ASXL1* point mutation in a leukemia cell line using CRISPR/Cas9 technology, resulting in restored protein function. This study presents a new strategy to illuminate the impact of oncogenic mutations on cellular function and may lay the foundations for a new approach to leukemia therapeutics (*e.g.* modified autoSCT). We provide proof-of-concept for gene correction via CRISPR/Cas9 technology in leukemic cells from patients with a myeloid malignancy.

S464

MUTATION ANALYSIS OF EPIGENETIC MODIFIERS IN CHRONIC PHASE CML USING NEXT GENERATION SEQUENCING

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Background: Chronic myeloid leukaemia (CML) originates from a single genetic aberration (BCR-ABL1); however the clinical disease is remarkably heteroge-

neous and the genetic mechanisms of resistance to tyrosine kinase inhibitors (TKI) are still poorly understood. Recently, we have identified consistent differences in genome-wide DNA methylation patterns in patients with chronic phase CML (CML-CP) compared to healthy controls, indicating an involvement of epigenetic mechanism in CML pathogenesis. Several epigenetic modifying enzymes have been found frequently mutated in other haematopoietic neoplasms.
Aims: The aim of this study is to analyse a panel of mutations in epigenetic modifiers in chronic phase CML using Ion Torrent next-generation sequencing. The panel design was based on a combination of gene expression analysis we generated and literature search for frequently mutated enzymes in leukaemia. Potential mutations involved in epigenetic deregulation of CML may be used as novel prognostic biomarkers for TKI response.
Methods: 53 samples from untreated patients with newly diagnosed CML-CP ($CD34^+$) who started treatment with imatinib were included in the study. Patients were classified as responders (n=26) /non-responders (n=27) based on 10% BCR-ABL1/ABL ratio at 3 months. 6 samples from GCSF mobilised healthy donors ($CD34^+$) were used as negative controls, to exclude false positive variants and 2 samples from CML-BC (blast crisis) as positive controls. A custom panel of 71 epigenetic enzymes was designed, containing 2002 amplicons. We used the Ion AmpliSeq Library, the Ion PGM Template OT2 200 and the Ion PGM Sequencing 200 kits, running 8 samples on a 318 Chip. Data were analysed using Ion Reporter (Life Technologies), while the web-based application Mutation Taster was used for the evaluation of the disease-causing potential of a variant.
Results: A mean of 636,170 mapped reads per sample with mean depth of 273 and mean coverage (at x20) of 95.9% was obtained, detecting mutations with high sensitivity. The filtering steps included exclusion of UCSC common SNPs, use of healthy controls for excluding false positive variants, exclusion of intronic variants, exclusion of variants called as polymorphisms by Mutation Taster, quality control including all variants with frequency of the mutated allele>20% and some variants with lower frequency based on QC, and exclusion of synonymous variants. 59 disease causing variants were identified in 34 genes including missense, nonsense and frameshift insertions. No difference of overall number of variants between responders/non-responders, however nonsense variants in ASXL1, IKZF1 and EP300, similar to ones found in CML-BC samples, were found only in non-responders. No variant was present in frequency more than 2/53 samples and were mutually exclusive between R-NR except for a missense variant in HDAC2, RUNX1 and a TET2 found in 5, 4, 3 samples respectively. At least one variant was present in 85% of NR and in 67% of R. The findings were validated using Sanger sequencing. For some genes, there was correlation of gain or loss of function mutation with differences in gene expression.
Summary and Conclusions: We demonstrate the feasibility of using the Ion Torrent PGM platform for mutation analysis of epigenetic modifiers in CML. The findings may be the first report for the presence of these mutations in CML-CP, which will allow a better understanding of the abnormal epigenetic changes in CML. Some mutations found in high frequency can be considered characteristic for CML whereas some others (found only in responders or non-responders) can be considered as novel prognostic biomarkers for IM response.

S465

RUNX1 MUTATION AND/OR DISRUPTION OF THE C-TERMINAL DOMAIN MAY BE CRITICAL FOR CHRONIC MYELOID LEUKAEMIA (CML) BLAST CRISIS TRANSFORMATION

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Background: Runt-related transcription factor 1 (Runx1)/ AML1 plays a crucial role in haematopoiesis and its dysfunction may contribute to leukaemogenesis. RUNX1 is frequently involved in chromosomal translocations including t(8;21), t(3;21), and t(16;21). Normal RUNX1 function is repressed by all truncated variants of RUNX1 that retain the entire Runt domain and lack the C-terminal domain. RUNX1 with various point mutations also has a dominant-negative effect on wild type and exhibits decreased transactivation activity; these have been found in AML, myelodysplastic syndromes and accelerated phase/blast crisis (AP/BC) of CML.
Aims: To explore the role of RUNX1 lesions in disease evolution of CML, we investigated RUNX1 splice variants and point mutations in CML chronic phase (CP) and BC.
Methods: The cases studied included 54 cases of Philadelphia chromosome positive (Ph^+) acute leukaemia (22 cases of myeloid, 21 lymphoid, 3 bi-phenotypic and 8 unknown lineage of leukaemia). Of these 54 cases, 35 evolved from previous CP CML and 19 were *de novo* Ph^+ acute leukaemia. Twenty cases of newly diagnosed CP CML were also studied. RNA was extracted and cDNA synthesised from whole blood white cells. First round PCR primers were designed to amplify the full length cDNA of the longest RUNX1 isoform 1c. Then two overlapping fragments were amplified by nested PCR primers. For several cases, it was found that there were PCR products of different size in addition to the PCR product of wild type RUNX1 1c. All PCR products of various sizes for each sample were gel-purified and subject to Sanger sequencing.
Results: A total of 18 types of transcript variant were found. Of these, 2 types (39 bp spliced out from exon3 and 99 bp spliced out from the end of exon10) were universally present and were excluded from this analysis. The 16 other types each had either all or the majority of the RUNT domain spliced out (named as Group 1 variants, 8 types) or had apparent aberrant C-terminal alteration (named as Group2 variants, 8 types). Group 2 variants were detected predominantly in Ph+ acute leukaemia cases, *i.e.* 8 of 54 Ph+ acute leukaemia cases. In contrast, Group 2 variants were only found in 1 of 20 CP cases and this one case was the only case that could not be controlled by imatinib treatment. The presence of Group1 variants was not significantly different between Ph+ acute leukaemia cases and CP cases. There were 35 types of nonsynonymous mutation and 14 types of synonymous mutation detected. All nonsynonymous mutations have been aligned to various single nucleotide polymorphism databases to exclude known polymorphisms. Nonsynonymous RUNX1 mutations were detected in of 20 of 54 cases of Ph+ acute leukaemia (37%). In contrast only 2/20 (10%) cases of CP CML had a nonsynonymous RUNX1 point mutation ($p=0.025$). Combining RUNX1 splicing variant Group 2 and point mutation together, 23/54 (43%) Ph+ acute leukaemia cases had either a Group 2 splice variant or point mutations, which were only present in 3/20 (15%) CML CP ($p=0.03$). 5/8 cases of CML who had transformed had point mutations at the BC stage. In 4 of these 5 cases new point mutations were only detected at the BC stage; 1 case had mutations that were present in the earlier CP sample. 6/8 cases of CML who had transformed had variants detected, and 3 of these had Group 2 splice variants that were not present in CP.
Summary and Conclusions: RUNX1 splicing variants with disrupted C-terminal domain and RUNX1 point mutations are predominantly found in Ph+ acute leukaemia and at the transformation of CML, but are uncommon in chronic phase; these lesions may therefore be crucial transformation events, and candidate biomarkers of transformation.

Gene therapy, cellular immunotherapy and vaccination

S466

OUTCOMES OF GENE THERAPY FOR B-THALASSEMIA MAJOR AND SEVERE SICKLE DISEASE VIA TRANSPLANTATION OF AUTOLOGOUS HEMATOPOIETIC STEM CELLS TRANSDUCED *EX VIVO* WITH A LENTIVIRAL BETA GLOBIN VECTOR

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Background: In patients with hemoglobinopathies, hematopoietic stem cell (HSC) gene therapy has the potential to induce production of functional β-globin in the red blood cell lineage with the aim of reducing or eliminating the symptoms of disease. Results for 2 subjects with β^0/β^E-thalassemia major treated in clinical study HGB-205 that were previously presented (EHA 2014, ASH 2014) suggested that transplantation with autologous $CD34^+$ cells transduced with the self-inactivating LentiGlobin BB305 lentiviral vector containing an engineered $\beta^{A\text{-}T87Q}$-hemoglobin (Hb) and early transfusion independence.
Aims: Herein, we provide additional follow-up data on these two subjects as well as early safety and efficacy data on the first subject with sickle cell disease (SCD) treated with gene therapy.
Methods: After obtaining informed consent, subjects with β-thalassemia major undergo HSC collection via peripheral blood apheresis, while subjects with severe sickle cell disease undergo HSC collection via bone marrow harvest. $CD34^+$ cells were selected and transduced with LentiGlobin BB305 lentiviral vector. Estimation of the mean *ex- vivo* vector copy number (VCN) was obtained by quantitative PCR performed on pooled colony-forming progenitors. Subjects underwent myeloablation with intravenous busulfan, followed by infusion of transduced $CD34^+$ cells. Subjects were monitored for overall safety including hematological engraftment, $\beta^{A\text{-}T87Q}$-globin expression (by high performance liquid chromatography) and transfusion requirements. Integration site analysis (ISA, by linear amplification-mediated PCR and high-throughput sequencing on nucleated cells) and replication-competent lentivirus (RCL) assays were performed. Prophylactic pRBC transfusions are continued in sickle cell subjects who are transfusion dependent pre-treatment to maintain HbS <30%, followed by gradual tapering over time.

Table 1. Demographics and transplantation outcomes.

Subject	Age (years) /gender	Genotype	BB305 Drug Product VCN[a]	BB305 Drug Product $CD34^+$ cell dose ($x10^6$ per kg)	Day of Neutrophil Engraftment	Drug Product-related Adverse Events	Day of last pRBC transfusion	Last Study Visit	Hb amounts at last visit (g/dL)
Subjects with β-thalassemia major									**HbA^{T87Q}/ Total Hb**
1201	18 F	β^0/β^E	1.5	8.9	Day +13	None	Day +10	12M	7.7/11.0
1202	16 M	β^0/β^E	2.1	13.6	Day +15	None	Day +12	9M	9.4/13.2
Subject with severe sickle cell disease									**HbA^{T87Q}/ HbS/HbF/Total Hb**
1204	13 M	β^S/β^S	1.2 / 1.0	5.6	Day +37	None	Day +88	4.5M	2.9/4.0/0.9/12.0

As of February 2015
[a] VCN, vector copy number; F=female; M= Male for gender, and months for day of last follow-up
^these authors contributed equally

Results: As of February 2015, 2 subjects with β^0/β^E thalassemia major (Subjects 1201 and 1202) and 1 subject with β^S/β^S severe sickle cell disease (Subject 1204, who was on prophylactic transfusion therapy for multiple veno-occlusive crises and acute chest syndrome) have undergone treatment. The outcome of these subjects to date is shown in Table 1. No subject has experienced a drug product related adverse event, and ISA analyses demonstrate highly polyclonal reconstitution without clonal dominance. Both β-thalassemia major subjects remain transfusion-free for 14 and 11 months respectively, post-treatment. Subject 1204, at his most recent visit (Month 4.5 post-treatment), has a total hemoglobin of 12.0 g/dl, of which 24% is HbA^{T87Q} (from 9.6% at Month 3) and 33% is HbS (from 14.7% at Month 3), with increasing levels of transduced cells detected in peripheral blood (PBL VCN of 2.4 at Month 4.5). This subject has not had a post-treatment hospitalization for a SCD-related event and his transfusion support is being tapered.
Summary and Conclusions: The subjects with β-thalassemia major remain transfusion-free for 14 and 11 months. The subject with SCD demonstrates increasing production of HbA^{T87Q} over time. Gene therapy using autologous HSC transduced with LentiGlobin BB305 lentiviral vector *ex vivo* is a promising approach for the treatment of patients with both β-thalassemia major and severe SCD.

S467

GENERATION OF EBV-SPECIFIC AND EBV-SPECIFIC, TCR-REPROGRAMMED HUMAN CD8+ CYTOTOXIC T LYMPHOCYTES WITH STEM CELL MEMORY AND CENTRAL MEMORY PROPERTIES BY MODULATING GLYCOLYTIC T CELL METABOLISM

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Background: Adoptive transfer of virus-specific and TCR- or CAR-reprogrammed cytotoxic T lymphocyzes (CTL) has advanced as a valuable cellular therapy for opportunistic viral infections, Epstein-Barr Virus (EBV)-mediated lymphoma and leukemia relapse. However, durable clinical responses are often hampered by limited capability of terminally differentiated, high avidity effector T cells (T_{EFF}) to establish sustained antileukemic immunity whereas less differentiated stem cell memory (T_{SCM}) and central memory (T_{CM}) T cells could be shown to elicit potent antitumor responses, prolonged survival and memory. Moreover, T_{SCM} and T_{CM} depend less on glucose consumption to drive oxidative phosphorylation (OXPHOS) as their primary source of adenosine triphosphate (ATP) production whereas T_{EFF} require additional aerobic glycolysis to generate the ATP needed.
Aims: In the current study, we therefore investigated means of modulating T cell metabolism to generate EBV-specific T_{SCM} and T_{CM} as well as EBV-specific $T_{SCM/CM}$ TCR-reprogrammed T cells.
Methods: Naïve $CD8^+CD45RA^+$ T cells isolated from peripheral blood mononuclear cells (PBMC) of healthy $HLA\text{-}A2^+$ donors using the Naive T cell isolation kit® (Milenyi Biotec, Bergisch Gladbach, Germany) and unselected (total) $CD8^+$ T cells were primed by autologous dendritic cells loaded with EBV-peptides followed by restimulation with peptide loaded autologous PBMC in the presence of low (1 mM) glucose, glutamine, an optimized cytokine cocktail and 1 mM of the non-metabolizable glucose analogue 2-deoxy-glucose (2-DG). Moreover, either galactose or 9-oleic acid was added to glucose deprived cultures. In addition to phenotypic and functional *in vitro* analyses by FACS, IFN-g ELISPOT- and ^{51}Cr-release assays, glucose uptake and lactate production was determined. OXPHOS and aerobic glycolysis were assessed by measuring oxygen-consumption rate (OCR) and extracellular acidification rate (ECAR), respectively, using a Seahorse Analyzer. Finally, the biological activity of EBV-reactive $T_{SCM/CM}$ was tested *in vivo* in NSG mice.
Results: We obtained strong expansion of EBV-reactive CTL expressing a $CD8^+CD45RA^+CD45RO^-CD95^+$ $CD27^+CD28^+CD62L^+CCR7^+T_{SCM}$ and $CD8^+CD45RA^-CD45RO^+CD95^+CD27^+CD28^+CD62L^+CCR7^+$ T_{CM} phenotype upon repetitive stimulation of naïve T cells in the presence of low glucose, glutamine and 2-DG when compared to CTL stimulated without 2-DG. This effect was also seen in total $CD8^+$ T cells although less pronounced. In contrast, supply of galactose or oleic-acid to glucose free (but glutamine containing) medium did not result in reduced T_{EFF} differentiation, suggesting OXPHOS by galactose converted to glucose and fatty acid oxidation. Moreover, 2-DG treated T_{SCM} and T_{CM} as well as total $CD8^+$ CTL showed less lactate production and ECAR as compared to untreated controls, confirming that differentiation to T_{EFF} requires aerobic glycolytic ATP production. Functional assays *in vitro* did not only reveal cytolytic activity of 2-DG treated EBV-T_{SCM} and T_{CM} comparable to controls but elicited superior migration properties of these cells when tested to untreated naïve or total $CD8^+$ T cells in a transwell- migration assay. We further showed that EBV-$T_{SCM/CM}$ can be successfully TCR-reprogrammed to recognize primary acute myeloid leukaemia (AML) blasts.
Finally, first adoptive transfer studies into NSG mice indicated that EBV-$T_{SCM/CM}$ generated in low glucose can persist and mount an immune response to EBV-transformed B cells engrafted into NSG recipients.
Summary and Conclusions: These studies demonstrate that modulation of T cell metabolism may be a promising approach to generate stem cell memory and central memory T cells *in vitro* for virus-specific and redirected immunity in adoptive cellular therapy.

S468

A THERAPEUTIC NKT CELL ADJUVANT-BASED VACCINE INDUCES AN EFFECTIVE ANTITUMORAL IMMUNE RESPONSE IN B-CELL LYMPHOMA

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Background: Type I natural killer T (NKT) cells are lymphocytes with unique

specificity for glycolipid antigens presented by nonpolymorphic CD1d receptor on dendritic cells (DCs). NKT cells play a central role in tumor immunology since they coordinate innate and adaptive immune responses. Activation of NKT cells with the prototypic lipid α-galactosylceramide (α-GalCer) stimulate INF-γ production and cytokine secretion (eg, IL-12, IL-17, IL-21) which contribute to the enhancement of T cell activation.
Aims: We evaluated the antitumor effect of a combination of DCs and irradiated tumor cells with the NKT cell agonist α-GalCer in a mouse model of B cell lymphoma.
Methods: The murine B cell lymphoma line 4TOO was used as tumor model. A therapeutic vaccine was generated by mixing DCs and irradiated 4TOO tumor cells (10^6 cells in a 1:1 ratio) in the presence of α-GalCer (2μg/mouse). A single dose of the therapeutic vaccine was injected iv. into Balb/c mice (n=10 per group) two days after tumor challenge ($4x10^5$ cells/mouse, iv), and mice were followed for survival. NKT cell expansion was analyzed by flow cytometry using a PE conjugated CD1d: α-GalCer analogue (PBS-57) loaded tetramer (NIH Tetramer Core Facility, Atlanta GA). Intracellular IFN-γ production on NKT cells and $CD4^+$ and $CD8^+$ T cells was detected by flow cytometry. One-way ANOVA was used for comparisons between different experimental groups. Differences in survival were analyzed using the log-rank test.
Results: Therapeutic vaccine eradicated B cell lymphoma in all treated mice, and was superior to any vaccine combination, including α-GalCer alone, irradiated tumor cells with DCs, and DCs with α-GalCer (100%, 10%, 0% and 50% of survival at day 100, respectively; p<0.001). Importantly, 90% of treated mice with the vaccine were resistant to a tumor rechallenge, suggesting the development of a memory immune response. In addition, the immune response was tumor-specific since all the mice were unable to reject a syngeneic A20 B cell lymphoma. A significant NKT cell expansion in the spleen and liver of vaccinated mice was observed compared with control groups (4.8 *vs* 1.2 fold expansion; p=0.02, and 3.8 *vs* 1.4 fold expansion; p=0.04, respectively). Moreover, this vaccination strategy induced an increase of IFN-γ secreting NKT cells (58.4% *vs* 39.5% (p=0.05) and of IFN-γ secreting T cells compared to control groups (25.7% *vs* 16.8% in $CD8^+$ cells (p=0,04), and 31.2% *vs* 14% in $CD4^+$ cells (p=0,04).
Summary and Conclusions: A therapeutic vaccine consisting of dendritic cells pulsed with tumor cells plus the NKT agonist α-GalCer efficiently eradicates B cell lymphoma in a therapeutic setting. This immune response is long-lasting, tumor-specific, and it is associated with an expansion of NKT cells in lymphoid organs and with an increase in IFN-γ secreting NKT and T cells. These data support the development of immunotherapy strategies in patients with B cell lymphoma using DCs and NKT cell agonists.

S469

ALLOGENIC ADOPTIVE IMMUNOTHERAPY OF ACUTE MYELOID LEUKEMIA (AML) BY TARGETING CD123 WITH CAR T-CELLS

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Background: Adoptive immunotherapy using autologous T-cells endowed with chimeric antigen receptors (CARs) has given rise to long-term durable remissions and remarkable objective response rates in patients with refractory leukemia, raising hopes that a wider application of CAR technology may lead to a new paradigm in cancer treatment. However, a limitation of the current autologous approach is that CAR T-cells must be manufactured on a "per patient basis".
Aims: To overcome this limitation we have developed a standardized platform for manufacturing T-cells from third-party healthy donors to generate allogeneic "off-the-shelf" engineered CAR+ T-cell-based frozen products.
Methods: This allogenic platform utilizes Transcription Activator-Like Effector Nuclease (TALEN) gene editing technology to inactivate the TCRa constant (TRAC) gene, eliminating the potential for T-cells bearing alloreactive TCR's to mediate Graft *versus* Host Disease (GvHD). We have previously demonstrated that editing of the TRAC gene can be achieved at high frequencies, yielding up to 80% of TCRab negative cells and allowing efficient production and purification of TCR-deficient T-cells that no longer mediate alloreactivity in a xeno-GvHD mouse model. As a safety feature, T-cells are engineered to co-express the RQR8 gene, with the aim of rendering them sensitive to the monoclonal antibody rituximab.
Results: Here, we have adapted this allogeneic platform to the production of T cells targeting CD123, the transmembrane alpha chain of the interleukin-3 receptor, which is expressed on tumor cells from the majority of patients with Acute Myeloid Leukemia (AML). In a first step, we have screened multiple antigen recognition domains in the context of different CAR architectures to identify effective CAR candidates displaying activity against cells expressing variable levels of the CD123 antigen. In addition, experiments in an orthotopic AML mouse model using UCART123 cells demonstrate important anti-tumor activity *in vivo*.
Summary and Conclusions: The ability to carry out large scale manufacturing of allogeneic, non alloreactive CD123 specific T-cells from a single healthy donor can offer the possibility of an off-the-shelf treatment that would be immediately available for administration to a large number of AML patients.

S470

UCART19, AN ALLOGENEIC "OFF-THE-SHELF" ADOPTIVE T-CELL IMMUNOTHERAPY AGAINST CD19+ B-CELL LEUKEMIAS

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Background: Autologous T-cells engineered to express chimeric antigen receptors (CARs) that target specific tumor antigens are known to be of high potential in treating different kinds of cancer. However, they must be generated on a "per patient" basis, thereby limiting the population of patients that could benefit from this approach. In particular, immune homeostasis may be affected in heavily pre-treated patients, such that autologous T-cells may be low in number, not fully functional, or unable to expand, thereby restricting the amount of cells that could be manufactured.
Aims: The use of allogeneic T-cells isolated from healthy third party donors could constitute an easy-to-scale-up alternative, producible in advance, with potential for standardized quality controls, better batch consistency, and immediate availability for administration to a larger number of patients.
Methods: We have developed a standardized platform for manufacturing T-cells from third-party healthy donors to generate allogeneic "off-the-shelf" engineered CD19-CAR+ T-cell-based frozen products. Our platform involves the use of transcription activator-like effector nucleases (TALEN), which mediate the simultaneous inactivation of two genes through genome editing. The knock-out of the TCR alpha gene eliminates TCR expression and is intended to abrogate the donor T-cell's potential for graft-*versus*-host disease (GvHD), while knocking out the CD52 gene makes donor T-cells resistant to the lymphodepleting agent alemtuzumab. In addition, our T-cells are engineered to co-express the RQR8 gene as a safety feature, with the aim of rendering them sensitive to the monoclonal antibody rituximab.
Results: We have obtained proof-of-concept for the application of this approach by manufacturing TCR/CD52-deficient RQR8+ and CD19-CAR+ T-cells (UCART19) using a good manufacturing practice-compatible process and have demonstrated that the resulting UCART19 cells were functional using *in vitro* assays. Furthermore we have demonstrated that the ability of UCART19 cells to engraft into an orthotopic human CD19+ lymphoma xenograft immunodeficient mouse model. UCART19 cells exhibited antitumor activity equivalent to that of standard CD19 CAR T-cells. We also demonstrated that UCART19 cells did not mediate alloreactivity in a xeno-GvHD mouse model. Finally, the effectiveness of the rituximab-induced depletion mechanism of $RQR8^+$ cells was shown in an immunocompetent mouse model.
Summary and Conclusions: This valuable dataset supports the development of allogeneic CAR T-cells, and UCART19 will be investigated in an exploratory, first-in-human, clinical trial where refractory/relapsed CD19+ B-cell leukemia patients are to be enrolled.

PRESIDENTIAL SYMPOSIUM

Best abstracts

S471

ELOQUENT-2: A PHASE 3, RANDOMIZED, OPEN-LABEL STUDY OF LENALIDOMIDE/DEXAMETHASONE WITH OR WITHOUT ELOTUZUMAB IN PATIENTS WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA

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Background: Elotuzumab, an immunotherapeutic monoclonal antibody (mAb), recognizes Signaling Lymphocytic Activation Molecule F7 (SLAMF7), a protein highly expressed on myeloma cells and natural killer cells. Elotuzumab targets and kills SLAMF7-expressing myeloma cells by direct activation of natural killer cells with minimal effect on normal tissues. Elotuzumab showed encouraging activity with lenalidomide/dexamethasone (Ld) in a Phase 1b/2 study in patients with relapsed/refractory multiple myeloma (RRMM).
Aims: ELOQUENT-2, a Phase 3 study (NCT01239797) compared efficacy and safety of elotuzumab plus lenalidomide/dexamethasone (ELd) *vs* Ld.
Methods: Patients with RRMM and 1-3 prior therapies were randomized 1:1 to ELd or Ld in 28-day cycles until disease progression or unacceptable toxicity: elotuzumab (10 mg/kg IV) was given weekly during Cycles 1 and 2 and then biweekly; lenalidomide (25 mg) was given on Days 1-21; dexamethasone was given weekly (40 mg or 28 mg PO+8 mg IV on elotuzumab dosing weeks). Written informed consent was obtained for all patients. Response/progression was assessed by an independent review committee using European Group for Blood and Bone Marrow Transplant criteria. Primary endpoints were progression-free survival (PFS) and overall response rate (ORR). Results of a prespecified interim analysis are reported.

Figure. Progression-free survival (PFS)

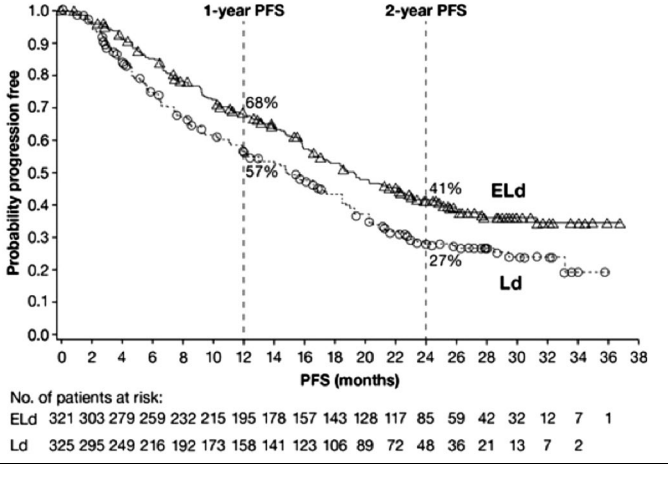

Figure 1. Progression-free survival (PFS).

Results: 646 RRMM patients were randomized (321 to ELd, 325 to Ld; median age 66 years [20% of patients ≥75 years old]; refractory to last therapy 35%). Median number of prior therapies was 2, including bortezomib 70%, thalidomide 48% and lenalidomide 6%. High-risk patients were included: del(17p) in 32%; t(4;14) in 9%. At the data cut-off (4 Nov 2014), 35% (ELd arm) and 21% (Ld arm) of patients remained on therapy; discontinuation was mainly due to disease progression (42% in the ELd arm, 47% in the Ld arm). Median number of cycles was 19 and 14 in the ELd and Ld arms, respectively. After a median follow-up of 24 months, PFS rate was superior in the ELd over the Ld arm: 68% *vs* 57% at 1 year and 41% *vs* 27% at 2 years. Hazard ratio for PFS was 0.70 (95% CI 0.57, 0.85; p=0.0004). Median (95% CI) PFS in the ELd arm was 19.4 (16.6, 22.2) months *vs* 14.9 (12.1, 17.2) months in the Ld arm (Figure 1). PFS benefit with ELd was consistent across key subgroups. ORR (95% CI) was 79% (74, 83) in the ELd arm *vs* 66% (60, 71) in the Ld arm (p=0.0002). Very good partial response or better was seen in 32.7% and 28.0% of patients in the ELd and Ld arms, respectively; partial response was seen in 45.8% and 37.5%, respectively. Grade 3-4 adverse events in ≥15% of patients include neutropenia (25% in the ELd arm, 33% in the Ld arm) and anemia (15% in the ELd arm, 16% in the Ld arm). Infections (any grade) occurred in 81% of patients in the ELd arm and 74% in the Ld arm. Exposure-adjusted infection rates were the same in both arms (incidence rate/100 person-years of exposure, 197). Infusion reactions occurred in 10% of patients with ELd (mostly Grade 1-2). There were 210 deaths (94 in the ELd arm, 116 in the Ld arm).
Summary and Conclusions: This is the first positive randomized Phase 3 trial with an immunotherapeutic mAb, assessing Ld with or without elotuzumab in RRMM. It showed a 30% reduction in risk of progression or death with ELd *vs* Ld. More patients remain on ELd *vs* Ld, and follow-up for long-term outcomes is ongoing. Infusion reactions were manageable. Elotuzumab, a mAb with a novel mechanism of action, showed improved PFS at 1 year and 2 years, with minimal added toxicity in combination with Ld *vs* Ld alone in patients with multiple myeloma.
^*Equal contribution: M. Dimopoulos and S. Lonial.*

S472

RECURRENT MTORC1-ACTIVATING RRAGC MUTATIONS IN FOLLICULAR LYMPHOMA

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Background: Follicular lymphoma (FL) is an incurable B-cell malignancy characterized by the t(14;18) and mutations in one or more components of the epigenome in virtually all cases. Whilst frequent gene mutations in signalling pathways, including JAK-STAT, NOTCH and NF-κB, have also been defined, the spectrum of these mutations typically overlaps with the closely-related diffuse large B cell lymphoma (DLBCL). Through a combination of discovery exome and extended targeted sequencing, we reveal recurrent somatic mutations in components of the mTOR pathway in FL patients.
Aims: We sought to identify novel gene mutations in FL that might serve as therapeutic targets.
Methods: Whole exome sequencing (WES) was conducted on 24 FL matched tumour/normal samples (mean coverage: 133x) from 5 patients that had multiple episodes of FL but have not transformed to a more aggressive disease. Prevalence screening and clonality assessment were performed by targeted deep sequencing of candidate and FL-associated genes, using Fluidigm-Miseq, in an extension cohort of 176 FL cases, with further Sanger sequencing in 329 related mature B-cell NHLs and 51 B-cell lymphoma cell lines. Functional analyses were carried out by expressing wild-type and mutant *RRAGC* in HEK-293T for signalling and co-immunoprecipitation experiments and purified wild-type and RagC mutant proteins were used in nucleotide-binding assays.
Results: Novel somatic mutations in *RRAGC* encoding a Rag GTPase protein (RagC) were identified in 4 out of the 5 untransformed FL patients analysed by WES. Clonal density plots demonstrated the *RRAGC* mutations resided within the dominant clone, with temporal analyses of multiple biopsies from the same individual showing stability of the *RRAGC* mutations during disease progression. Coding *RRAGC* mutations were identified in 28 of 176 cases (16%) from our FL extension cohort with mutations rare or absent in other mature B-NHL

entities. Mutations were heterozygous, predominantly missense (93%) and clustered within the nucleotide binding domain of RagC. The somatic origin of 10 of these mutations was confirmed by sequencing of matched constitutional DNA. A search for additional mutations in regulatory components upstream and downstream of the pathway uncovered co-occurring mutations in 2 subunits of the vacuolar H+-adenosine triphosphate ATPase (v-ATPase) complex, in more than half of *RRAGC* mutated cases. Together, Rag GTPases form a super-complex with the v-ATPase, Ragulator and SLC38A9 on the lysosomal surface and promote amino acid-mediated activation of the mechanistic target of rapamycin complex 1 (mTORC1). By transiently expressing RagC mutants ($RagC^{muts}$) in HEK-293T cells, we showed increased co-immunoprecipitation with a key regulatory component of mTORC1, raptor, compared to wild-type. Furthermore, stable overexpression of the $RagC^{muts}$ not only activated mTORC1 but rendered it resistant to leucine or arginine deprivation, as indicated by phosphorylation of a canonical mTORC1 substrate. Moreover, whilst all $RagC^{muts}$ were activating, mutations at amino acid residue Serine-75 led to mTOR activation by specifically disrupting nucleotide binding.
Summary and Conclusions: We report novel recurrent mutations in *RRAGC* and associated v-ATPase subunits, uniquely enriched in FL. The *RRAGC* mutations lead to activation of mTORC1 in the absence of amino acids. Overall, the emergence of frequent, gain-of-function *RRAGC* mutations that are clonally represented and maintained during progression provides an excellent candidate to be therapeutically exploited.

S473

DNMT3A R882 MUTATION PROMOTE CHEMORESISTANCE AND THERAPEUTIC RELAPSE THROUGH IMPAIRED DNA-DAMAGE SENSING
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Background: Although most patients with AML initially respond to anti-leukemic chemotherapy, the majority subsequently relapse and die of relapsed, refractory disease. Recent studies have suggested that therapeutic relapse is due to persistent, chemoresistant AML cells that survive initial therapy, and serve as a reservoir for genetic/epigenetic evolution and subsequent clinical relapse. Although many AML patients have evidence of residual leukemia at clinical remission, the mechanisms leading to chemoresistance and to the survival of AML cells in response to chemotherapy have not been delineated.
Aims: Investigate the link between specific mutations and minimal residual disease (MRD); deliniate molecular mechanism of therapeutic resistance in *DNMT3A*-mutant AML.
Methods: MRD was assessed by multi-parameter flow cytometry at day 28 in patients in clinical/pathologic complete remission. Novel genetic mouse model was used to study the role *DNMT3A* R882 mutation in AML pathogenesis and chemoresistance.
Results: Multivariate analysis revealed that *DNMT3A* R882 mutations were associated with an increased risk of MRD at the time of complete remission (p=0.007). By contrast, non-R882 mutations in *DNMT3A* did not predict for MRD, nor did other recurrent AML mutations. These data suggest an important role for *DNMT3A* R882 mutations in chemoresistance in AML, consistent with studies suggesting that *DNMT3A*-mutant, pre-leukemic stem cells persist after anti-leukemic chemotherapy. Next we generated a mouse model that conditionally expressed *Dnmt3a(mut)* from the endogenous locus. In agreement with clinical observations, *Dnmt3a(mut)* animals were characterized by expansion of the stem-cell-enriched $Lineage^{-}$ $Sca1^{+}$ $cKit^{+}$ (LSK) compartment. Consistent with enhanced clonal stem cell fitness in clonal hematopoiesis, we observed robust serial hematopoietic repopulation ability of *Dnmt3a(mut)* cells over 3-4 rounds of retransplantation *in vivo*. Notably, expression of *Dnmt3a*(mut) in hematopoietic cells from the endogenous locus did not induce AML *in vivo*. By contrast, we found that co-expression of mutant *Dnmt3a*, *Flt3-ITD* and *Npm1c* mutations, the most common "triad" of mutations observed in AML, resulted in a fully penetrant, short latency AML phenotype capable of propagating disease in secondary recipients. We next investigated the basis for *DNMT3A* R882 mutant-induced chemoresistance. Cells expressing *Dnmt3a(mut)* showed a survival advantage compared to wild-type controls and preserved clonogenic potential after *in vitro* and *in vivo* exposure to daunorubicin, but not in response to other DNA damaging agents. Of note, although *DNMT3A* mutant cells had attenuated pro-apoptotic signaling through the p53 pathway in response to anthracyclines, we observed increased single strand breaks and increased mutagenesis in *DNMT3A*-mutant cells consistent with impaired DNA-damage sensing in response to DNA strand torsion induced by daunorubicin. Most importantly, *Dnmt3a(mut)* cells fail to remodel their chromatin after anthracycline-induced DNA damage, with impaired nucleosome remodeling and reduced recruitment of the FACT chromatin remodeling complex subunit SPT-16 in response to anthacycline induced DNA torsion. This results in defective sensing of DNA breaks, attenuated pro-apoptotic signaling and reduced DNA repair, leading to increased mutagenesis in response to AML chemotherapy.
Summary and Conclusions: These data provide a novel mechanism by which mutant *DNMT3A* promotes chemoresistance in AML, and suggests the possibility of unique vulnerabilities in *DNMT3A*-mutant cells that can be exploited therapeutically to this common AML subset.

S474

ANALYSIS OF NEW GFI1B VARIANTS IN PATIENTS WITH INHERITED BLEEDING AND PLATELET DISORDERS
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Background: Recently, we found that a heterozygous mutation in *Growth Factor Independence 1B* (*GFI1B*) causes an autosomal-dominant bleeding disorder characterized by gray platelets.[1] Affected individuals exhibited thrombocytopenia and suffered from mild to severe bleeding episodes. Their platelets appeared gray in blood films, as they contained reduced numbers of α-granules. GFI1B is an essential transcription factor for megakaryocyte differentiation. It contains six C terminal zinc fingers (Znfs), of which 3-5 are essential for DNA binding. The mutation that we reported caused a truncation of Znf 5. The mutant protein lost its repressive function and inhibited the repressive capacity of the wild type GFI1B protein (dominant-negative effect). Platelets from affected individuals expressed CD34, which had not been reported for bleeding and platelet disorders (BPD) caused by other genetic aberrations.
Aims: Here, we study eight new *GFI1B* variants that we identified in cases with inherited BPD. We assessed platelet α-granule numbers and CD34 expression of these patients and CD34 expression of BPD cases with proven pathogenic mutations in FLI1, GATA1, NBEAL2 and RUNX1. We also investigated the repressive capacity of the GFI1B variants.
Methods: Variants found in *GFI1B* in 529 whole exome-sequenced BPD cases, enrolled by the BRIDGE-BPD consortium, were compared to ~60,000 reference genomes (1000 genomes and ExAC databases).[2] Platelet α-granule numbers were assessed by electron microscopy. CD34 expression was measured on GPIbα/CD42B positive platelets by flow cytometry. Luciferase repression assays using GFI1B binding sequences were performed as described before.[1] Informed consent was obtained from all patients.
Results: Eight new *GFI1B* variants were found in patients with BPD.[2] Four variants were located upstream of the Znfs (D23N, Q89fs, G139S and C168F). The other four variants were located in or close to Znfs 1 and 2 (H181Y, R184P, R190W and G198S). All variants were heterozygous except for C168F being homozygous. Most variants were extremely rare (<2 hits/121,198 alleles) or absent in the reference samples. Approximately half of the patients had thrombocytopenia. Reduced platelet α-granule numbers were found for patients carrying Q89fs and H181Y, but normal α-granule numbers were found for patients carrying R184P and R190W. Platelets from all tested individuals with GFI1B variants (H181Y, R184P and R190W) exhibited CD34 expression. NBEAL2, GATA1, RUNX1 and FLI1 mutated platelets were CD34 negative. We tested the repressive capacity of Q89fs, G139S, C168F, H181Y, R184P and R190W. Q89fs lacked all Znfs and did not repress in a luciferase reporter assay, whereas all missense variants did repress.
Summary and Conclusions: We found eight new *GFI1B* variants in families with BPD. Platelet α-granule numbers were reduced for two out of four variants, while all tested variants had platelet CD34 expression. The latter seems unique to GFI1B variants. Only the frameshift variant lacking the DNA binding Znfs lost its repressive capacity. Therefore, distinct molecular mechanisms may cause platelet abnormalities. The frameshift variant might inhibit the function of wild type GFI1B by quenching co-factors, whereas the missense variants might do this by occupying DNA while lacking the ability to bind certain co-factors. The identification of these new variants opens new avenues to understand how GFI1B dictates megakaryopoiesis at the molecular level.

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S475

COMBINATION OF GENE TRANSFER AND FORCED CHROMATIN LOOPING CONCURRENTLY DECREASES SICKLE CELL HEMOGLOBIN AND REACTIVATES THE SYNTHESIS OF FETAL HEMOGLOBIN IN PATIENT CELLS
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Background: The hemoglobinopathies, such as beta-thalassemia and sickle cell anemia (SCA), are characterized by mutations of the beta-globin gene resulting in either decreased or functionally abnormal hemoglobin (Hb) production. As bone marrow transplant is the only curative option for these patients, there is a strong need for new therapeutic approaches. Both beta-thalassemia and SCA represent ideal targets for gene therapy since introduction of a normal beta-globin gene can ameliorate the phenotype, as we and others have shown previously. In addition, overcoming the developmental silencing of the fetal gamma-globin gene represents an additional approach for the treatment of hemoglobinopathies. Here, we directly compare a recently established approach to activate the gamma-globin gene using forced chromatin looping with pharmacologic approaches to raise gamma-globin expression.

The beta-type globin genes are activated through dynamic interactions with the distal locus control region. This region physically contacts the developmental stage appropriate globin gene via chromatin looping, a process partially dependent on the protein Ldb1. We have shown that tethering Ldb1 to the human gamma-globin promoter with a custom designed zinc finger protein (ZF-Ldb1) can redirect loop formation from the beta- to gamma-promoter and potently reactivate gamma- globin gene expression in adult human erythroid cells (Deng *et al.*, Cell, 2012 and 2014).

Aims: Our objective is to compare this recently established approach to pharmacological induction of fetal hemoglobin (HbF) in cells with abnormal beta-globin function, as in Sickle Cell Anemia (SCA).

Methods: We isolated hematopoietic stem cells from blood of 11 SCA patients. These cells were expanded and differentiated into mature erythroid cells *in vitro*, and treated with a lentivirus expressing the ZF- Ldb1 transgene. 10 of these specimens were also treated with hydroxyurea, the only FDA-approved HbF inducer used in SCA patients, or with the experimental inducers decitabine, tranylcypromine, pomalidomide and butyrate.

Results: ZF-Ldb1 increased HbF synthesis in SCA erythroid cells up to 86% and, concurrently, reduced sickle Hb (HbS) below 15%, showing a balanced synthesis between alpha and beta like globins, and consistent with additional studies with healthy erythroid cells. In addition, the induction of HbF in cells treated with ZF-Ldb1 was roughly three times higher (+34%, at a dose of ~ one transgene copy per cell) than that observed using pomalidomide (+12%, 10μM). Decitabine and tranylcypromine had an intermediate effect (+9% for both drugs, 0.5 μM and 1.5 μM, respectively). Hydroxyurea and butyrate showed the lowest HbF increase (+2% and +3%, respectively, both 100μM). Importantly, pomalidomide treatment lowered to 46% cell viability, which remained unaltered in ZF-Ldb1 expressing cells. In related experiments, we compared the expression of a battery of genes known to regulate HbF levels (BCL11A, SOX6, KLF1 and C-Myb) in normal and SCA derived erythroid cells treated with ZF-Ldb1 or HbF inducers and compared to controls. Quantitative PCR analyses indicate reduced expression of KLF1 in and SOX6 in SCA specimens, in line with a higher HbF synthesis at steady state.

Summary and Conclusions: Given its efficacy and safety, lentiviral-mediated ZF-Ldb1 gene transfer has great therapeutic potential for SCA erythroid cells and appears superior to drug induction. Moreover, a reduced expression of HbF repressors in SCA cells might facilitate ZF-Ldb1-induced expression of HbF

Multiple myeloma - Biology

S476

EXOME SEQUENCING POINTS TO DIFFERENCES IN GENETIC INSTABILITY LEVEL IN MGUS COMPARED TO MM

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Background: Malignant transformation of normal to tumour cells is a multistep process followed by sequential aggregation of hits at different molecular levels. Genetic events including single nucleotide variants (SNVs), insertion-deletion changes (indels) as well as copy number alterations (CNAs) affect the phenotype of the tumour population and consequently patient prognosis. Transformation from a symptomless state, monoclonal gammopathy of undetermined significance (MGUS) to multiple myeloma (MM) can be used as a unique model for cancer development studies. To date, there is very little data regarding the mechanisms leading to disease progression at molecular level.

Aims: To perform exome sequencing together with SNP array analysis in MGUS patients to describe the premalignant phenotype and compared these to advanced tumour cells at the DNA level.

Methods: Overall, 33 and 69 MGUS patients were included in a WES and SNP array study, respectively. Plasma cells (PC) were isolated from bone marrow by FACSAria (BD Biosciences) system using CD138, CD19 and CD56 markers to obtain a pure abnormal PC population with a purity >90%. For WES, NEBNext kit and SureSelect Human All Exon V5 (Agilent) were used, samples were sequenced by HiSeq2000 (Illumina). Copy number alterations (CNAs) were tested by SurePrint G3 CGH+SNP, 4x180K (Agilent). Results were compared to 463 and 91 MM patients analysed by WES and SNP arrays, respectively.

Results: CNAs and acquired somatic gene mutations (SNVs) were detected in 68% (47/69) and 100% (33/33) of MGUS patients in comparison to 100% (91/91, $p<10^{-4}$) and 100% (463/463) of MM patients, respectively. However, the overall number of both CNAs and SN*Vs* per patient was significantly lower in MGUS (CNAs: median 2, range 0-15; SNVs: median 89, range 9-315) than in MM (CNAs: median 16, range 2-49, $p<10^{-18}$; SN*Vs* median 123, range 1-897, $p<10^{-4}$). Non-synonymous SN*Vs* (NS-SNVs) were present in 97% (32/33) cases with a median 19 (range 0-70) NS-SN*Vs* per patient. Overall, 42 genes were recurrently mutated in at least 2 patients and 2 non-large protein coding genes were mutated in at least 3 cases including *KLHL6* and *NPIPL2*. We identified 7 genes which were significantly mutated in MM in our previous study including *KRAS* (n=2), *HIST1H1E* (n=2) and *NRAS*, *DIS3*, *EGR1*, *LTB*, *PRKD2* (all n=1). *IGH* translocations were identified in 27% (9/33) of patients: t(11;14) in 12% (4/33), t(4;14) in 9% (3/33), t(14;16) in 3% (1/33) and t(14;20) in 3% (1/33). As previously described in MM, only one type of *IGH* translocation was found per patient and all 9 cases with *IGH* translocation did not have additional hyperdiploidy. We did not find any translocations involving *MYC* (8q24.21) or the light chain loci *IGK* (2p12) and *IGL* (22q11.2) and any mutations in *TP53*, *ATM*, *ATR* and *ZNFH4* genes involved in DNA repair pathway alterations which were identified as unfavourable factors to MM patients survival.

Summary and Conclusions: We have performed the first comprehensive analysis of MGUS patients using exome sequencing together with SNP arrays and described the main genetic events that are already present in this premalignant state. We proved that complex genetic instability is formed before clinical manifestation first at the gene level then at the chromosome level. Then, a number of random genetic hits increases to form a landscape for significant oncogenic hits driving the transition to a malignant state.

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S477

EXPRESSION LEVELS OF CD38 AND COMPLEMENT INHIBITORY PROTEINS CD55 AND CD59 PREDICT RESPONSE TO DARATUMUMAB IN MULTIPLE MYELOMA

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Background: Daratumumab (DARA), an anti-CD38 monoclonal antibody, induces lysis of multiple myeloma (MM) cells through complement dependent cytotoxicity (CDC) and antibody-dependent cellular cytotoxicity (ADCC). Immunotherapy with DARA is clinically effective, but there is a significant variability in quality of response among patients.
Aims: We examined potential determinants of DARA sensitivity before start of treatment, and mechanisms of resistance during therapy, with special emphasis on the target protein CD38. Since several studies have stressed the importance of CDC for therapeutic efficacy of antibodies, we also focused on the complement inhibitory proteins (CIPs), including CD46, CD55, and CD59.
Methods: We investigated a panel of cell lines, as well as patient samples by flow cytometry for CD38, CD46, CD55, and CD59 and assessed their sensitivity to DARA-mediated CDC *in vitro*. We also evaluated samples from relapsed/refractory MM patients treated with DARA as a single agent (GEN501 study).
Results: MM and lymphoma cell lines (n=33), as well as primary MM cells (n=31) expressing high levels of CD38 and low levels of CD55 and CD59 were significantly more sensitive to DARA-mediated CDC, compared to cells with low CD38 expression and high levels of CD55 or CD59. However, CD46 expression was not associated with CDC sensitivity. We also analyzed the expression of CD38 and CIPs on MM cells from 21 relapsed/refractory MM patients directly before start and during DARA treatment. The CD38/CD59 and CD38/CD55 ratios prior to treatment were predictive of clinical response. Also the sensitivity of pretreatment MM cells towards DARA-mediated CDC *ex vivo*, correlated positively with clinical response to DARA, expressed as maximum reduction in tumor load (R=0.70, *P*=0.005). At the time of progression during DARA therapy, there was a significant reduction of CD38 expression and significant increase in CD59 and CD55 expression on both bone marrow-localized and circulating MM cells, whereas CD46 levels did not change. As expected, at the time of relapse, a significant decrease in DARA-mediated CDC was found when compared to baseline values. Interestingly, before start of DARA therapy, two out of 21 patients had two coexisting populations of MM cells based on differential expression of CD55 and/or CD59. In both cases, the dominant relapsing clone was strongly positive for both CD55 and CD59, whereas these clones were present as minor populations before initiation of DARA treatment. Altogether, these data suggest that elevated CIP expression results in reduced sensitivity to DARA-mediated CDC, and that upregulation of CD55 and CD59 during DARA treatment may contribute to the development of resistance. With the intention to improve CDC sensitivity, we evaluated phospholipase C, which enzymatically removes the GPI-anchored CD55 and CD59 molecules from the cell surface. Indeed, loss of CD55 and CD59 improved DARA-mediated CDC sensitivity *in vitro*. Similarly, all-*trans* retinoic acid (ATRA) enhanced CD38 expression levels and reduced CD55 and CD59 expression resulting in a significant enhancement of CDC in MM cell lines and primary MM cells. Also in a humanized mouse model, ATRA significantly enhanced the anti-MM effect of DARA.
Summary and Conclusions: These results show that baseline CD38/CD55/CD59 expression may be useful to predict response to DARA, and that targeting CIPs could be a potential adjuvant therapy for DARA or other monoclonal antibodies with CDC activity.

S478

ADDITIONAL PROGNOSTIC VALUE OF CIRCULATING MIR-16 EXPRESSION OVER THE USUAL PROGNOSTIC PARAMETERS IN MULTIPLE MYELOMA PATIENTS

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Background: Current prognosis in multiple myeloma (MM) is based on International Staging System (ISS) and the presence of chromosomal abnormalities detected by Fluorescent *in situ* Hybridization (FISH). However, their ability to stratify patients is still suboptimal and novel biomarkers are required to better predict survival. We identified circulating miRNAs as a non-invasive biomarker to stratify MM patients.
Aims: To evaluate the role of circulating miRNA in combination with usual prognostic factors in MM. In details: 1) to confirm the prognostic value of circulating miRNAs in MM; 2) to investigate their relationship with classical prognostic parameters; 3) to evaluate the addition of circulating miRNAs into a prognostic model together with ISS and FISH.
Methods: Circulating miRNA expression has been evaluated in newly diagnosed MM patients enrolled in the GIMEMA clinical trial NCT#01063179. Patients were randomized to receive VMP (Velcade-Melphalan-Prednisone) or VMPT-VT (Velcade-Melphalan-Prednisolone-Thalidomide with Velcade-Thalidomide maintenance). We used NanoString assay to screen more than 800 miRNAs in serum at baseline and qRT-PCR to validate significant miRNAs as reported elsewhere (Rocci A *et al.*, *Leukemia* 2014). High and low expression of miRNAs were defined using the 233 patients median value as a cut-off. We defined high risk FISH as the presence of del(17p) or t(4;14) or t(14;16) and standard risk as all other patients. Proportional hazard regression model was used, stratified by maintenance (to remove the effect of different chemotherapy regimens), with ISS treated as time-dependent covariate.
Results: We analyzed serum from 233 evaluable patients, with a median follow-up of 71 months. Patient's characteristics are reported in Table 1 and are balanced by age, treatment received and maintenance. Ten miRNAs were significantly expressed in serum (≥100 counts in at least 20% of patients) and confirmed by miRNA qRT-PCR. High expression of circulating miR-16 was confirmed as a strong predictor of better PFS (median PFS 32 *vs* 26 months, p=0.020) and OS (median OS unreached *vs* 58 months, p=0.006) when compared to low miR-16 in univariate analyses. High expression of miR-25 showed a trend for improved survival (PFS p=0.063; OS p=0.064). In multivariate analysis including age (>75 yrs *vs* ≤75 yrs), FISH (standard *vs* high risk), isolated Del17 by FISH, and ISS (I *vs* II and I *vs* III), miR-16 was a strong predictor of OS (p=0.014). Grouping miR-16, ISS and FISH identified those patients with a poor prognosis (miR-16 low, ISS 3, high risk FISH) *versus* good prognosis (miR-16 high, ISS 1, standard risk FISH) with a median OS of 18.4 months *vs* unreached respectively (p=0.0003). MiR-16 expression divides patients with standard FISH (n=127) into those with good outcome (high miR-16, 5 yrs OS: 68%) and those with poor outcome (low miR-16, 5 yrs OS: 47%, p=0.012). High miR-16 identifies patients with better outcome independently from treatment arm, however its effect was strongest in patients receiving VMPT-VT: patients with high miR-16 have a better 5-years PFS (38% *vs* 21%, p=0.07) and 5-year OS (76% *vs* 47%, p=0.0009).

Table 1.

Patients	233
Age	
Median (range)	71 (56 – 85)
Gender	
Feale, %	49.8%
Male, %	50.2%
B2M (mg/L)	
Median (range)	4 (0.2 – 24.8)
≤ 3.5	74
>3.5	119
Data missing	40
Albumin (g/L)	
Median (range)	3.7 (1.3 – 5.1)
Data missing	30
ISS	
1	53 (28%)
2	77 (41%)
3	56 (31%)
Data missing	47
Creatinine (mg/dl)	
Median (range)	1.0 (0.5 -2.5)
Data missing	0
Hemoglobin (g/dL)	
Median (range)	10.5 (5.9 - 15.7)
Data missing	22
LDH (U/L)	
Median (range)	280 (106 – 1283)
Data missing	36
FISH abnormalities	
del13	98 (52%)
del17	29 (15%)
t(4;14)	38 (20%)
t(11;14)	31 (16%)
t(14;16)	9 (5%)
High risk	60 (32%)
Treatment Arm	
VMP	116 (50%)
VMP-VT	117 (50%)

Abbreviations: B2M - β_2-microglobulin; ISS - International Staging System; LDH - lactate dehydrogenase; FISH - Fluorescent *in-situ* hybridization; VMP - Bortezomib-Melphalan-Prednisone; VMPT-VT - Bortezomib-Melphalan-Prednisone-Thalidomide followed by maintenance therapy with Bortezomib-Thalidomide

Summary and Conclusions: Circulating miR-16 is a strong prognostic factor in newly diagnosed MM patients. It independently predicts overall survival in a model that includes ISS and FISH abnormalities. MiR-16 is independent from ISS and FISH and these three covariates together dichotomize the population in terms of overall survival.

S479

TARGETING EPITHELIAL TO MESENCHYMAL TRANSITION (EMT) THROUGH CXCR4 BLOCKADE IN MULTIPLE MYELOMA

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Background: Multiple osteolytic lesions are one of the main clinical features of patients with multiple myeloma (MM), thus suggesting the ability of clonal plasma cells to disseminate from bone to bone. The bone marrow (BM) homing process of MM cells is supported by the CXCR4/CXCL12 axis activation. Nevertheless, the role of CXCR4 in mediating MM cell bone metastasis, and the role of CXCR4-targeted therapy in inhibiting MM cell dissemination has not been previously reported.
Aims: 1) To define the pro-metastatic role of CXCR4 *in vivo*; 2) to investigate the mechanisms of CXCR4-mediated EMT both *in vitro* and *in vivo*; 3) and to test the anti-CXCR4 monoclonal antibody (Ulocuplumab) in inhibiting CXCR4-mediated EMT phenotype *in vivo*.
Methods: Gene set enrichment analysis and Atlas of Cancer Signaling Networks were used to study publically available gene expression dataset of primary BM-derived MM plasma cells (GSE24080; n=577; FDR<0.25; P<0.05). The functional relevance of CXCR4 in mediating MM cell dissemination was studied by generating CXCR4-gain and -loss-of-function expressing cell lines, using an *in vivo* model of MM cell dissemination from bone to bone. Modulation of EMT-related genes and proteins was tested both *in vitro* and *ex vivo*, using qRT-PCR, western blot, confocal miscroscopy, IHC and flow cytometry. The anti-tumor effect of Ulocuplumab was tested both *in vitro* and in an *in vivo* model of MM cell bone metastasis, bioluminescence imaging (BLI) and intravital confocal miscroscopy.
Results: We categorized BM-MM-derived CD138+ cells (GSE24080; n=577) according to their CXCR4 mRNA expression; and identified an EMT-related pathway to be significantly activated in high-CXCR4- *versus* low-CXCR4-BM-MM CD138+ cells. We next performed CXCR4-gain-of-function studies and demonstrated that CXCR4+ cells presented with changes in actin cytoskeleton, with protrusion of cell pseudopodia, sustained by modulation of EMT-related markers (Slug/Snail/Twist up-regulation; E-cadherin down-regulation), compared to control cells. These findings were recapitulated using an *in vivo* model of MM cell bone-to-bone metastasis: $CXCR4^+$ MM cells showed higher levels of bone-to-bone metastasescompared to control cells, as confirmed on harvested host femurs. Lower expression of human (h)-E-cadherin, and higher expression of h-Twist/h-Snail/h-Slug was confirmed within the BM of the host femurs. The monoclonal antibody, anti-CXCR4 (Ulocuplumab) exerted an anti-MM activity *in situ*, within the s.q. implanted bones; and also reduced MM cell dissemination from the implanted bone to the host bone. Importantly, Ulocuplumab modulated EMT-related genes in the MM cells that metastasized to the host bones, with enhanced mRNA expression of human (h)-E-cadherin, and reduced expression of h-Twist/h-Snail/h-Slug and h-CXCR4 in the BM cells harvested from the host bones of Ulocuplumab-treated mice. Similar findings were confirmed in an *in vivo* breast cancer model (MDA-MB-231 intra-cardiac injected). CXCR4-loss of function in MM cells led to inhibited BM homing and tumor growth *in vivo*; with prolonged survival compared to control mice injected with scramble-transfected control cells. Moreover, Ulocuplumab led to inhibition of MM tumor growth *in vivo*, using MM.1S- and RPMI.8226- xenograft s.q.models; with a synergistic effect when used in combination with lenalidomide or bortezomib.
Summary and Conclusions: Our study supports the pro-metastatic role of CXCR4, due to its effect in mediating EMT in both MM and breast cancer *in vivo* models, thus suggesting the potential role for using CXCR4-neutralizing agents in order to delay or prevent tumor cell metastasis to bones.

S480

OVEREXPRESSION OF ARGINASE AND PROK-2 MAKES NEUTROPHILS OF MULTIPLE MYELOMA DYSFUNCTIONAL AND IMMUNOSUPPRESSIVE

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Background: Multiple Myeloma (MM) is a plasma cell malignancy with a well documented immune dysfunction. However, the function of neutrophils in MM and monoclonal gammopathy of undetermined significance (MGUS) has been poorly investigated.
Aims: The objective of this investigation was evaluating the activation status of peripheral neutrophils (N) isolated from MM and MGUS patients.
Methods: Using oligonucleotide microarrays we first evaluated the gene expression profile (GEP) of granulocytes at the steady state in 10 MM, 5 MGUS and 8 healthy subjects matched for sex and age, identifying Arg-1 and PROK-2 among the first 10 genes differentially expressed in MM *versus* healthy granulocytes. Thus, we validated Arg-1 and PROK-2 by RT-PCR in a series of 60 newly-diagnosed MM patients, 30 MGUS and 30 healthy subjects. Activation status on N was then investigated in the same series, evaluating phagocytic and burst activity by commercial kit, surface expression of CD64, CD16, CD62L and CD11b, markers of neutrophil activation and reactive oxygen species (ROS) by flow cytometer. Finally, we tested the immunosuppressive properties of N isolated from MGUS or MM patients, through functional assays, based on *in vitro* co-culture of N isolated from patients and T-lymphocytes from healthy subjects.
Results: MM-N exhibit an increased expression of ARG-1 compared to MGUS and healthy subjects (25.5 *vs* 6.2 *vs* 1 fold changes in gene expression, p=0.003), confirmed by functional assay of enzymatic activity of ARG-1, positively correlated with advanced disease. PROK-2 expression was two times higher in MGUS than healthy subjects (p=.02) and up to ten times higher in MM (p=.001). In MM patients, increased levels of PROK-2 were positively associated with advanced bone disease and unfavourable cytogenetics. The phagocytic activity was reduced in MM-N compared to HS-N and MGUS-N (46% *versus* 75% *versus* 72%, p<0.0001 and p<0.0001 respectively). Oxidative burst was 71% in MM-N, lower than HS-N (85%, p=0.01) and MGUS-N (86%, p=0.01). The MM-N exhibit higher levels of ROS (MFI 113±9) compared to HS-N (MFI 46±7) and MGUS-N (MFI 62±8, p<0.0001 and p=0.0001 respectively). Expression of CD64 was significantly elevated in MM-N compared to MGUS-N or healthy subjects-N (p=0.01 and p=0.007 respectively) and was inversely correlated with phagocytic activity (p=0,01). After 72 hours, MM-N were able to reduce T-cell proliferation at both tested 1:2 and 1:8 ratios, while MGUS-N could induce a similar effect only at the highest ratio 1:8. This effect was partially reverted with the treatment of 200 μM nor-NOHA, an Arg-1 inhibitor.
Summary and Conclusions: Compared to controls, neutrophils obtained from MM patients have a reduced phagocytic activity, a greater expression of Arg-1 and PROK-2 and exhibit an immunosuppressive function on T lymphocytes. These features are only minimally present in neuthophils from MGUS patients

Optimization and innovation in treating aggressive lymphomas

S481

THE B CELL RECEPTOR SIGNALING OUTPUT IN BURKITT'S LYMPHOMA IS GENOTYPE-SPECIFIC AND IMPACTS SENSITIVITY TOWARDS BCR SIGNALING INHIBITORS

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Background: B cell antigen receptors (BCR) control important cell fate decisions of the B-lineage such as differentiation, proliferation and B cell survival. Recently, it was demonstrated that major subgroups of Burkitt lymphomas (BL) critically depend on BCR expression. The current treatment regimens consist of intensive chemotherapy in combination with anti-CD20 antibodies and show significant toxicity. Pharmacological interference with critical BCR signaling pathways might open up the possibility for targeted therapies in BL that are needed in particular for elderly patients and patients with endemic BL in developing countries.
Aims: Our study aims (i) to identify drug targets in BL, (ii) to validate selected drug targets *in vitro* and *in vivo* and (iii) to elucidate BCR induced processes.
Methods: We performed mass-spectrometry-based phosphoproteomics, transcriptome sequencing and bioinformatic analyses to elucidate BCR signaling networks in BL cell lines and patient-derived BL cells. Moreover, we validated identified drug targets using a compound library and BL xenograft models. Detailed functional analyses were performed for identifying the role of HSP90 in BL.
Results: We identified and quantified about 10.000 phospho-sites in various BL cell-lines and patient-derived BL cells, of which more than 1000 phospho-sites in almost 600 proteins were regulated in a BCR-dependent manner leading to dynamic transcription of more than 100 genes. The identified BCR signaling effectors belong to various functional groups, of which kinases, transcription factors and cytoskeleton regulators are most represented and bioinformatic analyses revealed that 83% of the identified BCR effectors were not described in the context of B cells or lymphoma before. Our data-sets allowed to investigate the dynamic interplay between receptor-triggered kinase activation, phosphorylation events and mRNA-/protein expression changes. While BCR-proximal signaling networks were concordantly regulated among genetically distinct BL cell-types, BCR-distal signaling turned out to be genotype-specific. Based on the detailed signaling information we established genotype-specific kinase/substrate/transcriptome networks that provide functional insights and information about druggable BCR-signaling pathways in BL. Predicted drug targets were furthermore validated in more than twenty BL cell lines using a compound screen that allowed us to correlate BCR signaling profiles with responsiveness to compounds targeting BCR signaling. For instance, heat shock protein 90 (HSP90) inhibition by small molecule inhibitors such as AT13387 induced apoptosis in most analyzed BL cell models and BL xenografts. This was achieved by disruption of BCR signal initiation due to destabilzation of spleen tyrosine kinase expression that turned out to critically depend on HSP90 function in BL. In contrast, compounds like Bruton's tyrosine kinase and protein kinase B inhibitors targeting the more heterogenous BCR-distal signaling networks induced apoptosis only in certain BL cell-types.
Summary and Conclusions: Taken together our study is a considerable complement to recent genetic studies that elucidated the mutational landscape in BL as it reveals mechanisms of signaling addiction to non-mutated druggable BCR effector proteins. Their further (pre)clinical evaluation as drug targets seems warranted.

S482

THE PRESENCE OF GENOMIC IMBALANCES IS ASSOCIATED WITH WORSE OUTCOME IN PATIENTS WITH BURKITT LYMPHOMA TREATED WITH DOSE-INTENSIVE CHEMOTHERAPY INCLUDING RITUXIMAB

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Background: The introduction of intensive cycles of high-dose immunotherapy with rituximab has improved the outcome and survival rates in patients with Burkitt lymphoma (BL). However, early relapse and refractoriness are current limitations of BL treatment and new biological factors affecting the outcome of the patients treated with dose-intensive chemotherapy including Rituximab have not been explored.
Aims: To characterize by array-based comparative genomic hybridization (aCGH) and next generation sequencing (NGS) the presence of genetic changes that could be predictive of the clinical response to Rituximab in a selected group of BL patients included in the Dose-Intensive Chemotherapy Including Rituximab (Burkimab trial) from the Spanish PETHEMA cooperative group.
Methods: Forty adolescent and adult BL patients were included. All of them were homogeneously treated according to Burkimab trial established by the PETHEMA group. The inclusion criteria were: a) diagnosis of BL or Burkitt-like lymphoma according to WHO criteria, b) *MYC*-rearrangement demonstrated by karyotype and/or fluorescence *in situ* hybridization c) age greater than 14 years. The segmentation analysis was performed using CGHweb tool. In addition, amplicon-based NGS was carried out to investigate the mutational status of *TP53* gene (4 to 11 exons). The 454 Life Sciences, a Roche company GS Junior system was used. The variant analysis was performed using Amplicon Variant Analyzer (AVA-Roche 454) and Sequence Pilot (JSI Medical Systems) software. The genomic alterations were associated with clinical, biological and survival parameters.
Results: CNAs were present in 97.5% of patients. The most common aberrations were gains on chromosomes 1q (33%), 7q (31%) and 8q (18%), whereas the losses predominantly involved chromosomes 9p (23%), 13q (23%), 15q (21%), 11q (15%) and 6q (10%). The losses on 11q, 13q, 15q or 17p (p=0.028) and poor performance status (ECOG≥2) (p=0.016) was associated with the poor response Burkimab. These losses were also associated with shorter four-year progression free survival (PSF) (50% *vs* 93%, p=0.012). Shorter DFS was observed in BL patients with losses on 11q (p=0.003), 15q (p=0.002) or 17p (p=0.03). The four-year DFS of patients with these losses was 56% *versus* 93% for patients without losses. Likewise, BL patients with losses on 11q (p=0.001) or 15q (p=0.02) had shorter OS than patients without these abnormalities; the four-year OS of patients with these losses was 60% *vs* 96% for patients without them. The ECOG≥2 was associated with a shorter PFS (p=0.02), DFS (p=0.036) and OS (p=0.012). The losses on 11q, 13q, 15q or 17p (HR, 5.506; 95%CI, 1.283-23.624; p=0.022) and ECOG≥2 (HR, 8.605; 95%CI: 1.044-70.900; p=0.045) were independent risk factors associated with shorter PFS. Losses in 11q, 15q or 17p were identified as risk factors independently associated with shorter DFS (HR, 4.921; 95%CI: 1.0-22.4; p=0.040) and OS (HR, 7.2; 95%CI: 1.2-41.5; p=0.026). In addition, the integrative analysis of aCGH and NGS showed that 26% (5/19) of patients carried at least one alteration in *TP53* gene, two cases had loss of 17p and three cases had missense mutations. *TP53*alt was associated with a poor response to Burkimab therapy (p=0.044) and a shorter four-year PFS: 84.6% *vs* 25% (p=0.022).
Summary and Conclusions: Losses of 11q, 13q, 15q, or 17p, as well as *TP53*alt are associated with a poor response to Dose-Intensive Chemotherapy Including Rituximab (Burkimab trial). These non-random losses could be used for the risk stratification of BL patients treated with Rituximab.

S483

SUBCUTANEOUS *VERSUS* INTRAVENOUS RITUXIMAB IN COMBINATION WITH CHOP FOR PREVIOUSLY UNTREATED DIFFUSE LARGE B-CELL LYMPHOMA: EFFICACY AND SAFETY RESULTS FROM THE PHASE IIIB MABEASE STUDY

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Background: Subcutaneous rituximab (R^{SC}) offers improved patient (pt) convenience and healthcare resource savings *versus* intravenous R (R^{IV}), with similar efficacy and safety outcomes in the SABRINA study in follicular lymphoma (FL). R^{SC} is approved in Europe and other countries for use in FL and diffuse large B-cell lymphoma (DLBCL).
Aims: To confirm the efficacy and safety of R^{SC} *versus* R^{IV} plus CHOP (cyclophosphamide, doxorubicin, vincristine and prednisone) for the treatment of DLBCL.
Methods: The open-label, randomised MABEASE study (NCT01649856) investigated the efficacy of R^{SC} *versus* R^{IV} plus CHOP in untreated DLBCL. Pts were randomised 2:1 to receive R^{SC} (R^{IV} 375 mg/m^2 cycle 1; R^{SC} 1400 mg fixed dose cycles 2-8) or R^{IV} (R^{IV} 375 mg/m^2 cycles 1-8), plus 6 or 8 cycles of CHOP every 14 or 21 days. Responses (complete response [CR], unconfirmed CR [CRu], partial response [PR], progressive disease [PD]) were determined by investigator assessment (Cheson 1999 criteria). Administration-related reactions (ARRs) were defined as R-related adverse events (AEs) that occurred ≤24 hours of administration. The primary endpoint was response rate at end of treatment (EOT). Safety was a secondary endpoint.
Results: In total, 576 pts were randomised (R^{SC} n=381; R^{IV} n=195; intent-to-treat [ITT] population); baseline characteristics were balanced between arms. A total of 572 pts (R^{SC} n=378; R^{IV} n=194) received ≥1 dose of R. Nine R^{SC} pts discontinued after cycle 1, due to AE (n=5), withdrawn consent (n=2), PD and protocol violation (both n=1); as both arms received R^{IV} during cycle 1, these pts were included in the R^{IV} arm for safety analyses (R^{SC} n=369; R^{IV} n=203). At EOT (R^{SC} n=342; R^{IV} n=177), response rates were similar between arms in the ITT population overall (R^{SC} 52%; R^{IV} 51%) and when pts were stratified by age (~50% for all groups; Table 1). AE rates were comparable between the R^{SC} and R^{IV} arms overall (92% *vs* 91%) and during cycle 1 (61% *vs* 64%); the most common AE overall was neutropaenia (R^{SC} 36%; R^{IV} 33%). During cycles 2-8, R^{SC} and R^{IV} pts had similar rates of grade ≥3 AEs (53% *vs* 50%) and serious AEs (SAEs; 34% *vs* 31%); the most frequent grade ≥3 AE was neutropaenia (R^{SC} 22%; R^{IV} 20%) and the most common SAE was febrile neutropaenia (R^{SC} 11%; R^{IV} 6%). ARR rates were similar between R^{SC} and R^{IV} pts overall (28%/arm), during cycle 1 (11% *vs* 14%) and cycles 2-8 (22% *vs* 20%). From cycle 2-8, the most common ARR by System Organ Class (SOC) was general disorders and administrative site conditions (R^{SC} 10%; R^{IV} 4%). Rates of grade ≥3 ARRs was similar between arms overall (2% *vs* 5%), during cycle 1 (0% *vs* 2%) and cycles 2-8 (2% *vs* 4%); from cycles 2-8, the most common grade ≥3 ARR by SOC was blood and lymphatic system disorders (R^{SC} 1%; R^{IV} 3%). In total, 52% (R^{SC}) and 54% (R^{IV}) of pts had R-related AEs; the most frequent R-related AE was neutropaenia (R^{SC} 14%; R^{IV} 16%). Overall, 8% (R^{SC}) and 9% (R^{IV}) of pts discontinued R due to AEs, most commonly infections (R^{SC} 2%; R^{IV} 3%). In total, 63 pts (11%) died (R^{SC} 10%; R^{IV} 13%). AEs led to death in 6% (R^{SC}) and 7% (R^{IV}) of pts, most commonly due to infections (2%/arm).

Table 1. Response rates at EOT.

Pts, n (%)		CR/CRu	PR	PD
ITT	R^{SC} (n=342)	178 (52)	103 (30)	13 (4)
	R^{IV} (n=177)	90 (51)	56 (32)	9 (5)
Age <60 years	R^{SC} (n=138)	64 (46)	48 (35)	7 (5)
	R^{IV} (n=74)	38 (51)	22 (30)	4 (5)
Age ≥60 years	R^{SC} (n=204)	114 (56)	55 (27)	6 (3)
	R^{IV} (n=103)	52 (50)	34 (33)	5 (5)

Summary and Conclusions: R^{SC} and R^{IV} plus CHOP had similar efficacy and safety outcomes in previously untreated DLBCL. R^{SC} offers convenience for pts and efficient utilisation of healthcare resources.

S484

MANAGEMENT AND OUTCOME OF PRIMARY CNS LYMPHOMA AT FIRST RELAPSE/PROGRESSION: ANALYSIS OF 256 PATIENTS FROM THE FRENCH LOC NETWORK

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Background: A significant proportion of primary CNS lymphoma (PCNSL) patients is refractory or relapses after first line therapy. Clinical presentation and therapeutic management of patients with relapsed/refractory (R/R) PCNSL are heterogeneous.
Aims: The aim of this study was to analyze the characteristics, management, and outcome of R/R PCNSL patients after first-line therapy.
Methods: We analyzed patients with R/R PCNSL who had been prospectively registered in the database of the French LOC network between 2011 and 2014.
Results: Among 563 PCNSL patients registered in the LOC database, we identified 256 patients with relapsed (N=93, 16.5%) or refractory (N=163, 29.0%) disease after a median follow-up of 9 [0.3-43.0] months. Median age was 68 [26-93] years. Most patients (92.6%) had received a methotrexate-based chemotherapy as first line treatment. First relapse/progression occurred after a median progression-free survival from diagnosis (PFS1) of 5.1 [0.3-35.8] months. Relapse/progression was asymptomatic in 25.5% of the cases, mostly diagnosed on systematic neuroimaging. Overall survival after relapse/progression (OS2) was 3.5 [0-29.6] months for the entire cohort. At first relapse/progression, 28.2% of the patients received palliative care. All of them died within 5 months with a median OS2 of 0.6 months. The remaining patients (71.8%) received salvage chemotherapy (methotrexate, cytarabine or ifosfamide-based regimens) without (79.5%) or with (20.5%) consolidation therapy consisting in radiotherapy (14.7%) or intensive chemotherapy followed by autologous stem cell transplantation (ICT+ASCT) (85.3%). Survival was significantly longer in patients receiving consolidation therapy with a median PFS2 (PFS from first relapse/progression) of 13.5 *vs* 2.6 months and a median OS2 not reached *vs* 6.7 months (p<0.01). In patients receiving ICT+ASCT, 44.8% (13/29) experienced a PFS2 longer than their PFS1. Survival was significantly worse in refractory patients and in relapsed patients with a PFS1<1 year (median OS2=2.1 and 3.7 months, respectively) compared to relapsed patients with a PFS1>1 year (median OS2 not reached, p<0.01) (Figure 1). Other prognostic factors were age (<*vs* >60 years), Karnofsky index (KI, >*vs* <70%), administration of a salvage therapy, and administration of Rituximab. In multivariate analysis, three prognostic factors remained statistically independent: performance status (KI), duration of first remission (PFS1) and administration of a salvage therapy.

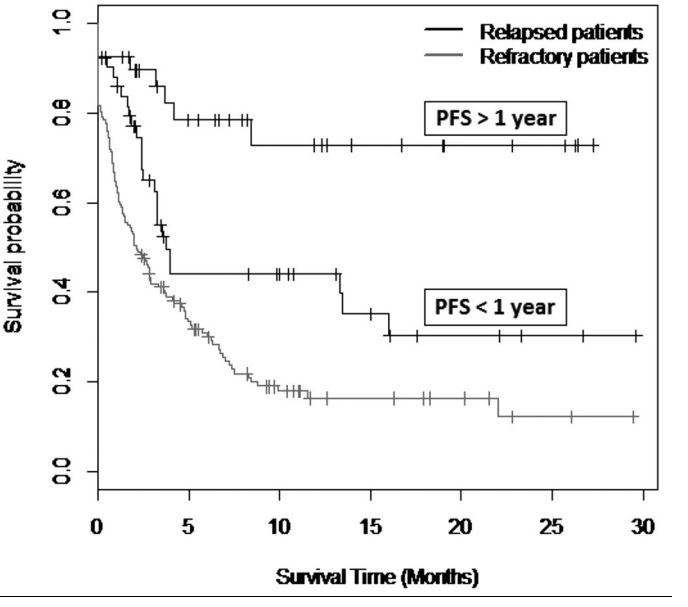

Figure 1.

Summary and Conclusions: One fourth of R/R PCNSL are asymptomatic underlining the need for systematic neuroimaging in surveillance. Duration of first remission (PFS1) is a strong prognostic factor. Salvage chemotherapy followed by consolidation with ICT+ASCT is associated with prolonged remission in a subset of patients.

S485

PATIENTS WITH HEAVILY PRETREATED DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL) WHO RESPOND TO ORAL SELINEXOR THERAPY SHOW PROLONGED SURVIVAL: UDPATED PHASE 1 RESULTS

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Background: The nuclear export protein XPO1 (CRM1) is overexpressed in all types of malignant lymphoma including DLBCL. Selinexor is a selective inhibitor of nuclear export (SINE) that inhibits XPO1 to force nuclear retention & activation of >10 tumor suppressor proteins such as p53, IkBa, FOXO & p21. Selinexor also reduces levels of c-myc, BCL2 & other oncoproteins. Current therapy for DLBCL leads to cures in >50% of patients (pts) after 1-2 lines of therapy. Pts with relapsed/refractory DLBCL have a very poor prognosis with expected median survival <1 year.
Aims: We previously reported efficacy & safety of selinexor in pts with relapsed/refractory Non-Hodgkin's Lymphoma (NHL) (J. Kuruvilla *et al*. ASH 2014). Here we correlate response to selinexor with progression-free (PFS) & overall (OS) survival of pts with heavily pretreated DLBCL.
Methods: Selinexor was administered orally (3-80 mg/m^2) for 8 or 10 doses in a 28-day cycle in this phase 1 study (NCT01607892). Response evaluation was performed in cycle 1 and 2 & then every 2 cycles. Comparison of survival rates was done by Kaplan Meier analysis using 10-weeks timepoint as landmark. All pts had heavily pretreated DLBCL (prior R-CHOP & additional therapies) with documented progressive disease on study entry.
Results: In 31 (of 33) pts with heavily pretreated DLBCL, the median number of prior therapies was 3 (range 1-11). The most common adverse effects (Grade 1/2) occurring *after* cycle 1 are fatigue (43%), anorexia (38%), nausea (29%), & dysgeusia (24%). Grade 3/4 adverse events occurring in ≥2 pts are thrombocytopenia (62%), neutropenia (24%), anemia (24%), fatigue (10%), hyponatremia (10%), syncope (10%), & confusion (10%). These are managed with dose interruption, platelet stimulators and/or standard supportive care. Among 31 pts treated with at least 1 cycle of selinexor, the overall response rate was 39% (12/31) with a median duration of response of ~7 months. 8 pts (26%) achieved partial response (PR), & 4 pts (13%) complete response (CR) confirmed by PET scan. Selinexor showed similar activity in GCB & non-GCB subtypes of DLBCL. The overall median PFS & OS for pts on study ≥10 weeks were 104 days & 155 days, respectively. In order to determine if best response of CR or PR to selinexor was associated with improved clinical outcomes, the PFS & OS of responding pts were compared with those of pts with stable (SD) or progressive (PD) disease. Comparison of OS & PFS for pts with objective responses *vs* those with SD & PD revealed a strong correlation between response & survival (N=24): Median PFS of pts with CR/PR *vs* SD/PD were 329 & 49 days respectively (p<0.001). Median OS of pts with CR/PR *vs* SD/PD were 571 & 108 days, respectively (p<0.01). Furthermore, 4 pts with CR remained on study for 217, 505+, 525+, & 650+ days.
Summary and Conclusions: Selinexor monotherapy shows significant anticancer activity in pts with heavily pretreated relapsed/refractory DLBCL. Objective responses to selinexor are durable & correlate with improved OS & PFS compared with pts who achieve SD or PD, suggesting that these responses are associated with clinical benefit. Clinical & molecular predictors of objective response to selinexor are being evaluated. A study of selinexor monotherapy (pts randomized to high *versus* low dose) in pts with heavily pretreated DLBCL is ongoing & combination studies are being initiated.

CML: Clinical trials

S486

EFFICACY AND SAFETY OF FRONTLINE NILOTINIB IN 1089 EUROPEAN PATIENTS (PTS) WITH CHRONIC MYELOID LEUKEMIA IN CHRONIC PHASE (CML-CP): ENEST1ST FINAL ANALYSIS

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Background: The Evaluating Nilotinib Efficacy and Safety in Clinical Trials as First-Line Treatment (ENEST1st) study evaluated the efficacy and safety of nilotinib in a large population of pts with newly diagnosed CML-CP using a network of 14 European Treatment and Outcome Study (EUTOS) standardized laboratories to monitor molecular response (MR).
Aims: Final results of ENEST1st, evaluated after all pts completed 24 mo of treatment or discontinued early, are reported.
Methods: ENEST1st was a phase 3b, open-label, multicenter study of nilotinib 300 mg twice daily in adults with newly diagnosed CML-CP. The primary endpoint was the rate of MR^4 (*BCR-ABL* ≤0.01% on the International Scale [*BCR-ABL*^{IS}] or undetectable *BCR-ABL* in cDNA with ≥10,000 *ABL* transcripts) at 18 mo. Results from 11 ancillary substudies, driven by national investigators and local study groups, will help shape the future management of CML. All pts provided written informed consent before entering the study.

Table 1.

Response	Nilotinib 300 mg Twice Daily 18 mo	24 mo
MR^4 at 18 or 24 mo, %		
All (n = 1052)[a]	38.4	40.4
Low EUTOS score (n = 876)[b]	38.9	41.4
High EUTOS score (n = 90)[b]	28.9	27.8
Low Sokal score (n = 367)[b]	44.4	44.7
Intermediate Sokal score (n = 395)[b]	35.7	39.7
High Sokal score (n = 191)[b]	29.3	31.4
Cumulative incidence by 18 mo or 24 mo, %		
MMR		
All (n = 1052)[a]	77.2	80.4
BCR-ABL^{IS} ≤1% at 3 mo (n = 615)[b]	91.4	93.0
BCR-ABL^{IS} >1% to ≤10% at 3 mo (n = 145)[b]	43.4	55.9
BCR-ABL^{IS} >10% at 3 mo (n = 23)[b]	21.7	34.8
MR^4		
All (n = 1052)[a]	48.7	55.2
BCR-ABL^{IS} ≤1% at 3 mo (n = 615)[b]	59.0	67.6
BCR-ABL^{IS} >1% to ≤10% at 3 mo (n = 145)[b]	20.0	24.1
BCR-ABL^{IS} >10% at 3 mo (n = 23)[b]	0	0
MR$^{4.5}$		
All (n = 1052)[a]	31.7	38.6
BCR-ABL^{IS} ≤1% at 3 mo (n = 615)[b]	39.5	47.0
BCR-ABL^{IS} >1% to ≤10% at 3 mo (n = 145)[b]	9.0	14.5
BCR-ABL^{IS} >10% at 3 mo (n = 23)[b]	0	0

MMR, major molecular response (*BCR-ABL*^{IS} ≤0.1%); MR$^{4.5}$, *BCR-ABL*^{IS} ≤0.0032% or undetectable *BCR-ABL* in cDNA with ≥32,000 *ABL* transcripts.
[a] Pts with typical b2a2 and/or b3a2 *BCR-ABL* transcripts and ≤3 mo of prior imatinib.
[b] n = number of pts in the indicated subgroup.

Results: In 26 European countries, 1089 pts were treated. Median age was 53 y, 59.0% of pts were male, 90.3% were Philadelphia chromosome positive, 97.0% had typical b2a2 and/or b3a2 *BCR-ABL* transcripts, and 16.9% were previously treated with imatinib for ≤3 mo. EUTOS scores were low in 82.6% and high in 8.6% of pts (8.7% missing). Sokal risk scores were low, intermediate, and high in 34.6%, 37.5%, and 18.1% of pts, respectively (9.8% missing). A total of 80.9% of pts completed 24 mo of treatment; 19.1% discontinued

early, most frequently due to adverse events (AEs; 11.7%). The MR[4] rate at 18 mo in all treated pts with typical transcripts and ≤3 mo of prior imatinib (n=1052) was 38.4% (95% CI, 35.5%-41.3%). Among pts with typical transcripts and without prior exposure to imatinib, 70.5%, 16.6%, and 2.6% of pts, respectively, achieved *BCR-ABL*^{IS} ≤1%, >1% to ≤10%, and >10% at 3 mo. MR rates are further detailed in the Table 1. Six pts (0.6%) progressed to accelerated phase/blast crisis (AP/BC) on study; 13 pts (1.2%) died by 24 mo, including one due to CML progression (16 mo after study drug discontinuation). The most common AEs of any cause were rash (21.4%), pruritus (16.5%), and headache (15.2%). Peripheral artery disease occurred in 1.9% of pts, ischemic heart disease in 3.4%, and ischemic cerebrovascular conditions in 0.8%; 0.6% of pts had pleural effusions. Grade 3/4 AEs related to hepatotoxicity and pancreatitis occurred in 0.4% and 0.6% of pts, respectively. Grade 3/4 thrombocytopenia and neutropenia occurred in 6.0% and 4.8% of pts, respectively. The most frequently observed grade 3/4 biochemical abnormalities were decreased phosphate (14.3%) and increased lipase (7.2%); glucose and lipid monitoring was not mandated in the study protocol.

Summary and Conclusions: In this large study, frontline nilotinib yielded rapid and high rates of response and a very low rate of progression to AP/BC; however, some pts discontinued treatment by 24 mo. As assessed with multicenter molecular monitoring, MR rates in this study provided prospective confirmation of the centrally reviewed MR rates in the pivotal ENESTnd study. This process contributed to the development of standardized definitions for deep MR. Despite the higher median age of pts in ENEST1st than in prior studies, safety results were consistent with the known safety profile of nilotinib. Relatively low rates of the most common AEs may reflect improvements in the management of nilotinib-treated pts. These results support the use of frontline nilotinib 300 mg twice daily in pts with CML-CP.

S487

ANALYSIS OF TREATMENT AND OUTCOME DATA OF 2904 PATIENTS FROM THE EUTOS POPULATION-BASED REGISTRY

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Background: Most of the knowledge about treatments and outcomes of patients with chronic myeloid leukemia (CML) originates from clinical trials. To get new and unbiased insights the European Treatment and Outcome Study (EUTOS) for CML collected population based data in 20 European countries.

Aims: The population-based registry was set up inside the infrastructure of the EUTOS to further explore the epidemiology, patient and disease characteristics, treatment and outcomes of CML in Europe. This work focuses on the treatment and early outcomes of CML in Europe.

Methods: The population-based registry aimed to identify and to document all newly diagnosed adult patients with Ph+ and/or BCR-ABL+ CML at any stage of disease in whole countries or specified regions in Europe. First-line treatment and treatment changes were documented. Cytogenetic responses are analyzed using cumulative incidences considering death and progression as competing risks.

Results: Overall 2904 Ph+ and/or BCR/ABL1+ adult CML-patients were registered. 94% of the patients were diagnosed in chronic phase (CP), 3.5% in accelerated phase (AP) and 2% in blastic phase (BP). 57% of patients were male. The median age was 56 years (18-99). Clonal chromosomal abnormalities (CCAs) in Ph+ cells were found in 11% of patients. 12% of the patients were at high risk of not achieving CCyR 18 months after start of therapy according to the EUTOS score. According to the Euro score 38% of patients were low risk, 51% intermediate risk and 11% high risk. The follow-up data of 2499 and the first-line therapy of 1986 patients were documented, for 1954 patients both, first-line therapy and follow-up is known. As first-line therapy 81% of patients received imatinib, 12% nilotinib, 3% dasatinib and 4% a treatment based on HU. Patients with high EUTOS or Euro risk and patients in AP and BP did not receive second generation tyrosine kinase inhibitors (TKIs) as a frontline therapy more often than patients with low EUTOS risk or Euro low and intermediate risk or in chronic phase (16% EUTOS low risk *vs* 13% EUTOS high risk, 16% euro low risk *vs* 14% Euro high or intermediate risk, 16% in CP *vs* 12% in AC or BP received second generation TKIs). Patients with CCAs in Ph+ cells, however, received more often second generation TKIs (22% *vs* 15%). Median time to first complete cytogenetic remission (CCyR) was 8 months for all patients. There were major differences observed between different EUTOS classes (high: 13 months *vs* low: 8 months), CCAs in Ph+ cells (with CCAs: 10 months *vs* without CCAs: 8 months) and treated with different medications first-line (Imatinib: 8 months *vs* second generation TKIs: 5 months). Survival probabilities 12 and 24 months after diagnosis were 95% and 92%, respectively. Stratified by Euro score the survival probability at 12 months was 97% for Euro low and intermediate risk patients and 96% for Euro high risk patients. At 24 months the survival probability was 96%, 93% and 91%, for Euro low, intermediate and high risk, respectively.

Summary and Conclusions: This is the first analysis of the treatment and outcome data from the EUTOS population-based registry, which provides an unselected sample of Ph+ and/or BCR/ABL1+ adult CML patients in Europe. Imatinib is the first choice in treatment of CML, while second generation TKIs as a first-line treatment are not very common. Median time to CCyR in the population is comparable to the one reported in patients enrolled in prospective randomized trials, while survival at 12 and 24 months are lower probably due to the selection of patients that are included in clinical trials.

S488

LONG-TERM OUTCOME OF ALTERNATING NILOTINIB 400 MG TWICE DAILY AND IMATINIB 400 MG ONCE DAILY AS FIRST-LINE TREATMENT OF CHRONIC MYELOID LEUKEMIA: A PHASE 2 STUDY OF THE GIMEMA CML WORKING PARTY

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Background: Imatinib (IM) is the standard treatment for Ph+ Chronic Myeloid Leukemia (CML) in early Chronic Phase (ECP). Nilotinib (NIL) has demonstrated superior efficacy to IM (phase 3 ENESTnd trial). The treatment with more than one TKI, according to the principles of cancer poly-chemotherapy, may improve the response rates and may decrease the frequency of drug-resistance. The combination of different TKIs is potentially toxic, difficult to be explored in the ECP setting, while the alternating administration of IM and NIL could be better tolerated.

Aims: To evaluate the response (either cytogenetic and molecular) and the long-term outcome of ECP Ph+ CML pts treated with the alternating administration of NIL and IM.

Methods: Phase 2 study (ClinicalTrials.gov. NCT00769327). Schedule: NIL 400 mg twice daily for the first 3 months; IM 400 mg daily for the next 3 months; then, NIL and IM rotating every 3 months, for a total duration of 24 months (study core). The primary end-point was the Complete Cytogenetic Response (CCyR) rate at 12 months. The pts remained on study unless both drugs were discontinued. Definition of failure: according to 2013 ELN recommendations; event: failure, permanent discontinuation of both drug for any reason, patient refusal of alternating schedule during the study core. All the analysis was performed according to the ITT principle.

Results: 123 pts were enrolled in 38 Italian hematologic Centers; median age 56 years (range 18-84); 33% low, 45% intermediate and 22% high Sokal score; 94% low EUTOS score; median follow-up 60 months. The CCyR rates at early milestones were: 68% at 3 months, 73% at 6 months and 67% at 12 months (primary efficacy variable). The cumulative CCyR rate by 60 months was 93%. The cumulative MMR rates by 12 and 60 months were 82% and 84%, respectively. The cumulative MR4.0 rates by 12, 24 and 60 months were 43%, 61% and 62%, respectively. At the end of the 60 months, 69% of pts were on study, being the majority on monotherapy with NIL (35%) or IM (23%), and only 11%

still on alternating schedule. Events were observed in 52 (42%) pts: 22 failures, 13 adverse events; 14 patient refusal; 3 others. Failures included: 7 (5.6%) progressions to accelerated/blast phase (AP/BP); 5 primary resistances; 9 secondary resistances. The progressions to AP/BP occurred after a median time of 10 months (4-25 months); 4 pts had an ABL mutation (2 T315I, 1 Y253H, 1 F359V); all pts subsequently died. Athero-thrombotic adverse events (AAE) were observed in 6 (4.8%) pts: 3 acute myocardial infarctions, 1 unstable angina, 1 peripheral arterial occlusive disease and 1 aortic atherosclerosis. All pts discontinued permanently NIL and were treated with IM (4 pts) or dasatinib (2 pts). Overall, 12 pts died: 7 for progression to AP/BP, 2 for a second malignancy, 1 after ASCT, 1 for pulmonary embolism, and 1 for cerebral hemorrhage. The 5-year overall survival, progression-free survival, failure-free survival and event-free survival were 90%, 90%, 78% and 57%, respectively.

Summary and Conclusions: The early response rates achieved with the alternating administration of NIL and IM seem to be higher compared to historical data with IM alone; however, no difference seems evident in the long-term. Moreover, we observed a high number of progressions to AP/BP, which can be only partially justified by the relevant proportion of pts at intermediate or high Sokal risk. If compared to the results of NIL as monotherapy in ECP CML, this final analysis, despite the lower rate of AAE, does not support the alternating schedule of NIL and IM as first-line treatment of ECP CML.

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S489

SPIRIT 2: AN NCRI RANDOMISED STUDY COMPARING DASATINIB WITH IMATINIB IN PATIENTS WITH NEWLY-DIAGNOSED CHRONIC MYELOID LEUKAEMIA-2 YEAR FOLLOW UP

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Background: SPIRIT 2 is the largest trial comparing imatinib 400mg with dasatinib 100mg daily.

Aims: The primary end point of the study is event-free survival at 5 years. A key secondary endpoint is rate of a major molecular response (MMR, MR3, BCR-ABL1/ABL ratio <0.1% international scale).

Methods: 814 patients were recruited from 144 hospitals in the UK between August 2008 and March 2013. 812 patients were randomised, 406 in each arm.

Results: With a median follow up of 37.4 months, 236 imatinib and 276 dasatinib patients still received study treatment. 133 patients discontinued due to non-haematological toxicity. 53/406 (13.1%) on the imatinib arm (predominantly gastrointestinal toxicity) and 80/406 (19.7%) dasatinib patients (predominantly thrombocytopenia). 45/814 patients discontinued due to sub-optimal response as assessed by the treating physician (not defined by the protocol). These were predominantly patients on imatinib (42 of 406, 10.3%) as compared to dasatinib-3 of 406, 0.7%. Pleural effusions occurred in 90/406 (22.2%) patients on dasatinib and 13 required drainage. There was no significant difference in arterial cardiovascular events: imatinib 3/406 (0.7%); dasatinib 9/406 (2.2%). The MR3 rate at 12 months was significantly (p<0.001) higher with dasatinib 237/406 (58.4%) compared to imatinib 175/406 (43.1%) patients. In contrast to previous studies, pleural effusion was not associated with a significantly higher rate of MR3. Accelerated phase (imatinib 1/406; dasatinib 2/406) and blast crisis (imatinib 7/406; dasatinib 4/406) were observed in 14 patients. 38 patients died and there was no significant difference in deat rate in the two arms (Figure 1).

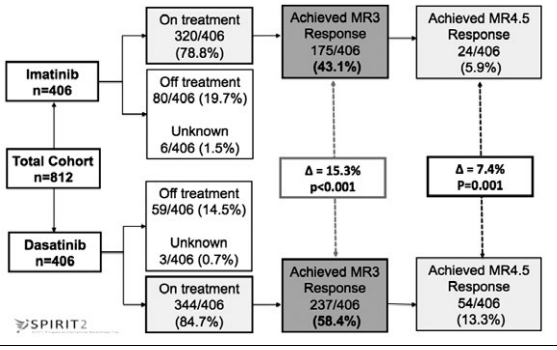

Figure 1.

Summary and Conclusions: In conclusion, dasatinib-treated patients have a significantly higher rate of molecular response at 1 year but so far there is no significant difference in rates of disease progression or overall survival. More patients abandoned imatinib than dasatinib due to investigator and/or patient concerns about sub-optimal responses. Further follow up will evaluate differences in the primary endpoint: event free survival at five years.

S490

BCR-ABL LEVELS AT LANDMARK TIMEPOINTS BETWEEN 1-3 YEARS OF IMATINIB ARE PREDICTIVE OF TIME TO A DEEP MOLECULAR RESPONSE AND CAN GUIDE THERAPY SWITCH DECISIONS WHERE TKI DISCONTINUATION IS THE GOAL

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Background: For patients (pts) with CML, achieving and maintaining a deep molecular response (MR^4 ≤0.01% or $MR^{4.5}$ ≤0.0032%) is necessary to minimize the risk of relapse after tyrosine kinase inhibitor (TKI) discontinuation, which is desirable for long term management of side effects, pregnancy and for economic reasons. For most pts, TKI therapy achieves an initial rapid BCR-ABL reduction, followed by a slow decline over many years. Thus, attaining a deep molecular response requires a long time. More potent TKIs administered as first line or after switch from imatinib (IM) yield higher rates of $MR^{4.5}$. IM treated pts who switched to nilotinib were indeed more likely to achieve $MR^{4.5}$ in the subsequent 2 years compared to continuing on IM (Hughes Blood 2014). However, decisions on treatment switch warrant careful consideration for a responding patient tolerating IM, who risks toxicity of second-line therapy. We analyzed BCR-ABL levels during IM therapy to determine the probability and timing of subsequent deep molecular response. This information could guide decisions on treatment switch if certain BCR-ABL response levels are associated with slow achievement of deep molecular response.

Aims: We 1) determined the probability of achieving MR^4 or $MR^{4.5}$ on IM, based on BCR-ABL levels attained; 2) assessed the time to reach these responses; and 3) identified subgroups where a switch to a more potent TKI may be warranted if timely TKI discontinuation is the goal.

Methods: 528 consecutive first line IM treated pts were examined. Pts were grouped by their BCR-ABL level at 12, 18, 24 and 36 months (mo) of IM and cumulative incidence of confirmed MR^4 and $MR^{4.5}$ by 8 years were calculated. The BCR-ABL groups were >0.01-0.1% and >0.1-1% for subsequent achievement of MR^4, and >0.0032-0.01%, >0.01-0.1% and >0.1-1% for $MR^{4.5}$.

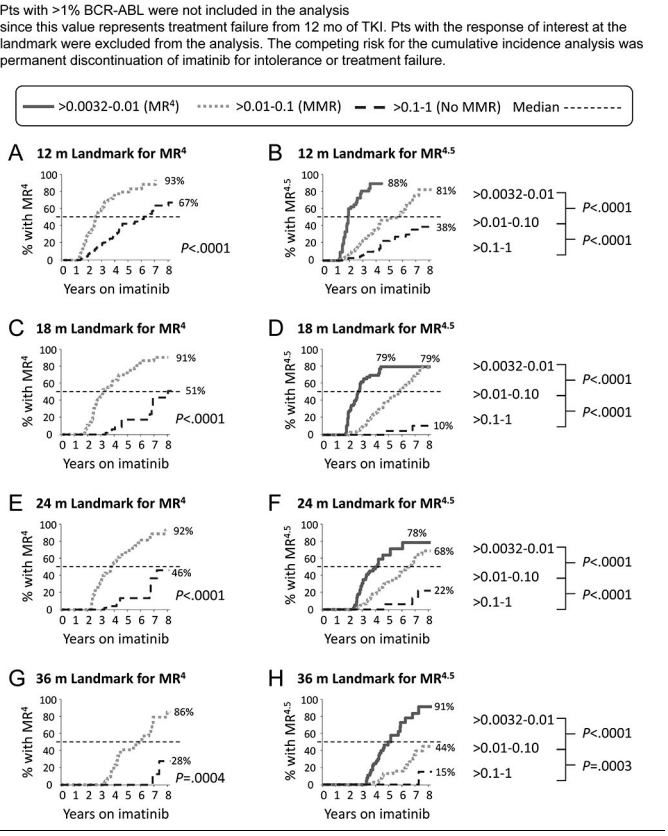

Figure 1. Cumulative incidence of MR^4 and $MR^{4.5}$ according to the molecular response level at landmark timepoints of imatinib therapy.

Results: By 8 years, the overall cumulative incidence of MR^4 and $MR^{4.5}$ were 66% and 53%. At landmark timepoints the subsequent rates of MR^4 or $MR^{4.5}$ were significantly associated with the BCR-ABL level, Figure 1. Rates at 8 years ranged from 91% $MR^{4.5}$ for pts with >0.0032-0.01% (MR^4) at 36 mo to 15% $MR^{4.5}$ for pts with >0.1-1% (no MMR) at 36 mo, *P*<.0001. The majority of pts with MR^4 at any of the landmarks achieved $MR^{4.5}$ within the subsequent 2 years with continued IM. The median time to achieve $MR^{4.5}$ for pts without an MMR at ≥12 mo was not reached with up to 7 additional years of IM. A substantial temporal lag in attaining the deep molecular responses was evident between the BCR-ABL groups for each landmark analysis. For example, for patients with MR^4 at 12 mo the median time to $MR^{4.5}$ was 23 mo after start of IM. However, for pts with BCR-ABL >0.01-0.1% at 12 mo (MMR) the median time to $MR^{4.5}$ was 69 mo after start of IM, a difference of >3.5 years (Figure 1B).
Summary and Conclusions: BCR-ABL levels are a powerful predictor of long term response. Whether switching therapy to achieve timely deep response will be associated with higher rates of treatment free remission compared with continuing on IM is currently unknown. Nevertheless, our data show that pts with <1% BCR-ABL without an MMR by 18-36 mo have a low chance of attaining $MR^{4.5}$, even with up to 6.5 additional years of IM, whereas most pts with MR^4 at any time achieved $MR^{4.5}$ with ~2 additional years of IM. These data provide timelines for the achievement of molecular response levels to clinicians and their IM-treated pts, and will guide decisions for potential therapy switch to optimise TKI discontinuation opportunities in pts where this is the goal of long term management.

Stem cell transplantation: Experimental

S491

LEUKEMIA RELAPSES AFTER ALLOGENENIC HSCT DISPLAY A DISTINCTIVE IMMUNE-RELATED SIGNATURE, WITH FUNCTIONALLY RELEVANT ALTERATIONS IN HLA CLASS II ANTIGEN PRESENTATION AND T CELL COSTIMULATION

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Background: Allogeneic Hematopoietic Stem Cell Transplantation (allo-HSCT) can cure Acute Myeloid Leukemia (AML) thanks to the synergistic combination of chemotherapy and antitumor immunity. Still, relapse after transplantation remains an unsolved issue for a large proportion of patients, warranting further investigation on the immunobiology of AML. Our group demonstrated that, after partially HLA-compatible HSCT, AML relapse is frequently due to the genomic loss of the HLA determinants targeted by alloreactive donor T cells (*Vago, N Engl J Med, 2009; Crucitti, Leukemia, 2014*), suggesting that relapse might represent an expression of immune veasion from the donor-derived immune system.
Aims: To identify and characterize novel mechanisms of post-transplantation leukemia relapse.
Methods: Serial AML samples purified from 9 patients at diagnosis, relapse after chemotherapy and relapse after allo-HSCT were employed for whole transcriptome profiling using Illumina microarrays. Deregulated genes were identified by pair-wise LIMMA analysis and used in an unsupervised gene ontology enrichment analysis. Results obtained by gene expression microarrays were confirmed in a validation cohort of 21 patients by *ad hoc* optimized molecular and cellular assays, including qPCR, immunophenotypic analysis, and functional *ex vivo* and *in vivo* experiments.
Results: A 110-gene signature (p<0.1) was able to discriminate between AML collected at disease diagnosis and at relapse after allo-HSCT. Most of the transcripts deregulated at post-transplantation relapse were involved in immune-related processes, and in particular in T cell costimulation and in the antigen presentation machinery. Conversely, no significant enrichment in immune-related processes was documented when comparing diagnosis with relapses after sole chemotherapy. We validated the significant upregulation of the PDL1 and B7H3 coinhibitory ligands on leukemic cells, accompanied by high levels of PD1 on the respective donor-derived T cells. Blocking this inhibitory axis by the usage of an anti-PDL1 monoclonal antibody, we rescued the ability of donor T cells to proliferate in response to the patient AML blasts. Even more evident were the *de novo* changes observed in the antigen presentation machinery at post-transplantation relapse: selective loss of surface expression of all HLA class II molecules and downregulation of their master regulator CIITA were detected in 4 out of 14 patients analyzed, occurring exclusively in patients transplanted from partially HLA-incompatible donors. SNP arrays documented no genomic rearrangement in HLA Class II genes or their regulators, suggesting an epigenetic origin of the alteration: accordingly, surface expression could be recovered upon culturing AML blasts in the presence of interferon-γ. Notably, loss of HLA-II expression on AML blasts abolished their recognition and killing by donor-derived T cells both *in vitro* and *in vivo*, which was recovered upon exposure of AML blasts to γ-interferon.
Summary and Conclusions: Our results demonstrate that the deregulation of immune-related processes, and in particular of the pathways involved in T cell-mediated allorecognition, is a distinctive feature of AML relapses after allo-HSCT. Identification of patient-specific mechanisms of immune evasion and relapse might be rapidly translated into personalized therapeutic approaches.

S492

AN INNOVATIVE HUMANIZED MOUSE MODEL TO INVESTIGATE THE INTERPLAY BETWEEN IMMUNE SYSTEM AND ACUTE MYELOID LEUKEMIA IN ALLOGENEIC HSCT

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Background: In spite of the efficacy of allogeneic Hematopoietic Stem Cell Transplantation (allo-HSCT) in curing Acute Myeloid Leukemia (AML), post-transplantation relapse remain a major issue. According to the "leukemia immunoediting" hypothesis relapse may be due to the outgrowth of immune-resistant leukemic variants upon the selective pressure of the transplanted immune system. Immunodeficient mouse models might represent a precious tool to provide insights into the immunobiology of this phenomenon.
Aims: In the present study, we set up a novel mouse-human chimeric model to characterize the biology of AML engraftment into mice and to model its interactions with adoptively transferred human T cells.
Methods: AML samples harvested at diagnosis from 26 patients were purified and infused into non-irradiated immunodeficient NOD/SCID γ-chain null (NSG) mice. Upon leukemia engraftment mice were sacrificed and purified leukemic cells were reinfused in serial recipients. To mimic immune pressure, mice received serial infusions of human T cells, either autologous or allogeneic (HLA-identical, HLA-matched, HLA-haploidentical or HLA-disparate) to the AML. At sacrifice, leukemic cells were FACS-purified and gene expression profile was analyzed using Illumina microarray. Deregulated genes were identified by pairwise LIMMA analysis. Gene Ontology and Gene Set Enrichment Analysis curated databases were interrogated to identify deregulated processes.
Results: Twelve out of 26 primary samples (46%) generated AML xenografts. Engraftment into mice significantly correlated with poor patient prognosis, and in particular with relapse after allo-HSCT (p=0.005). Infused leukemic cells reproducibly engrafted and exponentially expanded in mice, displaying a stable gene expression profile amongst littermates and upon serial transfer. Noticeably, serially transplanted AML exhibited an accelerated and more aggressive growth kinetic, with selective deregulation of genes involved in cell proliferation, myeloid differentiation and sister chromathide organization during mythosis.
Four selected AML xenografts were challenged *in vivo* with serial injection of T cells. We observed rapid and complete eradication of AML after treatment with HLA-disparate and HLA-haploidentical T cells from 20/20 treated mice. In all the cases, HLA matched cells harvested from unrelated donors were effective in controlling disease outgrowth. On the contrary HLA-identical T cells granted only temporary control in 6/10 mice, while autologous T cells were completely inefficacious in 14/14 mice. Leukemic blasts subjected to T cell-mediated immune pressure showed a specific and reproducible gene signature, with selective deregulation of genes involved in immune processes. Among the top-ranked upregulated genes we identified those related to response to interferons, comprising proteasome and immunoproteasome subunits, as well as molecules involved in antigen processing and presentation, comprising HLA Class I and II molecules. This data were further validated *in vivo*, observing in real-time the activation of this transcriptional program during the antileukemic immune response. Interestingly, in several experiments upon initial eradication we documented AML recurrence, with patterns which were highly reminiscent of post-transplantation relapses in human.
Summary and Conclusions: The model we set up represents a precious asset to mimic and decipher the complex dynamics of allo-HSCT, and thus to provide novel insights into the mechanisms of graft-*versus*-leukemia effect and relapse.

S493

MIR-153-3P, A NEW BIO-TARGET, IS INVOLVED IN THE PATHOGENESIS OF ACUTE GRAFT-*VERSUS*-HOST DISEASE THROUGH THE REGULATION OF INDOLEAMINE-2,3-DIOXYGENASE

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Background: To date, many studies investigating acute graft-*versus*-host disease (aGVHD) have focused on seeking potential biomarkers to initiate early intervention. MicroRNAs (miRNAs) have been reported to be promising diagnostic biomarkers for various types of diseases. A few studies have also demonstrated roles for miRNAs in the pathogenesis of aGVHD. However, data regarding the function of these miRNAs in human GVHD are limited. Using a miRNA polymerase chain reaction (PCR) chip, miR-153-3p was detected as a potential biomarker of aGVHD. Interestingly, indoleamine-2,3-dioxygenase (IDO) was found to be a potential target of miR-153-3p. Many previous studies have demonstrated that IDO plays important roles in the immunosuppression. Increased IDO expression may serve as a protective mechanism during aGVHD.
Aims: This study was aim to confirm the regulation of miRNA-153-3p on IDO both *in vitro* and mice model experiments, and to elucidate its role during GVHD development.
Methods: Plasma samples from 70 patients who underwent allogeneic hematopoietic stem cell transplantation (allo-HSCT) from Sep 2012 to Jun 2013 were prospectively collected at fixed time points from Peking University Institute of Hematology. RQ-PCR analysis was performed to examine the miRNA expression of plasma. BALB/c (H-2d) and C57BL/6 (H-2b) mice were used to establish the GVHD model. Antagomir and its control of miRNA-153-3p were administered via tail vein injection after allo-HSCT. Clinical and histological assessment of GVHD was performed. The expression levels of miRNA-153-3p and IDO in mice organs were also examined by Western Blot and RQ-PCR analysis.
Results: In this study, elevated plasma miR-153-3p levels at +7 d after transplant appeared to be a good predictor for subsequent aGVHD. IDO was found to be a potential target protein of miR-153-3p using bio-informative analysis. *In vitro* experiments confirmed that miRNA-153 could bind directly to the promoter region of IDO gene and affect its transcription and protein expression (above data have been reported on 2014 ASH meeting as a poster). In addition, the expression of plasma IDO was also lower in the aGVHD group at +7d compared to that of control group after allo-HSCT. To confirm the relationship between miR-153-3p and aGVHD, we established the MHC-mismatched murine GVHD model. Two groups received antagomir-control and antagomir-153-3p injection, respectively. The incidence and severity of the aGVHD were significantly decreased in the recipients of antagomir-153-3p infusion, as evidenced by the clinical GVHD scores (p<0.0001). Recipients of the antagomir-153-3p infusion survived longer compared to those of the antagomir-control infusion (log-rank test, p=0.0089). The pathological severity of aGVHD at day +21 in the liver and colon was also reduced in recipients who received antagomir-153-3p with positively stained granzyme B cell proportions as a marker. Besides, recipient mice of antagomir-153-3p displayed relatively higher IDO expression at the early stage after transplantation.
Summary and Conclusions: This is the first study to demonstrate, using *in vitro* and animal model experiments, that IDO can be directly regulated by miR-153-3p and that this miRNA might participate in aGVHD development by down-regulating IDO. Therefore, miR-153-3p may be a putative bio-target for a novel strategy for aGVHD intervention.

S494

IMMUNOSUPPRESSION BY MESENCHYMAL STROMAL CELLS DERIVED FROM HUMAN INDUCED PLURIPOTENT STEM CELLS: EVALUATION IN AN AGVHD MODEL

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Background: One major problem of allogeneic hematopoietic stem cell transplantation (HSCT) is acute Graft *versus* Host Disease (aGVHD). aGVHD has been managed until now with HLA matching and a constant evolving repertoire of immunosuppressive drugs. One alternative would be to generate in the host a permanent tolerance state toward the graft. Tolerant inducing cell therapy has been proposed with adult mesenchymal stromal cells (MSCs). *Ex vivo* isolated somatic MSCs have been implicated in immunoregulatory functions on cells from both the innate and adaptive immune system. They were proposed for cell therapies for treatment of aGVHD, Nevertheless, their use is restricted because of the few number that can be recovered from adult tissues, their limited *in vitro* expansion, and the absence of a full phenotypic characterization. Therefore other sources of well-defined and unlimited number of MSCs are needed, and MSCs derived *in vitro* from human Induced pluripotent stem cell (huIPS) would be a valuable tool for therapeutic approaches.
Aims: Because of our expertise in pluripotent stem cell differentiation, we generated huiPS-MSCs that present a strong immunosuppressive activity on allogeneic T cell responses. Our objectives are: 1) To evaluate and characterize *in vitro* this immunosuppression. 2) To validate *in vivo* these results using a xenoGVHD model.
Methods: To characterize the huIPS-MSCs i*n vitro,* FACS phenotyping and multipotency were tested. Their immunogenicity *in vitro* was monitored in co-cultures with allogenic peripheral blood mononuclear cells (PBMC). The *in vivo* immunosuppressive activity of huiPS-MSCs was evaluated using a xenoGVHD model in immunodeficient NSG (NOD/SCID/IL2rγKO) mice in which human PBMC were injected intra-peritonally. We established 3 groups: 1) huIPS-MSCs (control) 2) PBMC 3) PBMC+IPS-MSCs. We repeated huIPS-MSCs injection weekly with median number of injection n=3 (range 2-3). The activation state of human allogeneic T lymphocytes recovered from mice between 5 to 8 weeks after initial injection was evaluated and indicated the level of the xenoGVHD process and the efficiency of huiPS-MSCs to prevent it.
Results: a) *In vitro* characterization of huIPS-MSCs - As expected, the huiPS-MSCs were positive for CD73, CD90, CD105, HLA-I Ags and negative for CD45, CD34, HLA-II Ags and they were capable of differentiation into the classical mesenchymal-derived cells (osteoblast, chondrocytes and adipocytes). To test their immunosuppressive properties, we analyzed their action on the proliferation of human T lymphocytes stimulated in an allogeneic manner (Figure 1). The stimulation of PBMC in mixed lymphocyte reaction resulted in CD4 and CD8 T cell

proliferation (28±7% and 47±8%, respectively), which was significantly reduced in co-culture with huiPS-MSCs (4±2% and 10±2 respectively, n=3 p<0,05). We were able to demonstrate using blocking antibodies that part of the inhibition exerted by the iPS-MSCs is due to a) B7H1, a membrane receptor for the B7 family, known for its inhibitory action on the activation of T lymphocytes b) and B7H3 (from the same family) whose role remains controversial. b) *In vivo ch*aracterization of huIPS-MSCs - After sacrifice of mice, human circulating cells, those present in the peritoneal cavity and in the spleen were analysed by FACS. Mostly T lymphocytes were detected, and their number was significantly reduced in mice treated with huIPS-MSCs p<0,05. Intracytoplasmic labelling of recovered T cells showed that untreated mice displayed high percentages of human differentiated T cells producing IFN g and TNF a (typical of a inflammatory Th1 cytokine polarization profile), while little or none produced low inflammatory (IL-4) or anti-inflammatory (IL-10) cytokines. In contrast, in mice treated with the huiPS-MSCs, the proportion of T cells of the Th1 type was substantially reduced, while that of T cells producing IL-4 and/or IL-10 was slightly increased. In parallel, T cells expressing FoxP3 appeared (Figure 1).

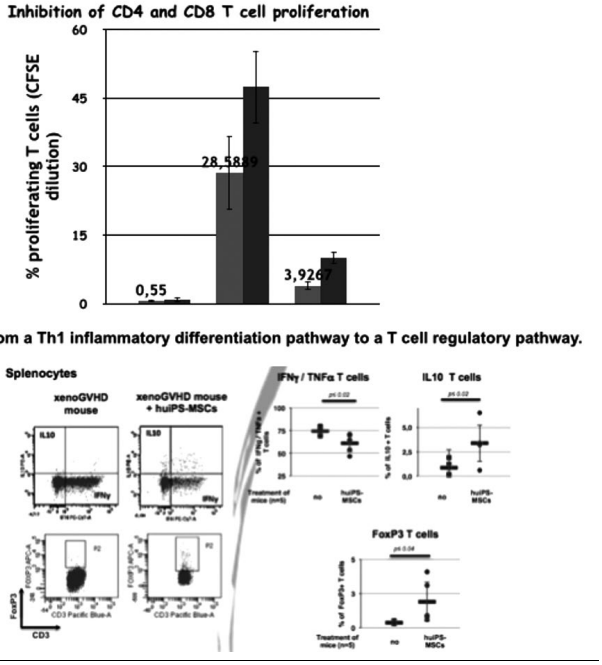

Figure 1.

Summary and Conclusions: We were able to generate immune-modulatory huiPS-MSCs that can be used to reduce activation of T cells in a xeno-aGVHD model through a switch from a Th1 inflammatory differentiation pathway to a T cell regulatory pathway. Our results may favor the development of new tools and strategies based on the use of pluripotent stem cells and their derivatives to prevent aGVHD but also for the induction of specific tolerance.

S495

IN VITRO-GENERATED MYELOID-DERIVED SUPPRESSOR CELLS (MDSC) PREVENT MURINE GRAFT-*VERSUS*-HOST DISEASE (GVHD) BY INDUCING TYPE 2 T CELLS WITHOUT DISABLING ANTI-TUMOR CYTOTOXICITY

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Background: Allogeneic bone marrow transplantation (BMT) is a curative treatment modality for hematopoietic malignancies such as acute and chronic leukemias and lymphomas. Mature donor T cells in the allograft support engraftment, promote early T cell immunity of the recipient and mediate the graft-*versus*-tumor (GVT) effect. However, these donor T cells are also responsible for the induction of graft-*versus*-host disease (GVHD) by destroying recipient tissue such as liver, skin, and bowel. Myeloid-derived suppressor cells (MDSCs) are a population of immature myeloid cells preventing T cell activation, proliferation and functions.
Aims: Therefore, we tested whether and how *in vitro*-generated MDSCs suppress GVHD development without disabling the GVT effect in allogeneic BMT models.
Methods: MDSCs were generated *in vitro* by culturing BM cells in the presence of GM-CSF and G-CSF. After 4 days more than 90% of the cells exhibited the $CD11b^{+}Gr\text{-}1^{+}$ MDSC phenotype. To test, whether and how MDSCs prevent GVHD, we transplanted allogeneic BM and spleen cells in lethally irradiated recipient mice in combination with *in vitro*-generated MDSCs and analyzed survival and allogeneic T phenotype and functions. By co-injection of syngeneic tumor cells, the effect of MDSC-treatment on the anti-tumor capacity of allogeneic T cells was analyzed.
Results: In-vitro generated MDSCs efficiently suppressed alloantigen-specific T cell proliferation *in vitro*. Transplantation of $1x10^{7}$ MDSCs together with allogeneic BM and spleen cells efficiently prevented clinical GVHD and attenuated histological GVHD. MDSCs expanded *in vivo* and invaded lymphatic and GVHD target organs and were still detectable 30 days after BMT. MDSC-mediated GVHD suppression was antigen-independent since transplantation of MHC class I deficient MDSCs prevented GVHD development comparable to wild type MDSCs. Inhibition of GVHD required the presence of MDSCs during T cell priming because transplantation of MDSCs one week after BMT was ineffective in GVHD prevention. Interestingly, MDSC treatment did not significantly reduce allogeneic T cell numbers in lymphoid and GVHD target organs or change their homing behavior. However, MDSCs skewed allogeneic T cells towards type 2 T cells up-regulating Th2-specific transcription factors and cytokines. Polarization towards type 2 T cell immunity was indispensable for GVHD prevention since MDSC-treatment failed to prevent GVHD when allogeneic STAT6-deficient T cells, which are unable to differentiate into Th2 cells, were transplanted. However, MDSC-mediated type 2 T cell polarization did not abrogate anti-tumor cytotoxicity of alloantigen-specific T cells since syngeneic thymoma tumor cells were efficiently eradicated.
Summary and Conclusions: Therefore, MDSC-induced Th2 polarization might be exploited in clinical settings for GVHD prophylaxis while simultaneously maintaining anti-tumor cytotoxicity.

Platelet and bleeding disorders

S496

DOWN-REGULATED BETA2-GPI IS ASSOCIATED WITH A REDUCED ABILITY TO MITIGATE COMPLEMENT ACTIVATION BY INHIBITING JNK/BID-DEPENDENT PLATELET DESTRUCTION IN THE PATHOGENESIS OF IMMUNE THROMBOCYTOPAENIA

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Background: It is widely accepted that immune complex-mediated classical complement pathway activation represents an important mechanism of platelet destruction in immune thrombocytopaenia (ITP) (Peerschke at al. Br J Haematol. 2010). However, not all patients diagnosed with ITP have detectable antibodies, suggesting a potential mechanism of complement activation in the plasma of ITP patients. Moreover, the mechanisms involved in complement-mediated platelets lysis are elusive. β2-glycoprotein I (β2-GPI) is a plasma glycoprotein that functions as a complement regulator (Gropp *et al.* Blood. 2011); however, there is no information available on the role of β2-GPI in patients with ITP to date.
Aims: The aim of the present study was to assess the contribution of β□-GPI in complement activation and the details of its underlying mechanism.
Methods: Twenty-four consecutive patients with primary ITP and 24 healthy donors were enrolled in this prospective study. The levels of plasma β2-GPI and serum C1q, C4d, C3b and C5b-9 were determined by ELISA in ITP patients and healthy donors. The deposition of the fore-mentioned complement components on immobilized heterologous platelets was quantified. The levels of C3 convertases in plasma and the phosphorylation of MAPKs (phospho-ERK, phospho-JNK, phospho-p38^{MAPK}) and Bid in C5b-9 mediated platelet lysis were determined using Western blot. To study the effect of β2-GPI on complement activation, β2-GPI was added to ITP plasma, C3 convertase activity was assayed via the determination of C3a generation, and serum levels of C5b-9 and its deposition and signalling proteins were re-evaluated.
Results: We first explored the significantly lower concentrations of β2-GPI in plasma from patients with ITP (80±21 ng/ml) compared with healthy donors (180±22 ng/ml) ($p<0.05$). Increased deposition of C5b-9 was observed, indicating the complement activation in ITP plasma. Evidence for enhanced classical complement pathway activation (C1q and/or C4d deposition) was noted in 45.8% of patient plasma samples, and alternative pathway activation (C3b) was observed in 37.5% of plasma samples. Additional evidence for enhanced complement activation was noted with increased serum levels of C3 convertases (C4b2b and C3bBb). β2-GPI plasma levels and C5b-9 serum levels were negatively correlated. The intensity of phospho-MAPKs bands revealed the over-expression of phospho-JNK, and detection of Bid exhibited similar results. SP600125 (JNK) and BI-6C9 (Bid) inhibitors significantly reduced complement-mediated JNK/Bid-dependent platelet lysis. Treatment of ITP plasma with β2-GPI *in vitro* resulted in a dose-dependent reduction of serum C5b-9 generation and platelet deposition as well as reduced phospho-JNK and Bid. Moreover, β2-GPI inhibited C3a generation and formed complexes with C3 in plasma.
Summary and Conclusions: This original observation highlights the role of β2-GPI in ITP in harbouring complement activation at the level of the C3 convertase and inhibiting JNK/Bid-dependent platelet destruction. The first mention of decreased β2-GPI in ITP is suggestive of abnormal complement activation. Our data provide important insights into immune dys-regulation via decreased plasma β2-GPI, which may be exploited in the pathogenesis of ITP.

S497

BLEEDING RISK OF SURGERY IN PATIENTS WITH INHERITED PLATELET FUNCTION DISORDERS (IPFD): OUTCOME OF 389 SURGERIES IN 205 PATIENTS

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Background: IPFD are a heterogeneous group of rare mucocutaneous bleeding diseases of variable clinical severity. Excessive bleeding at surgery is a feared complication for bleeding disorders. While studies on the bleeding risk of surgical procedures in other hemorrhagic disorders, like hemophilia or von Willebrand disease, have been conducted, very few and small studies have evaluated the bleeding risk associated with surgery in IPFD.
Aims: Our aim was to evaluate the bleeding complications of surgical procedures in patients with IPFD, the therapeutic approaches adopted for the prevention/treatment of hemorrhage and their efficacy.
Methods: Retrospective, multicentre worldwide study involving clinical centers managing IPFD. Participants were asked to examine retrospectively their patient records and to enroll all cases who had undergone surgery fulfilling strict inclusion criteria. Patients had to have a definite IPDF diagnosis, confirmed according to well-defined laboratory and/or molecular genetic criteria. All types of surgical procedures, including biopsies and dental extractions, were admitted. Bleeding tendency before surgery was scored by the WHO bleeding scale, while the extent of bleeding at surgery was assessed by the BARC classification, subjective evaluation from the surgeon, and/or duration of bleeding at surgery. Information about prophylactic antihemorrhagic preparation and emergency treatment of bleeding was requested.
Results: 205 patients (Age 5 to 86, 56.6% females) from 36 centers with 13 different forms of IPFD were enrolled: 43.4% had Glanzmann Thrombasthenia (GT), 22.4% Primary Secretion Defect (PSD), 6.8% combined α-δ granule deficiency (αδ-SPD), 5.4% Hermansky-Pudlak syndrome (HPS), 4.4% Gray platelet syndrome (GPS). Data from 389 surgeries were collected, the most frequent being dental extraction (26.7%), adenotonsillectomy (3.3%), appendectomy (2.8%), endoscopic polipectomy (2.8%). Major procedures, like cholecystectomy (2.6%), hernia repair (2.6%), gastrectomy (2.3%), hysterectomy (1.8%), were also reported. The frequency of excessive bleeding at surgery was 19.8% by subjective evaluation, 19.7% by the BARC classification and 23.1% by the combination of the two. High frequency of excessive bleeding was observed in GT (29.5% of 183 procedures), HPS (27.3% of 22) and α-δ SPD (12.5% of 32). Frequency of excessive bleeding in some forms remains uncertain due to the low number, but some of them (thromboxane A2 receptor defect, CalDAG, platelet-type von Willebrand Disease) appear to be at high risk. Excessive bleeding at surgery was significantly predicted by presurgical WHO bleeding score=3 (OR 8.1, 95% CI 1.1-61.8) and=4 (OR 28, 95% CI: 2.9-270.5). Prophylactic platelet transfusions were given in 38.5% of procedures and antifibrinolytic agents or DDAVP in 32.9%, and reduced significantly the risk of excessive bleeding (OR 0.3, 95% CI: 0.2-0.5). Emergency treatment was administered in 85.5% of the procedures with excessive bleeding (platelet transfusions in 50.6%, antifibrinolytic agents in 23.4%, FVIIa in 7.8%, DDAVP in 3.9%, other in 14.3%), and was successful in 81.8% of cases.
Summary and Conclusions: IPFD are associated with a significant surgical bleeding risk. The risk of excessive bleeding varies according to the diagnosis, with GT having the highest risk, and is predicted by the preoperative WHO bleeding score. Preoperative prohemostatic prophylactic treatments prevent excessive bleeding in most cases. Treatment of excessive bleeding with platelet transfusions and/or other measures stops excessive bleeding in most, but not all, treated cases.

S498

USEFULNESS OF TARGETED NEXT-GENERATION SEQUENCING IN INHERITED PLATELET DISORDERS WITH UNSPECIFIC PHENOTYPE

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Background: Inherited platelet disorders (IPDs) cause bleeding diathesis with varying severity. Due to their rarity and extreme heterogeneity, the diagnosis is complex, poorly standardized, time consuming and expensive. Based on clinical and laboratory findings, gene mutations may be assessed by means of conventional Sanger sequencing to diagnose the disease. However, in many cases a proper diagnosis and the genetic cause for the disease remain unknown. Next generation sequencing (NGS) enables the simultaneous analysis of large groups of candidate genes in IPDs and may be useful for rapid genetic diagnosis and allow optimal disease classification and understanding of the pathogenesis of the disease.
Aims: To improve the diagnosis of IPDs by using a next generation sequencing panel.
Methods: Forty patients with suspected IPDs were analysed by NGS panel to identify a pathogenic mutation causes them. Patients had abnormal bleeding symptoms, normal coagulation factors levels, platelet function testing pathological or thrombocytopenia without evidence of acquired causes. Two groups were established, first group, 19 patients with specific phenotype and 21 without a proper diagnosis or unknown phenotype. For the 70 genes associated with IPDs (figure1) baits were generated to tile 400 kb DNA, corresponding to the exons and splice sites of all known transcripts of the candidate genes. The bait library was tested by enriching candidate genes from 50 ng DNA using the Nextera Rapid Custom Enrichment system followed by massive parallel sequencing (Illumina).
Results: Genotype were determined in 16 of 19 patients with specific phenotype, therefore IPDs diagnosis were confirmed: 4 Von Willebrand Disease (VWD), 1 Glanzmann Thrombastenia (GT), 4 Bernard Soulier syndrome (BSS), 2 MYH9-related disease, 1 Hermansky Pudlack Syndrome, 1 Prekalicrein defiency, 2 Dysfibrinogenemia, 1 Signalling defect (GP6). Patients with unknown phenotype, were subdivided in patients with or without thrombocytopenia. In patients with thrombocytopenia diagnosis were indentified in 7/10: 2 BSS (*GP1BA*; c.463C>; c.1280_1291delCCTCAGAGCCCG); VWD-PT (*GP1BA*; c.733G>A); Bening Mediterranean macrothrombocytopenia (*GP1BB*; c.1A>T), Gray Platelet syndrome (*NBEAL2*; c.3422_3423insC), Wiskott-Aldrich Syndrome (*WAS*; c.802delC) and Thrombocytopenia FLNA-related (*FLNA*; c.3695C>T). In IPD patients without thrombocytopenia diagnosis were identified in 6/11: GT (*ITGA2B*; c.2063C>T); VWD 2N (*VWF*; c.2561G>A), mild Hemophilia A (*F8*, c.6623A>G), GATA-related disease (*GATA1*; C.865C>T), RUNX1-related disease (*RUNX1*; c.167T>T), and Defect ADP receptor (*P2YR12*, c.835G>A). No mutations were found in 3 patients with "Aspirin-like" syndrome. In fact, genotype were determined in 13 of 21; and 22 mutations have not previously been described in the literature. Types of mutations described were 33 missense and 8 frameshift (3 insertion, 5 deletion). All mutations identified by NGS were confirmed by Sanger sequencing. Once, diagnosis was determined, functional testing, flow cytometry and electron microscopy were done to establish a proper diagnosis of IPDs (Table 1).

Table 1.

IPDs	Genes = 70 / 1570 Exons
Transcription Factors	*HOXA11, MPL, RBM8A, RUNX1, GATA1, FLI1, STIM1, GFI1B*
Agonist platelet receptors	*P2RX1, P2RY1, P2RY12, TBXA2R, TBXAS1, ADRA2A, GP6, CD36 o GP4, DTNBP1, ITGA2*
Platelet granules	*NBEAL2, PLAU, HPS1, HPS3, HPS4, HPS5, HPS6, LYST, MLPH, USF1 BLOC1S3, BLOC1S6, AP3B1, VIPAS39, VPS33B, RAB27A, MYO5A,*
Cytoskeletal Assembly and Structural Proteins	*ACTN1, WAS, MYH9, FLNA, ANKRD26, GP1BA, GP1BB, GPV, A2M, GPIX, ITGA2B, ITGB3, ABCA1, ANO6, FERMT3, MASTL*
Signal transduction	*GNAI3, GNAQ, GNAS, PLA2G7, PLCB2PTS, GGCX, DPAGT1, DHCR24*
Others	*F8, VWF, KLKB1, C1QBP, CLEC3B*
Dysfibrinogenemia	*FGA, FGB, FGG, FIBCD1*

Summary and Conclusions: NGS by amplicon-based capture enables a fast diagnosis of IPDs. This tool cold be used ina clinical setting to facilitate the diagnosis of IPD displaying an unclear phenoptype.

S499

CLINICAL CHARACTERSTICS AND RISK FACTORS OF INTRACRANIAL HAEMORRHAGE IN PATIENTS FOLLOWING ALLOGENEIC HAEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Although cerebral complications and causes after allogeneic haematopoietic stem cell transplantation (allo-HSCT) are well documented, assessment of incidence rates and risk factors of intracranial haemorrhage (ICH) following allo-HSCT are less frequently reported (Najima *et al.*, Am J Hematol 2009).
Aims: The aim of this study is to determine the clinical characteristics, risk factors and prognosis of ICH following allo-HSCT.
Methods: A nested case-control study was conducted that included 175 subjects obtained from 2165 subjects who underwent HSCT in Peking University People's Hospital between Sept. 2004 and Jul. 2014. In total, 35 patients with ICH and 140 controls matched for age, sex, transplantation type and time after transplantation were identified (total n=175). The incidence of ICH was identified by searching hospital records of CT and MRI scans.
Results: Among the 2165 patients, 35 patients (1.6%) developed intracranial haemorrhage, including 29 cases (82.9%) of intraparenchymal haemorrhage (IPH), 2 cases (5.7%) of subdural haematoma (SDH), 1 case (2.9%) of subarachnoid haemorrhage (SAH), and 3 cases (8.6%) of multiple haemorrhage lesions in the brain parenchyma. The median time of appearance for cerebral haemorrhages was 129 days (range, 1-450 days). ICH patients exhibited unique characteristics compared with controls, including systemic infections, severe graft *versus* host disease (GvHD), and coagulation disorders. Multivariate analysis revealed that systemic infections, III-IV acute GvHD, lower platelet count and fibrinogen levels are independent risk factors for ICH among HSCT patients. The risk of ICH increases when platelet counts are below 13.2×10^9/L and fibrinogen is below 129.5 g/L. However, in addition to the causes indicated above, chronic GvHD, hypertension, central nervous system leukaemia, and INR were identified as risk factors by universal analysis. Transplantation-related mortality rates in the intracranial haemorrhage and control groups were 50% and 22.2%, respectively. The cumulative survival rates in the intracranial haemorrhage and control groups were 47.1% and 75.7% (P<0.001).
Summary and Conclusions: ICH is one of the most common cerebral complications after HSCT and is associated with a high mortality and reduced overall survival rate. Systemic infections, III-IV acute GvHD, lower platelet count, and lower fibrinogen levels are individual independent risk factors. To prevent ICH, it is useful to increase platelet counts and fibrinogen to safe levels.

S500

A LONGITUDINAL PROSPECTIVE STUDY EVALUATING THE EFFECTS OF ELTROMBOPAG (EPAG) TREATMENT ON BONE MARROW (BM) IN PATIENTS WITH CHRONIC IMMUNE THROMBOCYTOPENIA: FINAL ANALYSIS

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Background: EPAG, an oral thrombopoietin receptor agonist (TPO-RA), is approved for treating thrombocytopenia in adults with chronic immune thrombocytopenia (cITP) with insufficient response to prior therapy. TPO-RAs are associated with varying degrees of BM reticulin increases.[1] Due to lack of pretreatment evaluations, the incidence and clinical significance of these findings have not been established. Inconsistencies in specimen preparation, staining, and analysis across institutions further confound conclusions.
Aims: To assess the degree of BM fibrosis (reticulin and/or collagen) in cITP patients (pts) treated for up to 2 years with EPAG.
Methods: Informed consent was provided. BM biopsies were collected at baseline (prior to study treatment) and at 1 and 2 years on treatment. Specimens were centrally processed, stained for reticulin (silver) and collagen (trichrome), and reviewed by central independent hematopathologists for cellularity; megakaryocyte, erythroid, and myeloid quantity and appearance; trabecular bone quality; reticulin grade (European Consensus scale of marrow fibrosis [MF][2]); and presence of collagen.
Results: Of 162 pts analyzed, the median age was 42 years (range, 18-80), 104 pts (64%) were female, 50% were White, 20% East Asian, and 29% South Central Asian. A total of 77% were diagnosed with ITP ≥12 months before study entry. Prior ITP therapy was reported by 70%, and 8% received prior TPO-RA treatment. Of 162 pts, 44 withdrew from the study, 118 completed the study, and 93 had all 3 on-treatment biopsies. MF grades at each time point are shown in the Figure 1. Of the 3 pts with MF-2 at 1 year, 1 completed 2 years of treatment and had MF-2 at 22 days post-treatment and MF-1 at 288 days post-treatment. Another pt received 17 months EPAG and had MF-0 at 44 days post-treatment. The third pt did not have a follow-up biopsy. Of the 2 pts with MF-3, 1 had an on-treatment MF-0 biopsy 31 days after the MF-3 biopsy and at 2 years and 1 had MF-0 at 184 days post-treatment. At 2 years, no pt on

treatment had ≥MF-2. When comparing biopsies at 2 years *vs* baseline, 80 pts (86%) had no change in MF grade, 9 (10%) had a 1-grade increase from MF-0 to MF-1, and 2 (2%) had a 1-grade decrease from MF-1 to MF-0. All pts were negative for collagen at baseline. At 1 year, 5 pts (4%) were positive for collagen, 3 of whom had MF-2 or MF-3. At 2 years, 1 pt (1%) with MF-1 had collagen present. None of the on-treatment biopsies were prompted by an abnormal peripheral blood smear or done at the investigator's discretion for clinical symptoms suggestive of BM dysfunction. Cellularity was normal in 80%, 80%, and 76% of pts at baseline, 1 year, and 2 years, respectively. Trabecular bone thinning was found in 73 pts (50%) at baseline, 61 (50%) at 1 year, and 33 (38%) at 2 years, likely the result of prior steroid therapy.

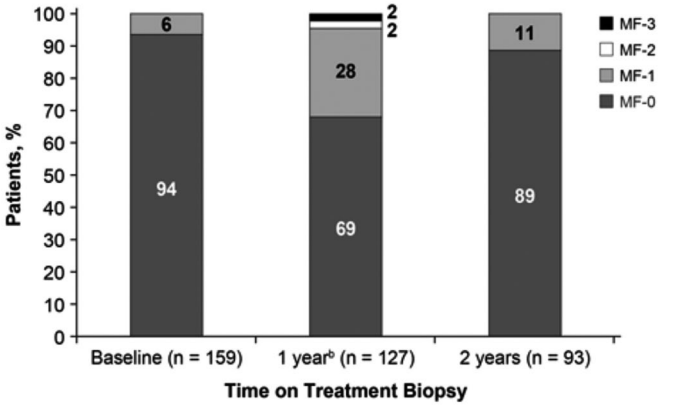

Figure 1. Maximum reticulin grade[a].

Summary and Conclusions: After 2 years of treatment, no increase in reticulin was observed in the majority of pts (86%) while few pts (10%) had a mild increase in reticulin. No pt had an on-treatment biopsy of ≥MF-2 at 2 years or clinical signs/symptoms indicative of BM dysfunction. Results were similar to those reported for EXTEND, an EPAG extension study (treatment duration up to 5.5 years[3]). These data suggest that treatment with EPAG is generally not associated with clinically relevant increases in BM reticulin or collagen. This study (NCT01098487) was funded by GlaxoSmithKline.

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Iron clinical and biology

S501

TP-0184 LOWERS HEPCIDIN LEVELS AND IS A POTENTIAL THERAPEUTIC FOR ANEMIA OF CHRONIC DISEASE

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Background: The liver peptide hormone, hepcidin, is known as a master regulator of bioavailable iron and red blood cell formation. Hepcidin expression is in part regulated by pro-inflammatory cytokines that ultimately signal through the activin-like kinase receptor 2 (ALK2) and SMAD transcription factors leading to the expression of hepcidin. In chronic inflammatory conditions, such as chronic kidney disease, rheumatoid arthritis, colitis, and in some forms of cancer, hepcidin levels remain high, lowering bioavailable iron leading to anemia. Hepcidin functions by binding to and inhibiting ferroportin, an iron pump that functions in macrophage-based iron recycling and intestinal iron uptake. Several studies demonstrated that lowering hepcidin could provide a novel approach for targeting this complicated clinical challenge. Current clinical strategies for these patients focus on red blood cell transfusions and the use of erythropoetin-based therapies. Neither of these approaches target the underlying mechanism behind the anemia associated with chronic inflammation and cancer.
Aims: The aim of this work is to develop a small molecule inhibitor of ALK2 for the treatment of hepcidin-driven anemia of chronic diseases.
Methods: Two animal models were primarily employed for these studies. First, turpentine oil (TO) was used to induce an acute inflammatory response in C57BL/6 mice with associated hepcidin-driven anemia. The animals were orally administered TP-0184 1 hour prior to TO treatment and then 8 hours later. Plasma and livers were collected 16 hours after TO treatment. Second, a TC-1 lung cancer syngeneic model was used in C57BL/6 mice. The TC-1 cells were injected intraperitoneally and allowed to establish for about 1 week. The mice were then administered TP-0184 repeatedly for 3-5 days, after which, plasma and livers were collected. Liver hepcidin levels were determined by RT-qPCR and plasma hepcidin levels by mass spectrometry. Iron levels were measured by ELISA.
Results: TP-0184 is a small molecule inhibitor of the kinase activity of ALK2 with an IC_{50} value of 5 nM. It has minimal activity against JAK2 (IC50=8540 nM), compared to other analogs within the same series. TP-0184 is effective at targeting hepcidin expression in a HepG2 cell culture model with an EC_{50} lower than 100 nM. It achieves this activity in HepG2 cells with no observable toxicities and in a pathway-specific manner. Furthermore, TP-0184 has been evaluated in multiple animal models of inflammation and anemia. TO was used to induce an acute inflammatory response in mice resulting in a 14-fold increase in liver hepcidin levels. Two oral doses of TP-0184 at 100 mg/kg, separated by 8 hours, was able to almost completely reverse the induction of hepcidin that was stimulated by the TO treatment. In this same TO model, TP-0184 was evaluated at doses as low as 25 mg/kg to further explore the lower limits of its efficacy and to establish a therapeutic window. Similarly, TP-0184 was tested in an anemia of cancer model utilizing TC-1 lung cancer cells. Tumor-bearing animals experienced a 3-fold increase in liver hepcidin expression, which was again completely reversed by dosing with TP-0184 at 25 mg/kg. In addition to the activity observed in these models, TP-0184 demonstrates favorable pharmacokinetic properties and good drug-like characteristics making it a viable candidate molecule to move into advanced preclinical testing, IND-enabling studies and formal clinical development.
Summary and Conclusions: TP-0184 is a potent and selective inhibitor of ALK2 with good activity in preclinical models of anemia associated with inflammation. We anticipate a clinical development strategy that focuses on anemia of chronic disease where an erythropoetin-sparing approach might offer significant clinical benefit both to patients and to health management institutions.

S502

HEME AND IRON CONTROL OF MACROPHAGE PLASTICITY IS PREVENTED BY THE HEME SCAVENGER HEMOPEXIN AND THE IRON CHELATOR DFO: LESSON FROM SICKLE CELL ANEMIA

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Background: Hemolytic disorders are associated with recurrent events of intra- and extra-vascular hemolysis. Under these conditions, circulating heme levels and erythrophagocytosis are increased resulting in heme and iron accumulation in macrophages of the reticuloendothelial system (RES). The heme scavenger Hemopexin limits heme accumulation in RES macrophages in heme-overloaded Hx-null mice, a model mimicking heme release in hemolytic

events. As a consequence cell activation as well as cytokine and ROS production is reduced.
Aims: Here we investigated, both *in vitro* and *in vivo*, whether heme and/or iron affect macrophage activation and polarization (*e.g.* M1 inflammatory *vs* M2 anti-inflammatory macrophages) and, whether this is prevented by heme/iron scavengers.
Methods: We characterized bone marrow-derived macrophages treated with aged RBCs (Red blood cells), heme or iron and analyzed the expression of iron-related genes and cytokines, ROS production and polarization markers. Additionally, we evaluated the effect of co-treatment with the heme scavenger Hemopexin or the iron chelator Desferrioxamine. Macrophage polarization was further assessed *in vivo*, in mice injected with heme or iron-dextran as well as in a mouse model of sickle cell disease, which is hallmarked by enhanced macrophage heme/iron retention. Finally the effect of Hemopexin administration on macrophage polarization was evaluated *in vivo*.
Results: Macrophages treated with RBC, heme or iron increase mRNA expression of HO-1, L-Ferritin and FPN and decrease TfR1 mRNA levels. This correlates with an increase of M1 markers (MHCII/CD86/TNFa) and a decrease of M2 markers (CD206/Arg1), indicating that heme and iron drive macrophage polarization towards a M1-like pro-inflammatory phenotype. Interestingly, heme/iron treatment further potentiates M1 polarization and shifts M2 macrophages to the M1 phenotype. Similarly, heme and iron affect macrophage plasticity *in vivo*, promoting macrophage polarization towards a M1 phenotype. These effects are reversible by the co-treatment with Hemopexin or Desferrioxamine both *in vitro* and *in vivo*. Additionally, the treatment with heme together with the anti-oxidant N-acetyl-cystein or the TLR4 inhibitor, TAK-242, rescued the up-regulation of some M1 markers and the down-regulation of some M2 markers, likely suggesting that both oxidative stress (induced by both heme and iron) and the activation of the TLR4 pathway (activated by heme) are responsible for heme-induced M1 polarization of macrophages. Interestingly, hepatic macrophages from sickle mice show an enhanced M1-like phenotype, correlating with increased iron accumulation in these cells and Hemopexin depletion. The administration of Hemopexin to sickle mice rescued the expression of M1 markers in liver macrophages to levels similar to control mice, indicating that heme scavenging reduces the pro-inflammatory state of macrophages, by reducing cell heme-iron loading.
Summary and Conclusions: Here we show that heme and iron induce the polarization of macrophages towards the M1 pro-inflammatory phenotype. This finding is of pathophysiological relevance in disorders associated with heme and iron loading in RES macrophages, such as sickle cell disease. We expect that the heme/iron-induced increase in cytokine production and ROS will significantly contribute to the enhanced inflammatory status of some hemolytic diseases. The administration of anti-oxidant and hemoglobin/heme scavenger could be beneficial to counteract the heme-induce pro-inflammatory status of macrophages in these disorders.

S503

FUNCTIONAL CHARACTERIZATION OF NOVEL ABCB6 MUTATIONS AND THEIR CLINICAL IMPLICATIONS IN FAMILIAL PSEUDOHYPERKALEMIA

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Background: Isolated Familial Pseudohyperkalemia (FP) is a dominant red cell trait characterized by cold-induced slow 'passive leak' of red cell K+ into plasma, first described in a large Scottish family from Edinburgh (Stewart GW, *et al.*, 1979). Although in freshly obtained blood samples plasma [K+] was normal, it was increased when measured in blood stored at or below room temperature. This trait was unaccompanied by clinical symptoms or signs except for mild abnormalities of red cell shape. Functional gene mapping and sequencing analysis of the candidate genes within the 2q35-q36 critical interval in three multigenerational FP families with 20 affected individuals identified two novel heterozygous missense mutations in the ABCB6 gene that cosegregated with disease phenotype (Andolfo I. *et al.*, 2013). The two genomic substitutions altered two adjacent nucleotides within codon 375 of ABCB6, a porphyrin transporter that in erythrocyte membranes bears the Langereis blood group antigen system (Krishnamurthy PC, *et al.*, 2006; Helias V, *et al.*, 2012).
Aims: In this study we analyzed three additional families and report the first functional characterization of ABCB6 mutants towards understanding the pathogenic mechanism of FP.
Methods: DNA was obtained for genetic analysis from affected and unaffected family members, after signed informed consent, according to the Declaration of Helsinki. The search for mutations was performed by direct sequencing of the *ABCB6* gene. cDNAs encoding full-length wildtype ABCB6 were cloned in pcDNA3.1 vector. The novel point mutations found in our patients: c.1361T>C, p.V454A; c.826C>T, p. R276W; c.2168G>A, p.R723Q were introduced into pcDNA3.1-ABCB6 by site-directed mutagenesis. WT and mutants constructs were transfected into HEK-293 cells for 72h. The cells were maintained at 30°C to evaluate the effects of reduced temperature. After transfection, the cells were incubated in a medium containing ^{86}rubidium ($^{86}Rb^+$) as a tracer for K^+. ^{86}Rb was determined in cell lysate, and K was determined in the supernatant by atomic absorption spectrometry.
Results: In one Bolivian patient we found the homozygous mutation c.1361T>C, p.V454A. In one patient from Cardiff we found two heterozygous mutations in trans: c.826G>T, p. R276W and c.2168G>A, p.R723Q. In Ireland family we found the heterozygous mutation c.826G>T, p. R276W. All these mutations are annotated in public databases as single nucleotide variants (SNVs), suggesting that many patients with FP could be present in donor blood population and are predicted to be damaging by *in silico* analysis by PolyPhen2 and SIFT tools. V454A, R276W, and R723Q as well as the previously identified, R375Q and R375W, were expressed into HEK-293 cells. Expression analysis of all mutants showed no alterations in levels of expression of mutant RNA or polypeptide, and molecular modeling predicted minimal structural changes resulting from the novel missense mutations. However, measurement of ouabain- and bumetanide-resistant net cation flux demonstrated a greater loss of cell K from ABCB6 mutant-expressing cells compared to ABCB6 WT-expressing cells. The coexpressed R276W/R723Q mutations, in particular, elicited greater efflux of cellular K^+ into extracellular medium than did the other mutants studied.
Summary and Conclusions: Our findings demonstrate that missense mutations in *ABCB6* lead to increased cellular K^+ efflux as exhibited in RBCs of FP patients. Storage of FP blood can cause a significant increase in blood K^+ levels, with serious clinical implications mostly in neonates and infants receiving large-volume transfusions of whole blood. Furthermore, the prevalence of FP might be underestimated, since patients with FP can be asymptomatic and thus undetected in the donor population. In the future, genetic tests for FP could be added to blood donor prescreening.

S504

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

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Background: Dysregulated iron homeostasis causes a wide spectrum of diseases including hepatic fibrosis and cirrhosis, endocrinopathy and cardiac failure. This is evident not only in hereditary hemochromatosis, a genetically inherited primary iron overload disorder, but also in many dyserythropoietic syndromes (*e.g.* beta-thalassemia or congenital *dyserythropoietic* anemias) in which the ineffective eyrhtropoiesis drives an abnormal request for iron causing secondary iron overload.
Systemic iron levels are balanced by the hepatic iron hormone hepcidin and its "receptor" ferroportin (FPN) to prevent the pathological consequences of iron overload or iron deficiency. Hepcidin binding to the iron export channel FPN reduces dietary iron uptake in duodenal enterocytes and iron recycling from aging erythrocytes in reticuloendothelial macrophages. Despite the importance of these two cell types as iron exporters, their single contribution in maintaining systemic iron content has not yet been elucidated.
Aims: The aim of this study is to quantify the individual contributions of macrophage iron recycling and dietary iron uptake to systemic iron levels. This knowledge is an important prerequisite to develop specific pharmacological strategies to limit iron export in iron-related disorders.
Methods: Central to this study is a novel mouse line that expresses a FPN mutant protein that is unable to bind to hepcidin FPN(C326S) (Altamura *et al.*, Cell Met. 2014). Constitutive disruption of the hepcidin/FPN regulatory loop causes increased serum and hepatic iron levels and a dramatic decrease in the splenic and duodenal iron content. By using the cre/lox technology, we generated mice expressing the FPN(C326S) mutation only in duodenal enterocytes (Villin-Cre/FpnC326S) or in macrophages (Lyz-Cre/FpnC326S) to dissect the contribution of the two iron-exporting cell types in generating systemic iron overload. 10-week old C57BL6/J congenic male mice have been analyzed. Hematological parameters have been measured using the Scil-Vet blood analyzer while serum iron levels have been assessed using the SFBC and UIBC iron kits (Biolabo). Gene expression analysis has been performed using SYBR-green qRT-PCR. All mouse breeding and animal experiments were approved by and conducted in compliance with the guidelines of the EMBL Institutional Animal Care and Use Committee.
Results: Mice carrying the FPN(C326S) mutation specifically in the duodenum have the same alterations in hematological parameters found both in hemochromatotic patients and in constitutive FPN(C326S) mice, showing increased hemoglobin (Hb), hematocrit (HCT) and mean corpuscular volume (MCV). Serum iron content and transferrin saturation are strongly increased mirroring a hemochromatotic phenotype. Interestingly, Lyz-Cre/FpnC326S mice of unregulated macrophage iron export failed to show both hematological and serum iron alterations, providing the first indication that dietary iron uptake could be the major responsible mechanism in causing systemic iron overload in hemochromatosis. Hepatic iron measurement revealed a strong iron accumu-

lation only in Villin-Cre/FpnC326S mice. This, together with the systemic iron overload, causes an increase in circulating hepcidin levels triggering the FPN degradation in reticuloendothelial macrophages and causing splenic iron overload. Lyz-Cre/FpnC326S mice, instead, have splenic iron deficiency due to the presence of the FPN(C326S) unregulated iron channel that continuously exports iron from reticuloendothelial macrophages.
Summary and Conclusions: Our results show for the first time that unregulated duodenal iron export is the major contributor in the generation of systemic iron overload. This finding opens new insights in developing pharmacological strategies aimed to specifically limit dietary iron import for the treatment of primary and secondary iron overload diseases.

S505

REGULATORY NETWORK GATA1-MEDIATED ON SEC23B GENE IN ERYTHROID CELLS

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Background: Mutations in *SEC23B* gene cause the congenital dyserythropoietic anemia II (CDA II), an autosomal recessive disorder with ineffective erythropoiesis. Most of patients show biallelic mutations, according to the pattern of autosomal recessive inheritance. However, the 14% of the cases exhibit an incomplete pattern of inheritance, with a monoallelic *SEC23B* mutation. Mutations in deep regulatory regions of the *SEC23B* gene as well as in CDA-related loci could be hypothesized as further pathogenetic mechanisms of CDA II.
In *SEC23B* monoallelic patients we postulated the occurrence of a second mutation in *GATA1* gene, assuming that it could be involved in the regulation of SEC23B expression. GATA1 transcription factor is a key regulator of the erythro- and thrombopoiesis. Indeed, mutations in this gene have been already associated to specific CDA variants, as X-linked dyserythropoietic anemia and thrombocytopenia (XLTDA).
Aims: Our aims are: (i) to perform a mutational screening of *GATA1* genomic sequence in *SEC23B* monoallelic patients, as well as in those without *SEC23B* mutations; (ii) to characterize the promoter region of *SEC23B* and (iii) to analyze GATA1-mediated regulation of SEC23B expression.
Methods: Genomic mutational screening, gene and protein expression analyses were performed as described (Russo *et al.*, 2014; Russo *et al.*, 2013). In order to characterize *SEC23B* promoter region, 10 overlapping fragments covering a 3500 bp upstream region of the gene were cloned upstream the luciferase gene into PGL3 vector. Putative binding sites for GATA1 (GATA1bs) in the promoter sequence of *SEC23B* were predicted by MatInspector and PROMO web server tools. GATA1 cDNA was cloned into the expression vector pcDNA3.1. All mutants were obtained by site-direct mutagenesis. Direct binding of GATA1 to the GATA1bs was assayed by chromatin immunoprecipitation (ChIP) in both K562 and HEL cells at 6 days of erythroid differentiation.
Results: In our cohort we found one XLTDA patient with the hemizygous mutation GATA1-Gly208Arg. Two *SEC23B* monoallelic patients showed the single nucleotide variant rs113966884 G/A in GATA 1 5'upstream region. Both GATA1 variants resulted in a reduced gene expression, which in turn correlates with a decrease of SEC23B expression. By luciferase assay of the deletion mutants we identified the promoter region of the *SEC23B* gene (HuSEC23B/3.44). Ten putative GATA1bs were predicted. However, direct GATA1 binding were confirmed for only two sites, GATA1bs/HuSEC23B-2475 and -463. The co-transfection of GATA1bs/HuSEC23B-2475 and -463 mutants and GATA1 WT showed a reduction of 30-40% luciferase activity respectively, compared to WT sequence. Finally, we analyzed the GATA1-mediated regulation of SEC23B expression by co-transfection of several GATA1 mutants (GATA1/G208R, GATA1/R216W, GATA1/D218G, and GATA1/V205M) and the HuSEC23B/3.44 in HEK-293 cell line. We demonstrated a marked reduction of HuSEC23B/3.44 luciferase activity induced by the mutants G208R and R216W, which are the causative variants related to XLTDA and congenital erythropoietic porphyria (CEP), respectively.
Summary and Conclusions: This study provided new insights into the molecular mechanisms of SEC23B regulation. We also suggested an explanation of the variability of phenotypes GATA1-related by means of the crosstalk of this gene with *SEC23B*. Moreover, the identification of transcriptional regulatory elements in *SEC23B* promoter could allow the definitive diagnosis of CDAII patients with peculiar clinical phenotypes or those with incomplete mutation pattern in *SEC23B*.

MDS - Clinical

S506

PROGRESSIVE ANEMIA CAN BE DETECTED MORE THAN 2 YEARS PRIOR TO THE DIAGNOSIS OF MYELODYSPLASTIC SYNDROME: RESULTS FROM THE PRIMARY CARE SETTING IN DENMARK

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Background: Myelodysplastic syndrome (MDS) is a clonal hematopoietic disorder, characterized by inefficient hematopoiesis and peripheral blood cytopenias. Cytopenias are frequently observed in the primary care setting, where the clinical presentation of the patient determines further investigations instituted by the general practitioner (GP). Cytopenias have diverse origins such as inefficient hematopoiesis due to hematological malignancy, but also carcinomatosis, vitamin deficiency, immune dysfunction and viral infections. In primary care, MDS is a rare cause of cytopenias. However, if untreated they are persistent, and important predictors of survival in MDS according to the revised international prognostic scoring system (IPSS-R).
Aims: The aim was to investigate the association between pre-diagnostic cytopenias in primary care patients and the characteristics and prognosis of subsequently occurring MDS with focus on the hemoglobin concentration.
Methods: Between 2000 and 2010, GPs in the Copenhagen area were all served by one laboratory, The Elective Laboratory of the Capital Region (ELCR). The Copenhagen Primary Care Differential Count *(CopDiff)* database based on ELCR data contains information on all complete blood cell counts requested by GPs for 555,039 individuals during this period. Cancer occurrence within the *CopDiff* population has been obtained by linkage to the Danish Cancer Registry, which is known to have valid and almost complete registration of cancer cases in Denmark. For the present study, we included cases of MDS (ICD-10=D46) between 2000 and 2010Consecutive hemoglobin counts obtained prior to diagnosis were modeled using linear regression models. An estimate of the time point at which hemoglobin levels fell below the sex specific threshold, was derived from this regression. In this model patients with secondary MDS were excluded, n=3.
Results: We identified 284 patients with MDS of whom 221 had undergone primary care laboratory work-up in the preceding 10 years. The remaining patients (n=63) only had laboratory workup performed after diagnosis and were excluded from the present study. Hence, we included 221 patients with an average of four prediagnostic complete blood counts measured (SD=4,5). Anemia prior to MDS diagnosis was observed for 69% of men and 84% of women (p=0.01). Using the linear regression model, we estimated that male patients in average dropped below the hemoglobin reference range 780 days prior to diagnosis. The corresponding estimate for women was 250 days. At diagnosis women had estimated hemoglobin levels of 5.5 mmol/L (8.8 g/dL) and men of 5.1 mmol/L (8.3 g/dL). The proportion of patients presenting with prediagnostic thrombocytopenia and neutropenia were similar between men and women (Table 1).

Table 1.

Patients	Female (n=103)	Male (n=118)
Median age at diagnosis, years	74	73
Average number of complete blood cell counts prior to diagnosis of MDS per patient, (SD)	4.1 (3.4)	3.8 (3.8)
Anemia (hemoglobin ? <7 mmol/L ? < 8mmol/L) n (%)**	71 (69)	99 (84)
Thrombocytopenia (platelets < 150-10^9/L), n (%)	62 (60)	72 (61)
Neutropenia (neutrophils < 1.8 10^9/L) , n (%)	40 (39)	46 (39)
Mean interval to diagnosis of MDS in days*	250	780
Mean hemoglobin at diagnosis, predicted	5.46	5.14
Mean Charlson score	0.83	0.68

*Days are counted from the day the hemoglobin count was below sex specific reference interval
**P=0.01

Summary and Conclusions: Cytopenia is a cardinal finding in MDS. In this study we demonstrate that men on average are anemic two years prior to diagnosis. This is significantly longer than for women. According to the IPSS-R we know that the severity of anemia is a risk factor, and we speculate that this risk factor is an indirect marker of disease duration. If so, it would emphasize the need for earlier diagnosis to improve prognosis, especially in male patients. We are currently performing subgroup analysis according to bone marrow histology, cytogenetics and IPSS-score. This will elucidate if the duration of prediagnostic anemia *per se* has prognostic impact. Furthermore we will investigate these patterns in subgroups of low- and high-risk MDS and estimate prediagnostic neutro-and thrombocytopenia as well. These data will be presented at the meeting.

S507

CLINICAL AND MOLECULAR PREDICTORS OF RESPONSE TO ERYTHROPOIESIS STIMULATING AGENTS (ESA) IN LOWER RISK MDS PATIENTS

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Background: Up to 50% of lower risk MDS respond to ESA. While response can be predicted by serum(s) EPO level and transfusion dependence (TD), it is unknown if molecular findings are also prognostic.
Aims: We studied prognostic factors of erythroid response (HI-E, IWG 2006 criteria) to ESA in 90 lower risk MDS treated with ESA in France (GFM), Italy (FISM) and Germany (GMDS) with a focus on molecular mutations.
Methods: We studied prognostic factors of erythroid response (HI-E, IWG 2006 criteria) to ESA in 90 lower risk MDS treated with ESA in France (GFM), Italy (FISM) and Germany (GMDS) selected on the availability of all the following: sEPO, serum ferritin (SF), marrow slides for review (especially of dysplasia). In 79 of them, mutational analysis of 39 genes recurrently mutated in myeloid malignancies was performed on marrow cell DNA collected before ESA treatment, using an AmpliSeq approach (Life Technologies), and confirmed by Sanger method when the variant allele frequency was found upper than 20%.
Results: Median age was 74 years, with 56% males, 26% RBC TD patients, median Hb level 9.5 (range 6.2-11) in non RBC-TD patients, median sEPO level 59 U/l (range 9-402), median SF 411 µg/l (range 11-1856), WHO diagnosis: 21% RA; 24.4% RAEB-1; 24.6% RARS; 24.4% RCMD; 2% del(5q); 3.6% MDS-U. IPSS was low (45.3%) and int-1 (54.7%), IPSS R: very low (6.6%), low (59.7%) and int (33.7%). 85% pts had dyserythropoiesis, 62% dysgranulopoiesis, 67% dysmegakaryopoiesis. HI-E, was 64.5% and median response duration 19.8 months. Clonal and subclonal mutations were found in: SF3B1 (40.5% patients), TET 2 (35.4%), ASXL1 (31.4%), DNMT3A (20.2%), U2AF1 (10.1%), SRSF2 (8.8%), IDH1/IDH2 (7.4%), RUNX1 (2.5%), STAG2 (11.4%), NRas (3.7%), KRas (3.7%), BCOR (3.7%), Jak2, PHF6, and CBL in 1 pt each. 86.1% patients had at least one mutation (median number 2,range 0-6). As previously described by others, SF3B1, DNMT3A and TET2 co-occurred frequently. ASXL1 and DNMT3A seemed to be mutually exclusive and SRSF2 never co-occurred with SF3B1.The number of mutations was not correlated with the degree of dysplasia. By univariate analysis, male gender, sEPO level >100U/L, IPSS-R (int *vs* very low and low) were correlated with worse HI-E. Patients with ≤2 mutations had better HI-E (OR: 0.32, p=0.02), but none of the most frequent mutations (SF3B1, ASXL1, TET2, DNMT3A), age, karyotype and importance of dysplasia had any impact on HI-E. In an adjusted logistic regression model, male gender, sEPO level>100U/L and RBC- TD, but not number of mutations, were significantly correlated with poorer HI-E.
Summary and Conclusions: Presence of >2 mutations predicted worse HI-E to ESA in lower risk MDS. However, in multivariate analysis, mutational analysis lost its prognostic value compared to more conventional factors, especially RBC-TD and sEPO level.

S508

ANALYSIS OF PROGNOSTIC MARKERS IN 615 PATIENTS WITH THERAPY-RELATED MYELODYSPLASTIC SYNDROMES-ARE CURRENTLY AVAILABLE SCORING SYSTEMS SUITABLE IN THIS PATIENT GROUP?

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Background: Prognostication in myelodysplastic syndromes (MDS) has recently been improved by the revised International Prognostic Scoring System (IPSS-R). However this score, as the original IPSS, was developed analyzing primary, untreated patients (pts) only. Data on its usefulness in pts with therapy-related MDS (tMDS) is limited.
Aims: We analyzed 615 pts from Spanish, German, Swiss, and Austrian centers diagnosed 1975-2015.
Methods: Complete data to calculate the IPSS/-R was available in 446 pts. Prognostic impact of features was analyzed by uni- and multivariable models and estimated by a measure of concordance for censored data (Dxy).
Results: Median age was 67 years. According to WHO classification 2% of pts had 5q-syndrome, 6% RA, 3% RARS, 27% RCMD, 9% RCMD-RS, 16% RAEB-1, 18% RAEB-2, 6% CMML-1, 2% CMML-2, 3% MDS-U, and 8% AML (RAEB-T). Cytogenetics were 47% good, 14% intermediate, and 39% poor according to IPSS, and 2% very good, 44% good, 17% intermediate, 16% poor, and 21% very poor according to IPSS-R. Regarding prognostic risk groups 19% exhibited IPSS-low, 33% int-1, 30% int-2, and 18% high, while the IPSS-R was very low in 8%, low in 26%, intermediate in 16%, high in 22%, and very high in 27%. Regarding the primary disease most frequent diagnoses were NHL 19%, breast cancer 16%, myeloma 10%, Hodgkin's disease, and AML 6% each. 75% of pts received chemotherapy and 47% received radiotherapy. Most pts received combination regimen containing alkylating agents in 59%, topoisomerase inhibitors in 35%, antitubulin agents in 28%, and antimetabolites in 38%. Latency periods varied broadly (≤3 yrs 22%, >3-≤6 yrs 26%, >6-≤12 yrs 31%, >12 yrs 21%). Median follow-up from MDS diagnosis was 56 months, median survival 17 months. After MDS diagnosis 30% of pts received disease altering treatment, including stem cell transplantation in 17%. Features with influence on survival and time to AML in univariable analysis included age, FAB, WHO, IPSS, IPSS-R, cytogenetic risk, platelets, marrow and peripheral blasts, ferritin, fibrosis, year of primary diagnosis. Predominantly influence on survival was seen for year of MDS diagnosis, hemoglobin, LDH, and use of alkylating agents. A latency period >12 years showed higher risk of AML. Neutrophil count, use of chemo or radiotherapy as well as other chemotherapeutic agents had no influence on both outcomes. Our results indicate that both the IPSS (Dxy 0.26 for survival, 0.35 for AML), and IPSS-R (Dxy 0.32 for both) perform moderately in tMDS, but not as well as in primary MDS. Adjusting prognostic models to tMDS seems therefore required. Score versions including peripheral blasts perform somewhat better. Separate score versions for survival and time to AML would give differing weights to most features. Hemoglobin and cytogenetics would get more weight for survival, while marrow blasts would be more important regarding AML. Another issue is the possible integration of data on primary disease/therapy (Figure 1).

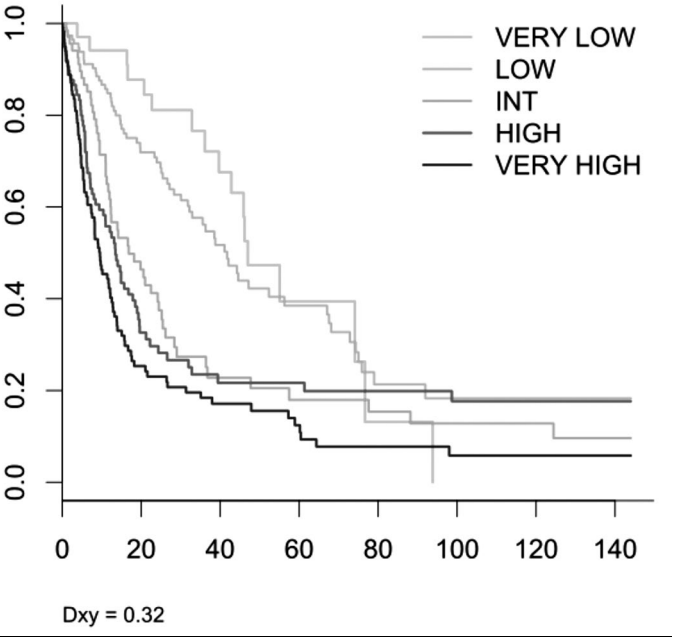

Figure 1. Survival by IPSS-R.

Summary and Conclusions: In contrast to early publications on tMDS, where aberrant cytogenetics were described in >90% of pts and prognosis was seen uniformly poor, surprisingly we find good risk karyotypes in a relatively large number. Although some cases might be unrelated to previous therapy and poor

risk cytogenetics are still overrepresented, this indicates, different types of tMDS exist. Our analysis shows that indeed many variables exhibit a prognostic influence in tMDS. About one third of our pts were treated for MDS. However, censoring/leaving them out would not show a representative cohort. Further analyses are performed to propose an optimized scoring system for tMDS.

S509

LUSPATERCEPT INCREASES HEMOGLOBIN AND REDUCES TRANSFUSION BURDEN IN PATIENTS WITH LOW OR INTERMEDIATE-1 RISK MYELODYSPLASTIC SYNDROMES (MDS): PRELIMINARY RESULTS FROM THE PHASE 2 PACE-MDS STUDY

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Background: Luspatercept (ACE-536) is a fusion protein (modified activin receptor IIB-IgG Fc) being investigated for the treatment of anemias with ineffective erythropoiesis. MDS patients have increased GDF11 levels (Suragani, Nat Med 2014) and aberrant Smad2,3 signaling in the bone marrow. Luspatercept binds TGF-β superfamily ligands, including GDF11, inhibits Smad2,3 signaling, and promotes late-stage erythroid differentiation, distinct from ESAs. In a healthy volunteer study, luspatercept was well-tolerated and increased hemoglobin (Hb) levels (Attie, Am J Hematol 2014).
Aims: This is an ongoing, phase 2, multicenter, open-label, dose-finding study to evaluate the effects of luspatercept on anemia in patients (pts) with low/int-1 risk MDS (IPSS classification). Study outcomes include erythroid response of increased Hb in low transfusion burden (LTB) pts (<4 RBC units/8 weeks), reduced transfusions in high transfusion burden (HTB) patients (≥4 RBC units/8 weeks), safety, PK, and PD biomarkers.
Methods: Inclusion criteria include age ≥18 yr, anemia, defined as being HTB or LTB with Hb <10.0 g/dL, EPO >500 U/L or nonresponsive/refractory to ESAs, no prior azacitidine or decitabine, and no current treatment with ESA, G-CSF, GM-CSF, or lenalidomide. Luspatercept was administered by SC injection once every 3 weeks in sequential cohorts (n=3-6 each) at dose levels ranging from 0.125 to 1.75 mg/kg for up to 5 doses with a 3-month follow-up. Treatment of an expansion cohort is ongoing, with individual pt dose titration allowed. Patients completing this study may enroll into a 12-month extension study.
Results: Enrollment is complete for 27 pts in the dose escalation cohorts and 31 pts in the expansion cohort. Preliminary safety and efficacy data (as of 23Feb2015) were available for 44 pts (19F/25M, 15 LTB/29 HTB). Mean age was 68.1 yr, 61% had prior EPO therapy, and 21% had prior lenalidomide. 35 (80%) patients had ≥15% ring sideroblasts (RS) in bone marrow. The mean baseline Hb for LTB patients was 8.6 g/dL, ranging from 6.8 to 10.1 g/dL. LTB pts treated at 0.75-1.75 mg/kg (n=13) had a 77% response rate for the primary endpoint (Hb increase ≥1.5 g/dL for ≥2 weeks) and a 69% IWG HI-E response rate (Hb increase ≥1.5 g/dL over 8 weeks). The mean (SD) maximum change in hemoglobin was 2.7 (1.1) g/dL in the higher dose groups compared with 0.9 (0.1) g/dL in the lower dose groups. HTB patients treated at 0.75-1.75 mg/kg (n=22) had a 50% HI-E response rate (reduction of ≥4 RBC units over 8 weeks). Higher response rates were observed in pts with ring sideroblasts and/or mutations of SF3B1 (data to be presented). Of the patients with RBC transfusions during the 8 weeks prior to treatment, 10/28 (36%) patients were transfusion-free for ≥8 weeks during treatment. Luspatercept was generally well-tolerated. Adverse events were mostly mild-moderate. The most frequent related adverse events (>10% patients) were bone pain, headache, diarrhea, myalgia, and pain in extremity.
Summary and Conclusions: Based on preliminary data in Low/Int-1 MDS patients, luspatercept treatment at therapeutic dose levels for 3 months led to HI-E response for increased Hb levels and/or decreased transfusion requirement in the majority of patients, with a favorable safety profile. Higher response rates were observed in patients with ring sideroblasts and/or SF3B1 mutations. These data strongly support further evaluation of luspatercept in patients with low to intermediate risk MDS.

S510

A PHASE 2, DOSE-FINDING STUDY OF SOTATERCEPT (ACE-011) IN PATIENTS (PTS) WITH LOWER-RISK MYELODYSPLASTIC SYNDROMES (MDS) AND ANEMIA REQUIRING TRANSFUSION

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Background: Anemia, a hallmark of MDS, is challenging to treat, particularly after failure of erythropoiesis-stimulating agents (ESAs). Sotatercept (ACE-011) is a novel and first-in-class activin type IIA receptor fusion protein that acts on late-stage erythropoiesis to increase release of mature erythrocytes into circulation (Carrancio S, *et al.* Br J Haematol 2014;165:870-82).
Aims: This phase 2, open-label, dose-finding study aims to determine a safe, tolerable, and effective dose of sotatercept in anemic pts with lower-risk MDS.
Methods: The primary endpoint was erythroid hematological improvement (HI-E; modified International Working Group 2006 criteria). Secondary endpoints included rate of RBC transfusion independence (TI) ≥8 weeks and safety. Eligible pts had International Prognostic Scoring System-defined Low or Intermediate-1-risk MDS (Greenberg P, *et al.* Blood 1997;89:2079-88) and anemia, with no response, loss of response, or low chance of response to ESAs. Pts received subcutaneous sotatercept at 0.1, 0.3, 0.5, 1.0, or 2.0 mg/kg every 3 weeks for up to 8 doses; pts with clinical benefit may receive treatment beyond 8 doses until meeting protocol discontinuation criteria. Informed consent was obtained from all pts. ClinicalTrials.gov identifier: NCT01736683.
Results: As of February 4, 2015, a total of 59 MDS pts were enrolled: 7, 6, 21, 20, and 5 in the 0.1, 0.3, 0.5, 1.0, and 2.0 mg/kg groups, respectively. Overall, 66% of pts were male, median age was 71 years, and median time from diagnosis 4 years. Pts received a median 6 RBC units (range 0-16) in the 8 weeks prior to treatment; 50 (85%) received ≥4 RBC units (high transfusion burden; HTB) and 9 (15%) received <4 RBC units (low transfusion burden; LTB). In this heavily pre-treated cohort, 56 (95%) pts were previously treated with ESAs, 31 (53%) with hypomethylating agents, 27 (46%) with lenalidomide, and 26 (44%) with other MDS treatments. Of 53 pts evaluable for efficacy, 23 (43%) achieved HI-E: 0 of 7 (0%), 4 of 6 (67%), 9 of 20 (45%), and 10 of 20 (50%) pts in the 0.1, 0.3, 0.5, and 1.0 mg/kg groups, respectively. Review of efficacy data for the 2.0 mg/kg dose group is ongoing. Of 45 evaluable HTB pts, 6 (13%) achieved RBC-TI ≥8 weeks; median duration of longest response was 67.5 days, 107 days, and 122.5 days in the 0.3, 0.5, and 1.0 mg/kg dose groups, respectively. Of 8 evaluable LTB pts, 5 (63%) achieved RBC-TI ≥8 weeks and a mean hemoglobin increase of ≥1.5 g/dL sustained for ≥8 weeks; duration of RBC-TI for responders ranged from 175-472+ days. Sotatercept was generally well tolerated. The most common adverse events (AEs), all grades, were asthenia/fatigue (n=24), peripheral edema (n=12), and diarrhea (n=12). Eighteen (31%) pts had ≥1 grade 3 or 4 AE; of these, 3 (5%) were reported as suspected to be study drug-related (1 each with grade 3 pain in extremity, grade 3 hypertension, and grade 4 acute myeloid leukemia, all in the 0.5 mg/kg dose group). Overall, 4 pts discontinued due to suspected treatment-related AEs: 1 each with grade 2 hemolytic anemia, grade 2 hypertension, grade 2 muscular weakness, and grade 2 increased blood pressure with grade 2 diarrhea in the 0.3, 0.5, 1.0, and 2.0 mg/kg groups, respectively.
Summary and Conclusions: Data from this ongoing study suggest sotatercept was well tolerated, with promising evidence of clinical activity in this cohort of heavily pre-treated, anemic, lower-risk MDS pts who had failed or were refractory to prior ESAs.
PF and AFL contributed equally to this abstract as senior co-authors.

AML outcome and clinical trials

S511

CONTINUED IMPROVEMENT IN SURVIVAL OF ACUTE MYELOID LEUKEMIA PATIENTS AND AN APPLICATION OF THE LOSS IN EXPECTATION OF LIFE

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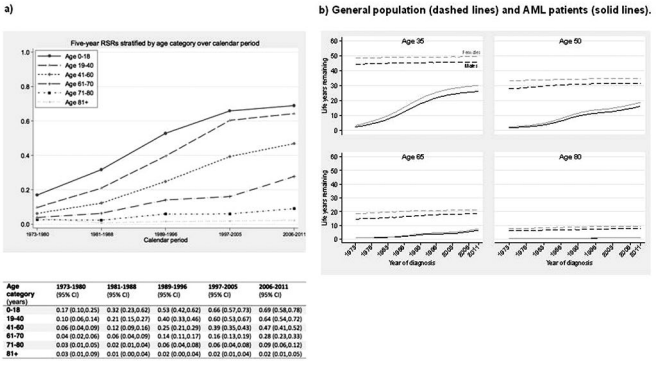
Background: Survival in acute myeloid leukemia (AML) is increasing, but most survival data come from clinical trials. National registries provide population-based survival data. Relative survival ratios (RSRs) provide a measure of total excess mortality associated with AML irrespective of whether the excess mortality is directly or indirectly because of AML. A new statistical method, loss in expectation of life (LEL), has recently been developed and provides an alternative for presenting survival.
Aims: We previously evaluated RSRs in Swedish AML patients 1973 -2005 and found a large improvement in survival for younger patients, while less improvement was seen among those 71+ years.[1] We now extend the study to include patients diagnosed -2011 aiming to investigate whether there was a continuous improvement in survival and whether any improvement was seen among elderly. The aim was also to present trends in survival using LEL.
1 Derolf *et al.*, Blood 2009
Methods: AML patients in the Swedish Cancer Registry 1973-2011 were included. Date of death was obtained from the Register of Causes of Death. RSRs and LEL were used to quantify survival. RSR is defined as the all-cause survival of the patients under study divided by the expected survival of a comparable group from the general population. LEL is calculated by subtracting the life expectancy for a patient from the life expectancy of a matched subset of the general population. This is interpreted as the number of life years lost due to an AML diagnosis. 5-year conditional LEL measures the LEL for patients conditional on the fact that they have survived 5 years since diagnosis.
Results: 11598 AML patients (51% males; 2212 diagnosed 2006-2011) were included. Median age was 69 years. 10 and 69 allogeneic SCTs were perfomed in patients 61-70 years (EBMT register data) in the two last calendar periods, respectively. The increase in RSRs for 1973-2005 continued into the additional 6 years for all ages except patients 81+ years (Figure 1). The increase was most pronounced for patients 61-70 years; 0.16 (95% CI: 0.13-0.19) and 0.28 (95% CI: 0.23-0.33) in 1997-2005 and 2006-2011, respectively. Among patients 41-60 years RSRs increased from 0.39 (95% CI 0.35-0.43) to 0.47 (95% CI 0.41-0.52). The greatest improvement in survival 1973-2005 was seen among patients ≤40 years; now the increase in survival was modest. LEL decreased for all AML patients 1973-2011 but most for those aged 35 and 50 years (Figure 1). A 35-year old male diagnosed in 1973 lost, on average, 41.6 (95%CI: 40.7-42.5) life years whereas a 35-year old male diagnosed in 2011 is predicted to lose 19.5 (95%CI: 16.4-22.5) life years. 5-year conditional LEL decreased for younger patients during the study period suggesting less long-term complications of applied treatment and fewer relapses. However, young patients surviving 5 years still have their lives shortened by about two years.



Age category (years)	1973-1980 (95% CI)	1981-1988 (95% CI)	1989-1996 (95% CI)	1997-2005 (95% CI)	2006-2011 (95% CI)
0-18	0.17 (0.10,0.25)	0.32 (0.23,0.42)	0.53 (0.42,0.62)	0.66 (0.57,0.73)	0.69 (0.58,0.78)
19-40	0.10 (0.06,0.14)	0.21 (0.15,0.27)	0.40 (0.33,0.46)	0.60 (0.53,0.67)	0.64 (0.54,0.72)
41-60	0.06 (0.04,0.09)	0.12 (0.09,0.16)	0.25 (0.21,0.29)	0.39 (0.35,0.43)	0.47 (0.41,0.52)
61-70	0.04 (0.02,0.06)	0.06 (0.04,0.09)	0.14 (0.11,0.17)	0.16 (0.13,0.19)	0.28 (0.23,0.33)
71-80	0.03 (0.01,0.05)	0.02 (0.01,0.04)	0.06 (0.04,0.08)	0.06 (0.04,0.08)	0.09 (0.06,0.12)
81+	0.03 (0.01,0.09)	0.01 (0.00,0.04)	0.02 (0.00,0.04)	0.02 (0.01,0.04)	0.02 (0.01,0.05)

Figure 1. Temporal trends in survival of AML patients illustrated by a) relative survival rations stratified by age category over calendar period; and b) temporal trends in the life expectancy of the general population and AML patiens.

Summary and Conclusions: AML survival continued to improve except among patients 81+ years. Most improvement was seen among patients 61-70 years. In Sweden at least 85% of patients of this age are treated with induction chemotherapy. Our results suggest clear benefit from intensive treatment including allogenic SCT. Posakonazol profylaxis was introduced during the last calendar period and risk stratification has improved, both are factors that may also contribute to increased survival in all age groups.
RSR is useful for comparisons of survival but we believe that using the LEL and comparing survival in terms of years, rather than proportions, is more intuitive and useful for communicating survival statistics.

S512

CLINICAL OUTCOME IN OLDER AML PATIENTS NOT FIT FOR INTENSIVE CHEMOTHERAPY: RESULTS OF THE POPULATION-BASED AMLSG-BIO (NCT01252485) REGISTRY STUDY OF THE GERMAN-AUSTRIAN AML STUDY GROUP

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Background: Comparative population-based outcome data in AML of older patients not fit for intensive chemotherapy are rare. Currently available treatment options outside clinical trials are best supportive care (BSC), decitabine (DAC), azacitidine (AZA), and low-dose cytarabine (LDAC).
Aims: To assess clinical outcome in patients older than 60 years not fit for intensive chemotherapy within the registry/molecular-screening trial AMLSG BiO (NCT01252485).
Methods: Between 2011 and 2014 a total of 2651 patients (median age 72 years, range 60- 94) have been registered. Molecular screening was performed within 48hours for *NPM1* and *FLT3* mutations as well as for the fusion-genes *PML-RARA*, *RUNX1-RUNX1T1*, *MYH11-CBFβ*. Treatment and outcome data were documented based on medical records and case record forms.
Results: Of the 2651 patients, n=1104 (42%) received intensive chemotherapy, n=88 (3%) AZA, n=162 (6%) DAC, n=250 (9%) LDAC, n=229 (9%) BSC, n=818 (31%) unknown. Of the 729 patients treated non-intensively n=176 (LDAC, n=134; DAC, n=40; AZA, n=1) were treated in clinical trials. Of the remaining 553 patients, the distribution according to treatment was n=116 LDAC, n=122 DAC, n=87 AZA and n=228 BSC. There was no difference in the age distribution between the groups BSC, AZA, DAC, LDAC (p=0.22, median 76 years, range 60-92), cytogenetic risk group according to ELN recommendations (high, 26%; intermediate, 72%; low, 2%; p=0.35), HCT-CI score <3 (p=0.50), but significantly more patients with ECOG performance status >1 (p<0.001) were in the BSC group, however, without difference between the treatment-groups AZA, DAC, LDAC (p=0.76). Patients with a *NPM1* mutation were more frequently (p<0.001) found in the BSC (23%) and the LDAC (22%) group compared to AZA (5%) and DAC (10%). Overall, *FLT3*-ITD and *FLT3*-TKD were detected rarely and mutually exclusive (3% each) without a difference between the groups (p=0.87, p=0.89). Significantly (p<0.001) higher white blood counts (WBC) at diagnosis were present in the BSC (median, 27/nl) and LDAC (median, 29.6/nl) compared to AZA (median, 3.5/nl) and DAC (median, 5.0/nl), no differences between groups were present for platelets (p=0.97) and hemoglobin (p=0.60). Median, one-year and two-year survival according to the 4 groups (BSC, AZA, DAC, LDAC) are given in the Table 1.

Table 1.

	BSC	AZA	DAC	LDAC
Median survival (months)	0.9	9.7	10.1	3.5
One-year survival	12%	45%	46%	29%
Two-years survival	2%	13%	8%	14%

In a Cox regression model including all patients receiving therapy (AZA, DAC, LDAC), overall survival was significantly influenced by cytogenetic high-risk category (HR, 1.8; p=0.006), ECOG performance status <2 (HR, 0.5;

p=0.0008), treatment (reference LDAC) with AZA (HR, 0.37; p=0.00003), DAC (HR, 0.38; p=0.00008), but not by NPM1 (p=0.72) and FLT3 (p=0.40) mutational status. In univariable comparisons, treatment with AZA and DAC resulted in a significantly better survival of patients with high-risk cytogenetics (p=0.001), normal cytogenetics (p=0.03), ECOG performance status <2 (p=0.0007), HCT-CI score <3 (p=0.0006) and low WBC (<4.0/nl).
Summary and Conclusions: In older patients with AML treatment with AZA or DAC is in the short-term perspective of one to two years superior to LDAC. However, results beyond 2 years are still dismal for all treatment options.

S513

A COMPARISON OF LIMITED CONSOLIDATION CHEMOTHERAPY THERAPY OR NOT, AND DEMETHYLATION MAINTENANCE OR NOT IN OLDER PATIENTS WITH AML AND HIGH RISK MDS: LONG TERM RESULTS OF THE UK NCRI AML16 TRIAL

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Background: In older patients (>60yrs) with AML or high risk MDS (>10% marrow blasts), the UK NCRI AML16 trial compared two induction courses of DA *vs* DClo +/- gemtuzumab ozogamicin (GO). The benefit of GO has been reported (Burnett *et al.* J Clin. Oncol *30*(32); 3924-31, 2012). The trial also compared adding etoposide or ATRA to DA induction in a 2x2 design, and considered the optimal number of courses of treatment and the use of maintenance therapy.
Aims: Following 2 courses of induction patients with at least a partial remission (<15% marrow blasts) after course 1, and in CR after course 2 were eligible to be randomised to one course of consolidation (Dauno 50mg/m^2 d 1, 2$^+$ara-C 100mg/m^2 bid d 1-5) or not; patients in remission after course 2 could be randomised to maintenance (Azacitidine 75mg/m^2 for 5 d x9 every 6 wks) or not.
Methods: From 8/2006-5/2012, 1880 patients entered the intensive induction options: 573 patients were randomised between 3 *vs* 2 courses in total, and 530 between maintenance and not. Of those randomised for consolidation 474 entered CR after course 1 and 99 achieved PR after course 1 and were in CR after course 2. Of the 530 patients entering the maintenance randomisation, 421 were in CR post course 1. A total of 453 patients entered both questions. Randomisations were stratified by induction chemotherapy and other demographics to ensure balance. The demographics of those entering the post induction options were: median age 67y (53-84); 77% de novo, 14% secondary, 8% high risk MDS; 4%, 82%, 14% favourable, intermediate, poor risk cytogenetics: 18% *FLT3*-ITD and 30% *NPM1* mutant*:* 32%, 40%, 28% Wheatley good, standard, poor risk. Follow-up is complete to 1st January 2014 (median 50.4 months).
Results: For all trial entrants the overall response rate was 69% (CR 59%, CRi 10%) and 5-yr OS was 14%. There were no significant outcome differences between induction arms. There was no difference in 5-year survival by consolidation randomisation (consolidation 25% *vs* not 22% HR 0.92 (0.75-1.12) p=0.4). Similarly, there was no difference in 5-year OS in those who did (24%) or did not (20%) receive maintenance (HR 0.93 (0.76-1.14) p=0.5). In the 226 patients allocated consolidation there was no difference in 5 year survival whether they received maintenance (26%) or not (21%) (p=0.7). Of 227 patients allocated no consolidation there was a non-significant benefit of maintenance (5yr OS 27% *vs* 18%, p=0.15), but no significant interaction (p=0.5). During the study marrow samples were tested for MRD by immuno-flow analysis (Freeman S. *et al.* J Clin. Oncol. *31;* 4123-4131, 2013) with a sensitivity of 1 in 10^4. This showed that for patients in CR after course 1, MRD positivity was the most powerful predictor of survival in multivariable analysis. In the 74 MRD -ve patients who received consolidation or not, there was a trend for improved OS (36% *vs* 26%) for recipients of consolidation (p=0.09); however, in the 74 patients who were in CR but MRD +ve, survival was significantly inferior with consolidation (11% *vs* 27%; p=0.03). In the 73 MRD -ve patients in the maintenance randomisation, there was a significant survival benefit for maintenance (40% *vs* 13%, p=0.003), but no difference for MRD +ve patients (20% *vs* 23%, p=0.9). There was no additional benefit from both consolidation and maintenance irrespective of MRD status.
Summary and Conclusions: Overall, neither a 3rd course nor demethylation maintenance improved survival. However in the ~50% of patients who achieved CR with course 1 who were MRD -ve, OS was improved by consolidation or maintenance but not incrementally by both. MRD appears to identify chemosensitive patients who benefit from additional treatment whereas the chemoinsensitive do not.
Acknowledgements: This study received research support from Cancer Research UK. Celgene provided Azacitidine for the trial.

S514

A COMPARISON OF DAUNORUBICIN/ARA-C *VERSUS* DAUNORUBICIN/CLOFARABINE AS INDUCTION TREATMENT IN OLDER PATIENTS WITH AML AND HIGH RISK MDS: LONG TERM RESULTS OF THE UK NCRI AML16 TRIAL IN 806 PATIENTS.

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Background: In older patients (>60yrs) the UK NCRI AML16 trial compared two courses of DA *vs* DClo induction with or without gemtuzumab ozogamicin (GO). The benefit of the addition of GO has been reported (Burnett *et al.* J Clin. Oncol *30*(32);3924-3931, 2012).
Aims: Based on a feasibility study a schedule of Clofarabine 20mg/m^2 d 1-5 was combined with Daunorubicin (Dauno) 50mg/m^2 d 1-3 for two courses, and compared with Dauno 50mg/m^2 d 1,3,5+ara-C 100mg/m^2 bid d 1-10 (course 1) or d 1-8 (course 2). Subsequently, patients with at least a partial remission (<15% marrow blasts) after course 1, and CR after course 2 were eligible to be randomised to one course of consolidation (Dauno 50mg/m^2 days 1, 2$^+$ara-C 100mg/m^2 bid days 1-5); patients in CR could be randomised to azacytidine maintenance or not.
Methods: From 8/2006-12/2009, 806 patients were randomised between DA and DClo, of whom 683 entered the GO randomisation. The median age was 67y (56-84); 72% had de novo, 17% secondary, and 11% high risk MDS; 4%, 73% and 23% had favourable, intermediate, poor risk cytogenetics; 14% were *FLT3*-ITD and 20% *NPM1* mutant; 30%, 34%, 36% had good, standard, poor Wheatley risk. Following the two courses of induction, 263 patients were randomised to 2 *vs* 3 courses (138 from DA and 125 from DClo; 42% were Wheatley good risk, 37% standard risk, 21% poor risk). 274 were randomised to maintenance or not (137 from DA and 137 from DClo). The post induction interventions will be reported separately, but are taken into account in this assessment of induction treatments. Follow-up is complete to 1/1/2014 (median FU 66 months).
Results: In the DA *vs* DClo randomisation, the overall response rate (ORR) was 68% (CR 61%, CRi 7%) and 5-year OS 14%. The ORR was not different between DA: 71% (CR 64%, CRi 6%) and DClo 66% (CR 58%, CRi 8%), OR 1.26 (0.94-1.70) p=0.12, with 60 day all-cause mortality of 15% and 14% respectively. Remission rates after course 1 were higher with DA (54% *vs* 47%, OR 1.33 (1.01-1.76) p=0.04). There were no significant differences between DA and DClo at 5 years in RFS (14% *vs* 15%, HR 0.98 (0.82-1.18) p=0.9), CIR (78% *vs* 71%, HR 0.92 (0.76-1.12) p=0.4), death in CR (8% *vs* 14%, HR 1.50 (0.90-2.49) p=0.12), survival from CR (20% *vs* 22%, HR 0.96 (0.79-1.16) p=0.6), survival from relapse (4% *vs* 7%, HR 0.92 (0.75-1.13) p=0.4) or OS (15% *vs* 14%, HR 1.04 (0.89-1.21) p=0.6). The schedules were equi-toxic, and although neutrophil and platelet recovery was quicker in the DA arm in both courses DClo patients received significantly less transfusion support, days on antibiotics and hospitalisation. There was no evidence of interaction between the two induction randomisations, or between the DA *vs* DClo randomisation and any demographic feature. There was no evidence of interaction between the number of courses (3 *vs* 2) or receipt of maintenance or not. 135/410 patients in the clofarabine induction randomisation who achieved CR post course 1had information for MRD by immuno-flow analysis (Freeman S. *et al.* J Clin. Oncol. *31;* 4123-4131, 2013), Of patients in morphological CR after the first induction course 48% of DA and 47% of DClo patients were MRD -ve which resulted in a survival of 25% and 37% respectively. For those who were in CR but were MRD +ve the survival was 14% for DA and 19% for DClo patients.
Summary and Conclusions: DA and DClo in induction give similar outcomes in older patients, with equivalent toxicity, and slightly lower resource usage on the DClo arm. Of patients in CR after course 1, as defined by immunoflow MRD +ve.
Acknowledgements: This study received research support from Genzyme and Cancer Research UK.

S515

DASATINIB (DAS) IN COMBINATION WITH CHEMOTHERAPY AND AS MAINTENANCE IN CORE-BINDING FACTOR (CBF) ACUTE MYELOID LEUKEMIA (AML): A PHASE IB/IIA STUDY OF THE GERMAN-AUSTRIAN AML STUDY GROUP (AMLSG)

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Background: The outcome in CBF-AML [AML with t(8;21)(q22;q22) or inv(16)(p13.1q22) or t(16;16)(p13.1;q22)] has been largely improved by using repetitive cycles of high-dose cytarabine (HiDAC) for postremission treatment. However, still only half of the CBF-AML patients (pts) have a favorable long term outcome, indicating the need for improved therapeutic approaches. Dasatinib (DAS) is a multikinase inhibitor with activity against the KIT kinase, which is highly expressed in CBF-AML and mutated in around one third of the cases. In preclinical studies DAS inhibited growth and survival of AML progenitor cells, enhanced the sensitivity to chemotherapy and induced *in vitro* differentiation of AML cells (Dos Santos *et al.*, Blood 2013; Fang *et al.*, PLoS One 2013). Anecdotally, DAS also induced differentiation of blasts in an AML patient with t(8;21) after single-drug treatment (Chevalier *et al.*, Leukemia 2010).
Aims: The primary endpoint in the open-label AMLSG 11-08 study (NCT00850382) was to assess the feasibility of DAS after intensive induction and postremission chemotherapy, and as single agent in 1-year maintenance therapy in pts with newly diagnosed CBF-AML.
Methods: Adults [≥18 years (yrs)] with CBF-AML received induction with daunorubicin (60 mg/m^2/d, d1-3) and cytarabine (200 mg/m^2/d, d1-7); DAS 100 mg/d was added on days 8-21; pts with partial remission could receive a second induction cycle. Pts achieving a complete remission (CR) or CR with incomplete hematologic recovery (CRi), were intended to receive four cycles of HiDAC [cytarabine 3 g/m^2 (in pts >60 yrs 1 g/m^2) q12h, d1,3,5); DAS 100 mg/d was added on days 6-28 of each cycle. Maintenance with DAS 100 mg/d was continued for 1 year after completion of chemotherapy or until relapse.
Results: Eighty-nine pts [median age 48.5 yrs; males, n=47; t(8;21), n=37; inv(16), n=52] were included in the study. The median baseline WBC in AML with t(8;21) and inv(16) was 9.2 Giga (G)/l and 34.1 G/l, respectively. The current median follow-up is 2.9 yrs. The overall CR/CRi rate was 93% (83/89); CR/CRi rate in AML with t(8;21) and inv(16) was 94% and 92%, respectively. Overall, there were 2 cases with refractory disease and 5 cases with early/hypoplastic death. The median time until recovery of neutrophils (>0.5 G/l) and platelets (>20 G/l) after induction cycles was 27d and 23d, respectively, and after the consolidation cycles 22d and 21d, respectively. To date, a total of 64 serious adverse events (SAEs) with suspected causal relationship to DAS have been reported. Among them the most frequent SAEs were infection-related (n=23); they only rarely occurred during maintenance phase (n=2). Pleural/pericardial effusion and liver toxicity CTC ≥3° was reported in 11% (10/89) and 10% (9/89) of the pts, respectively. There was no significant difference in event-free (EFS) and relapse-free survival between AML with t(8;21) and inv(16), but overall survival (OS) was superior (P=.04) in AML with inv(16) (OS at 3-yrs: 80.5% *vs* 63.4%). When compared with an AMLSG historical control group, there was an improvement in EFS (P=.04), but so far not for OS (P=.4).
Summary and Conclusions: There was no unexpected excess in toxicity by the addition of DAS to intensive induction and postremission therapy as well as of single-agent maintenance. The preliminary data indicate efficacy of the treatment when compared to historical controls. Based on the favorable toxicity profile and the encouraging efficacy data, a confirmatory phase III trial with DAS (AMLSG 21-13; NCT02013648) has been initiated.

Biological pathway deregulation in B Cell Precursor ALL

S516

ABERRANT EXPRESSION OF THE STEM CELL MICRORNA-126 INDUCES B CELL MALIGNANCY

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Background: MicroRNAs are essential regulators of normal and malignant hematopoiesis. We previously described the function of miR-126 in HSC where it regulates the balance between quiescence and self-renewal (Lechman *et al.*, 2012).
Aims: We aimed at studying the role of miR-126 in induction and maintenance of high-grade B cell malignancies in mouse and humans.
Methods: We exploited the following techniques: Transduction of murine bone marrow (BM) cells and human primary ALL blasts with lentiviral vectors (LV); LV integration site (IS) analysis by linear amplification mediated PCR and mapping on the murine genome; RNA sequencing; Immunofluorescence staining
Results: We here report a novel role for miR-126 in the induction and maintenance of high-grade B cell malignancies. By ectopically expressing miR-126 in transplanted BM cells, we observed that up to 60% of mice (n=71) developed B cell tumors. LV IS analysis revealed that all tumors were monoclonal. We then tracked back the leukemic clone to different hematopoietic lineages prospectively purified from the mice 2-6 months before disease onset. IS sharing between normal lineages and the leukemic clone suggests a stem or multipotent progenitor cell origin for most tumors. Importantly, we show that miR-126 is the direct cause for the induction and maintenance of leukemia, since (i) leukemogenesis is abolished when miRNA expression is inhibited by doxycycline (doxy) using a tetracycline-repressible miR-126 cassette, and (ii) established symptomatic leukemia completely regresses when miR-126 is switched off by doxy. We then performed RNA sequencing on blasts purified *ex vivo* before and 24 to 72h after doxy administration. Gene sets that changed were significantly enriched in migratory functions, cancer pathways and cell cycle regulators. Strikingly, there was a near-complete overlap with pathways that resulted significantly regulated by miR-126 in a second RNAseq dataset obtained from human cord blood (huCB) CD34+ cells following lentiviral miR-126 overexpression or knockdown. Many genes converged on the p53 pathway in both murine B-ALL and huCB CD34+ cells. Indeed, overexpression of miR-126 in huCB CD34+ cells physically displaced p53 from the nucleus and caused a significant reduction of its transcriptional activity. We identified a dozen of genes involved in p53 regulation that were directly targeted by miR-126 or miR-126*, suggesting extensive cooperative regulation of this central tumor suppressor pathway by miR-126. We further studied the function of miR-126 in Philadelphia+ human primary B acute lymphoblastic leukemia (Ph+ B-ALL) where miR-126 expression ranges from low to high in individual patients. We stably knocked down miR-126 in primary blasts from five Ph+ B-ALL patients using a miR-126 sponge LV. Following xenotransplantation, we observed increased apoptosis and impaired engraftment after primary and secondary transplantation in 4 out of 5 diseases (miR-126/KD: n=32 mice; Ctrl: n=37 mice), demonstrating the relevance of miR-126 in human B-ALL.
Summary and Conclusions: Down-regulation of miR-126 could be exploited as therapeutic strategy in ALL, since it would deplete leukemic cells while expanding normal HSC, two ways to restore normal hematopoieis.

S517

COMPOSITE INDEX FOR RISK PREDICTION IN RELAPSED CHILDHOOD ACUTE LYMPHOBLASTIC LEUKAEMIA

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Background: Somatic genetic abnormalities are key initiators and drivers of disease in acute lymphoblastic leukaemia (ALL). Several chromosomal abnormalities have proven clinical utility as prognostic and predictive biomarkers at initial diagnosis. However, the role of genetic biomarkers in relapsed ALL is less well understood and has rarely been studied comprehensively within a clinical trial.
Aims: To evaluate the role of genetics in predicting outcome among children with relapsed B-cell precursor ALL treated on the international trial, ALLR3.
Methods: We analysed cytogenetic, copy number alteration (CNA) and sequence mutation data at relapse in representative cohorts of patients. Patients with a very early relapse (<18 months from first diagnosis) and those patients with an isolated marrow relapse who had an early relapse (<6 months from stopping frontline therapy) were treated as clinical high risk (HR) whereas all other patients were treated as clinical standard risk (SR).
Results: Clinical HR patients accounted for 25% of the cohort and had a significantly inferior overall survival (OS) compared to SR patients: 25% (95% CI 15-37) v 65% (57-72), p<0.0001. A total of 427 patients were assigned to predefined cytogenetic risk groups which were predictive of survival post-relapse in both univariate and multivariate analysis adjusting for clinical risk: good risk (GR) cytogenetics (*ETV6-RUNX1*, high hyperdiploidy) 5 years OS 68% (60-75); intermediate risk (IR) cytogenetics (*TCF3-PBX1*, *IGH* translocations, B-other ALL) 47% (38-55); and HR cytogenetics (*BCR-ABL1*, *MLL* translocations, near haploidy, low hypodiploidy, iAMP21, *TCF3-HLF*) 26% (14-40), p<0.001. However, the prognostic effect of cytogenetic risk group was strongest within the clinical SR group. A representative cohort of 240 patients with marrow involvement was screened for CNA and mutations affecting key genes in ALL. Over 75% patients harboured at least one CNA or mutation: *CDKN2A/B* (39%), *IKZF1* (22%), *PAX5* (20%), *TP53* (17%), *ETV6* (16%), *KRAS* (12%), *NRAS* (12%), *NR3C1* (9%), *PAR1* (8%), *PTPN11* (8%), *RB1* (4%), *EBF1* (4%), *BTG1* (4%), *FLT3* (4%), *CBL* (1%). Cox models adjusted for clinical risk revealed that only four genes were associated with outcome. Patients with a *TP53* alteration or a deletion of either *NR3C1 or BTG1* had an inferior progression free survival (PFS) with hazard ratios of 2.07 (95% CI 1.20-3.58), p=0.009 and 2.26 (1.38-3.70), p=0.001, respectively. In addition, cytogenetic GR patients with a *NRAS* mutation had an inferior PFS compared with other GR cytogenetic patients 2.54 (1.24-5.22), p=0.01. The integration of clinical and cytogenetic risk groups with *TP53*, *NR3C1*, *BTG1* and *NRAS* gene status revealed three groups: (1) Favourable-clinical SR patients with GR cytogenetics and without a *TP53, NR3C1, BTG1* or *NRAS* abnormality; (2) Intermediate-clinical SR patients with GR cytogenetics and a *TP53, NR3C1, BTG1 or NRAS* abnormality plus clinical SR patients with IR cytogenetics; and (3) Adverse-all clinical and cytogenetic HR patients. The three groups accounted for 35%, 35% and 30% patients, respectively, and had markedly distinct OS rates: 78% (61-89), 56% (46-65) and 27% (19-35), p<0.001, respectively. Multivariate Cox models including variables for treatment and minimal residual disease did not materially alter the results. Receiver operating characteristic (ROC) curve analysis revealed that the new index had significantly greater predictive power than clinical risk alone for both PFS and OS: area under the curve (AUC)=0.73 v 0.67, p=0.02 and 0.75 v 0.69, p=0.03, respectively.
Summary and Conclusions: In conclusion, we have integrated key genetic information with clinical risk to improve risk prediction in relapsed ALL and propose a three-tier index which could be used to develop risk-directed therapy in future trials.

S518

CENTRAL NERVOUS SYSTEM ACUTE LYMPHOBLASTIC LEUKEMIA IS MEDIATED BY VASCULAR ENDOTHELIAL GROWTH FACTOR PROVIDING A NOVEL TARGET FOR DIRECTED THERAPY

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Background: In acute lymphoblastic leukemia (ALL), involvement of the central nervous system (CNS) is associated with adverse prognosis. Despite non-detectable CNS involvement in many cases, CNS-directed therapy is indispensible for relapse free survival indicating subclinical CNS manifestation in many patients. However, CNS therapy coincides with increased risk for secondary neoplasms and impaired cognitive skills in ALL survivors.
Aims: In this study, we aimed to characterize mechanisms mediating CNS involvement in ALL and to identify targets for CNS leukemia directed treatment.
Methods: Primary B-cell precursor (BCP) ALL cells were transplanted onto NOD/SCID mice. At onset of disease related morbidity, human ALL engraftment was analyzed in bone marrow (BM), spleen (S), peripheral blood (PB), and meninges of the recipients by flowcytometry staining for huCD19. Transcriptome profiles of ALL cells isolated from meningeal and BM infiltrates (8 pairs of 8 CNS+ ALLs) were analyzed. Gene expression was validated by qPCR. Proliferation, survival and trans-endothelial migration was analyzed in genetically modified (down-regulation or overexpression) ALL cells and in response to recombinant protein or an antagonizing antibody. In an *in vivo* model of CNS+ ALL, inhibition of CNS manifestation was evaluated.
Results: At onset of leukemia manifestation in recipients transplanted with BCP ALL, meningeal infiltration was detected along with ALL engraftment in BM, S and PB in a subset of samples (CNS+) in contrast to absent CNS involvement despite full-blown leukemia in BM, S and PB in others (CNS-). In line, magnetic resonance imaging revealed meningeal enhancement in CNS+ recipients. By expression profiling, the gene coding for *vascular endothelial growth factor A (VEGF)* was identified to be highly expressed in leukemia cells isolated from CNS as compared to BM-derived cells. Interestingly, VEGF was reported to mediate cell survival, vascular permeability and trans-endothelial cell migration and increased levels of VEGF protein were described in cerebrospinal fluid collected from CNS+ ALL and AML patients. Differential *VEGF* expression was confirmed in independent cohorts. VEGF receptor 1 expression was detected on all ALL cells. However, ALL cell proliferation and survival was not affected by VEGF overexpression, down-regulation, or exposure to VEGF or the antagonizing antibody bevacizumab. Further, we addressed the paracrine effect of VEGF on endothelial cells mediating trans-endothelial leukemia migration. Upon VEGF incubation, brain endothelial cells (bEND.3) showed induction of downstream VEGF receptor 2 signaling activity mediating cellular permeability. In a transwell assay, we identified VEGF-dependent trans-endothelial migration of BCP ALL cells (Nalm-6) with significantly increased or decreased migration in the presence of VEGF or bevacizumab through bEND.3 monolayers. Moreover, we investigated the effect of VEGF inhibition by bevacizumab on CNS leukemia manifestation in 3 patient-derived primografts (4 experiments including 1 replicate) *in vivo*. Most interestingly, in all 4 experiments anti-VEGF treatment significantly reduced the leukemia load exclusively in the CNS but not in BM, S and PB organ compartments indicating that the CNS+ phenotype is mediated by VEGF and can be controlled by VEGF inhibition.
Summary and Conclusions: We identified VEGF as a mediator of trans-endothelial leukemia cell migration and CNS manifestation in ALL. Most importantly, *in vivo* inhibition of VEGF by bevacizumab significantly decreased involvement of CNS ALL indicating a novel therapeutic strategy to control CNS leukemia.

S519

DEREGULATION OF SNO-RNAS IN THE GENOMICALLY IMPRINTED PRADER-WILLI LOCUS IN ERG-RELATED PEDIATRIC B CELL PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: "ERG-related" childhood B cell precursor acute lymphoblastic leukemias (BCP-ALL) are characterized by a specific gene expression signature and by recurrent *ERG* intragenic deletion. ERG-related patients have a favourable outcome, despite a high incidence of *IKFZ1* deletions. Nothing is known about non-coding (nc) RNA regulation in this leukemia subtype.
Aims: To investigate the main features of ERG-related patients by integrative genomic and gene expression analysis in an Italian cohort of B-others ALLs enrolled in the AIEOP ALL 2000 therapeutic protocol with emphasis on ncRNAs.
Methods: 143 BCP-ALL patients lacking known genomic aberrations or a hyperdiploid karyotype ("B others") were assigned to the ERG-related subtype according to their gene expression profile (GEP) and *ERG* and *IKFZ1* aberrations were assessed by genomic analyses. A representative sub-cohort of "B others" including 8 ERG-related and 16 non-ERG-related samples were profiled by ncRNA microarray (Mirna1.0, Affymetrix) and validated by qRT-PCR.
Results: Non supervised clustering of 143 B-others patients identified a cluster of 35 samples (24.5%) in which 15 (45.5%) carried the *ERG* intragenic deletion. None of patients outside this "ERG-related" GEP cluster had *ERG* deletions. Similar to previous reports from BFM and EORTC childhood ALL protocols for the *ERG* deleted subgroup, our GEP defined ERG-related patients were characterized by more common *IKZF1* deletions (35.3% compared with 26.2% in non-ERG related) and a favourable outcome, with a cumulative relapse incidence at 5 years of 6%±4.1 *vs* 25.7%±4.3, p-value 0.03; (3/35 *vs* 29/108 relapses). Analysis of ncRNAs revealed two interesting findings: Upregulation of the oncogenic miR-125b-2 cluster (hsa-miR-125b, -125b-2*, -99a, let-7c) and the related hsa-miR-100, hsa-miR-125a-3p, and a specific signature of snoRNAs tightly clustered within the ERG-related group. Strikingly the differentially expressed snoRNA probe sets (adjusted p-value <0.05) were located mainly in the Prader-Willi syndrome (PWS) locus at chromosome 15q11.2, a locus controlled by genomic imprinting. These snoRNAs are processed from a paternally expressed transcript that includes the PWS imprinting center upstream from *SNURF/SNRPN*. Specifically, there was up-regulation of SNORD109a,

SNORD64, SNORD107 and 11 snoRNAs of the SNORD116 cluster (SNORD116-11, 14, 15, 16, 17, 20, 21, 22, 23, 24, 25) and downregulation of a subset of SNORD116 snoRNAs (SNORD116-4, 6, 10, 12, 13, 26) in ERG-related patients. Validation by qRT-PCR confirmed microarray findings.

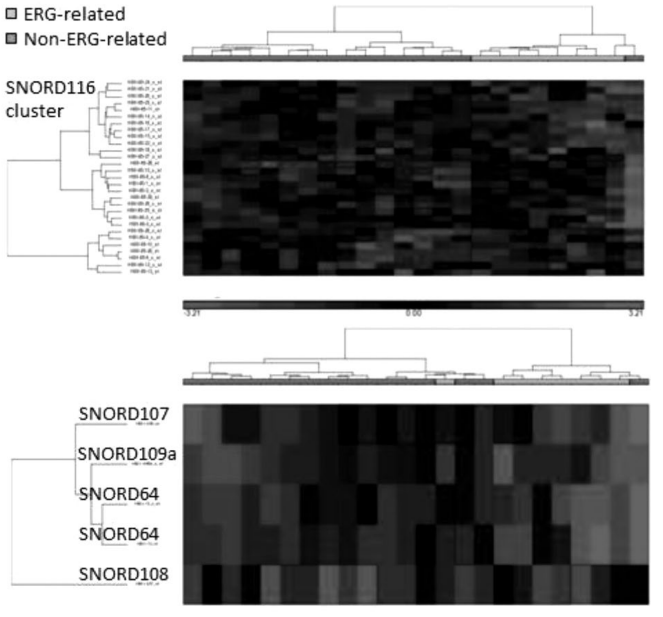

Figure 1.

Summary and Conclusions: ERG-related GEP defines a phenotypically unique subgroup in which only half carry *ERG* deletions, suggesting another underlying pathogenic mechanism beyond ERG intragenic deletion. We have identified a ncRNA signature specific of ERG-related BCP-ALL that can be used for differential diagnosis of these patients. While loss of imprinting associated with modified snoRNA expression was reported in acute promyelocytic leukemia, this is the first report of abnormal expression of snoRNAs in childhood BCP-ALL. The specific clustering of these snoRNAs within the imprinted PWS locus suggests a potential epigenetic mechanism underlying ERG-related ALLs.

S520

GENOMIC AND DRUG RESPONSE PROFILING OF FATAL TCF3-HLF-POSITIVE PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA IDENTIFIES RECURRENT MUTATION PATTERNS AND NOVEL THERAPEUTIC OPTIONS

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Background: The translocation t(17;19)(q22;p13) results in the fusion of the transactivating domain of the B cell transcription factor TCF3 and the DNA binding domain of the hepatic leukemia factor HLF. TCF3-HLF defines a rare subtype of ALL that is currently incurable. In contrast, the fusion of the same portion of TCF3 to the PBX1 gene (t(1;19)) results in a leukemia subtype with a favorable prognosis. TCF3-HLF ALL constitutes a paradigm of treatment refractory leukemia in which the oncogenic fusion protein drives programs that are fundamental for the malignant process. However genetic engineering experiments in mice did not recapitulate disease, suggesting that the cellular context in which the translocation occurs is critical for transformation.
Aims: We explored the genetic landscape of ten TCF3-HLF positive ALL cases in comparison to TCF3-PBX1 positive ALL using primary diagnostic samples and corresponding patient derived leukemia xenografts in order to analyze whether TCF3-HLF is associated with characteristic patterns of genomic lesions and to establish a well characterized humanized mouse model for functional investigations.
Methods: We used an integrated multi-OMICs approach to identify mutation and gene expression patterns in TCF3-HLF- and TCF3-PBX1-positiveALL. To compare drug response profiles of treatment resistant TCF3-HLF-positive and responsive TCF3-PBX1-positive ALL we established a xenograft model of patient derived leukemia. At least two biological replicates per case were xenografted in NOD/SCID/IL2rγ^{null} (NSG) mice. Drug response profiles to a customized library of >100 therapeutic compounds were obtained and *in vivo* validation studies were performed using this model.
Results: In TCF3-HLF-positive ALL, intragenic deletions in the lymphoid transcription factor PAX5 or somatic mutations in the non-translocated allele of TCF3 (acting upstream of PAX5), but not in any of the IFKZ family members, were recurrent and frequently observed in conjunction with RAS pathway aberrations. The pattern of co-occurring mutations was not overlapping in TCF3-PBX1 ALL, supporting the notion that completely different genetic interactions may be required for disease pathogenesis. The profile of mutations was maintained in the corresponding xenografts. Sequence features at the translocation breakpoints suggested RAG mediated recombination and an already lymphoid-committed cell of origin, but the TCF3-HLF-positive ALL transcriptome was enriched for stem cell and myeloid features, consistent with reprogramming towards a hybrid, more drug-resistant hematopoietic state. This gene expression signature was maintained in matched patient-derived xenografts. TCF3-HLF-positive ALL revealed a distinct drug-response profile with resistance to some agents commonly used in ALL therapy, but sensitivity towards glucocorticoids and some therapeutic agents in clinical development. Striking on-target sensitivity was achieved with the BCL2-specific inhibitor ABT-199 indicating BCL2-dependency of TCF3-HLF-positive ALL. Our results show, that ABT-199 acts synergistically with conventional chemotherapeutic agents, including dexamethasone and vincristine, providing a rationale for experimental therapy.
Summary and Conclusions: The molecular portrait of TCF3-HLF-positive ALL indicates that the initiating event occurs in cells committed to the lymphoid lineage and that leukemogenesis requires a restricted pattern of additional genetic lesions. Maintenance of dominant genomic and transcriptomic patterns of both TCF3-translocated subtypes in the corresponding xenografts provides the basis for systematic functional investigations. Integration of drug response profiling revealed recurrent patterns of resistance and new options for the treatment of this deadly disease.

POSTER SESSION

Acute lymphoblastic leukemia - Biology 2

P521

SPHINGOSINE KINASES ARE REQUIRED FOR IL-7-MEDIATED SIGNALING IN T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Acute lymphoblastic leukemia (ALL) is the most common pediatric malignancy. T-cell ALL is associated with high risk and poor prognosis. We have shown that IL-7, produced in the leukemia milieu by stromal cells, can accelerate leukemia progression *in vivo* and has a relevant contribution to the viability and proliferation of T-ALL cells (Silva *et al.*, Cancer Res 2011). Moreover, IL-7/IL-7R-mediated signaling may partake in leukemia development, as demonstrated by the identification of IL-7Rα gain-of-function mutations in around 9% of T-ALL patients (Zenatti *et al.*, Nat Genet 2011). Sphingosine Kinases (SPHKs), of which 2 isoforms have been described (SPHK1 and 2), are lipid kinases that promote cell viability by phosphorylating sphingosine and thereby regulating the ceramide/sphingosine 1-phosphate (S1P) rheostat. SPHKs play a fundamental role in many signaling pathways associated with cancer, although their involvement in leukemia development is not fully understood.
Aims: The aim of this study was to elucidate the crosstalk of SPHK isoenzymes and IL-7-mediated effects on pediatric T-ALL leukemogenesis.
Methods: SPHKs expression was determined by qRT-PCR, and activity measured by a luminescence-based assay. To test the involvement of SPHKs on IL-7-mediated signaling we incubated D1 (IL-7-dependent, thymocyte-like), TAIL7 (IL-7-dependent, T-ALL), HPB-ALL (IL-7-responsive, T-ALL) and DND-41 (IL-7Rα mutant, T-ALL) cell lines in the presence of IL-7 and/or SPHK1- or SPHK2-specific pharmacological inhibitors, SKi-II and K145 respectively. Cell viability was determined by flow cytometry analysis (FSCxSSC distribution and Annexin V/7AAD staining). Maintenance of mitochondrial membrane potential was measured by TMRE staining. Caspase 3 cleavage and activation of signaling pathways were measured by western blot. Proliferation was determined by evaluation of DNA synthesis upon ^{3}H-thymidine incorporation.
Results: We first observed that SPHK1, but not SPHK2, is increased in T-ALL patient samples as compared to normal controls. Analysis of SPHK activity revealed that IL-7 positively regulates SPHK activity without significantly affecting its expression. Furthermore, inhibition of SPHK1 or SPHK2 prevented the activation of both PI3K/AKT and JAK/STAT pathways in all cell lines studied, as determined by the phosphorylation status of key members of each pathway. These results suggest that SPHK activity is fundamental for the activation of pro-survival pathways derived from IL-7/IL-7R-dependent activation. In accordance, signaling downstream from IL-7 stimulation or IL-7Rα mutant activation was not able to improve leukemia cell viability upon SPHK inhibition. The antagonistic effect of SPHK inhibition on IL-7-mediated effects associated with increased apoptosis, decreased mitochondrial membrane potential and increased caspase-3 cleavage. Importantly, we found that both inhibitors completely prevented IL-7 viability effects not only in T-ALL cell lines but also in primary leukemia cells collected from patients at diagnosis. Moreover, T-ALL patient samples were significantly more sensitive to SPHK inhibition than their normal counterparts. Finally, we observed that T-ALL cell line proliferation upon IL-7 stimulation or IL-7Rα mutational activation was abrogated in the presence of SPHK inhibitors.
Summary and Conclusions: Our study identifies SPHK1 and 2 as essential modulators of IL-7-dependent activation of pro-survival and proliferative pathways in T-ALL, opening new possible therapeutic avenues in this pathology.

P522

MIR-146B IN T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA: A POSSIBLE TUMOR SUPPRESSOR ROLE

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Background: Regardless of improved therapy regimens, acute lymphoblastic leukemia from T-cell origin (T-ALL) is still not a curable disease. Therefore, the field currently faces the challenge of creating more efficacious therapies, rationally-designed and less toxic. A better understanding of the pathogenesis of the disease, namely molecular analysis of the common genetic alterations in leukemic cells, may be the solution to understand why some cases fail to respond to chemotherapy and to improve selective targeting of leukemic cells without long-term effects on the normal tissues. The transcription factor TAL1 is down-regulated early in T-cell development and over-expressed in more than 60% of pediatric T-ALL cases. TAL1 is able to either activate or repress the expression of downstream targets as part of a transcriptional complex.
Aims: This work aimed to contribute to a better comprehension of the biology of T-ALL, namely in relation to the means by which TAL1 exerts its oncogenic functions. Having shown previously that microRNA genes are regulated by TAL1 at the level of transcription (Correia *et al.*, *Leukemia* 2013), we hypothesized that some of these miRNAs may be part of the oncogenic network triggered by TAL1 ectopic expression in leukemia. We focused on miR-146b-5p and evaluated the functional and molecular effects of its deregulation by TAL1 in the context of T-ALL.
Methods: The expression of miR-146b-5p in T-ALL cell lines was determined by real time-PCR using LNA primers, whereas in T-ALL patients and normal counterparts it was analyzed from publicly available data (GSE51908 and Mets *et al.*, *Leukemia* 2014). To verify the functional effects of miR-146b aberrant expression in T-ALL we manipulated its levels in T-ALL cells using lentiviral transduction. *In vitro* functional assessment of the effects of miR-146b-5p modulation in T-ALL cell lines was performed by analysis of proliferation by cell counts, of viability by flow cytometry, and of migration and invasion by transwell assays. For the *in vivo* experiment ten NOD/SCID mice were either injected with CCRF-CEM cells over-expressing miR-146b or with mock transduced cells.
Results: The miR-146b-5p was found to be down-regulated by TAL1 in cell lines (Correia *et al.*, *Leukemia* 2013). We now further verified that pediatric T-ALL patients over-expressing TAL1 display significantly lower miR-146b-5p levels than other T-ALL patients (p=0.002). Moreover, T-ALL patients significantly express lower levels of miR-146b-5p compared to normal cells (p<0.0001). This led us to hypothesize that miR-146b-5p has a tumor suppressor role in this disease. Our results point to a direct down-regulation of miR-146b-5p by TAL1. Although no clear differences were found concerning cell viability or proliferation, miR-146-5p reduced expression improved the migration and invasion capacities of T-ALL cells. Moreover, we showed that over-expression of miR-146b in human T-ALL cells significantly increased the survival of recipient mice (p=0.039) in a xenotransplant model of human leukemia.
Summary and Conclusions: We have shown that miR-146b-5p is a functionally relevant TAL1 microRNA target gene, whose down-regulation may contribute to T-ALL by modulating leukemia cell motility and disease aggressiveness.

P523

IL-7 ACTIVATES THE JAK/STAT5/PIM1 KINASE AXIS THEREBY MEDIATING VIABILITY AND GROWTH OF T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA CELLS

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Background: T-cell acute lymphoblastic leukemia (T-ALL) constitutes, in general, an aggressive subset of ALL, the most frequent childhood malignancy. Although risk-adjusted chemotherapeutic regimens are currently extremely effective, their efficacy is associated with significant long-term side effects and those cases that relapse have dismal prognosis. New, more specific, therapies are therefore required. To achieve this goal it is essential to have a better understanding of T-ALL biology, including the contribution of tumor microenvironmental factors for leukemia progression. Interleukin 7 (IL-7) is produced in the bone marrow and thymus. While IL-7 is essential for normal T-cell development, there is also considerable evidence that it can partake in leukemia expansion. Previously, we have shown that IL-7 promotes T-ALL expansion *in vivo* (Silva *et al.*, Cancer Res. 2011) and leukemia cell survival and proliferation *in vitro* by activating PI3K/Akt/mTOR signaling pathway (Barata *et al.*, J Exp Med. 2004), consequently downregulating $p27^{kip1}$ and upregulating Bcl-2. However, it is also known that T-cell lymphomas arising spontaneously in IL-7 transgenic mice depend on STAT5 activity and IL7R gain-of-function mutations, found in around 10% of T-ALL patients (Zenatti *et al.*, Nat Genet. 2011), drive JAK/STAT5 pathway activation.
Aims: To investigate whether the JAK/STAT5 pathway may be involved in the IL-7/IL-7R pro-leukemia effects in human T-ALL.
Methods: We used an IL-7-dependent leukemia T-cell line (TAIL7), an IL-7-responsive T-ALL cell line (HPB-ALL) or primary T-ALL samples collected at diagnosis as cellular models. We used pharmacological inhibitors of JAK3 (WHI-P131), STATs in general (parthenolide), STAT5 in particular (N-((4-Oxo-4H-chromen-3-yl)methylene)nicotinohydrazide) and PIM1 (Smi-4a). Analysis of viability, cell size, cell cycle, surface CD71 and Bcl-2 expression was performed by flow cytometry. Signaling pathway activation, STAT5, PIM1 and cell cycle protein expression was performed by western blot analysis. Proliferation was assessed by ^{3}H-Thymidine incorporation. STAT5 ChIP-seq and RNA-seq were performed on TAIL7 cells.
Results: We show that IL-7 induces JAK1/3-STAT5 pathway activation, STAT5 DNA binding and transcriptional activity. To test the potential clinical applicability of these findings, we treated TAIL7 cells or primary T-ALL samples with inhibitors of JAK3, STATs in general or STAT5 in particular. All inhibitors abrogate IL-7-mediated T-ALL cell viability, growth and proliferation, with complete inhibition of IL-7-induced downmodulation of $p27^{kip1}$, upregulation of cyclin A and increased transferrin receptor (CD71) surface expression. Interestingly, IL-7-dependent Bcl-2 upregulation is not affected by STAT5 inhibition. To understand how STAT5 mediates the survival effects of IL-7 in T-ALL cells without affecting Bcl-2, we performed STAT5 ChIP-seq together with RNA-seq. Cross-analysis of the data revealed that IL-7 drives transcription of the serine/threo-

nine kinase PIM1 via STAT5. Notably, inhibition of PIM1 kinase activity abrogates IL-7-mediated T-ALL cell growth, viability and proliferation.
Summary and Conclusions: Overall, our results indicate that a JAK/STAT5/PIM1 axis is mandatory for IL-7/IL-7R-mediated T-ALL cell survival. Furthermore, these results indicate that JAK/STAT5 pathway inhibitors can eliminate T-ALL cells and that STAT5 plays a major role in mediating IL-7/IL-7R signaling effects in T-ALL cells, therefore constituting a promising target for therapeutic intervention in this malignancy.

P524

JAK2 MUTATIONS ARE HIGHLY ENRICHED IN CRLF2-REARRANGED B-ALL CASES WITH A PH-LIKE GENE SIGNATURE

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Background: High expression of *CRLF2* occurs in 5-15% of adult and pediatric B-ALLs, and commonly results from a cryptic deletion that juxtaposes *CRLF2* to the promoter of *P2RY8* (*P2RY8-CRLF2*), or from the translocation of *CRLF2* to the immunoglobulin heavy chain locus (*IGH-CRLF2*), bringing *CLRF2* under the control of *IGH* enhancer elements. *CRLF2* overexpression alone is not sufficient for leukemic transformation, requiring additional oncogenic lesions, and a large proportion of *CRLF2*-rearranged cases harbor activating mutations in *JAK2*, and less commonly in *JAK1*. To date, the prognostic significance of *CRLF2* rearrangements (*CRLF2*-r) remains debatable, although recent reports demonstrate that *CRLF2*-r are enriched in Ph-like ALL, a high-risk sub-type. In this study we show that Ph-like B-ALL cases with *CRLF2*-r represent a distinct genetic sub-group that may reflect underlying differences in secondary cooperating lesions and disease progression.
Aims: The aims of this investigation are to characterize the genetic and biological differences between Ph-like and non-Ph-like *CRLF2*-r B-ALL cases, to better understand the prognostic significance and targetable pathways in these patients that have previously been grouped in one class.
Methods: Patients with high *CRLF2* expression detected by Taqman Low Density Arrays (TLDA), were screened for *P2RY8-CRLF2* and *IGH-CRLF2* rearrangements by RT-PCR and FISH, respectively. Ph-like ALL cases were identified by TLDA analysis using a customized 9-gene signature. gDNA from *CRLF2*-r samples was analyzed for *JAK1/2* mutations by Sanger sequencing, and where possible, phospho-flow cytometry detecting pathway activation of downstream kinase targets was used to assess sensitivity to JAK inhibitors *in vitro*.
Results: We have screened >300 B-ALL samples from our Australian cohort (age between 0.5 and 75 years) and identified 27 *CRLF2*-r cases (*P2RY8-CRLF2* n=14 and *IGH-CRLF2* n=13). Interestingly, the frequency of *IGH-CRLF2* rearrangements increased with age. Activating *JAK2* mutations were found in 10/27 cases (R683G/S n=4; F694L n=1; D873N n=1 and D875N n=4) and consistent with prior reports all cases harboring *JAK2* mutations that were tested, responded to JAK inhibitors *in vitro*. No *JAK1* mutations were identified in this cohort. Importantly, TLDA analysis showed that 20/27 cases classified as Ph-like, and these samples also expressed significantly higher levels of *MUC4* and *GPR110* than the non-Ph-like *CRLF2*-r cases, despite no significant difference in *CRLF2* levels. Most strikingly, the activating *JAK2* mutations were restricted to the Ph-like patients. Of note a rare non-synonymous polymporphism in *JAK2* (R1063H) was detected in one of the non-Ph-like patients. Importantly, these results suggest that *JAK2* mutations are highly enriched in Ph-like *CRLF2*-r cases, likely indicating differences in disease etiology, and highlighting the heterogeneity of ALL which can be observed within a subset of patients that harbor identical translocations, and which have previously been analyzed as one group. We are currently using NGS approaches to further elucidate the genetic landscape of *CRLF2*-r patients, with a particular focus on the Ph-like patients lacking *JAK2* mutations and the non-Ph-like subset of B-ALLs.
Summary and Conclusions: We propose that Ph-like and non-Ph-like *CRLF2*-r cases compose prognostically and biologically distinct groups of B-ALL patients that should be studied as independent entities, possibly addressing some of the discrepancies previously observed in large studies of *CRLF2* deregulation in ALL. Importantly, further study of these sub-groups may provide critical leads for the development of novel targeted therapies.

P525

CLONAL ORIGINS OF HIGH-HYPERDIPLOID ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Monozygotic twins with concordant leukemia provide a unique model to analyze the timing and early genetic steps of leukemogenesis. Our previous studies on twins and cord blood samples revealed that pre-leukemic clones of high-hyperdiploid acute lymphoblastic leukemia (HHD ALL) are prenatal in origin.
Aims: Identification of the fetal cell type which is transformed in HHD ALL.
Methods: Diagnostic bone marrow samples from three monozygotic twin pairs concordant for HHD B-cell precursor ALL were screened for all varieties of immunoglobulin (*IG*) and T-cell receptor (*TCR*) gene rearrangements, which are hallmarks of lymphoid cell development, using a combination of targeted next-generation sequencing and conventional screening approaches. Identical rearrangements shared by twin siblings and indicative of the *IG/TCR* status of the cells giving rise to pre-leukemic clones were identified. Previous genome-wide SNP-array analysis indicated trisomy of chromosome 14 in all six patients. The study was conducted in accordance with the Declaration of Helsinki.
Results: In total, 23 rearrangements were identified in the three twin pairs. Clonal rearrangements were detected in *IGH*, *IGK*, *IGL*, *TCRD*, *TCRG* and *TCRB* genes in 6, 2, 1, 3, 1 and 2 patients, respectively. At least two different rearrangements were found in each child. Seven identical junctions shared by the siblings were observed with the following distribution: twins 1A/B *IGH* VDJx2 (non-functional); 2A/B *IGH* DJx2 and 3A/B *IGH* VDJx1 (non-functional), *IGK* VKdex1 and *TCRD* VD2-DD3x1. All these rearrangements must have occurred in the pre-leukemic cell population formed *in utero*. The majority of clonal markers proved to be patient-specific and is likely to have arisen post-natally in twin-specific sub-clones.
Summary and Conclusions: To date, only conventional screening of *IG/TCR* gene rearrangements was reported in a limited number of twins with concordant HHD ALL. We have carried out a comprehensive screen for these rearrangements in a cohort of twin pairs, also using advanced genomics. Our data suggest that the pre-leukemic clone spawned *in utero*, and shared by the twins, has the *IG* and *TCR* feature of precursor B-cells in all twin pairs tested. These results add to the accumulating evidence that the formation of the hyperdiploid karyotype can arise *in utero*, probably as an initiating mutation and it most likely occurs in early B-lineage progenitors at the stage of or after RAG-dependent *IGH* rearrangement. Therefore, the pre-leukemic clone expands preferentially in the pro- or pre-B lineage compartment, initially in one twin and spreads into the sibling via shared circulation. Clonally descendent cells with self-renewal stem cell activity are sustained in both twins after birth as independent targets for a number of secondary genetic hits - copy number changes and sequence mutations - essential for clinical development of ALL.

P526

INTEGRATED ANALYSIS OF JAK/STAT, RAS/AKT AND NOTCH1 PATHWAYS IN T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA: PROGNOSTIC AND THERAPEUTIC IMPLICATIONS

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Background: We previously reported a high rate of mutations in the JAK/STAT and RAS/AKT pathways in refractory/early relapsed T-cell acute lymphoblastic leukemia (T-ALL) cases analyzed by RNAseq (Gianfelici *et al.*, ASH 2013). Conversely, the NOTCH1/FBXW7 pathway was less frequently affected.
Aims: Screening of JAK/STAT, RAS/AKT and NOTCH1/FBXW7 was performed to confirm their prognostic impact in T-ALL. *In vitro* assays were carried out to assess the sensitivity of primary cells to specific inhibitors.
Methods: The study included 49 adult T-ALL cases (median age 37 years, range 16-59) enrolled in 2 consecutive Italian GIMEMA ALL protocols, for which genomic material was available. Both refractory and responsive cases were included. Hotspot mutations of *JAK3*, *JAK1*, *IL7R* and *STAT5B* for JAK/STAT signaling, *N/K-RAS*, *PTEN* and *FLT3* for RAS/AKT and *NOTCH1/FBXW7* were analyzed by Sanger sequencing. Overall survival (OS) and disease-free survival (DFS) were estimated using the Kaplan-Meier method. The JAK1/2 inhibitor Ruxolitinib and the JAK3 selective inhibitor Tofacitinib were tested on primary diagnostic cells. Following 72 hours of incubation, proliferation and cytotoxicity were determined using the MTT assay.
Results: Eight T-ALL cases (16%) harbored JAK/STAT mutations, namely *JAK1* (n=3), *JAK3* (n=3), *STAT5B* (n=2) and *IL7R* (n=3). All *STAT5B* mutants carried concomitant mutations in *JAK3* (1 of them also had a *JAK1* mutation), whereas the *IL7R* positive patients had no additional mutations of the same pathway. RAS/AKT alterations were found in 10 cases (20%), namely *PTEN* (n=5), *N/K-RAS* (n=4) and *FLT3* (n=1). NOTCH1/FBXW7 mutations were found in 28 cases (57%), in 16 without additional alterations, while in 12 a concomitant mutation in JAK/STAT (n=8) or *N/K-RAS* (n=4) was documented. We observed an enrichment of JAK/STAT and RAS/AKT alterations within the group of patients developing

an early relapse or refractoriness: 6/8 and 7/10, respectively. A significantly shorter OS and DFS was observed in patients harboring JAK/STAT mutations compared to patients without alterations in the pathway: 15.7 *vs* 27.3 months (p=0.04) and 11 months *vs* median not reached (p=0.002), respectively. Similarly, a significantly shorter OS and worse DFS were observed in cases with RAS/AKT alterations compared to patients without alterations in the pathway: 7.9 *vs* 27.3 months (p=0.0108) and 4.3 *vs* 33.2 months (p=0.0005), respectively. Conversely, OS and DFS were significantly better for cases harboring NOTCH1/FBXW7 mutations alone compared to wild-type patients or cases with concomitant JAK/STAT or RAS/AKT mutations: median not reached *vs* 18.7 *vs* 15.7 months, respectively, (p=0.0325), and median not reached *vs* 14.5 *vs* 9.8 months, respectively (p=0.0015). Primary cells from *JAK1* mutated patients proved sensitive to Ruxolitinib, whereas viability was not affected by Tofacitinib, documenting a selective sensitivity to Ruxolitinib. Furthermore, primary cells carrying *JAK1* and *JAK3* mutations in association with a *STAT5B* alteration were poorly sensitive both to Ruxolitinib and to Tofacitinib.

Summary and Conclusions: This study documents the negative prognostic impact of JAK/STAT and RAS/AKT mutations in T-ALL, at least when standard chemotherapy regimens are used. Notably, the favorable impact of NOTCH1/FBXW7 was overcome by the presence of concomitant JAK/STAT or RAS/AKT mutations. Finally, *in vitro* experiments demonstrate that inhibitors directed specifically against the underlying lesions may be effective on primary T-ALL cells.

P527

DISTINCT ALL-ASSOCIATED JAK3 MUTANTS EXHIBIT DIFFERENT CYTOKINE-RECEPTOR REQUIREMENTS AND JAK-INHIBITOR SPECIFICITIES

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Background: JAK1 and JAK3, which are intracellular tyrosine kinases associated to cytokine receptors, are recurrently mutated in acute lymphoblastic leukemia. These mutations are activating and scattered through the kinase and pseudokinase domains, which contrasts with the unique pseudokinase V617F mutation in JAK2 that is prevalent in myeloproliferative neoplasms. JAK1 and JAK3 associate with heterodimeric receptors such as IL-7R or IL-9R, in which JAK1 is appended to the specific chain and JAK3 to the common gamma chain.

Aims: To study the role of cytokine receptor complexes in mediating the oncogenic activity of JAK3 mutants.

Methods: STAT5 transcriptional activity downstream JAK3 mutants was assessed by measurements of luciferase expression in HEK293 cells or JAK1-deficient U4C cells upon transient co-transfection of appropriate cDNA constructs with a reporter construct harboring STAT5-responsive elements upstream a luciferase gene. Same experiments were conducted for JAK1 and JAK2 mutants. *In vivo* transforming characteristics of JAK3 mutants were studied by transplantation of mouse bone marrow progenitor cells transduced with retroviral vectors expressing JAK3 mutants in irradiated Balb/c mice. JAK3 mutants' sensitivity to JAK inhibitors was assesed by treating growth factor-independent Ba/F3 cells obtained after stable transduction with JAK3 mutants with increasing JAK inhibitors concentrations and measuring their proliferation using a tritiated thymidine incorporation assay. Phosphorylation of STATs and JAK proteins in these cells was also analyzed by western blotting.

Results: Our study demonstrated that $JAK3^{V674A}$ and the majority of other JAK3 mutants need to bind to a functional cytokine receptor complex in order to constitutively activate STAT5. However, one mutant, $JAK3^{L857P}$, was unexpectedly found to not depend on such receptor complexes for its activity, which was induced without receptor or JAK1 co-expression. Introducing a mutation in the FERM domain that abolished JAK-receptor interaction did not affect $JAK3^{L857P}$ activity, while it inhibited the other receptor-dependent mutants. The same cytokine receptor independence as for $JAK3^{L857P}$ was observed for homologous L^{857} mutations of JAK1 and JAK2 and for $JAK3^{L875H}$. This different cytokine receptor requirement correlated with different functional properties *in vivo* and with distinct sensitivity to JAK inhibitors. Transduction of murine hematopoietic cells with $JAK3^{V674A}$ led homogenously to lymphoblastic leukemias in mice. In contrast, transduction with $JAK3^{L857P}$ induced various types of lymphoid and myeloid leukemias, illustrating a broader oncogenic potential related to the absence of cytokine receptor restriction. Ruxolitinib, which preferentially blocks JAK1 and JAK2, abolished the proliferation of cells transformed by $JAK3^{V674A}$, which requires a functional JAK1-JAK3 associated receptor complex, yet proved much less potent on cells expressing $JAK3^{L857P}$. These particular cells were, in contrast, more sensitive to JAK3-specific inhibitors.

Summary and Conclusions: Altogether, our results have demonstrated that different JAK3 mutations can induce constitutive activation through distinct mechanisms, suggesting that an improved understanding of these mechanisms could offer new therapeutic perspectives.

P528

XIAP EXHIBITS AN ESSENTIAL ROLE FOR PATIENTS' ALL CELLS GROWING *IN VIVO* AS SHOWN BY A NOVEL TECHNIQUE FOR STABLE KNOCKDOWN IN PATIENT-DERIVED XENOGRAFT (PDX) ALL CELLS

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Background: Acute lymphoblastic leukemia (ALL) is a frequent disease in children and adults. XIAP, the X-linked inhibitor of apoptosis, is an anti-apoptotic protein which is frequently and highly overexpressed in hematological malignancies. Up-regulation of XIAP was shown to be associated with inferior prognosis of patients in various different types of tumors. No data exist so far on XIAP's role for growth and survival of hematological malignancies *in vivo*.

Aims: We aimed at characterizing the role of XIAP for survival and growth of patient-derived xenograft (PDX) acute lymphoblastic leukemia (ALL) cells *in vivo*. Towards this aim, we had to establish a novel technique allowing stable knockdown in PDX ALL cells.

Methods: Primary tumor cells from ALL patients were transplanted into severely immune-compromised NSG mice. PDX ALL cells were lentivirally transduced with a construct allowing stable knockdown in these cells. We decided to embed the shRNA in the context of a miRNA background using miR30 as it allowed expressing the shRNA under a Pol II promoter and coupling it to the expression of transgenes. We expressed different fluorochromes such that expression of blue indicated cells with control knockdown and expression of green cells with knockdown of XIAP. Additionally, we expressed either a codon optimized version of Firefly luciferase or a membrane bound form of Gaussia luciferase which require different substrates, Luciferine and Coelenterazine, respectively. Expression of luciferases enabled *in vivo* imaging for highly sensitive, reliable and continuous follow up of the growth of each subpopulation *in vivo.* Several established B-ALL and T-ALL cell lines were transduced in the same way. Taken together, we expressed the different luciferases and colors such that control cells and cells with knockdown of XIAP could be distinguished *in vivo* using dual luciferase imaging and post mortem using flow cytometry. We finally injected both populations into a single mouse and monitored the distribution of the two populations over time.

Results: Knockdown of XIAP in PDX ALL cells lead to the reduction of the XIAP protein by more than 90% as shown by Western Blot. Knockdown remained stable over three passages indicating a highly stable knockdown by our novel technical approach. In cell lines, down-regulation of XIAP expression did not affect proliferation of different T- and B-ALL cell lines *in vitro*, nor engraftment and development of preB-ALL NALM6 cells in NSG mice. In contrast, down-regulation of XIAP expression significantly reduced tumor load of PDX ALL samples *in vivo*, as measured both by dual luciferase imaging *in vivo* and by *ex vivo* quantification of the leukemic subpopulations using flow cytometric analysis of the different colors.

Summary and Conclusions: We established a technique allowing efficient silencing of target genes in PDX ALL cells growing in mice together with convenient readout systems. Our data show that the role of XIAP is highly different in cell lines and in PDX ALL cells. Targeting XIAP by knockdown significantly reduces leukemia burden in PDX ALL cells *in vivo.* Our novel technical approach for knockdown in PDX ALL cells *in vivo* enables the preclinical testing of the role of putative therapeutic targets in PDX cells. Cell lines represent unsuitable models for predicting the role of XIAP in PDX ALL cells *in vivo*. Upregulation of XIAP constitutes an essential oncogenic alteration in PDX ALL cells; XIAP represents a promising target for treatment of ALL.

P529

EXPRESSION OF GATA3 DEPENDS ON GENOTYPE VARIANT AT RS3824662 AND IS ASSOCIATED WITH CLINICAL COURSE OF B-CELL CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Polymorphic site at rs3824662 in *GATA3* gene is a susceptibility locus for newly identified subtype of BCP-ALL associated with poor outcome called *BCR-ABL1*-like.
Aims: The main purpose of the study was to investigate an association between the genotype variant at rs3824662 of *GATA3* gene and *GATA3* expression level in leukemic cells at diagnosis of childhood ALL as well as genetic and clinical features of pediatric ALL.
Methods: Between November 2010 and January 2015, 452 pediatric patients (median age 8.5 yrs; median follow-up 2.3±1.7 yrs), with newly diagnosed BCP-ALL treated according to ALL-IC BFM09 protocol in 15 centers of the Polish Pediatric Leukemia/Lymphoma Study Group were enrolled into the study. Patients with BCR/ABL gene fusion, MLL rearrangements, hypodiploidy, Down syndrome were excluded from analysis. MRD was measured at day 15 of induction therapy using FCM with EuroFlow 8-color antibody panels (n=358). Targeted copy number screening of selected 23 loci was performed on available DNA samples (n=448) by using the MLPA methods. Determination of genotype at polymorphic locus rs3824662 was performed in all the patients' samples using allelic discrimination assay. *GATA3* mRNA expression was quantified by Q-PCR using FAM-MGB probe.
Results: Genotype distribution in studied group: AA: n=26 (6%); CA: n=148 (33%); CC: n=278 (62%). Genotype variant AA at rs3824662 was associated with higher level of *GATA3* mRNA expression compared to CA and CC variant, with mean -0.11; -1.45 and -3.88 respectively (p=0.02).
We have not succeeded in defining microdeletion profile specific for polymorphic variant. Among AA homozygotes 27% are *IKZF1* deletion carriers, whereas among CA and CC genotypes 16% and 13% respectively. However, after exclusion of TEL/AML rearrangements and hyperdiploidy AA homozygous were more likely to be carriers of *IKZF1* deletion risk allele compared with patient without this defect (p=0.03). Homozygotes AA showed higher WBC level at diagnosis of childhood ALL compared to CA and CC variant, with median 13.8 [1000/μL] (quartiles: 7.3-50.0); 13.2 (q: 4.90-44.70); 9.7 (q: 4.40-25.00) respectively, p=0.05 and were older with median 7.6 (q: 4.20-12.11); 5.0 (q: 3.11-9.31); 4.3 (q: 2.75-7.68) and with p=0.05). There was no difference in terms of MRD level at day 15 between patients with specific genotype in *GATA3* gene.
Summary and Conclusions: The expression of *GATA3* gene in leukemic cells was associated with genotype variant at rs3824662 in the study cohort. Furthermore, carriers of AA variant at rs3824662 of *GATA3* showed clinical features at ALL diagnosis related with poor treatment outcome.

P530

PATIENT'S SINGLE STEM CELL CLONES OF ACUTE LYMPHOBLASTIC LEUKEMIA SHOW DRUG RESISTANCE AND SLOW PROLIFERATION IN MICE *IN VIVO*

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Background: Acute lymphoblastic leukemia (ALL) consists of heterogeneous cell populations and the most aggressive subpopulation determines prognosis and outcome in each patient. Each subpopulation relies on a different ALL stem cell and a better understanding of challenging subclones is intensively required.
Aims: We aimed at functionally characterizing single stem cell clones *in vivo* regarding growth behavior and drug resistance and at correlating clone-specific functional characteristics with genetic features using a sample from a child with ALL growing in mice.
Methods: Primary tumor cells from a 5-year old girl with hyperdiploid ALL at first relapse were transplanted into severely immune-compromised mice and lentivirally modified to express the fluorochromes red, green and blue at different amounts and combinations (RGB marking, Weber *et al.*, 2011). Single cell clones were generated by limiting dilution transplantation and their uniqueness was verified by LM-PCR. Single cell clones were re-mixed for *in vivo* assays and analyzed one-by-one by flow cytometry where they could be distinguished from each other using their unique color expression. *In vivo* experiments were performed to evaluate spontaneous growth rate as well as drug resistance. Single cell clones and bulk cells were genetically analyzed for their exome, methylome and gene expression (RNA sequencing).
Results: 8 single stem cell subclones were generated and compared between each other and bulk cells. When several combinations of two clones were mixed within the same recipient mice, one clone overgrew the other in different amounts indicating that different clones grow at different rates. *In vitro*, chemosensitivity differed between several clones tested, especially regarding stimulation with glucocorticoids, including resistant clones. Two clones were mixed and transplanted into several mice and some mice were treated with glucocorticoids while others received buffer. One clone showed a significantly reduced sensitivity towards *in vivo* glucocorticoid treatment compared to the second clone indicating selective drug sensitivity in single cell clones. Exome sequencing and DNA methylation profiling revealed that passaging a bulk sample results in few changes only, while recurrent additional mutations in two clones suggest a common ancestor subclone. Individual clones showed divergent mutational and methylation signatures compared to the parental bulk sample. Glucocorticoid resistant clones show more alterations compared to sensitive clones.
Summary and Conclusions: Taken together, combining the individualized xenograft mouse model with genetic engineering allowed multicolor marking and generating viable single cell clones for functional characterization *in vivo*. Within the heterogeneous tumor bulk, aggressive subclones exist showing slow tumor growth or drug resistance which is associated with inferior genetic characteristics. Our studies allow the functional characterization of challenging subclones *in vivo* in order to develop efficient novel treatment approaches to eliminate aggressive stem cell clones in ALL.

P531

A RARE SUBPOPULATION OF QUIESCENT, DRUG RESISTANT STEM CELLS IN PATIENTS' ALL CELLS GROWING IN MICE

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Background: While ALL cells display major genetic heterogeneity upon clonal evolution, leukemia initiating potential is homogenously shared between many, if not all ALL cells. Nevertheless, drug resistant residual disease might persist after successful treatment cycles suggesting functional heterogeneity in ALL in response to therapy. We decided using quiescence as benchmark in searching for a clinically relevant subgroup of ALL cells.
Aims: We aimed at evaluating whether patients' ALL cells growing in mice exhibit differences in proliferation rate, drug sensitivity and stem cell behavior and whether a challenging subpopulation of stem cell might exist which is quiescent and drug resistant.
Methods: We engrafted patients' primary ALL cells in NSG mice. To monitor ALL growth patterns, we established a technique allowing for unbiased re-isolation of small numbers of human ALL cells from murine bone marrow. Towards this aim, we lentivirally transduced patient-derived ALL cells to express NGFR, a red fluorochrome and luciferase. A combined MACS/FACS procedure based on the expression of the transgenes enabled more than 10.000-fold enrichment of human ALL cells from murine bone marrow.
Results: CFSE labeling revealed a rare subpopulation of *in vivo* long-term quiescent cells which had not divided, even if tumor mass had increased by 3 orders of magnitude and 50% of the time from cell injection to animal's death by leukemia had passed. Re-transplantation into secondary recipients revealed that quiescent cells converted into cycling cells and vice-versa suggesting a major plasticity of patients' ALL cells *in vivo*. Limiting dilution transplantation assays revealed that both quiescent and cycling cells exhibited similar, high stem cell frequencies which were not further increased in quiescent cells. *In vitro*, quiescent and cycling cells displayed similar sensitivity towards cytotoxic drugs after 3 days of stimulation. In contrast and clinically most importantly, quiescent ALL cells displayed severe resistance against *in vivo* treatment of mice with cytotoxic drugs used in routine treatment of ALL patients; while the amount of cycling cells was decreased by more than 1 order of magnitude, the amount of quiescent cells remained completely unchanged.
Summary and Conclusions: Our data suggest that ALL consists of functionally heterogeneous cells. Non cell-autonomous factors and stimuli determine important functions of ALL cells. As the study was restricted to analysis of bone marrow, bone marrow niche factors determine both growth rate as well as drug sensitivity of patient-derived ALL cells *in vivo*. We conclude that uncoupling ALL cells from the bone marrow niche might represent an attractive approach for bringing these cells back into the cell cycle and sensitizing them towards chemotherapy. In summary, genetic engineering of ALL xenograft cells enabled the sensitive isolation and enrichment of ALL cells from murine bone marrow. We identified a small subpopulation of *in vivo* long-term quiescent and drug resistant cells in patient-derived ALL cells which obtain their phenotype from external stimuli exerted from the bone marrow niche. These cells might be important targets for therapy and should be considered when evaluating novel therapeutic approaches to treat ALL.

Acute lymphoblastic leukemia - Clinical 2

P532

PROGNOSIS IN OLDER/ELDERLY PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKAEMIA DIAGNOSED 2005-2012: RESULTS FROM A SWEDISH POPULATION-BASED STUDY

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Background: The knowledge of treatment results in older patients with acute lymphoblastic leukemia (ALL) is modest and derives mostly from clinical studies where selection mechanisms are present.
Aims: The aim of this study was to assess the outcome of treatment according to national guidelines in an unselected cohort of older/elderly ALL patients.
Methods: Data regarding all patients aged 55-85 years diagnosed with ALL from 2005 to 2012 in Sweden was obtained from the Swedish Acute Leukaemia Registry and the Swedish Acute Lymphoblastic Leukaemia Registry. Both are truly population-based registries with prospectively reported information including: date of diagnosis, phenotype, WHO performance status (WHO 0-4), treatment intention (induction therapy or palliative treatment), stem cell transplantation and dates of morphologic complete remission (CR)/relapse/death. Statistical analysis was carried out with SPSS v 22 (IBM). Overall survival (OS) was defined as time from diagnosis until last follow up or death. OS was calculated by the Kaplan-Meier method. Univariable and bivariable analyses regarding prognostic factors were performed using the Cox proportional hazards regression. Logistic regression was used to determine factors related to treatment decisions. Vital status was obtained until the 22nd of August 2014.
Results: In total, 182 patients (90 males and 92 females) with a median and mean age of 68 years were reported. Median follow up time of survivors was 61 (range: 20-115) months. Immunophenotype was reported for 172 patients; B-ALL 142 (82%), Burkitt-leukemia 17 (10%), and T-ALL 13 (8%). The median age in these groups was 67, 74 and 70 years, respectively. Induction therapy was given to 144 (79%), resulting in CR for 110/144 (76%) patients. Three year OS was 27 (95% CI, 20-33)% for all patients, 33 (95% CI, 25-41)% for patients receiving induction therapy, and 3 (95% CI, 0-8)% for patients reported as receiving palliative treatment (p<0.001, logrank test). The corresponding median OS was; 10 (95% CI, 6-14), 15 (95% CI, 11-19), and 1 (95% CI, 0-2) months respectively. Allogeneic stem cell transplantation was performed upfront in 19 patients (mean age 60; range: 55-66 years). CR frequency and OS for different age and WHO performance status groups are displayed in Table 1.
Treatment intention, age (as continuous variable), WHO performance status (0-1 *versus* 2-4), and gender were identified as significant prognostic factors for OS. The proportion of patients receiving induction treatment was negatively correlated to age and WHO performance status (p<0.001). Among 14 patients >80 years only three received induction therapy and none survived beyond one year. Although a significant difference in OS was found for the whole population regarding gender, sub-group analysis revealed that the difference was mainly located in the 55-64 year cohort (p<0.01, adjusted for WHO status) and was demonstrated also within the B-ALL cohort separately.

Table 1.

Age	N	OS 3y (95% CI) %	Induction therapy N (%)	CR %	OS 3y (95% CI) %
55-64 y:	69	41 (29-53)	67 (97)	79	42 (30-54)
Males	31	25 (9-40)	30 (97)	77	26 (10-42)
Females	38	55 (38-71)**	37 (97)	81	56 (40-72)**
65-74 y	68	24 (14-34)**	58 (85)	78	28 (16-40)
75-85 y	45	9 (1-17)**	19 (42)	63	16 (1-32)*
WHO 0-1	127	31 (23-40)	111 (87)	75	35 (26-44)
WHO 2-4	53	17 (7-27)**	32 (60)	84	28 (12-44)

* p< 0.05, **p≤ 0.01 log-rank test

Summary and Conclusions: We conclude that intensive ALL treatment could be given successfully to an older population up to at least 75 years of age. Age and performance status are important factors for decisions regarding treatment intensity and outcome. The difference in OS between males and females aged 55-64 years is striking and will need further evaluation. Elaborate studies regarding given treatments and risk factors are ongoing.

P533

GENOME-WIDE ANALYSIS OF WDR5 BINDING REVEALS ITS EFFECT ON TUMORIGENESIS IN ACUTE LEUKEMIA

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Background: Mixed-lineage leukemia (MLL) fusion proteins are potent inducer of leukemia. It is reported that MLL fusion proteins lost their histone modification activity. WD repeat domain 5 (WDR5) is the conserved component in the multi-subunit complex of SET1 family enzymes with direct methyltransferase activity. WDR5 itself may also have histone H3 trimethyl Lysine 4 (H3K4me3) methyltransferase activity. We observed that WDR5 was significantly increased in the patients with acute leukemia.
Aims: To identify the global binding profiling of WDR5 and H3K4me3 in leukemia cells and to explore the role of WDR5-mediated H3K4 methylation in the leukemogenesis.
Methods: Bone marrow samples from 120 patients with newly diagnosed acute leukemia, including 60 cases of acute lymphoblastic leukemia (ALL) and 60 cases of acute myeloid leukemia (AML), were collected between June 2008 and July 2013 at the First Affiliated Hospital of Nanjing Medical University. All the patients provided their written informed consent in accordance with the Declaration of Helsinki before enrollment in the study. The study was approved by the Institutional Review Board of the Nanjing Medical University. Quantitative PCR was performed to detect the expression of WDR5 in the patients and 40 normal controls. ChIP-Seq analysis for WDR5 and H3K4me3 were done and ChIP-seq libraries were sequenced in two MLL-rearranged leukemia cell lines RS4:11(MLL-AF4) and THP-1(MLL-AF9). The peaks were called using Cisgenome 2.0 and SISSRS. The overlapping of WDR5 peaks and H3K4me3 peaks was analyzed.
Results: We found more than 10,000 H3K4me3 peaks in two detected MLL-rearranged leukemia cell lines RS4:11 and THP-1 which express MLL fusion proteins. This result indicated that H3K4me3 is highly genomic enriched in the cells and suggested that there is a mechanism responsible for the genomic recruitment of H3K4me3. Moreover, we examined the expression of the WDR5 in patients with acute leukemia and found WDR5 high expression in this cohort of patients. We also observed the interaction of WDR5 with the MLL protein in the MLL-rearranged cells by co-immunoprecipitation. We further identified the global genomic binding of WDR5 in RS4:11 and THP-1 cells by ChIP-seq and detected more than 2000 binding peaks in the two leukemia cells. Importantly, we found the genomic co-localization of WDR5 binding with H3K4me3 enrichment and WDR5 with H3K4me3 binds to the promoter regions of important genes involved in leukemogenesis and hematopoiesis. Our data suggested that WDR5-mediated H3K4 methylation plays an important role in the leukemogenesis.
Summary and Conclusions: WDR5 expression significantly increased in adult ALL and AML patients. We demonstrated global enrichment of H3K4me3 binding around WDR5 binding peaks and co-existence of WDR5 and H3K4me3 binding in promoter region of oncogenes in MLL-rearranged leukemia cells.

P534

IMPACT OF STEROID DOSE AT INDUCTION PHASE OF PEDIATRIC ALL ON MINIMAL RESIDUAL DISEASE AND SURVIVAL OF THE PATIENTS: A PROSPECTIVE RANDOMIZED STUDY

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Background: Previous studies have demonstrated apoptosis induction effect of high dose methylprednisolone (MPZ) on lymphoblasts. In a previous study, high dose MPZ (900 mg/m2) during induction therapy of children with acute lymphoblastic leukemia (ALL) was compared with standard dose prednisolone (60 mg/m2) and increased cure rates were shown with high dose MPZ therapy.
Aims: Aim of the study is to compare effectiveness of two different high doses of MPZ, both doses being higher than the standard doses, during induction phase of therapy.
Methods: Pediatric patients newly diagnosed with ALL between 2008 and 2014 were enrolled. Patients were randomized for high doses of MPZ treatment, which was given for 7 days before commencement of induction phase of St Jude Total XV protocol, at doses of 10 mg/kg/day or 20 mg/kg/day, po. The steroid dose was tapered to 5 mg/kg/day and 10 mg/kg/day, in each group respectively by the 2nd week and concomitant chemotherapy was initiated. By the 3rd week of diagnosis, steroid dose was tapered to 2 mg/kg/day in both groups and continued at this dose upto the end of induction phase. Minimal residual disease (MRD) levels were measured by flow cytometry at 15th, 22nd, and 42nd days of induction. Event-free (EFS) and overall survival (OS) rates were calculated with Kaplan-Meier analyses.
Results: A total of 154 pediatric patients with newly diagnosed ALL were enrolled in the study. When the patients who were initiated full dose chemotherapy earlier, without a 1 week steroid only course, were excluded, a total of 141 patients were randomized to each dose group (n=78 and n=63 patients in 10

and 20 mg/kg/day groups, respectively). Patient characteristics of the study group were summarized in Table 1. Median age of patients was 5 (1-19) years, male/female ratio was 1.4. Majority of the patients were CALLA+ B cell ALL (80.5%) and t(9;22) positivity was detected in 7 (4.5%). CNS involvement was present in 6 (3.9%) of the patients . The two dose groups were similar in terms of age at diagnosis, gender, flow type, CNS and t(9;22) positivity. The MRD levels on 15th, 22nd, and 42nd days between two groups were statistically indifferent (Table 1). To investigate the effect of MPZ dose on MRD of 42nd day, effects of age, initial white blood cell count, t(9;22) positivity and neutropenic fever were adjusted using ANCOVA test, and still there wasn't statistical difference between these groups (p=0.83). Median follow-up was 33 (1-85) months. Five year OS of patients treated with 10 and 20 mg/kg/day MPZ were 85±5% and 92±4%, respectively (p=0.29). Five year EFS of patients treated with 10 and 20 mg/kg/day MPZ were 83±6% and 86±7%, respectively (p=0.34).

Table 1. Comparison of MRD levels based on initial MPZ dose

	10 mg/kg MPZ*	20 mg/kg MPZ	p
MRD on 15th day	1 (0-814)	1 (0-896)	0.92
MRD on 22nd day	0 (0-323)	0.5 (0-378)	0.14
MRD on 42nd day	0.5 (0-959)	0.5 (0-1449)	0.80

** Median (range); MPZ, methylprednisolone; MRD, minimal residual disease.*

Summary and Conclusions: Related to our published institutional previous experience of the better outcomes of patients at higher doses of steroid at induction; we aimed to find out whether lesser doses MPZ were still effective, although still higher than the standard recommended doses. Our study indicates that the survival rates and MRD levels are similar among patients treated initially with 10 mg/kg/day MPZ compared to 20 mg/kg/day.

P535

IKZF1 DELETION AND PROGNOSIS OF ADULTS WITH COMMON B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: The role of IKZF1 deletions is extensively-evaluated in children with ALL and is the most common genetic maker associated with a poor prognosis. Few data are available on the role of IKZF1 deletions in adults with common B-cell ALL.
Aims: To Interrogate the impact of IKZF1 deletion on therapy-outcomes of adults with common B-cell acute lymphoblastic leukemia.
Methods: 144 adults with common B-cell ALL achieved complete remission were tested for IKZF1 deletion. Deletions in IKZF1 were detected using multiplex RQ-PCR, multiplex fluorescent PCR, sequence analysis and multiplex ligation-dependent probe amplification (MLPA). All subjects received chemotherapy and some also received an allotransplant and tyrosine kinase-inhibitors, especially those with BCR/ABL. Multivariate analyses were done to identify associations between IKZF1 deletion and other variables on cumulative incidence of relapse (CIR), leukemia-free survival (LFS) and survival.
Results: IKZF1 deletion was detected in 66/144 subjects (49%). Amongst subjects achieving complete remission those with IKZF1 deletion had a higher 5-year CIR (39% [38-40%] *vs* 19% [18-20%]; *P*=0.010) and worse 5-year LFS (34% [16-52%] *vs* 60% [42-74%]; *P*=0.009) and survival (48% [32-62%] *vs* 76% [58-87%]; *P*=0.001, Figure1). In multivariate analyses IKZF1 deletion was independently associated with an increased relapse (relative risk [RR]=2.8, [1.4-5.8]; *P*=0.005), a higher risk of treatment-failure (inverse of LFS; RR=2.3, [1.3-4.2]; *P*=0.007) and a higher risk of death (RR=3.0, [1.5-6.3]; *P*=0.003). The adverse impact of IKZF1 deletion on outcomes was stronger in subjects without *vs* with BCR/ABL and in subjects receiving chemotherapy-only *vs* an allotransplant.

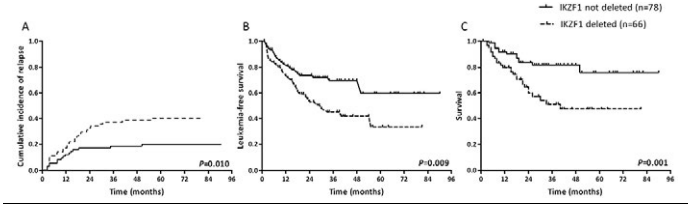

Figure 1. A) Cumulative incidence of relapse (CIR), B) leukemia-free survival (LFS) and C) survival of subjects with and without IKZF1 deletion.

Summary and Conclusions: IKZF1 deletion was independently-associated with a higher relapse risk and worse LFS and survival in adults with common B-cell ALL after adjusting for other prognostic variables and differences in therapies. These data suggest IKZF1 deletion may be a useful prognostic variable in adult common B-cell ALL, especially in persons without BCR/ABL and those receiving chemotherapy-only.

P536

EXTENSIVE PHENOTYPIC CHARACTERIZATION AND CYTOKINE PRODUCTION OF WHOLE BLOOD AT DAY 29 OF INDUCTION CHEMOTHERAPY FOR PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Despite significant improvements in cure rates, recurrent acute lymphoblastic leukemia (ALL) causes 10% of childhood cancer deaths and the need for new treatment strategies has not diminished. Although ALL has long been viewed as a poor target for immune therapy, it has recently been reported that the peripheral blood absolute lymphocyte count (ALC) at completion of induction chemotherapy (Day 29) is prognostic for outcome. This finding suggests that the immune environment during therapy may play a role in protecting against disease progression. It is currently unknown, however, whether ALC is simply a marker of bone marrow recovery or whether particular immune cell subsets at this early time-point confer a beneficial effect.
Aims: To address this question, we have initiated a study to evaluate the lymphocyte composition as well as cytokine production after stimulation of whole blood with a panel of Toll-like receptor (TLR) ligands. Peripheral blood was obtained at Day 29 from children undergoing therapy for precursor B cell ALL at BC Children's Hospital. The goal of this study is to correlate the size and responsiveness of the immune cell subsets with ALC in order to identify potential immune-mediators of the high ALC-associated favorable outcome.
Methods: The study was approved by the University of British Columbia Ethics Board. Freshly collected whole blood was immediately processed for i) multicolour flow cytometry and ii) 24 hour stimulation with a panel of Toll-like receptor (TLR) ligands. We next performed an extensive phenotypic characterization of 20 immune cell subsets and also measured cytokine production in the supernatant of TLR ligand-stimulated whole blood.
Results: To date we have evaluated 11 patients for T cells (5 subsets), B cells (6 subsets), NK cells (3 subsets), NKT cells (2 subsets), dendritic cells (2 subsets), monocytes, and granulocytes. Although we have not yet identified a single lymphocyte population that significantly correlates with ALC, clear trends, most notably with myeloid dendritic cells, have emerged that suggest that ALC may be indicative of specific immune activity. TLR ligand-induced cytokine production is currently being quantified to determine whether higher ALC correlates with a specific pattern of response.
Summary and Conclusions: While a large-scale prospective study to validate prognostic significance of ALC in children with high-risk ALL is underway, to our knowledge our ongoing study is the first to investigate the specific immune cell composition of Day 29 peripheral whole blood for significant links with ALC. The identification of the mechanism(s) underlying ALC-associated outcomes may reveal novel strategies for enhanced immune-mediated control of ALL after chemotherapy.

P537

NONE OF THE COMMON RISK FACTORS IS SIGNIFICANT IN T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA TREATED ACCORDING TO NON-INTENSIVE BUT NON-INTERRUPTIVE PROTOCOL ALL-2009: RESULTS OF THE RUSSIAN ALL STUDY GROUP

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Background: T-cell ALL is a distinct subgroup of ALL with better prognosis than B-cell ALL, especially with high-dose chemotherapy and allogeneic HSCT

in the majority of high risk patients (pts). RALL has initiated a prospective multicenter trial for adult Ph-negative ALL based on non-intensive but non-interruptive protocol "ALL-2009" (ClinicalTrials.gov public site; NCT01193933). The therapy was unified for all pts, but in T-cell ALL autologous hematopoietic stem cell transplantation (auto-HSCT) after non-myeloablative BEAM conditioning followed by prolonged maintenance was scheduled as late intensification. Allo-HSCT was planned to be an option for high risk pts with sibling donors.
Aims: Assess risk factors significance in T-cell acute lymphoblastic leukemia treated according ALL-2009 protocol.
Methods: From Jan 2009, till Dec 2014, 30 centers enrolled 263 ALL pts, including 90 (34,2%) T-cell ALL cases. Median age was 28 years (15-56 years), 33f/57 m; early T-cell (TI/II) phenotype was verified in 44 (49%), mature (T-IV) - in 11 (12%), thymic (TIII, CD1a+) ALL - in 35 pts (39%). T-lymphoblastic lymphoma (T-LBL) was diagnosed in 18,4%, CNS disease - 13,4%, mediastinal tumor - 57,3%. Cytogenetics was done in 61% of pts (n=55): 9% (n=5) had no mitosis, 45,5% (n=25) - normal karyotype, 45,5% (n=25) - different aberrations. 23% of pts (n=21) were in the standard risk, 77% (n=69) - in the high risk group (WBC >100; EGIL T-I-II-IV; LDH >2N; late CR). MRD levels by PCR analysis for clonal TCR rearrangements (10-3) before auto-HSCT were studied only in the minority of pts (n=7). The analysis was performed in Dec 2014. The median follow-up was 27,4 mo.
Results: CR rate in 86 available for analysis patients was 89,5% (n=77), induction death was registered in 5,8% (n=5), resistance - in 4,7% (n=4). CR was achieved after prephase in 16% (n=12) and after the 1st phase of induction - in 56% of pts (n=43). There was 28% of late responders (n=22). CR rate was higher -100% - in 34 thymic T-ALL pts in comparison with 85,7% in 42 early T-ALL and 70% in 10 mature T-ALL pts due to higher early death rate (7,1% and 10%, respectively) and more resistant forms (4,2% and 20%, respectively). Allo- HSCT was done in 6 pts (TI/II=4, T-IV=2) at a median of 7,2 mo from CR, all are alive without relapse. Only 28 of 77 (36%) CR pts underwent HSCT due to logistics problems and refusals. Auto-HSCT was done at a median time of 6 mo from CR and pts proceeded to further maintenance. We compared disease-free survival (DFS) and probability of relapse incidence (RI) in transplanted pts and those who survived in CR ≥6 months (land-mark) receiving only chemotherapy (n=27). Land-mark analysis demonstrated the essential benefit of auto-HSCT: DFS from time of transplantation was 100% and from land-mark for chemotherapy group - 66% (p=0,03). There were no relapses after auto-HSCT. RI at 5 years in the chemotherapy group constituted 21% (p=0,047). The MRD before HSCT was negative in 6 of 7 evaluated pts. At 5 years overall survival (OS) for the whole group constituted - 66%, DFS - 76% (Figure 1). In a multivariate analysis no relation was found between OS or DFS or RI and age, gender, initial risk group, T-LBL, leukocytosis, immunophenotype, late response, CNS disease, treating center.

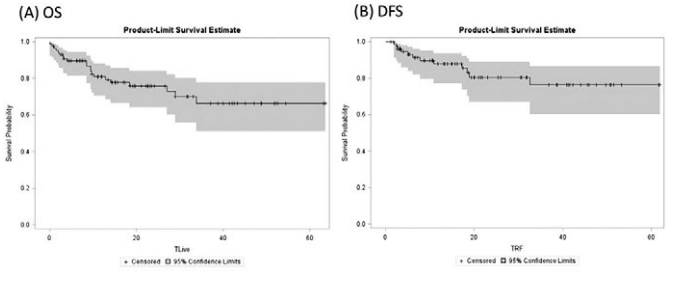

Figure 1.

Summary and Conclusions: Though small our data demonstrate that non-intensive, but non-interruptive treatment approach is effective. Auto-HSCT after BEAM conditioning followed by maintenance provides an adequate alternative to allo-HSCT. OS and DFS were not found to be depended on various common risk factors.

P538

OSTEONECROSIS IN AIEOP ALL 2000 AND 2006R TRIALS

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Background: Osteonecrosis (ON) is a potentially disabling complication in children and adolescents treated for acute lymphoblastic leukaemia (ALL), that could negatively impact on the quality of life (QoL).
Aims: We retrospectively assessed the incidence of ON and sequelae on patients enrolled in 2 consecutive ALL trials (ALL 2000 and 2006R) of the Italian Association of Pediatric Hematology and Oncology (AIEOP), from September 2000 to December 2011.
Methods: Using ad hoc report forms, data on 3691 ALL patients were reviewed in AIEOP participating centers in order to identify ON occurrence, ON-related symptoms, radiological findings, treatment, risk factors and outcome. Patients with Ph+ ALL, infants or who underwent an hematopoietic stem cell transplant (HSCT), in first remission or after relapse, were excluded from the study. Descriptive statistics and chi-square test for association were used. Cumulative incidence of ON were estimated adjusting for competing risks and compared with the Gray test.
Results: Overall, 99 ON (2.7%) were identified of whom 82% were symptomatic. ON was diagnosed during induction/consolidation therapy in 12% of patients, during reinduction in 24% and during maintenance or after therapy discontinuation in 64% of cases. The 5-year cumulative incidence of ON was significantly different by age, being 9.9% (n=77) in 781 patients ≥10 years *vs* 0.8% (n=22) in those under this age (p <0.001). Within each of these two age groups, the incidence of ON did not differ by gender, white blood cell count at onset, immunophenotype B or T lineage, type of steroid induction (dexamethasone or prednisone) and was not greater in patients treated in the high risk arm or in patients who received cranial radiotherapy. Table 1 summarizes the characteristics of patients aged ≥10 years. ON incidence in our study does not seem related to other risk factors such as obesity or thyroid dysfunctions. Involved-site was single in one-third of cases; the most frequent sites were hip, knee, ankle and shoulder. ON (unilateral or bilateral) in these sites was diagnosed in 64%, 51%, 35% and 9% of patients, respectively. ON-related treatment was performed in 34 (41%) patients, including: arthroprothesis' intervention (n=21), therapy with bisphosphonates (n=9) or other non-invasive procedures, such as physiokinesitherapy (n=4). At the last follow-up visit, 81% of patients were asymptomatic, 12% had persistent pain without functional limitations and 7% had pain with limitations of daily activities.

Table 1. Characteristics of patients aged ≥10 years.

	ON		No ON		TOTAL
	N.	*%*	*N.*	*%*	*N.*
Total	77	*9.9*	704	*90.1*	781
Gender					
Male	39	*8.4*	426	*91.6*	445
Female	38	*12.0*	278	*88.0*	316
p-value=0.09					
WBC count					
<20000	56	*11.2*	443	*88.8*	499
≥20000- <100000	16	*9.3*	157	*90.7*	173
≥100000	5	*4.6*	104	*95.4*	109
p-value=0.10					
Immunophenotype					
Non T	62	*10.2*	547	*89.8*	609
T	13	*7.8*	153	*92.2*	166
Not known	2	*33.3*	4	*66.7*	6
p-value=0.36					
Final risk					
Standard	13	*8.9*	133	*91.1*	146
Medium	55	*11.5*	425	*88.5*	480
High	9	*5.8*	146	*94.2*	155
p-value=0.11					
Steroid in induction					
Dexa	16	*9.0*	161	*91.0*	177
Pred	61	*10.2*	535	*89.8*	596
p-value=0.64					

Summary and Conclusions: This study confirms that ON is a challenging problem, with possible consequent morbidity that could compromise the QoL. ON occurrence was not related to the type of treatment but closely influenced by older age at diagnosis. An early detection of ON symptoms and the intro-

duction of innovative therapies to prevent sequelae for children or adolescents treated in ALL trials are needed.

P539

HDAC EXPRESSION PATTERNS ASSOCIATE WITH HIGH-RISK FEATURES IN PEDIATRIC LEUKEMIA

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Background: Altered expression of histone deacetylases (HDACs) is a common feature in cancer, including hematological malignancies. Thus, HDAC inhibitors have rapidly emerged as promising targets in cancer therapeutics (Stubbs, 2015). However, expression patterns of HDACs in childhood leukemia have been scarcely studied.
Aims: To analyze the expression of HDAC isoforms in different subtypes of pediatric leukemia and correlate them with prognosis and clinic-biological features.
Methods: We evaluated the mRNA gene expression profile of class I, II and IV HDAC genes (HDAC 1-11) by quantitative PCR in 54 leukemic pediatric samples and a pool of non-neoplastic samples as control. Samples were obtained from the Biobank of our hospital after appropriate informed consent was signed by patients and/or legal tutors. Our series included 35 boys and 19 girls diagnosed with acute leukemia (median age 5.4 years, range 0-16.7), treated according to Spanish Pediatric Hemato-Oncology Cooperative Group protocols in a single center. Nineteen (35%) cases were B-cell precursor acute lymphoblastic leukemia (pre-B-ALL), 22 cases T-cell ALL and 13 patients had acute myeloblastic leukemia (AML). The HDAC expression levels in different groups of patients were compared by the Mann-Whitney test. The level of significance was set up at $p<0.05$. The analyses were performed with SPSS 22.0.
Results: We found high expression levels of class I HDAC isozymes (HDAC 1, 2, 3 & 8) in leukemic samples compared to non-neoplastic samples, as previously reported (Gruhn, 2013; Moreno, 2010). We also noted a trend towards a downregulation of HDAC10 in all leukemia subtypes. A higher expression of HDAC1, HDAC2, HDAC3, HDAC4 & HDAC11 and a lower expression of HDAC5 and HDAC9 was observed in T-ALL compared to pre-B-ALL. HDAC11 was significantly overexpressed in AML cases. The class I HDACs HDAC1 & HDAC3 expression correlated with age, with patients >10 years old expressing the highest levels. In contrast, infant patients (<1 year-old) had a significant lower expression of HDAC7 ($p=0.022$). Interestingly, 3/4 cases with a pro-B phenotype had low levels of HDAC7, in line with our recently published data (Barneda-Zahonero, 2015). Of note, patients with MLL rearrangement had significantly higher expression of HDAC9 ($p=0.001$). Male patients had higher expression of HDAC4 ($p=0.008$). We did not find a correlation between HDACs expression and WBC count levels at diagnosis. In general, overexpression of all HDACs isoforms was associated with worse outcome. Noticeably, in T-ALL patients HDAC5 and HDAC9 expression levels above Q3 upper quartile significantly correlated with worse overall survival (OS) ($p=0.03$ and $p=0.04$, respectively). T-ALL cases with high HDAC1 had inferior OS (91% *vs* 51%), but the low number of patients precluded the finding of statistical significance. In summary, high expression of HDACs isoforms in leukemic patients correlate with worse outcome and older age. In addition, some isozymes associated with specific features. Thus, T-cell phenotype cases overexpressed HDAC1-4 & HDAC11 and underexpressed HDAC5 & 9. However, those T-ALL cases with high HDAC5 & 9 levels did worse, with inferior OS. HDAC7 levels were lower in infant patients and in those pre-B-ALL cases with pro-B phenotype. Finally, MLL rearranged cases showed higher expression of HDAC9.
Summary and Conclusions: We have observed a different pattern of HDACs expression in different subtypes of pediatric leukemia. Some of these profiles identified high risk patients who could benefit from a targeted treatment with HDAC inhibitors.

P540

DEVELOPMENT OF AN NGS ASSAY FOR IGK THAT CAN BE COMBINED WITH IGH FOR IDENTIFYING CLONAL POPULATIONS IN LYMPHOID MALIGNANCIES

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Background: Previously we developed next generation sequencing assays for the detection and characterization of clonality in B-cells, targeting the *IGH* locus with single step PCR approach followed by sequencing and analysis. These *IGH* assays were shown to be both sensitive and robust in a number of different laboratories and have demonstrated utility for tracking residual disease. *IGK* is also an important locus that can be used for the identification and tracking of clonality, as *IGK* rearrangements are stable markers that are retained even in lambda light chain expressing B-cells, as Kde rearranges to Vk elements as well as to the recombination signal sequence (RSS) in the Jk5-Ck intron (INTR) prior to rearrangements within *IGL*. Accordingly, IGK is an ideal marker for tracking residual disease in B-lineage ALL.
Aims: The development of an assay for *IGK* that can be combined with *IGH* that will increase the chance of identifying clonal populations in lymphoid malignancies such as non-Hodgkin's Lymphoma and tracking a variety of B-cell tumours.
Methods: Genomic DNA from peripheral blood, tonsil and bone marrow aspirates were tested for *IGK* gene rearrangements. Optimized V and J consensus primers targeted all *IGK* V and J region gene segments that are rearranged in lymphoid cells as well as the Kde rearrangements that precede lambda selection. Multiplex PCR master mixes simultaneously amplified *IGK* V-J gene rearrangements and incorporated sample identifying indices into amplicons, which facilitated combining and sequencing libraries generated testing up to 12 samples and controls in each run. Amplicon products were purified, quantified, the concentrations adjusted, pooled, and the harmonized libraries were loaded into the OneTouch 2. The enriched emulsion PCR libraries were sequenced using Ion 316 Chip Kit v2s and Ion PGM Sequencing 400 Kits. Data were analysed using Invivoscribe's proprietary bioinformatics software (included with the test kits) on a standard personal computer. The bioinformatics package allows for *IGH* and *IGK* libraries with the same barcodes to be analysed on the same run. The PGM platform allows a sustainable, rapid turn around time, less than 36 hours from primary sample to result.
Results: Data generated with the LymphoTrack® IGK assay and bioinformatics package reproducibly identified clonality and DNA sequences of *IGK* Vk-Jk, as well as Vk-Kde and INTR-Kde, gene rearrangements. Automated data outputs include frequency distributions and V-J gene usage. DNAs from cell lines serially diluted into polyclonal tonsil DNA, confirmed the linearity (R^2 >0.99) and low run-to-run variance of the assay. This assay may be used with genomic DNA from peripheral blood, bone marrow and FFPE. In addition to the assay development, the break point of the INTR element was also identified.
Summary and Conclusions: We have previously developed *IGH* and *TRG* assays for the IonTorrent PGM. We now add an NGS clonality assay for *IGK* that identifies clonal *IGK* Vk-Jk, as well as Vk-Kde and INTR-Kde, rearrangements and associated specific rearranged DNA sequences to the repertoire that can either be used in reflex testing to further characterise samples that are difficult to characterize by *IGH* alone. Alternatively, a more time and cost efficient approach would be to run both assays at the same time on samples to be run on the same sequencing run. These assays can be used to both detect and monitor lymphoproliferative disease. A further advantage is that the assays identify clonal lymphocyte populations by their unique DNA sequences that can subsequently be used to track clones in residual disease testing.

P541

SIGNIFICANCE OF RECURRENCE OF MINIMAL RESIDUAL DISEASE (MRD) DETECTED BY MULTI-PARAMETER FLOW CYTOMETRY (MFC) IN PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) IN MORPHOLOGICAL REMISSION

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Background: Minimal residual disease (MRD) assessment is now established as an important predictor of outcome in both pediatric and adult patients (pts) with acute lymphoblastic leukemia (ALL). Availability of novel agents able to eradicate MRD is a further factor emphasizing the need for MRD monitoring in these pts.
Aims: To determine the significance of MRD relapse as detected by multi-parameter flow cytometry (MFC) in pts with ALL after achieving an MRD negative status following induction and consolidation therapy.
Methods: Between January 2003 and August 2013, 585 newly diagnosed pts with ALL (excluding Burkitt's leukemia/lymphoma) were treated with regimens including the hyperCVAD backbone or Augmented BFM. 540 (92%) achieved complete remission (CR), of whom, 490 (91%) became MRD negative as assessed by MFC. 47 pts (41 with B-ALL, 6 with T-ALL) developed recurrence of MFC detectable disease while still in morphological CR and are subjects of this study. MRD was assessed by 4-color (and since 2009, 6-color) flow cytometry at CR and multiple time points thereafter, as clinically indicated.
Results: Median age was 43 years (yrs)(range,18-72 yrs), 27 (57%) were male. Median laboratory values at initial presentation were: WBC 7.3 K/uL (range, 0.6-303.8) and bone marrow (BM) blasts 89% (range, 26-98). Cytogenetics at initial presentation were: 11 diploid, 14 Philadelphia positive (Ph+), 6 insufficient metaphases/not done, 15 miscellaneous, and 1 with 11q23 rearrangement. Chemotherapy regimens included: 42 HCVAD +/- other (10 HCVAD, 8 HCVAD+Dasatinib, 10 HCVAD+Rituximab, 6 HCVAD+Imatinib, 5

HCVAD+Nelarabine, 3 HCVAD+Ofatumumab) and 5 Augmented BFM. Median Event-free survival (EFS) of the whole group was 24 months (mos) (range, 3.7-138 mos); median OS, 30 mos (range, 3.7-138 mos). Thirty-nine (83%) pts subsequently developed morphological relapse (32 BM, 3 BM+ central nervous system (CNS), 3 CNS, and 1 non-CNS extramedullary), after a median of 110 days (range, 3-540 days) from detection of MRD recurrence. Median EFS for this group was 24 mos (range, 7.6-72 mos), and median OS was 30 mos (range, 9-82 mos). Eight pts did not have morphological relapse including 5 pts who died in complete morphological remission, with only 3 pts alive in continued CR. Of the 5 pts who died prior to relapse, 4 had undergone stem cell transplantation (SCT) (2 related, 1 cord, and 1 haplotype), and died of post-SCT complications. 3 pts (2 B-ALL, 1 T-ALL,) are still alive and in CR despite having had MRD recurrence, all with normal karyotype and all having undergone matched unrelated donor(MUD) SCT with a median OS of 3.8 yrs (range, 3 -11.5 yrs).

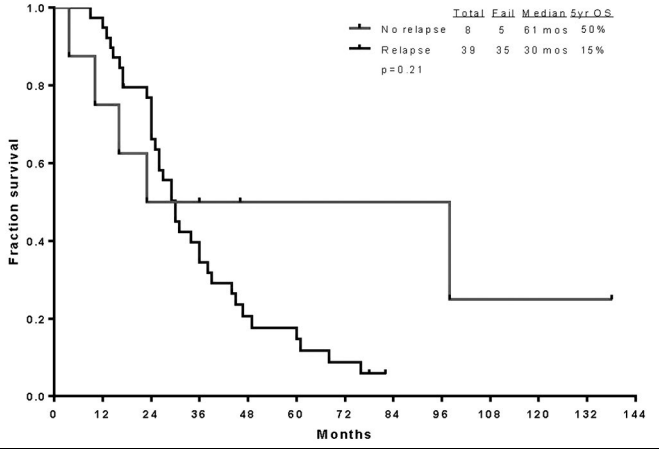

Figure 1. Overall survival for pts with MRD relapse with no morphological relapse (n=8) *versus* pts with MRD relapse followed by morphological relapse (n=39).

Summary and Conclusions: In this single institution retrospective analysis, the vast majority of pts with ALL who achieved MRD negative CR, followed by MRD relapse at any time point, subsequently experienced morphological relapse, suggesting that MRD relapse at any time point during a patient's follow-up is associated with a high risk for relapse. Although allogeneic SCT rescued some patients with ongoing long-term remission, it was associated with mortality in others. Prospective studies of long-term MRD assessments throughout a patient's treatment course, together with less toxic strategies to eradicate MRD, are warranted.

P542

IMPROVED SURVIVAL IN ADULT PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) IN THE NETHERLANDS: A POPULATION-BASED STUDY ON TREATMENT, TRIAL PARTICIPATION AND SURVIVAL, 1989-2012

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Background: Pediatric ALL has shown an impressive improvement in survival over the last decades. The outcome in adult patients is far less optimistic with current survival rates in several published trials reaching 60% only. Large, comprehensive population-based studies among unselected adult ALL patients are lacking.
Aims: We conducted a population-based study to assess trends in treatment, trial participation and survival among adult ALL patients diagnosed in the Netherlands from 1989-2012.
Methods: We identified 1833 over 18-year-old ALL patients (median age 49 years) from the nationwide Netherlands Cancer Registry (NCR) with follow-up until February, 2014. Patients were categorized into 4 calendar periods (1989-1994, 1995-2000, 2001-2006 and 2007-2012) and 5 age groups (18-24, 25-39, 40-59, 60-69 and ≥70 years). Data on treatment (ie, supportive care only, chemotherapy (CT) and stem cell transplantation (SCT)) were retrieved from the NCR. We obtained data on the type of SCT (ie, autologous (autoSCT) or allogeneic SCT (alloSCT)) from the HOVON SCT working party, and data on trial participation from the HOVON and EORTC cooperative groups. To correct for the life expectancy of the general population, relative survival rates (RSRs) were computed as a measure of patient survival. Overall survival (OS) according to treatment by calendar period was estimated by the Kaplan-Meier method.

Results: The annual age-standardized incidence remained stable over time at around 0.6/100,000 with a consistent male predominance (54% males). Overall, 38%, 41%, 49%, 72% and 43% of the patients in the 5 age groups received CT, 22%, 15%, 10%, 1% and 0% received autoSCT, 38%, 41%, 31%, 10% and 0% received alloSCT, while 2%, 3%, 9%, 17% and 57% received supportive care only, respectively. The application of alloSCTs increased over time among patients up to 70 years and was steadily introduced in the treatment of patients aged 60-69 years during the mid-1990s. Treatment remained essentially unchanged over time among patients aged ≥70 years.
When clinical trials were open for accrual, the inclusion rate was 67%, 66%, 55% and 58% in the first 4 age groups, respectively. No trials were available for patients aged ≥70 years.
Relative survival (RS) improved over time as 5-year RSRs (95% CI) were 19% (15%>23%) in 1989-1994 and 37% (32%>42%) in 2007-2012 (Figure 1A). This improvement was confined to patients up to 70 years, and was most pronounced among patients up to 40 years, especially in the most recent calendar period (Figure 1B). RS worsened sharply with age. 5-year RSRs (95% CI) for patients diagnosed in 2007-2012 were 75% (63%>84%), 57% (44%>68%), 37% (27%>47%), 22% (13%>31%) and 5% (1%>18%) for the 5 age groups, respectively. Among 18-39-year-olds, 5-year OS was 25% and 68% for those who received CT (Figure 1C) and 46% and 66% for recipients of an alloSCT (Figure 1D) in the first and last calendar periods, respectively.

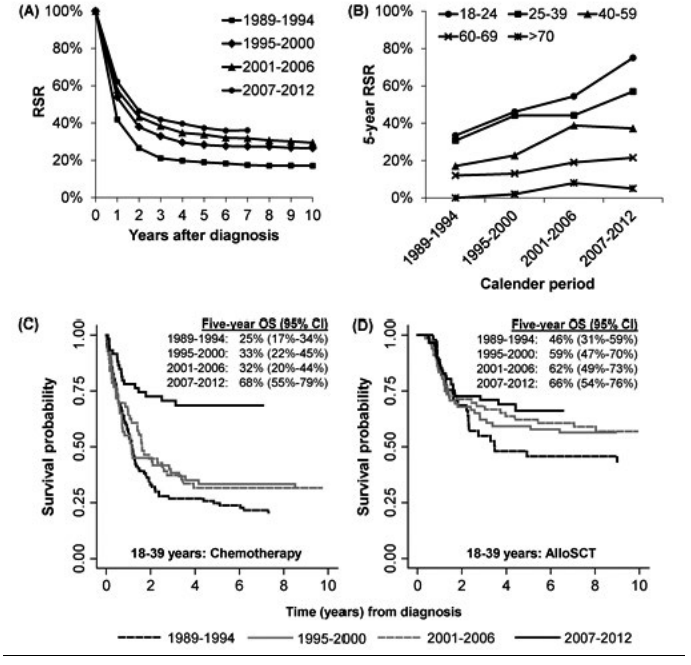

Figure 1.

Summary and Conclusions: We show that survival of unselected adult ALL patients up to 70 years of age improved over time in the Netherlands. The intensification of adult chemotherapeutic approaches based on more intensive, pediatric protocols implemented since 2007 and the increased application of alloSCT along with improved supportive care, are most likely important factors contributing to this improvement. The accrual into clinical trials slightly decreased with age, with no trials available for patients aged ≥70 years yet. Novel, and more targeted treatment options with a tolerable toxicity profile are needed to further improve outcome, particularly in the elderly.

Acute myeloid leukemia - Biology 2

P543

A DISTINCTIVE LONG-NON CODING RNA SIGNATURE CHARACTERIZES ACUTE MYELOID LEUKEMIA WITH TRANSLOCATION T(8;16)(P11;P13) AND MYST3-CREBBP REARRANGEMENT

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Background: Long Non-coding RNAs (lncRNAs) have recently emerged as important actors in the regulation of multiple cellular processes including cancer. Acute myeloid leukemia (AML) with translocation t(8;16)(p11;p13) (t(8;16) AML) is a subtype with specific clinical and biological characteristics including a specific gene (Camós *et al.*, 2008) and a microRNA (Díaz-Beyá *et al.*, 2013) expression profile. In this translocation, *MYST3* on chromosome 8p11 fuses with *CREBBP* on chromosome 16p13.3. The MYST3-CREBBP fusion protein is able to interact with multiple transcription factors producing a disturbed transcriptional program. However, the lncRNA expression pattern of t(8;16) AML has not been described yet.
Aims: The main objective of this study was to characterize the expression pattern of lncRNAs in t(8;16) AML in comparison to other AML subtypes.
Methods: Forty six AML patients, 4 normal bone marrow (NBM) and 3 CD34+ NBM samples were included in the study. Patient samples included a wide diversity of different AML subtypes: intermediate-risk cytogenetics AML (IR-AML, n=18), acute promyelocitic leukemia (APL, n=4), t(8;21) AML (n=4), inv(16) AML(n=2), t(6;9) AML (n=7), monosomal karyotype AML (n=4), t(3;3) AML (n=1), t(9;11) AML (n=1) and t(8;16) AML (n=5). Within IR-AML, 7 harbored an *FLT3*-ITD, 5 *NPM1,* 7 *CEBPA* and 6 *DNMT3A* mutations. The lncRNA expression was studied using Affymetrix® Human Gene 2.1 ST platform which includes 9698 lncRNAs transcripts. The filtering and normalization of the array data was performed using oligo package from Bioconductor. Statistical analyses were performed with TiGR MultiExperiment Viewer, BRB tools and R. The Transcription factor Affinity Prediction Web Tool was used to determine the putative transcription factors binding to the differentially expressed lncRNAs promoters.
Results: The hierarchical cluster analysis showed that all 4 NBM clustered together, as well as all 3 CD34+ NBM according to their lncRNA expression. Interestingly, all 5 t(8;16) AML samples clustered together, as well as the 3 APL, the 7 t(6;9) AML and 5 out of 7 cases with *CEBPA* mutations. Afterwards, we focused on t(8;16) AML and the supervised analysis identified an specific 113-lncRNA signature. Interestingly, when we analyzed which transcription factors (TF) had motifs overrepresented in the promoters regions of the t(8;16) AML lncRNA signature, we identified motifs for GATA2 (p<0.001) and Evi1 (p=0.04). These two TFs have been described to interact with *CREBBP,* one of the partners involved in t(8;16) AML. Of note, 4 overexpressed lncRNAs of the signature (*linc-HOXA11, HOXA11-AS, HOTTIP* and *NR_038120*) were located in the *HOXA* genomic region, previously found upregulated in t(8:16) AML. Since several studies suggest an active crosstalk between microRNAs and lncRNAs, we correlated the expression of these lncRNAs with the microRNA profile. We found significant correlations between *linc-HOXA11* and miR-222* (R^2=0.996, p=0.003), *HOXA11-AS* and miR-let-7c (R^2=0.994, p=0.006), *HOTTIP* and miR-196b*(R^2=0.958, p=0.041), *NR_038120* and miR-486-3p (R^2=0.999, p=0.0004), and miR-19a (R^2=0.953, p=0.04).
Summary and Conclusions: t(8;16) AML harbors a characteristic lncRNA signature. Some of the lncRNAs of this signature are located in the *HOXA* genomic region, and correlate with several of the distinctive microRNAs of this entity. Interestingly, we have identified *in silico* GATA2, which interacts with CREBBP, as the most significant TF that could regulate this lncRNAs signature. Further investigation is warranted to determine the importance of this lncRNA signature.
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P544

NEW JAK2 HETEROZYGOUS LOSS: A ROLE IN OVERALL SURVIVAL IN ACUTE MYELOID LEUKEMIA PATIENTS

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Background: Acute Myeloid Leukemia (AML) is a hematologic malignancy that originates in hematopoietic stem and myeloid progenitor cells. Age and karyotype have been recognized as the most prominent prognostic factors in AML patients. Novel array-based technique-single-nucleotide polymorphism (SNP) microarray can detect cytogenetic lesions involve mostly structural alterations with losses or gains of chromosomic material. Those chromosomic abnormalities are predictive of response and are very important to define therapeutic strategies. SNP microarray can detect copy-neutral loss of heterozygosity (CN-LOH), which plays a role in oncogenesis by duplicating oncogenes, inhibiting tumor suppressors, and stimulating improper epigenetic programming.
Aims: Our objective is to evaluate the prognostic impact of these genetic alterations on clinical outcome.
Methods: We analyzed 58 AML patients by high-density CytoScan HD Array (Affymetrix, Inc). The results obtained by SNP Array, were analyzed by Chromosome Analysis Suite (ChAS) v1.2 (Affymetrix Inc.) and Nexus Copy Number™ v7.5 (BioDiscovery).
Results: We treated 58 AML patients (pts) with a median age of 52 years, of which 9 were insensitive pts and 49 sensitive to therapies. The median age at diagnosis was 51,3 years (range: 19-89 years), and they have a female/male ratio of 53% and 47%, while the median follow up is of 28,5 and 34,4 months respectively. Copy Number Alterations (CNAs) were detected in all patients affecting all the chromosomes, in particular our cohort of pts present a percentage of CNA, divided as follow: 42% of LOH, 19% of Copy Number (CN) gain, 39% CN of loss. By SNP array analysis we found that several important genes were preferentially deleted: ADAM3A (62,2% pts; Chr 8), JAK2 (34,5% pts; Chr 9), HRAS (8,6% pts; Chr11), WT1 (20,7% pts; Chr 11), FLT3 (13,8% pts; Chr 13), SIRPB1 (41,4%; Chr 20) and CRLF2 (50% pts; Chr X). We observed a significant involvement of the Chromosome X, infact in several leukemia-related genes as ATRX (43,1% pts), BCOR (34,5% pts), GATA1 (39,7% pts), KDM6A (43,1% pts), PHF6 (29,3% pts), ZRSR2 (31% pts), STAG2 (29,3% pts) are concurrent events of loss and LOH: this double lesion may inactive their function as transcriptional regulators. While genes preferentially involved in amplifications are PRDM16 (12% pts), KIT (24,1% pts), IKZF1 (12% pts), MYC (8,6% pts), KRAS (8,6% pts). Moreover we focused on a particular heterozygosis loss of *JAK2*, detected in 5/58 (8,6%) pts, which goes from 5080280 bp to 5098786 bp, involving half of exon 17 and exons 18, 19, 20, 21 and 22. This region include the second tyrosine-kinase domain required for a correct function of the protein. We showed that a group of patients which present this deletion have an overall survival rate better than the group with an amplification of the gene (p-value <0,01) (Figure 1). None CNV in this region of JAK2 had been described in hematopoietic tissue before.

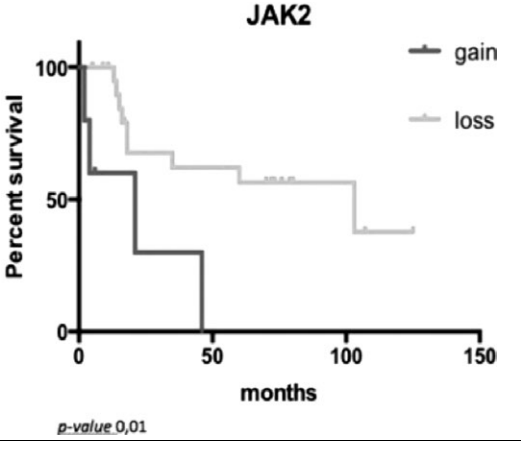

Figure 1.

Summary and Conclusions: By SNP arrays we have identified Copy Number Alterations involving important cancer genes in AML and we showed that a new deletion involving *JAK2* may have a role in overall survival. Future prospective will be to correlate other cancer genes alterations and mutations with the prognosis of AML, in order to identify new biomarkers relevant for the disease.
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P545

SELECTIVE DEATH OF LEUKEMIC STEM CELLS INDUCED BY DEFERASIROX

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Background: Despite high remission rate after therapy, only 30-40% of acute

myeloid leukemia (AML) patients survive 5 years after diagnosis. The main cause of treatment failure is thought to be insufficient eradication of leukemic stem cells (LSC). Identifying drugs that can efficiently eradicate LSCs is therefore imperative. HIF1α is essential for LSC maintenance and targeting HIF1α selectively eliminates LSC. Deferasirox is an iron chelator used to reduce chronic iron overload in patients receiving long-term blood transfusions.
Aims: To study the ability of deferasirox to induce apoptosis and to target HIF1α in AML LSCs.
Methods: We isolated $CD34^+CD38^-$ LSC-like cells from the leukemic progenitor CD34+ KG1a cell line using CD38 magnetic separation. We isolated the phenotypically primitive leukemic $CD34^+CD38^-CD123^+$ cells and the progenitor $CD34^+CD38^+CD123^+$ cells from bone marrow samples of newly diagnosed AML patients using a double CD34 & CD38 magnetic separation. Cell proliferation and apoptosis were analyzed by WST-1 and FACS assays, respectively. Synergism was calculated by Chou.T.C combination index. HIF1α expression was measured by real-time PCR and Western blot assays.
Results: $CD34^+CD38^-$ LSC-like cells, sorted from the KG1a cell line, exhibited increased sensitivity to deferasirox with an IC50 of 1.3μM compared to 8.9μM calculated for the more mature $CD34^+CD38^+$ cells. The LSC-like cells, which were more sensitive to deferasirox, were less sensitive to ARA-C compared to the $CD34^+/CD38^+$ cells. Deferasirox was >2-fold more efficient in inducing apoptosis in the CD34+CD38− cells, compared to the CD34+CD38+ cells (74±7.2% & 32±6.2% apoptosis, respectively). Deferasirox demonstrated a synergistic cytotoxic effect with ARA-C on both the CD34+CD38− and CD34+CD38+ KG1a cell fractions. Similar results were observed with $CD34^+CD38^-CD123^+$ LSCs and $CD34^+CD38^+$ $CD123^+$ progenitor cells isolated from AML patients. As shown with the cell line, AML patient LSCs were 2-fold more sensitive to deferasirox treatment showing a 62±15% induction of cell death compared to only a 34±9% induction in leukemic progenitor cell death. The increase in cell death was accompanied by an increment of reactive oxygen species (ROS) levels which was more prominent in the LSCs compared to the progenitor cells. Furthermore, deferasirox enhanced the cytotoxic effects of ARA-C on both the LSCs and the leukemic progenitor cells. These data indicate that deferasirox is highly cytotoxic to AML cells; however, it is significantly more specific to AML stem cells. Since deferasirox is an NFkB inhibitor which regulates HIF1α levels, and since HIF1α is selectively activated in AML stem cells and is essential for maintenance of these cells, we studied the effect of deferasirox on HIF1α expression. We found that HIF1α was downregulated transcriptionally (~40% reduction) and translationally (~55% reduction) in the LSC-like cells while it was upregulated in the $CD34^+CD38^+$ KG1a cells following deferasirox treatment. These data hint that AML-LSCs are selectively targeted by deferasirox.
Summary and Conclusions: We describe a novel and unique anti-LSC property of deferasirox, which was originally developed as an iron chelator. We found clinically relevant concentrations of deferasirox to be cytotoxic *in vitro* to AML cells but even more potent against LSCs. We believe that deferasirox exerts its cytotoxic effect, at least in part, by downregulating HIF1α levels in the LSC population. Pending further characterization, deferasirox can be considered as a potential therapeutic agent for eradicating LSCs.

P546

ADDITIONAL MOLECULAR ABERRATIONS LEADING TO LEUKEMIC TRANSFORMATION IN PATIENTS WITH FAMILIAL PLATELET DISORDER

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Background: Familial platelet disorder with predisposition to acute myeloid leukemia (FPD/AML) is a rare autosomal dominant disorder characterized by quantitative and qualitative platelet abnormalities and considered as a model of genetic predisposition to hematological malignancies. FPD/AML syndrome is caused by inherited *RUNX1* mutations (21q22) reported so far in less than 40 pedigrees. However, evolution remained very heterogeneous since only 30-40% of such patients develop hematopoietic neoplasms, usually after a long period.
Aims: In this context, it is likely that the acquisition of secondary molecular events functions as a driver to promote malignant transformation, thus the FPD/AML syndrome constitutes a unique multi-step leukemogenesis model *in vivo*. Recently, acquired mutations of *CDC25C* and *GATA2* have been reported in 53% and 23% of FPD/AML patients respectively (Yoshimi *et al.*, Nat Commun, 2014), particularly during disease progression.
Methods: We studied 24 patients from 15 FPD/AML families identified from 2005 to 2014. Twelve of them progressed to leukemic stage: 9 patients developed AML and 3 patients developed T-ALL. Cytogenetics and next-generation sequencing (MiSeq, Illumina®) of 44 genes (including *CDC25C* and *GATA2*) were performed for all patients. For patients who progressed to leukemic stage, samples were collected at the time of diagnosis and during complete remission allowing to confirm the acquired status of mutations.
Results: Characteristics of the patients studied are presented in the following Table 1.

Table 1.

Pedigree/patient	Sex	Germline RUNX1 alteration	Leukemic transformation	Age at leukemia diagnosis	Acquired RUNX1 alteration (leukemic stage)	Other acquired mutations	Loss of acquired alterations in complete remission
1/II:1	M	p.R177Q	No	NA	No	NA	NA
1/II:2	M	p.R177Q	No	NA	No	DNMT3A†	NA
1/II:3	M	p.R177Q	AML	60	p.A160T	FLT3-ITD, PHF6, KIT	Yes
1/III:1	M	p.R177Q	T-ALL	28	No	PHF6, WT1, NOTCH1	Yes
2/I:1	F	p.Q308RfsX259	AML	55	p.G138PfsX12	-	Yes
2/II:1	F	p.Q308RfsX259	No	NA	No	NA	NA
2/II:2	F	p.Q308RfsX259	T-ALL and t-AML 5 years later	24	No	t(1;3)(p36;q26) and KRAS at t-AML stage§	Yes
3	F	Complete deletion of RUNX1	AML	12	Duplication of RUNX1-deleted chromosome	-	Yes
4	F	p.R139X	AML	48	p.R139X (UPD)	KRAS, RAD21	Yes
5/II:1	M	p.P218S	No	NA	No	NA	NA
5/II:2	M	p.P218S	No	NA	No	NA	NA
6/I:2	M	p.G108V	T-ALL	14	No	WT1, FLT3-ITD, FLT3-TKD	Yes
7	F	p.D305TfsX262	AML	37	Duplication of RUNX1-mutated chromosome	SRSF2, WT1, TET2	NA (early death)
8	F	p.H377PfsX191	AML	12	p.S114P	SF3B1	Yes
9	F	p.G108V	No	NA	No	NA	NA
10	M	p.G143RfsX43	AML	36	p.K83Q	BCORL1	Yes
11/II:1	M	p.T169R	No	NA	No	NA	NA
11/II:2	F	p.T169R	No	NA	No	NA	NA
11/I:1	M	p.T169R	No	NA	No	NA	NA
12	F	Complete deletion of RUNX1	No	NA	No	NA	NA
13	F	p.T219RfsX8	AML	43	p.T219RfsX8 (UPD)	CBL, MPL, TP53, WT1, del(11)(q21)	Yes
14/I:1	F	p.T121HfsX9	No	NA	No	NA	NA
14/II:1	M	p.T121HfsX9	No	NA	No	NA	NA
15	M	p.A129E	AML	42	Duplication of RUNX1-mutated chromosome	SRSF2	Yes

†Acquired mutation at FPD stage without leukemic transformation.
§Not studied at T-ALL stage.

Summary and Conclusions: Acquisition of a secondary genetic or molecular event was identified in all patients of our cohort. Acquired mutations involved genes implicated in tyrosine kinase pathway, epigenetic, spliceosome and transcription regulation. In addition to germline *RUNX1* mutation, a second aberration of *RUNX1* was found in all patients who developed AML, in line with our previous report (Preudhomme *et al.*, Blood, 2009). Four patients acquired mutation in the second allele and 4 patients had duplication of the abnormal copy by uniparental disomy (n=2) or acquired trisomy 21 (n=2). On the other hand, patients who developed T-ALL were younger and did not acquire second aberration of *RUNX1*. Interestingly, *CDC25C* or *GATA2* mutations were not identified in our cohort, in contrast with Yoshimi *et al.*

P547

LEUKEMOGENIC MLL-ENL FUSIONS INDUCE ALTERNATIVE CHROMATIN STATES TO DRIVE A FUNCTIONALLY DICHOTOMIC GROUP OF TARGET GENES.

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Background: MLL fusion proteins provoke leukemogenic transformation by inappropriate activation of downstream target genes.
Aims: To identify MLL-ENL subordinate transcripts with importance for malignant development, primary hematopoietic precursor cells containing a conditional MLL-ENL knock-in were used in NGS experiments to monitor the molecular process driving the transition from a transformed, self-renewing to a normal, differentiating cell.
Methods: Nascent RNA-seq and ChIP-seq identified 165 high confidence MLL-ENL target genes.
Results: Correlation of individual transcription rates with cellular physiology suggested that about 40% of MLL-ENL targets play an important role in transformation thus assigning them a "driver" status with the remainder likely to act mainly as "bystanders". Next to well-known MLL-fusion targets like the *HoxA* cluster hitherto unknown MLL-ENL controlled regulatory units could be identified. Many of those supported pro-transformation traits like accelerated proliferation, reduced differentiation and inhibition of apoptosis. In addition mechanistic insight was gained from ChIP patterns that were linked to activation by MLL-ENL. MLL-ENL targets fell in two distinct clades characterized by unique MLL-ENL binding arrangements and chromatin configurations. In an "ME5" group the fusion protein bound at the transcription initiation site, RNA synthesis rates were directly proportional to H3K79 methylation and extensive transcriptional pausing was suggested by RNA-PolII Ser2-P distribution. In contrast, hallmarks of an "ME3" group were a location of MLL-ENL downstream of the transcription termination site, transcription rates that were inversely correlated to H3K79 methylation and an unusual RNA PolII accumulation associated with the occurrence of non-coding transcripts. This chromatin-based dichotomy was

mirrored by a functional separation into DNA and RNA related activities for ME5 and ME3 genes respectively.
Summary and Conclusions: As current small-molecule approaches aim to eliminate exclusively H3K79 methylation they may not be sufficient for complete leukemia eradication. Our results suggest that targeting both subsets of MLL-ENL subordinate genes may be potentially more efficient for treatment of MLL fusion induced leukemia.

P548

GENOMIC LANDSCAPE OF CORE-BINDING FACTOR ACUTE MYELOID LEUKEMIA

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Background: Core-binding factor (CBF) acute myeloid leukemia (AML) includes AML with t(8;21)/*RUNX1-RUNX1T1* chromosomal translocation and AML with inv(16)/t(16;16)/*CBFB-MYH11* chromosomal rearrangement. These recurrent genetic abnormalities are both associated with the disruption of the CBF, a heterodimeric transcription factor involved in hematopoiesis. Although the fusion proteins appear to be crucial for the leukemogenic process, considerable experimental evidences indicate that they are no sufficient to induce AML on their own. CBF-AML accounts for about 15% of de novo AML and is associated with a relatively favorable prognosis due to the high sensitivity to standard chemotherapy with high complete remission rates. Nonetheless, relapse incidence may reach 40% in such patients.
Aims: In this context, identification of additional molecular events is considered of great interest to improve our understanding of CBF-AML leukemogenesis, better predict clinical outcome and identify novel therapeutic approaches.
Methods: This study focused on 123 patients with CBF-AML including 68 AML with t(8;21) and 55 AML with inv(16)/t(16;16). Patients were enrolled in the 2 French therapeutic trials ELAM02 (n=68; median age: 10 years [4-17]) and CBF2006 (n=55; median age: 40 years [18-60]). All patients were studied at the time of diagnosis by next-generation sequencing (MiSeq, Illumina®) includnding 57 genes well-known in hematological malignancies.
Results: Identified mutations are indicated in the Figure 1 according to the CBF-AML subtype. Mutations involving genes from the tyrosine kinase pathways (i.e *KIT*, *NRAS*, *KRAS* or *FLT3*) were the most frequent aberration and were found in both subtypes as previously reported. Interestingly, mutations involving genes that control chromatin modifications (i.e *ASXL1*, *ASXL2*, *EZH2*, *KDM6A*) or implicated in the cohesin complex (*i.e. RAD21*, *SMC1A*, *SMC3*, *STAG2*, *NIPBL*) were frequent especially in the t(8;21)-AML subtype, whereas they were nearly absent in the inv(16)/t(16;16)-AML subtype. Finally, the two CBF-AML subtypes showed greatly distinct mutational profiles.

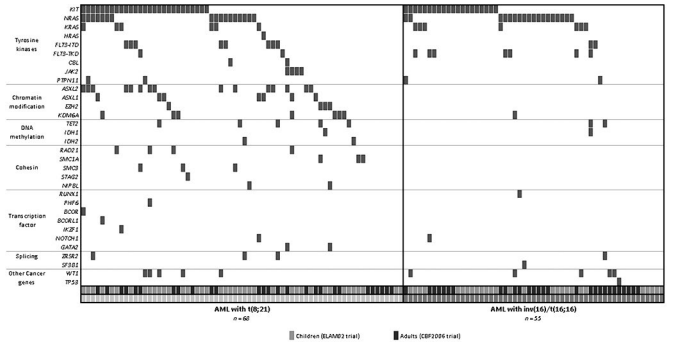

Figure 1.

Summary and Conclusions: Due to similarities between their molecular and prognostic features, t(8;21) and inv(16)/t(16;16)-AML are usually grouped and reported together in clinical studies. However, considerable experimental evidences have highlighted that they represent two distinct entities and should be considered separately for further studies. Particularly, t(8;21)-AML is characterized by the high frequency of mutations of genes involved in the epigenetic machinery and the cohesin complex. It is currently known that polycomb proteins functionally interact with cohesin to control transcription. Together, these results suggest an important pathway in t(8;21) leukemogenesis.
Note: Increase of our cohort size and correlations with clinical outcome are in progress to identify prognostic markers.

P549

PRO-METASTASIS PHOSPHATASE PRL-3 TRANSFORMS LEUKEMIA STEM CELLS AND PROMOTES LEUKEMOGENESIS IN AML VIA ACTIVATION OF SOX4/LIN28B/LET-7 SIGNALING

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Background: The reversible phosphorylation and dephosphorylation of tyrosine residues on proteins is mediated by protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs). This process regulates a wide range of signalling pathways and dysregulation of these signalling pathways are common biochemical phenomena in cancer cells. Protein-tyrosine phosphatase of regenerating liver 3 (PRL-3, encoded by *PTP4A3* gene) is a member of VH1-like PTP family with dual-specificity. Elevated expression of PRL-3 is associated with metastasis in solid tumors. Acute myeloid leukemia (AML) arises from abnormal clonal hematopoietic cells characteristic with uncontrolled proliferation and blocked differentiation of immature myeloid cells. A larger body of evidence supports the notion that leukemia stem cell (LSC), a distinct subpopulation of AML cells with self-renewal capacity, initiates and sustains the bulk leukemia. The molecular mechanisms underlying the transformation of LSC are not fully understood yet. We and others identified that PRL-3 is over expressed in patients with acute myeloid leukemia (AML) and serves a poor prognostic factor.
Aims: The aims of this study were to explore the role of PRL-3 in transformation of LSC in AML and to investigate the molecular mechanism by which PRL-3 was regulated.
Methods: Whole genome microarray was used to compare the transcriptome of a pair of iosgeneic cell line TF1-pEGFP *vs* TF1-hPRL-3. Colony formation assay and serial replating assay were employed to examine the *in vitro* self-renewal capacity of AML cells. To assess the *in vivo* function of PRL-3 in leukemogenesis, we performed limited serial dilution and bone marrow transplantation of TF1-hPRL-3 cells in nonobese diabetic/severe combined immunodeficiency (NOD/SCID) mice.
Results: We first show that ectopic expression of PRL-3 promotes proliferation, colony forming capacity of AML cells *in vitro* and leukemogenicity *in vivo*. Next, we conduct gene expression profiling of TF1-pEGFP and TF1-hPRL-3 cell lines and reveal upregulation of some stemness factors such as LIN28B, c-KIT, IGF2BP1 and downregulation of mature cell surface marker CD36 and CD38 by PRL-3. Importantly, gene-set enrichment analysis (GSEA) of this microarray result revealed a significant enrichment of genes identified in a previously defined gene expression signature of leukemia stem cell (LSC SIGNATURE_SAITO) and hematopoietic stem cell (HSC SIGNATURE_EPPERT). Notably, we unravel that LIN28B, a stem cell reprogramming factor, represses let-7 microRNA family in TF1-hPRL-3 cells. Furthermore, we identify IGF2BP1 as a novel target of let-7 miRNA. Through combination of shRNA targeting with serial replating assay and xenotransplantation mouse models, we validate that this regulatory axis is critical to PRL-3-medidated LSC phenotype. We also demonstrate SOX4 is a mediator to elevate LIN28B expression. LIN28B overexpression is associated with poor prognosis in a cohort of AML patients.
Summary and Conclusions: In conclusion, our investigation has identified a critical regulatory PRL-3/SOX4/LIN28B/let-7 networks, which play an important role in transformation of LSC and development of AML. Thus, we believe the novel regulatory uncovered in our study may represent the “Achilles’ heel” of LSC in AML and apprises the possibility of eliminating LSC in a molecular subset of AML in which PRL-3/LIN28B appears pivotal for the transformation.

P550

RATIONAL TARGETING OF NOTCH SIGNALLING IN ACUTE MYELOID LEUKAEMIA

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Background: Growing evidences from both preclinical and clinical investigations reveal Notch signalling as critical for the development of many cancers, and for their response to chemotherapy. Our lab has previously showed that Notch inhibition was efficient to abrogate stromal-induced chemoresistance in CLL and B-ALL. In contrast, the role of Notch in AML and its contribution to the crosstalk between AML cells and bone marrow stroma remains controversial: that makes unclear which could be the best therapeutic strategy (activation or inhibition) to target Notch in AML.
Aims: In this study, we sought to validate Notch signalling as potent successful therapeutic target in AML. We tested different modulators for their ability to influence Notch signalling at different levels, including the interaction ligand-receptor, the transactivation of the receptors and the transcriptional activation of the pathway. We then analysed the effects of Notch modulators, alone or in synergy with chemotherapeutic agents, on AML cells in culture or co-culture with bone marrow mesenchymal stromal cells (BM-MSCs).

Methods: AML blast cells were obtained from bone marrow samples (33) and peripheral blood (22) of AML patients. BM-MSCs were expanded from bone marrow of 20 healthy donors (BM-MSCs) and of 20 AML patients (BM-MSCs*). *In silico* analysis, RT-qPCR, FACS analysis and western immunoblotting were used to study the expression of Notch receptors and ligands, as well as Notch activation status, in AML cells and BM-MSCs. AML cells were cultured or co-cultured with BM-MSCs or BM-MSCs* at 10:1 (AML:BM-MSCs) ratio in presence or absence of Notch modulators including: recombinant human Jagged-1 and Jagged-2; anti-Notch-1, -2, -3, -4, anti-Jagged-1, -2 and anti-DLL-3 blocking antibodies; gamma secretase inhibitor-IX, -XII (GSI-IX, GSI-XII) and Notch transcription factor inhibitor (SAHM1). The chemotherapeutic agents used were: cytarabine, etoposide and idarubicin. Cell viability was evaluated by Annexin-V/Propidium Iodide (PI) and MTT assays; proliferation and cell cycle were assessed through CFSE dilution and PI method, respectively.
Results: *In vitro* and *in silico* analysis showed that disregarding FAB classification or molecular pattern, AML cells expressed Notch receptors and ligands. Notably, 50% of AML samples showed basal Notch signalling activation as demonstrated by the detection, at mRNA and protein level, of HES-1. The activation of Notch signalling using recombinant Jagged-1 or Jagged-2 did not lead to any changes neither in AML cell survival nor in cell proliferation. On the contrary, the pan blockade of Notch, at the membrane level by GSIs or by the combination of Notch blocking antibodies, or, at the transcriptional level using SAHM1, inhibited AML cell proliferation in culture. Notably, BM-MSCs* showed higher level of Notch1 and Jagged1 as compared to BM-MSCs. Interestingly, we observed that BM-MSCs*, but not BM-MSCs, stimulated AML proliferation and the pan Notch inhibition was sufficient to revert this phenomenon. In addition BM-MSCs* were more efficient to rescue AML cells from apoptosis induced by chemotherapeutic agents. Pan Notch signalling blockage by either GSI-XII or combination of Notch receptor-blocking antibodies in presence of chemotherapeutic agents, significant lowered the supportive role of BM-MSCs towards AML blasts; conversely SAHM1 has a slight effect. The specific blockade of Notch-1, -2, -3 or Jagged-1, -2 reduced partially the chemoresistance, while blockade of Notch-4 or DLL-3 rescued totally the chemosensitivity of primary AML cells in co-culture with BM-MSCs.
Summary and Conclusions: These results suggest that the inhibition of Notch signalling may represent a potential therapeutic strategy to improve AML treatment, overcoming bone marrow stromal-mediated anti-apoptotic and chemoresistance effects. Clinical application of GSIs is limited due their toxic and multiple off targets. In this study we showed that blocking antibodies were sufficient to recapitulate GSIs effects, making them a useful and less harmful strategy to target Notch signalling.

P551

ARSENIC TRIOXIDE AND ALL-TRANS-RETINOIC ACID TARGET NPM1 MUTANT ONCOPROTEIN LEVELS AND INDUCE APOPTOSIS IN NPM1-MUTATED AML CELLS

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Background: Acute myeloid leukemia (AML) is the most common acute leukemia in adults and a molecularly heterogeneous disease. Standard chemotherapy±hemopoietic-stem-cell transplantation can cure about 40-50% of younger adults and 10-15% of elderly patients (>60 years old). Thus, it is clearly needed to develop novel therapeutic approaches. At present, the only highly effective molecular targeted therapy for AML is based on all-*trans*-retinoic acid (ATRA) and/or arsenic trioxide (ATO) in acute promyelocytic leukemia (APL) with *promyelocytic leukemia* (*PML*)-*retinoic acid receptor a* (*RARa*) gene rearrangement. *Nucleophosmin* (*NPM1)* gene mutations represent the most frequent genetic lesion in AML accounting for about 30% of adult AML. NPM1 is an ubiquitously expressed nucleolar phosphoprotein with nuclear-cytoplasmic shuttling activity, involved in multiple cellular functions and essential for life. *NPM1* mutations in AML are heterozygous and lead to NPM1 wild-type haploinsufficiency and cytoplasmic accumulation of the leukemic NPM1 mutant protein. Because *NPM1* mutations are common, stable and represent a founder genetic lesion in AML, they are an appealing therapeutic target. However, whether and how the NPM1 mutant oncoprotein is druggable remain to be established.
Aims: Here we explored whether NPM1 mutant protein levels could be affected by treatment with drugs such all-*trans*-retinoic acid (ATRA) or arsenic trioxide (ATO) which have been shown to target specific oncoproteins (*i.e.* PML-RAR-alpha, Bcr-Abl) and display some anti-leukemic activity *in vitro* and in patients in AML cells carrying *NPM1* gene mutations.
Methods: For these purposes, we treated *in vitro* either AML cell lines (OCI/AML3 and IMS-M2, carrying *NPM1* gene mutation A, and OCI/AML2 and U937, with wild-type *NPM1*) or primary AML cells from patients (25 *NPM1*-mutated and 19 *NPM1*-wild type AML) with ATRA, ATO or combination of either drug and analyzed their effects on cell growth and apoptosis and on levels of NPM1 either mutant or wild-type protein. To explore the underlying mechanisms, we used pretreatment with the proteasome inhibitor MG132, to analyze the role of the proteasome machinery in protein degradation, and with *N*-Acetyl-*L*-Cysteine (NAC), to analyze the role of reactive oxygen species (ROS) which are known to be elicited by treatment with ATO.
Results: We found that whilst ATRA exerts a more general anti-leukemic effect in non-APL AML cells including *NPM1*-mutated AML, ATO displays a peculiar anti-leukemic activity in *NPM1*-mutated as compared to *NPM1*-wild type AML, and that this activity can be potentiated by ATRA. Interestingly, we found basally a partially altered PML nuclear bodies (NBs) organization in *NPM1*-mutated AML, which can be restored by treatment with ATO. Strikingly, ATO, as well ATRA, induced proteasome-dependent NPM1 mutant oncoprotein degradation in either cell lines or primary AML patients cells, relocalized NPM1 wild type protein in the nucleus with rearrangements of nucleoli, and increased daunorubicin-sensitivity. All these events resulted to be dependent on ROS, being prevented by pretreatment with the ROS-scavenger NAC. Collectively, these findings are reminiscent of the ATRA/ATO-induced PML/RARα degradation in APL.
Summary and Conclusions: Altogether, our data show that NPM1 mutant is targeted by ATO and, at least in some cases, also by ATRA and, although probably not the only mechanism, its downregulation might contribute to the pro-apoptotic and/or anti-proliferative effects of these drugs in *NPM1*-mutated AML. This suggests that ATO and ATRA are potential drugs for a molecular targeted-based therapy in *NPM1*-mutated AML leading to NPM1 mutant oncoprotein degradation. These findings provide experimental evidences for further exploring ATO/ATRA in preclinical *NPM1*-mutated AML *in vivo* models and a rationale for exploiting these compounds in chemotherapic regimens in clinics.

P552

OVEREXPRESSION OF INTEGRIN A9 COOPERATES WITH THE LOSS OF RUNX1 AND RUNX3 IN LEUKEMOGENESIS

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Background: Chromosomal translocations associated with RUNX family transcription factors, such as t(8;21) and inv(16), are frequently observed in human acute myeloid leukemia (AML). These chromosomal abnormalities produce fusion proteins which suppress function and/or expression of both RUNX1 and RUNX3. We previously reported that *Runx1$^{fl/fl}$;Runx3$^{fl/fl}$;Mx1-Cre^{+}* [double knockout (DKO)] mice exhibited two paradoxical lethal phenotypes: bone marrow failure and myeloproliferative disorder. These contradictory manifestations were shown to be caused by defect in DNA repair pathway. Accumulation of non-repaired DNA damage leads to massive cell death, while occasional acquisition of oncogenic mutations result in leukemia development.
Aims: In this study, we aim to identify cooperative genetic hits which cooperate with *Runx1* and *Runx3* deficiency in leukemogenesis.
Methods: To identify such cooperative genetic alterations, retroviral insertional mutagenesis (RIM) was performed on *Runx1;Runx3* DKO mice. DKO and their control mice were injected with Moloney murine leukemic virus (MoMuLV) and monitored for disease presentation. Bone marrow transplantation and colony forming assay were conducted to validate the cooperativity. To correlate findings in mice to human AML, publicly available gene expression datasets were also analyzed.
Results: *Runx1;Runx3* DKO mice showed shorter disease latency in RIM as compared to the control mice. Although MoMuLV inoculation commonly induce T cell leukemia/lymphoma, majority of the *Runx1;Runx3* DKO mice developed biphenotypic leukemia with myeloid features. Retrovirus integrations into integrin α9 (*Itga9)* locus, which resulted in Itga9 overexpression, were exclusively found in *Runx1;Runx3* DKO mice. We confirmed that overexpression of Itga9 in *Runx1;Runx3* DKO cells increased the incidence of leukemia. In human AML, high expression of ITGA9 was found in AML cells with t(8;21) and inv(16) and associated with a poor prognosis.
Summary and Conclusions: We identified Itga9 as a cooperative hit with *Runx1* and *Runx3* deficiencies in leukemogenesis. This synergism was confirmed in human RUNX-related leukemias. Better understanding of the cooperating mechanism between abnormalities in ITGA9 and RUNX transcription factors will give further directions in developing therapies for human leukemias.

Acute myeloid leukemia - Biology 3

P553

EUROPEAN NETWORK NGS-PTL PRELIMINARY DATA: WHOLE EXOME SEQUENCING IDENTIFIES MUTATIONS OF ALDH2, RETSAT, HSPG2, CHPF, AND OTHER METABOLIC GENES AS A NOVEL FUNCTIONAL CATEGORY IN ACUTE MYELOID LEUKEMIA

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Background: Next Generation Sequencing studies identified 9 functional categories of mutations in acute myeloid leukemia (AML), with >99% of cases having at least one of those mutations (*Ley et al.* NEJM 2013). However, multiple genetic hits participate to AML pathogenesis, and metabolic dysregulations, as the one induced by *IDH1/2* mutations, play oncogenic functions (*Ward et al.* Cancer Cell 2010).
Aims: To define novel functional categories of AML mutations affecting relevant and druggable biological processes, with focus on genetic determinants of metabolic plasticity.
Methods: Out of 455 whole exome sequencing (WES) cases from onco-hematological patients collected in the NGS-PTL project, we analyzed 37 AML cases, belonging to our cohort of 239 *FLT3*-WT samples (886 AML total). We performed 100 bp paired-end WES (HiSeq2000, Illumina) and mapped the sequenced reads with Burrows-Wheeler Aligner. Variants where called with MuTect or GATK for single nucleotide variant (SNV) and indels detection, respectively (>90% confidence). Gene expression profiling (GEP) was performed using HTA2.0 microarray (Affymetrix) on 56 bone marrow samples, including AML (≥80% blasts) and healthy controls.
Results: We detected an average of 26 somatic variants per patient (range 7-65) by WES. Gene ontology annotation identified 8 novel relevant functional categories of mutated genes: transcription, translation and post-translational modifications, protein degradation, cytoskeleton, cell cycle, DNA damage, cell survival and metabolism. Since metabolic pathways are promising targets for tailored therapies (*e.g.* IDH1/2 and glutaminase inhibitors), we focused our analysis on them. We identified 82 variants (74 SNVs, 2 frameshift and 4 nonframeshift deletions, 2 stopgains) targeting 70 genes involved in metabolism, with 78% of patients carrying at least one mutation in a metabolic gene and 35 variants rated as damaging by CONDEL algorithm. Among mutations in metabolic genes, the most represented pathways were amino acids, lipids, CoA and nucleotides metabolism, transport and bioenergetics pathways. Notably, *IMPDH2*, a mediator of MYC-induced proliferation involved in nucleotide interconversion, was mutated and overexpressed in our AML cohort (*p=0.01*), suggesting a potential oncogenic function. Moreover, *ALDH2*, a regulator of hematopoietic stem cell functions which is involved in multiple metabolic pathways and associates with metabolic remodeling, was mutated and 2-fold downregulated in AML blasts. Seven genes were mutated in 5-8% of samples: *RETSAT, HSPG2*, *CHPF*, *ABCA2*, *ND1*, *APOBR*, *NAAA*. *RETSAT*, *HSPG2* and *CHPF* mutations were predicted as "drivers" by DOTS-Finder tool. Bioenergetic pathways were affected by mutations in glycolysis and gluconeogenesis (*GPI*, *ITPA*), oxidative phosphorylation (*ND1*, *ND4*, *ND5*, *CYTB*), pentose phosphate pathway (*H6PD*, *PGLS*).
Patients carrying mutations in the bioenergetic pathway showed a strong trend towards reduced overall survival, which did not associate with unfavorable molecular mutations.
Summary and Conclusions: Metabolism is the most represented class of mutated genes (8.6% of variants) in our *FLT3*-WT AML cohort after signaling, leading us to propose a novel functional category. Our data suggest that, along with mutations in established oncogenes and tumor suppressors involved in metabolic control (*KRAS*, *TP53,* MYC pathway), a number of genetic determinants participate to leukemia metabolic plasticity and oncogenic mutations of metabolic enzymes may drive leukemogenesis, impact on patients' survival and become novel targets for personalized therapies.
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GS and AP: equal contribution.

P554

THE PAN-CLASS I PI3 KINASE INHIBITOR, NVP-BKM120, DEMONSTRATES ANTI-LEUKEMIC ACTIVITY IN ACUTE MYELOID LEUKEMIA

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Background: Aberrant activation of PI3K/Akt/mTOR pathway is a common feature of acute myeloid leukemia (AML) patients contributing to chemoresistance, disease progression and unfavourable outcome. Therefore, inhibition of this pathway may represents a potential therapeutic approach in AML. NVP-BKM120 (BKM120) is a pan-class I PI3K inhibitor which exerts anti-proliferative and cytotoxic effects on several solid tumors and hematological malignancies by inhibiting PI3K/Akt/mTOR activity.
Aims: To evaluate the pre-clinical activity of BKM120 on AML cell lines and primary samples.
Methods: BKM120 (Novartis) was tested on AML cell lines (U937, NB-4, HL-60/Mx2, OCI-AML2, HL-60, OCI-AML3, THP-1, MOLM-13 and KG-1) and on 15 primary AML samples. Given the role of PI3K/Akt/mTOR in cell metabolism, BKM120 was then combined with dichloroacetate (DCA), a glycolitic modulator, on selected AML samples. Cell cycle and apoptosis were analyzed by Acridine-Orange and AnnexinV (AnnV)/PI staining, respectively. Signaling modulations and metabolic changes were evaluated by western blot and by the XF24 Flux analyzer, respectively. *In vivo* experiments were performed on non-obese diabetic severe combined immunodeficient interleukin-2 receptor g-null mice.
Results: Basal expression and phosphorylation levels of PI3K/Akt/mTOR pathway components were initially assessed on AML cell lines and primary samples. Despite some heterogeneity, all cell lines shared the constitutive activation of PI3K/Akt/mTOR axis. Moreover, 7 of the 9 primary samples tested showed a higher phospho/total Akt ratio than normal mononuclear cells (MNCs) and displayed p-mTOR (S2448 and S2481) and p-4EBP1 (T37/46) overexpression, suggesting the aberrant activation of PI3K/Akt/mTOR pathway. BKM120 exposure resulted in a dose-dependent dephosphorylation of Akt (S473) and Gsk3α/β (S21/9) in all cell lines tested and affected, at higher doses, mTOR activity inducing the dephosphorylation of mTOR (S2448 and S2481), p70S6K (S371) and 4EBP1 (T37/46). Blockade of PI3K/Akt/mTOR signaling inhibited cell growth (IC50s: 0.7-1.2μM) and induced a significant ($p<0.005$) dose- and time-dependent apoptosis in all cell lines. Cytotoxicity was preceded by a temporary G_2/M block that was rapidly followed by induction of apoptosis, as demonstrated by the increase of the subG_0/G_1 peak at 72h. Efficacy of BKM120 was then confirmed on primary samples: at 144h, AnnV$^+$ cells increased from 19.5±9.6% (vehicle) to 48.0±23.6% ($p<0.001$) at 5μM. Dephosphorylation of Akt (S473), achieved in 3/3 primary samples, supported the target inhibition. Conversely, BKM120 failed to show considerable cytotoxicity on normal and PHA-activated MNCs (8.0% and 4.4% apoptosis net increase at 5μM, respectively). Metabolic perturbations induced by BKM120 were then assessed on AML cells demonstrating a dose-dependent reduction of basal and maximal respiration as well as ATP production in both cell lines and primary samples. Furthermore, BKM120 strongly synergized (CI<0.6) with DCA to trigger apoptosis at lower doses on AML cell lines and primary samples. Finally, *in vivo* administration of BKM120 in a xenotransplant mouse model of AML markedly inhibited leukemia progression and induced a significant ($p<0.001$) improvement of overall survival.
Summary and Conclusions: Our data demonstrated that BKM120, as single agent or in combination with other drugs (*i.e.* glycolytic modulators), has a significant anti-leukemic activity towards AML cell lines and primary samples, thus supporting its clinical evaluation as a therapeutic agent for AML patients.

P555

ACUTE MYELOID LEUKEMIA DERIVED MACROPHAGE MIGRATION INHIBITORY FACTOR DRIVES INTERLEUKIN-8 PRO-SURVIVAL SIGNALS IN THE TUMOR MICROENVIRONMENT

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Background: Despite recent significant advances in the understanding of the biology of acute myeloid leukemia (AML) the clinical outcomes for the majority of AML patients, particularly the elderly, remain poor. There is increasing evidence that the AML microenvironment plays an important role in AML survival and proliferation. The bone marrow microenvironment is composed of multiple cell types that via direct cell-to-cell interactions and soluble factors promote the survival of leukemic cells and affect their response to therapy. *In vitro*, primary AML blasts rapidly undergo spontaneous apoptosis, however we and others have shown that the same primary AML cells survive when cultured on bone marrow derived mesenchymal stem cells (BM-MSC). Furthermore dis-

ruption of the interaction between AML cells and BM-MSC increases AML cell sensitivity to chemotherapy. These observations indicate that the pro-survival apoptotic defect in AML is not cell autonomous but highly dependent on extrinsic signals derived from the microenvironment.
Aims: Our aim is therefore to investigate the bidirectional interaction of AML cells and BM-MSC.
Methods: Primary AML blasts and primary AML BM-MSC were obtained from patient's bone marrow. Primary AML blasts $1x10^6$ were co-cultured on confluent primary BM-MSC. Conditioned medium was then collected from these cultures and analyzed using Proteome Profiler Human XL Cytokine Array and target specific ELISAs. Real-time PCR was also used to verify the array data.
Results: Using the XL Cytokine array we found that IL-8 and MIF increased significantly in the media from AML/BM-MSC co-cultures compared to media from either AML or BM-MSC alone. The observed changes in IL-8 and MIF were confirmed by ELISA assays. RT-PCR was used to measure MIF and IL-8 gene expression from RNA extracted from AML or BM-MSC cultured alone or in combination. Results indicate that MIF is upregulated in AML in response to BM-MSC co-culture and IL-8 was upregulated in BM-MSC in response to co-culture with AML. Next we used recombinant MIF and IL-8 as well as inhibitors of MIF (ISO-1) and IL-8R (SB225002) to define their specific role in BM-MSC/AML co-cultures. Recombinant human MIF increases IL-8 mRNA and protein expression in BM-MSC but not in AML. Moreover, the MIF inhibitor, ISO-1, inhibits AML induced IL-8 expression and secretion by BM-MSC. Finally, we report that IL-8 protects primary AML from spontaneous apoptosis *in vitro* which is reversed by the IL-8R inhibitor, SB225002.
Summary and Conclusions: BM-MSC respond to signals from AML cells which results in changes in the expression of pro-survival cytokines. Hence, BM-MSC and AML cells act together in the formation of the pro-tumoral microenvironment. MIF/IL-8 regulated BM-MSC/AML crosstalk may represent a novel target for future AML therapies.

P556

XPO1 INHIBITION USING SELINEXOR SYNERGIZES WITH CHEMOTHERAPY IN ACUTE MYELOID LEUKEMIA (AML) BY TARGETING DNA REPAIR GENES

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Background: Targeting nuclear exporter receptors such as Exportin 1 (XPO1) is a novel approach to restore tumor suppressor function in AML. Selinexor, a selective inhibitor of XPO1, is currently being tested in a Phase 1 clinical trial in AML. Preliminary data indicate that selinexor is well tolerated, safe and active in AML patients. However, considering the molecular complexity of AML, it is unlikely that this disease can be cured with monotherapy and therefore we asked whether adding already established effective drugs such as Topo IIα inhibitors to selinexor will enhance or improve its anti-leukemic effects in AML.
Aims: To investigate the anti-leukemic activity and mechanisms of selinexor in combination with Topo IIα inhibitors in AML cell lines and patients blasts.
Methods: IC_{50} values for selinexor and Topo IIα inhibitors were determined using MTS assays in AML cells lines and patients. Synergy was calculated using the Chou-Talalay method.
Results: To evaluate whether the combination of selinexor and Topo IIα inhibitors (idarubicin, etoposide and mitoxantrone) induce synergy, we treated the AML cell lines MOLM-13 and MV4-11 with selinexor and each of the Topo IIα inhibitors separately at two-fold dilutions of their individual IC_{50} values, and measured cell proliferation at 48 hours. Concomitant treatment of selinexor with Topo IIα inhibitors resulted in synergy (combination index (CI) values <1) in all cell lines. Treatment of AML patients blasts (n=3) with selinexor and idarubicin at 4-fold dilutions of their individual IC_{50} values showed synergy as well. High-throughput studies on protein expression in tumor cells after selinexor treatment indicate that several DNA damage repair proteins are down-regulated. Based on these data, we reasoned that the synergistic effects of selinexor with Topo IIα inhibitors could be explained in part by selinexor induced down-regulation of DNA repair proteins thus preventing leukemia cells from repairing chemotherapy-induced DNA damage. A dose dependent reduction in mRNA (RT-PCR) and protein levels (WB) of the DNA damage repair genes *Chk1, Rad51, MSH2* and *MSH6* were observed as early as 6 hours after selinexor treatment and before apoptosis was observed (50 to 200 nM). *Chk1 and Rad5* mRNA and protein levels decreased the most (2 and >10 fold decrease respectively) and was caused by decrease MYC binding to these genes promoters which resulted in reduced transcriptional activation. Similar depletion of these DNA repair proteins was also observed in 4 AML patient samples incubated with selinexor at IC_{50} concentrations. Topo IIα inhibitors cause double stranded DNA breaks which can be repaired by the homologous repair (HR) pathway. HR is governed by multiple proteins that include Rad51. To measure the ability of cells to carry out HR in the presence of selinexor, we used the HeLa DR cells with an HR integrated plasmid. These cells carry two copies of inactive Green Fluorescent Protein (GFP) genes integrated into the genome. The cells are treated with an enzyme ICSE1 that cuts at 1 site within the GFP. If HR occurs then there is repair of the double strand break and GFP events are observed. Using this assay, when Hela DR cells are treated with selinexor, we observed a 7 fold decrease in the number of GFP expression compared to control cells ($P<0.05$). Animal studies are currently undergoing.
Summary and Conclusions: Our data showed that selinexor synergizes with anthracyclines to kill AML blasts by targeting DNA repair. Therefore, we propose to combine selinexor with Topo IIα inhibitors in a Phase 1 clinical trial.

P557

MAPPING WHOLE-EXOME SEQUENCING DATA OF ACUTE MYELOID LEUKEMIA PATIENTS INTO KEGG PATHWAYS

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Background: Acute Myeloid Leukemia (AML) is a highly heterogeneous disease and a complex network of events contribute to the pathogenesis. Recently, functional categorization of mutated genes in AML identified 9 classes of affected genes. However, how genomic alterations cooperate to induce AML, the pathways affected by the mutated genes and their prognostic value are still unknown.
Aims: To better stratify AML patients and identify novel molecular biomarkers based on the mapping of genomic alterations in biological pathways and correlation with clinical outcome.
Methods: We sequenced the exome of 33 cases of AML at diagnosis using the Illumina Hiseq2000 platform. We usedthe GATK and MuTect tools to call mutations and we selected variants with a minor allele frequency (MAF) lower than 0.05. We mapped variants in biological pathways using KEGG database and we identified putative driver genes with DOTS-finder and MutSig.
Results: A validation rate equal to 89% (26 validated variants out of 29) was obtained by Sanger sequencing analysis of variants. Our cohort included 16 patients belonging to the intermediate risk cytogenetic prognostic group and 17 patients belonging to the high-risk one (*p=0.002*). For each patient, we mapped the mutated genes into KEGG pathways and we performed unsupervised clustering analysis based on the represented KEGG pathways. We identified 4 groups of patients characterized by significant differences in the number of mutated pathways (*p=0.032*), with an average of 74 (group 1), 58 (group 2), 28 (group 3) and 9 (group 4) mutated pathways per patient. We identified the PI3K-Akt signalling as the top mutated pathway (23 out of 33 patients). Notably, the frequency of patients with mutations in that pathway differed among groups: 100% of patients in group 1 and 2, *versus* 62% and 20% of patients in group 3 and 4, respectively. Moreover, we were able to identify a common mutated gene in group 1, *KRAS,* which was defined a putative driver gene by both DOTS-finder and MutSig tools. On the other hand, 6 out of 8 patients of group 2 had *TP53* mutations . Group 3 and 4 showed a heterogeneous distribution of mutated pathways: the top mutated pathways in group 3 were the PI3K-Akt signalling and the Metabolic pathways, with 6 patients with mutations out of 8; whereas the top mutated pathway in group 4 counted 5 out of 10 patients with mutations in the Metabolic pathway. Klapan-Meier survival curves showed a significant correlation of the 4 groups with clinical outcome (*p=0.047*), with group 2 having an extremely unfavourable prognosis, as expected from *TP53* mutated patients. Subclassification of group 3 and 4 patients according to molecular characterization and correlation with clinical outcome are ongoing.
Summary and Conclusions: In summary, by mapping WES data into KEGG pathways, we were able to distinguish 4 groups of patients. Two of them were characterized by distinct molecular biomarkers able to stratify patients with different prognosis. *KRAS* was identified as putative driver gene in AML by two different tools, underling its importance in the disease pathogenesis. Interestingly, our analysis defined a potential association between two patients lacking *TP53* mutations and *TP53*-mutated cases (in group 2), in terms of KEGG pathway clusterization, which could be defined only by omics analysis. Our results confirm the different prognostic risks of AML patients with *KRAS* or *TP53* mutations and highlight the relevance of WES data interpretation according to mutated pathways for patient stratification at diagnosis.
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AP and GS: equal contribution.

P558

PBX3 AND MEIS1 COOPERATE THROUGH MULTIPLE MECHANISMS TO SUPPORT HOX-INDUCED MURINE LEUKEMIA

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Background: Hox homeobox transcription factors drive leukemogenesis efficiently only in the presence of Meis or Pbx proteins.
Aims: In this study we investigated the importance of a cooperation of Meis1 and Pbx3 for Hoxa9 mediated transformation.
Methods: Protein-protein interactions, protein-stability and half-life as well as DNA binding capability and transformation efficiency were tested *in vitro* and *in vivo*.
Results: A biochemical analysis of the Meis1-Pbx3 cooperation revealed that in the absence of Pbx3, Meis1 stability was greatly reduced. As shown by a deletion analysis Meis1 degradation was contingent on a motif coinciding with the Pbx-binding domain. Either deletion of this sequence or binding to Pbx3 prolonged Meis1 survival by preventing poly-ubiquitination and proteasome mediated decay. Meis1 break-down could be also blocked directly by inhibition of the ubiquitin proteasome system indicating a tight posttranscriptional control. In addition, Meis1 and Pbx3 cooperated genetically as overexpression of Pbx3 induced endogenous Meis1 transcription. These functional interactions translated to *in vivo* activity. Blocking Meis1/Pbx3 dimerization abrogated the ability to enhance proliferation and colony forming cell numbers in primary cells transformed by Hoxa9. Furthermore, expression of Meis1 target genes *Flt3* and *Trib2* was also dependent on Pbx3/Meis1 dimerization. This correlated with the requirement of Meis1 to bind Pbx3 in order to form high affinity DNA/Hoxa9/Meis1/Pbx3 complexes *in vitro*. Finally, kinetics and severity of disease in transplantation assays indicated Pbx3/Meis1 dimers as rate-limiting factors for Hoxa9 induced leukemia.
Summary and Conclusions: Here we show that Pbx3 and Meis1 form stable dimers and we present evidence that this dimers are the actual functional units whose formation is rate limiting for efficient leukemia induction by Hoxa9.

P559

THE MODULAR NETWORK STRUCTURE OF THE MUTATIONAL LANDSCAPE OF ACUTE MYELOID LEUKEMIA

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Background: The development of acute myeloid leukemia (AML) is associated with accumulation of acquired genetic alterations in hematopoietic progenitor cells. The study performed by the TCGA consortium has enabled for the discovery of recurrent mutations in 23 genes in AML. However, many patients with normal karyotype carry no mutations in any of the currently recognized driver genes associated with leukemogenesis, suggesting that AML has relevant changes not yet defined.
Aims: We performed whole-exome sequencing (WES) of tumor-normal matched samples to identify new driver mutations in AML patients with normal karyotype lacking *NPM1, CEBPA* and *FLT3*-ITD mutations. Novel candidate genes, together with other previously described,[1] were resequenced in an independent cohort of intermediate-risk AML patients. . The obtained results were further *in silico* analysed.
Methods: WES was performed on matched samples from 7 *de novo* AML patients, lacking cytogenetic alterations, *NPM1, CEBPA* and *FLT3*-ITD mutations (discovery cohort). Library preparation and exome capture were performed according to the manufacturer protocol for sequencing with an Illumina platform. WES data were analyzed using and in-house bioinformatic pipeline. Candidate variants were validated using an Ion AmpliSeq™ Custom Panel in an Ion Torrent platform (Life-Technologies). Furthermore, a custom panel of 87 genes (55 genes from in-house results and 32 genes reported to be mutated in ≥2% of patients in the TCGA study[1]) was analysed in an independent validation cohort of 100 patients with intermediate-risk AML using SureDesign Tool (Agilent). To prioritize the best candidate genes we studied the significance of the network in which they participate. Samples were provided by the Hospital La Fe Biobank.
Results: In the discovery cohort, we identified 94 SN*Vs* non-synonymous coding mutations and 8 small indels, with an average of 30 mutations per sample (range 22-37). We confirmed 60% of the candidate variants affecting 56 genes. Among them, 15 genes had been shown previously to be recurrent. Over the validation cohort we detected a mean of 6.81 mutations per sample (range 1-17). We identified a total of 193 variants affecting 65 different genes, been 41 mutated in more than one patient. After prioritization we found 9 individual genes with a a higher frequency in our cohort than expected in the 1000g database and with a strong potential of being involved in AML pathogenesis ($P \leq 0.01$). To improve our knowledge of the interactome of AML, these genes were also analyzed for functional enrichment of gene ontology terms and grouped according to their biological function. As a result, we defined a network in which 27 out of the 41 genes selected by harboring recurrent deleterious mutations were significantly more connected that the random expectation ($P < 0.05$).
Summary and Conclusions: This study shows a comprehensive analysis of AML combining WES with a custom panel of targeted genes by NGS. We identified recurrent deleterious mutations in 9 genes with a strong potential to be involved in AML pathogenesis and have defined a functional module in the interactome of intermediate-risk AML patients.

P560

INVESTIGATING THE ROLE OF BONE MARROW ADIPOCYTES IN REGULATING SURVIVAL AND PROLIFERATION OF ACUTE MYELOID LEUKEMIA

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Background: Most patients diagnosed with acute myeloid leukemia (AML) die of the disease despite currently available chemotherapy. The bone marrow (BM) microenvironment is a key player supporting AML blast survival. The BM microenvironment consists of many cell types not directly of the hematopoietic lineage, including bone marrow adipose tissue (MAT). MAT accounts for approximately 50% of BM volume in the axial skeleton of adult humans. Adipocytes store energy primarily as triglyceride which can be released following lipolysis as free fatty acid (FFA) and are taken up by cells for conversion into energy. How this energy source is utilised locally in the bone marrow by AML has not been well characterised to date.
Aims: The aim of this study is to determine the role of human bone marrow adipocytes in the support of AML blast survival *in vitro* and their protection against chemotherapy-induced apoptosis.
Methods: Primary AML blasts and primary AML BM mesenchymal stem cells (MSC) were obtained from patients' bone marrow. BM derived MSC were differentiated *in vitro* into adipocytes (MSC-Ad) and co-cultured with primary AML blasts. BrdU incorporation, annexin V and PI staining were used to determine proliferation and apoptosis of AML blasts. Lipolysis was measured by determining free fatty acid and glycerol release. Dodeconic acid fluorescent fatty acid analog (DFFAA) was used to measure uptake of FFA by AML blasts. Western blot analysis was used to determine the level of proteins associated with triglyceride breakdown; hormone sensitive lipase (HSL), phosphorylated HSL and fatty acid binding protein 4 (FABP4). Cytarabine and daunorubicin were used to determine the chemotherapy-induced apoptosis.
Results: Our results show AML blasts co-cultured with MSC-Ad have increased proliferation and reduced apoptosis by day 6 compared to AML blasts in monoculture. Cell cycle analysis shows a higher percentage of AML blasts from MSC-Ad co-culture in the M and G2 phase compared with monoculture. Furthermore we found that MSC-Ad had enhanced lipolysis in response to AML co-culture. We observed increase in free fatty acid and glycerol release in AML/MSC-Ad co-culture media compared to media from MSC-Ad alone. The uptake of FFA was then examined in AML blast co-culture with MSC-Ad. Flow cytometry analysis shows an increase in fluorescence (DFFAA) within AML blasts when co-cultured with MSC-Ad loaded with DFFAA. In addition, Western blot analysis of HSL, an enzyme that hydrolyzes triglycerides of the lipids, shows an increase in phosphorylation. FABP4 which is a lipid transport protein is also increased in MSC-Ad when cultured with AML blasts. Finally, chemotherapy induced apoptosis was significantly reduced in AML/MSC-Ad co-culture compared to AML blasts on their own.
Summary and Conclusions: Here we report that MSC-Ad upregulate lipolysis in response to co-culture with AML blasts. Furthermore MSC-Ad support the survival and proliferation of AML blasts and provide a protective microenvironment against standard cytotoxic chemotherapy. We hypothesize that pro-survival metabolic activity in AML blasts is supported by AML directed transfer of free fatty acid from adipocytes in the bone marrow microenvironment and that targeting this interaction may provide the foundation of novel micro-environment directed treatment strategies for AML in the future.

P561

COMPARATIVE FUNCTIONAL ANALYSIS OF THE MOLECULAR NETWORK OF 7 SELECTED MLL FUSION PROTEINS

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Background: The Mixed Lineage Leukemia gene (MLL) is a frequent target of

chromosomal rearrangements in human malignancies. Many MLL-rearrangements are associated with pediatric leukemias with poor clinical outcomes. Balanced translocations result in the fusion of the MLL gene to over 65 different fusion partner genes, leading to the production of novel chimeric proteins. Critical effectors of distinct MLL fusion proteins have previously been identified, and some of them were shown to hold great potential for targeted therapies. However, it is not clear if these effectors are conserved among all MLL fusion proteins or if different molecular mechanisms of transformation exist for distinct MLL fusion proteins.
Aims: We aim to delineate common critical effectors of 7 selected MLL fusion proteins that are presumed to differ in their oncogenic mechanisms (MLL-AF1p, MLL-AF4, MLL-AF9, MLL-CBP, MLL-EEN, MLL-ENL, MLL-GAS7).
Methods: Stable cell lines allowing for inducible expression of 7 selected, affinity-tagged MLL fusion proteins were prepared and expression of transgenes was verified by qRT-PCR and Western Blotting. Affinity purification coupled to mass spectrometry (AP-MS) identified novel interactors of 7 MLL fusions protein complexes. To assign functional information to the candidate genes we employed a pooled RNAi screening approach. The MLL-AF9-positive MOLM-13 cell line was transduced with pools of viral vectors allowing for the expression of 6 shRNA targeting the same candidate gene. Using this setup we have established a screening methodology that is suitable for positive and negative selection readouts. This dual read-out highly increases the significance of true hits and strongly decreases the false positive rate.
Results: Advanced statistical filtering using a novel, improved algorithm developed by us yielded a densely connected protein-protein interaction network of >950 proteins around 7 MLL fusions, including many known MLL-interactors. 128 proteins were found to interact with ≥5 of the 7 MLL-fusions. This list of conserved MLL-interactors is highly enriched for proteins with a function in chromatin metabolism and transcriptional control. RNAi screening performed in a 'mini-pool' format was used to functionally dissect the conserved MLL-fusion interactome. As a result, 35 novel interactors of MLL fusion proteins were selected for further validation experiments.
Summary and Conclusions: In conclusion, we developed a robust experimental pipeline allowing for the functional characterization of cellular effects of MLL fusion proteins in a comprehensive and comparative manner, which will contribute to further insight into the molecular mechanisms of MLL-fusion dependent leukemogenesis.

P562

RETINOIC ACID AND ARSENIC TRIOXIDE TRIGGER DEGRADATION OF MUTATED NPM-1 RESULTING IN APOPTOSIS OF AML CELLS

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Background: *Nucleophosmin-1 (NPM-1)* is the most frequently mutated gene in acute myeloid leukemia (AML). Addition of retinoic acid (RA) to chemotherapy was proposed to improve survival of some of these patients.
Aims: Investigate the efficacy and molecular mechanism of action on arsenic and retinoic acid (RA) combination on *NPM-1* mutated AML
Methods: AML derived cell lines and freshly isolated blasts from AML patients were treated *in vitro* with Retinoic acid, arsenic trioxide and their combination. Five elderly AML patients with normal karyotype, *NPM-1* mutation, without *FLT-3 ITD*, who were judged unfit to chemotherapy, received RA/arsenic on a compassionate basis.
Results: We found that in AML cell lines or primary samples, RA or arsenic trioxide synergistically induce proteasomal degradation of mutant NPM-1, leading to differentiation and apoptosis. *NPM-1* mutation not only delocalizes NPM-1 from the nucleolus, but also disorganizes promyelocytic leukemia (PML) nuclear bodies. Combined RA/arsenic treatment significantly reduced bone marrow blasts in three patients and restored the sub-nuclear localization of both NPM-1 and PML.
Summary and Conclusions: These findings could explain the proposed benefit of adding RA to chemotherapy in *NPM-1* mutant AMLs, and warrant a broader clinical evaluation of regimen comprising a RA/arsenic combination.

Acute myeloid leukemia - Clinical 3

P563

CLINICAL SAFETY AND ACTIVITY OF AG-120, A FIRST-IN-CLASS, POTENT INHIBITOR OF THE IDH1 MUTANT PROTEIN, IN A PHASE 1 STUDY OF PATIENTS WITH ADVANCED IDH1-MUTANT HEMATOLOGIC MALIGNANCIES

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Background: Somatic mutations in the metabolic enzymes isocitrate dehydrogenase (IDH) 1 and 2 occur in a spectrum of solid and hematologic malignancies. Mutant IDH1/2 in cancer cells results in the neomorphic production of the oncometabolite, D-2-hydroxyglutarate (2-HG), which impairs cellular differentiation via an epigenetic mechanism. AG-120 is a first-in-class, oral, potent, reversible and selective inhibitor of mutated IDH1 protein. In IDH1-mutant primary human blast cells cultured *ex vivo*, AG-120 lowered 2-HG and restored cellular differentiation. We report preliminary results from the ongoing, first-in-human, phase 1, open-label, single-arm study of AG-120 (NCT02074839).
Aims: Primary objectives are to evaluate the safety, tolerability, and maximum tolerated dose (MTD), pharmacokinetics (PK), pharmacodynamics (PD), and clinical activity.
Methods: Patients with advanced, IDH1-mutant, hematological malignancies including relapsed or refractory (RR) acute myeloid leukemia (AML), elderly untreated AML who are not candidates for standard therapy, and recurrent or refractory myelodysplastic syndromes, are eligible to receive continuous, single-agent, oral AG-120 once (QD) or twice (BID) daily in 28-day cycles. Informed consent is obtained prior to entry. Sequential cohorts are being enrolled, with expansion cohorts planned. Blood and bone marrow samples are collected for PK/PD assessment. Response is assessed on Days 15, 29, and 57, and every 56 days thereafter. Objective responses are investigator assessed using International Working Group criteria.

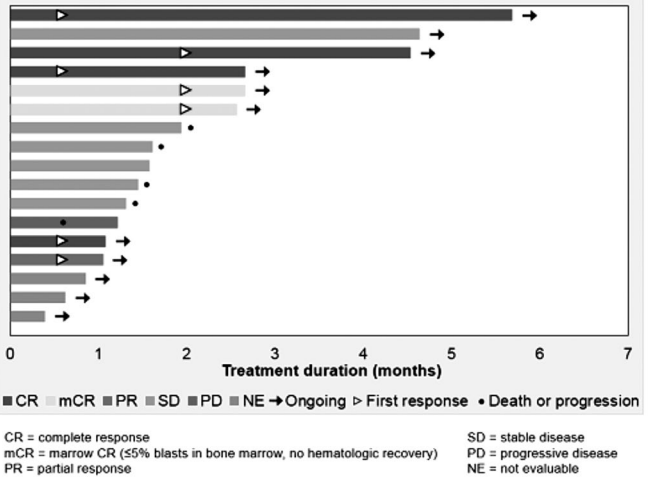

Figure 1. Duration of AG-120 treatment and best overall response.

Results: As of 17 October 2014, 17 patients had received AG-120 and 11 remained on study. All had RR AML (8 men/9 women), median age 73 years (range, 42-87) and median number of prior chemotherapy regimens 2 (range, 1-5). Doses administered were 100 mg BID (n=4), 300 mg QD (n=4), 500 mg QD (n=4), and 800 mg QD (n=5). Median treatment duration at data cut-off was 1.6 months (range, 0.4-5.7). Therapy has been well tolerated and the MTD has not yet been reached. Fourteen patients experienced adverse events (AEs), the most common (>20% of patients) were nausea, fatigue, dyspnea, vomiting, pyrexia, and cough. Serious AEs occurred in 8 patients: 1 differentiation syndrome, 1 tongue edema and grade 3 QT prolongation (dose-limiting toxicity; 800 mg QD), and 6 non-drug-related deaths, 5 of which occurred after discontinuation of AG-120 due to progressive disease and 1 due to disease-related intracranial hemorrhage while on treatment. Preliminary PK analyses showed high plasma expo-

sure and drug accumulation following oral administration and a mean half-life of >32 hours. PD analyses showed up to 98% inhibition of plasma 2-HG at all dose levels. Objective responses were observed in 7/14 efficacy-evaluable subjects (4 CR, 2 marrow CR, 1 PR), with durable responses of up to 5.7 months, and all responders remain on treatment (Figure 1). Six patients had stable disease and 1 had disease progression. Consistent with preclinical data, responses were associated with differentiation of myeloblasts into mature cell types. Dose escalation continues and updated safety, efficacy, and PK/PD data will be presented. As of 20 February 2015, 41 patients have been treated in 5 dose escalation cohorts from 100 mg BID to 1200 mg QD.

Summary and Conclusions: AG-120, a first-in-class, oral, potent, selective inhibitor of mutant IDH1, has been well tolerated to date. Durable single-agent responses of up to 5+ months have been observed. IDH1 inhibition resulted in reduced 2-HG and profound cell differentiation effects in some patients. Updated data from this ongoing study will be presented.

P564

TOSEDOSTAT PLUS LOW DOSE CYTARABINE COMBO INDUCES A HIGH RATE OF RESPONSES THAT CAN BE PREDICTED BY GENETIC PROFILING IN AML: FINAL RESULTS OF A PHASE II MULTICENTER STUDY

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Background: Elderly patients with acute myeloid leukemia (AML) have an extremely poor outcome. In addition, intensive induction chemotherapy is often unsuitable. Tosedostat is a new, orally bioavailable inhibitor of members of the M1 and M17 classes of aminopeptidases that includes the zinc-dependent aminopeptidases. Tosedostat was proven to be effective as single agent, with a manageable safety profile, in both de novo and relapsed AML.

Aims: We assumed that the addition of tosedostat to low-dose cytarabine may improve the response rate and remission duration over what is expected with chemotherapy or tosedostat alone.

Methods: This was a phase II, prospective, multicenter study, designed according to Fleming's method. Fixing the lowest acceptable rate as 10% and the successful rate as 25%, with a significance level a=0.05 and a power 1-b=0.80, the sample size was estimated in 33 patients. Thirty-three patients (median age 75 years) received Tosedostat 120 milligrams orally once daily from day 1 to day 240, coupled with intermittent low-dose cytarabine given subcutaneously at 20 milligrams twice/day from day 1 to day 10 of each cycle. Courses of cytarabine were repeated every 4 weeks in the absence of disease progression or unacceptable toxicity, up to 8 cycles. To identify possible biomarkers associated to sensitivity/resistance, global gene expression profiling (GEP, Affymetrix Transciptome Array 2.0) was performed on purified AML cells obtained from 29 patients.

Results: Median white blood cell count at diagnosis was $3.05x10^9$/l (range: $0.26\text{-}24.53x10^9$/l). Seventeen out of 33 patients had an intermediate karyotype, 13/33 an unfavorable karyotype and 3/33 were not evaluable. Sixteen patients had a *de novo* AML, whereas 17 had a secondary AML. Induction-period mortality was 12%, with 4 deaths occurring in aplasia. According to intent-to-treat, the CR rate was 45.4% (15/33 patients). Interestingly, 3/33 additional patients obtained a partial response, translating in an impressive overall response rate of 54.4%. In addition, 4/33 patients remained in stable disease for a median time of 9 months (range: 4-14). Seven patients did not respond at all and died with progressive disease after having received a median of 2 cycles of cytarabine and 45 days of tosedostat. In responding patients, the median time for the achievement of best response was 74 days (range 22-145). Statistical analysis showed that responding patients (CR+PR) had a longer median overall survival than non-responders (P=0.018). Ten out of 18 (55%) responding patients are still in remission (8CR, 2 PR) after a median follow-up of 319 days (range: 47-548); 2 patients are still alive with stable disease. Twenty-one patients died [while in aplasia (4), due to resistant disease (7) or due to progressive disease (8)] after a median CR duration of 5 months (2-17). As far as GEP analysis was concerned, we studied 29 cases and identified a molecular signature associated with the clinical response (CR *vs* no CR). Based on that, an algorithm to predict the clinical response was developed and validated.

Summary and Conclusions: The tosedostat and low-dose cytarabine combo produced a CR rate superior to what expected (45.4% *versus* 25%), and thus met the primary endpoint of study. Moreover, the achievement of CR could be efficiently predicted by the gene expression pattern. Further, potential biomarkers were identified by GEP. The study was registered at European Union Drug Regulating Authorities Clinical Trials (EudraCT) n.2012-000334-19.

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P565

LOW-DOSE LENALIDOMIDE AND CYTARABINE COMBO PRODUCES A HIGH COMPLETE REMISSION RATE THAT CAN BE PREDICTED BY GENETIC PROFILING IN AML PATIENTS AGED ≥70 YEARS: FINAL RESULTS OF A PHASE II STUDY

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Background: Outcome for patients aged ≥70 years with acute myeloid leukemia (AML) is extremely poor. Intensive induction chemotherapy is frequently unsuitable. Thus, novel therapies are needed.

Aims: We designed a prospective phase II study to assess the efficacy of the low-dose lenalidomide and cytarabine combo in patients with AML aged ≥70 years.

Methods: The study was designed according to the MiniMax design. The primary outcome was the complete remission rate (CR+CRi) according to the SWOG criteria. Fixing the lowest acceptable rate (P0) as 17% and the successful rate (P1) as 30%, with a significance level alpha=0.05 and a power 1-beta=0.90, the sample size was estimated to be 66 patients. Sixty-six patients (median age 76 years) were enrolled in the study. Median white blood cell count at diagnosis was $3.9x10^9$/l (range: $0{,}45\text{-}49{,}7x10^9$/l). Thirty-six out of 66 patients had an intermediate karyotype, 25/66 an unfavorable karyotype and 5/66 were not evaluable. Twenty-eight patients had a *de novo*, whereas 38 patients had a secondary AML. Patients received low-dose lenalidomide (10 mg/day orally, days 1-21) and low-dose cytarabine (20 mg/m^2 twice daily subcutaneously, days 1-15). Therapy was repeated every 6 weeks, up to 6 cycles. To identify possible biomarkers associated to sensitivity/resistance, global gene and miRNA expression profiling (Affymetrix Transciptome Array 2.0) was performed on purified AML cells obtained from 26 patients.

Results: After the first cycle of therapy, 8/66 patients died in documented aplasia. One patient died during cycle 1 (acute heart failure). The cumulative induction-period mortality was 13%. Three patients are too early. According to intent-to-treat, the CR rate was 36.3% (24/66 patients), the PR rate was 3% (2/66) with an overall response rate (ORR) of 39.3%. Four patients died in CR, one in PR, whereas 6/26 responding patients are still in CR after a median follow-up 358,5 days (range: 89-1787). The remaining 15 patients who achieved a CR/PR relapsed after a median time of 12 months and died due to progressive disease. Statistical analysis showed that responding patients had a longer median overall survival than non-responders (P <0.0001). Interestingly, cytogenetic risk and bone marrow blats at diagnosis were not predictive of CR. Conversely, by studying the miRNA and gene expression profile in 26 patients, we identified a molecular signature, including 306 genes and 3 miRNA associated with the clinical response (CR *vs* no CR). Noteworthy, the involved genes belonged to relevant functional categories such as NF-kB cascade, protein kinase signaling and immune response. Based on the expression of such genes/miRNA, treatment response could be predicted with high accuracy (88.5%, 23/26 samples correctly classified), with 87% sensitivity, 91% specificity, and 93% positive predictive value for responders. Moreover, we developed an algorithm to predict response based on the expression of 16 genes only, that correctly identified 14/14 CR patients.

Summary and Conclusions: The low-dose lenalidomide and cytarabine combo produced a CR rate superior to what expected when designing the trial (36.3% *versus* 30%), and thus met the primary endpoint of study. Moreover, the achievement of CR could be efficiently predicted by the gene expression pattern. The combo warrants further investigation in a randomized, phase III trial. The study was registered at EMA with the EUDRACT no 2008-006790-33.

Acknowledgements: Celgene is acknowledged for providing Lenalidomide for the patients.
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P566

OVERALL SURVIVAL (OS) AND CLINICAL OUTCOMES IN OLDER PATIENTS WITH ACUTE MYELOID LEUKEMIA (AML) TREATED WITH AZACITIDINE (AZA) OR INTENSIVE CHEMOTHERAPY (IC) IN THE AZA-AML-001 STUDY

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Background: There is no universally accepted approach to AML treatment (Tx) for older pts. IC is recommended for pts aged ≥60 years with favorable prognostic features (NCCN guidelines, 2014). However, many older pts can not tolerate IC. Such pts often receive low-dose cytarabine (LDAC), which is associated with a median OS of only ~5 months (Burnett, *Cancer*, 2007; Kantarjian, *JCO*, 2012). There is an unmet need for tolerable Tx options that prolong OS to a similar or greater extent than IC. The phase 3, randomized AZA-AML-001 study compared AZA with conventional care regimens (CCR) in older pts with AML. Before randomization, pts were preselected to receive 1 of 3 CCR per investigator choice of preferred Tx option: IC, LDAC, or best supportive care only. Pts were then randomized to receive AZA or CCR and received their preselected Tx.
Aims: To compare OS and clinical outcomes with AZA *vs* IC in the subgroup of pts in AZA-AML-001 preselected to receive IC before randomization.
Methods: Pts aged ≥65 years with newly diagnosed *de novo* or secondary AML (>30% bone marrow [BM] blasts) and ECOG PS 0-2, WBC ≤15x10^9/L, and intermediate- or poor-risk cytogenetics were enrolled. Pts received IC (cytarabine IV x7days (d)+an anthracycline IV x3d, with ≤2 subsequent cycles) or AZA (75 mg/m^2/d SC x7d/28d cycle). OS and 1-year survival were estimated using Kaplan-Meier methods. OS was compared between Tx groups by log-rank test. Hazard ratios (HRs) and 95% CI are from an unstratified Cox proportional hazards model. Rates and durations of complete remission (CR) and CR with incomplete blood count recovery (CRi) (IWG 2003), and RBC transfusion independence (TI) in pts transfusion-dependent at baseline, were assessed. Rates of grade 3-4 treatment-emergent adverse events (TEAEs), defined as new or worsening AEs during Tx, were assessed. To account for differences in Tx exposure, TEAE incidence rates (IR) per 100 pt-years of Tx exposure are reported.
Results: Of all pts in AZA-AML-001 (N=488), 87 (18%) were preselected to receive IC (AZA n=43, IC n=44). Median number of Tx cycles of AZA was 8 (range 1-24) and of IC was 2 (1-3). At baseline in the AZA and IC groups, median ages were 71 yrs (AZA range 65-79, CCR 65-81); 16% and 18% of pts, respectively, had ECOG PS of 2; median BM blasts were 72% (7-100%) and 70% (6-100%); median WBC count was 3.8x10^9/L (1-15) and 2.2x10^9/L (1-90); and 35% and 34% of pts had poor-risk cytogenetics. Median OS was comparable in the AZA and IC groups: 13.3 *vs* 12.2 months, respectively (HR=0.85 [95% CI 0.52, 1.38], p=0.5032) (Figure 1). One-year survival with AZA was 55.8% (95% CI 39.8%, 69.1%) *vs* 50.9% (35.2%, 64.6%) with IC (Δ4.9%; 95% CI -16.2%, 26.0%). In the AZA and IC groups, respectively, 30% and 36% of pts attained CR, and 12% and 11% achieved CRi. Median durations of CR+CRi in the AZA and IC groups were 17.3 (95% CI 3.7, not reached) and 19.8 (8.2, 26.3) months, respectively. RBC TI rates with AZA *vs* IC were 57% *vs* 35%, respectively. Proportions of pts with grade 3-4 TEAEs and [IRs] in the AZA and IC groups, respectively, were: anemia 12% *vs* 14% [14 *vs* 45]; neutropenia 30% *vs* 33% [37 *vs* 99]; febrile neutropenia 33% *vs* 31% [40 *vs* 92]; thrombocytopenia 23% *vs* 21% [29 *vs* 64]; and (any) infections 49% *vs* 50% [60 *vs* 149].

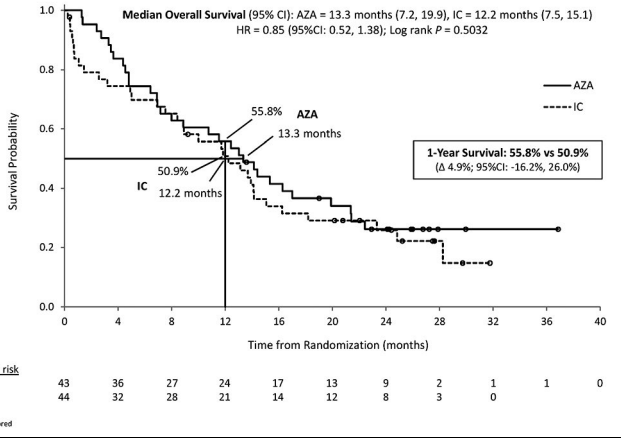

Figure 1. Overall survival in patients preselected to intensive chemotherapy.

Summary and Conclusions: AZA and IC were associated with comparable OS, 1-year survival, and rate and duration of remission in these older pts with AML. AZA was better tolerated than IC, with lower incidence rates of hematologic TEAEs and infections. AZA may be a good option for older pts with AML who are fit for IC but choose not to undergo high-intensity Tx.

P567

Abstract withdrawn

P568

UPDATED RESULTS FROM A PHASE 2 STUDY OF PRACINOSTAT (P) IN COMBINATION WITH AZACITIDINE (AZA) IN ELDERLY PATIENTS WITH ACUTE MYELOID LEUKEMIA (AML)

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Background: Elderly AML patients, deemed unsuitable for intensive therapy, have limited treatment options. We previously reported a high initial response rate in the first stage of a phase 2 study of P plus AZA in this population (ASH 2014). This report presents updated results, which include additional patients.
Aims: The study was designed to evaluate the efficacy and safety of the combination of pracinostat and azacitidine in elderly patients with AML
Methods: Eligibility includes previously untreated AML (≥ 20% bone marrow blasts), age ≥ 65 years, unsuitable for intensive therapy due to co-morbidities and/or AML related features, and intermediate or high-risk cytogenetics. Study therapy includes P, 60 mg p.o. 3 alternate days/week for 3 weeks plus AZA, 75 mg/m^2 /day 1-7 or day 1-5 and 8-9 either s.c. or i.v. with cycles repeated every 28 days until progressive disease, lack of response, or intolerance. The primary endpoint is CR+CR_i+ morphologic leukemia free state (MLFS) per IWG criteria. Response assessments occur at the end of cycle 1 or 2 then every other cycle or when clinically indicated. A Simon 2-stage statistical design is utilized with the following assumptions: null=0.10, alternate=0.25, α=0.10, power=0.90. Stage 1 n=27 and total stage 2 n=40.
Results: Between 12/2013 and 12/2014 50 patients from 15 study sites were enrolled. At this time, 47 are evaluable for efficacy. Baseline disease characteristics for all patients include: median age 75 (range 66-84); 32 *de novo* AML, 13 evolved from AHD, 5 treatment-related; 28 intermediate-risk and 20 high-risk cytogenetics and 2 unknown; baseline bone marrow blast counts ranged from 20% to 89% with a median of 40%. The primary endpoint of CR +CRi +MLFS has been observed in 22/47 evaluable patients (47%) to date, including 14/47 (30%) CR. Current median duration of response is 15+ weeks (range: 1- 48+ weeks). Disease progression has not been observed in any responders. 30 patients continue on study (range 11- 52+ weeks). The 60-day all-cause mortality rate is 10% (5/50). Median overall survival has not been reached. Treatment emergent adverse events (TEAEs) Grade ≥3 seen in >5% of patients: febrile neutropenia 30%; thrombocytopenia 22%; neutropenia 10%; cellulitis 10%; anemia 8%; fatigue 8%; sepsis 6%, and pancytopenia 6%. TEAE's leading to study therapy discontinuation: peripheral motor neuropathy (1), parainfluenza (1), atrial fibrillation/prolonged QTc/(1), subdural hematoma after a fall (1), and sepsis (3).
Summary and Conclusions: P plus AZA produces a high rate of durable responses in this AML population. Updated data, including EFS and OS estimates, will be presented at the meeting. These phase 2 data warrant definitive evaluation in a phase 3 study.

P569

AG-221, AN ORAL, SELECTIVE, FIRST-IN-CLASS, POTENT INHIBITOR OF THE IDH2 MUTANT ENZYME, INDUCED DURABLE RESPONSES IN A PHASE 1 STUDY OF IDH2 MUTATION-POSITIVE ADVANCED HEMATOLOGIC MALIGNANCIES

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Background: Somatic mutations in the metabolic enzymes isocitrate dehydrogenase (IDH) 1 and 2 occur in a spectrum of solid and hematologic malignancies. Mutant IDH1/2 in cancer cells results in the neomorphic production of the oncometabolite, D-2-hydroxyglutarate (2-HG), which impairs cellular differentiation via an epigenetic mechanism. AG-221 is a first-in-class, oral, potent, selective, reversible inhibitor of mutant IDH2. Data from the ongoing, first-in-human, phase 1, open-label, dose escalation study (NCT01915498) are reported.
Aims: To assess safety and define the maximum tolerated dose (MTD) of AG-221 and inform the dosing schedule for expansion cohorts and future phase 2 studies. Secondary objectives include preliminary evaluation of clinical efficacy and measurement of pharmacokinetic/pharmacodynamic (PK/PD) markers and cellular differentiation.
Methods: Patients with IDH2 mutation-positive advanced hematologic malignancies (acute myeloid leukemia [AML] or myelodysplastic syndromes) receive oral AG-221 once or twice daily in continuous 28-day cycles. Informed consent is obtained prior to entry. Dosing began at 30 mg twice daily and increased in subsequent cohorts. Response is determined by blood and bone marrow examinations according to International Working Group criteria. PK/PD analyses of AG-221 and 2-HG are conducted.
Results: As of 1 Oct 2014, 73 patients have received AG-221 (median age 67 years [range, 33-90], 53% men, 75% with relapsed/refractory [RR] AML) and 38 (52%) remain on therapy, with 45 (62%) evaluable for efficacy. Median treatment duration is 1.9 (range, 0.1-8.8) months, and 18 patients have been on study for ≥4 months. There are 10 dose cohorts, MTD has not been reached, and the highest daily cumulative dose is 300 mg. The most common adverse events (AEs) were nausea, pyrexia, diarrhea and fatigue, with 81% of patients experiencing any AE, including AEs that were not related to AG-221. There have been 4 AEs leading to discontinuation. The majority of serious AEs (SAEs) were disease-related, with 13 patients experiencing 21 treatment-related SAEs, including leukocytosis in 3 patients. Of 11 deaths reported, 2 were assessed as possibly related to treatment. AG-221 demonstrated adequate plasma exposure, with a mean half-life >40 hours. After multiple AG-221 doses, plasma 2-HG was reduced from the elevated levels observed at baseline, with inhibition of up to 98% in subjects with the IDH2-R140Q mutation. Objective responses were observed in 25 (56%) efficacy-evaluable patients, defined as having Day 28 bone marrow biopsy information available (6 CR, 4 marrow CR, 5 CR with incomplete platelet or hematologic recovery, 10 PR). There were durable responses of up to 8.8 months and 5 responders proceeded to transplant (Figure 1). As of 20 Feb 2015, 145 patients have been enrolled, 104 in dose escalation and 41 in four expansion arms of 1) age ≥60 years (y) with RR AML, or any age if relapsed after stem cell transplant (SCT); 2) age <60 y with RR AML excluding relapse after SCT; 3) age ≥60 y with untreated AML declining standard therapy; 4) any hematologic malignancy not eligible for arms 1-3.

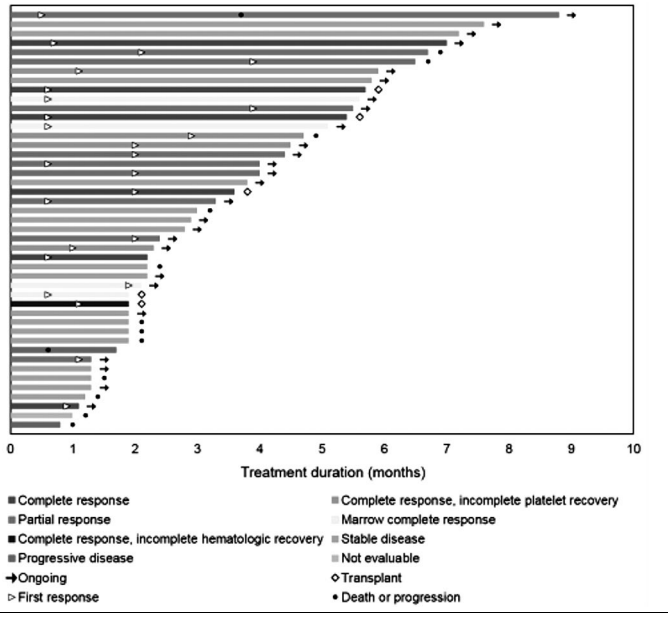

Figure 1. Duration of AG-221 treatment and best overall response, all efficacy evaluable patients as of 1 October 2014.

Summary and Conclusions: In patients with IDH2 mutation-positive advanced hematologic malignancies, AG-221 therapy was well tolerated, displayed favorable PK/PD properties, and resulted in durable remissions of up to 8.8 months. These results continue to validate mutant IDH2 as a therapeutic cancer target and demonstrate the safety of AG-221. Updated clinical results will be presented.

P570

ORAL TETRA-ARSENIC TETRA-SULFIDE FORMULA IS BETTER THAN INTRAVENOUS ARSENIC TRIOXIDE IN TREATING ACUTE PROMYELOCYTIC LEUKEMIA: A SYSTEMATIC REVIEW AND META-ANALYSIS OF RANDOMIZED CONTROLLED TRIALS

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Background: The chromosomal aberration t(15;17) plays a central role in the development of acute promyelocytic leukemia (APL) and results in the formation of PML/RARa fusion protein. Arsenic trioxide (ATO) and all-trans retinoic acid (ATRA) target the PML and RARa proteins respectively, which made APL changes from a highly fatal disease to a highly curable disease. However, ATO must be intravenously administered in a hospital setting. The development of an orally active arsenic-containing formulation with comparable efficacy and adverse effects is highly desirable. Increasingly clinical trials and articles demonstrated that an oral tetra-arsenic tetra-sulfide (As4S4) treatment alone can serve as a highly effective and safe remission induction and maintenance therapy.
Aims: To estimate the efficacy and safety of Oral As_4S_4 -containing formula named the Realgar-*Indigo naturalis* formula (RIF), combined with ATRA therapy in APL.
Methods: Cochrane Library, PUBMED, EMBASE, Chinese National Knowledge Infrastructure, and WanFang Data were searched for randomized controlled trials (RCT). Oral RIF+ATRA (RIF group) *versus* intravenous arsenic trioxide (ATO)+ATRA (ATO group) as treatment of APL, the complete remission (CR) rate and the incidences of adverse events of two groups were compared. Meta-analysis was conducted to generate combined odds ratios (ORs) with 95% confidence intervals (CIs) by RevMan 5.2.
Results: Data from 5 RCTs involving 343 APL patients were identified. For the CR rate, the RIF group and the ATO group showed a similar efficacy in the different treatments (95.57% *vs* 93.33%). For CR rate of RIF group *vs* CR rate of ATO group, ORs increased with the year of clinical trials (2008,OR:0.7; 2011,OR:2.97 and 2013 OR:3.72), which suggested RIF as the first-line treatment of APL stabilized and improved year by year. Besides, time to CR, 2-year DFS, 3-year DFS and mortality also showed that oral RIF+ ATRA had effect no worse than intravenous ATO+ATRA. At the end of consolidation therapy, the PML-RARα transcript was undetectable in any patient in both the RIF group and ATO group (two RCT were involved which was 113 patients). Notably, although there were no statistical difference in 3 articles which mentioned adverse events affecting the liver, meta-analysis suggested remarkable difference: happening of hepatic dysfunction in RIF group were lower than ATO group (OR=0.57, 95%CI 0.34-0.95, P=0.03). Moreover, rate of headache and infection in RIF group were also lower than ATO group, (OR=0.06, 95%CI 0.01-0.46, P=0.007) and (OR=0.34, 95%CI 0.14-0.32, P=0.02), respectively. Means of medical cost for postremission treatment in 15 months was 10701.9 RMB for RIF group, compared to 49957.5 RMB for ATO group. The cost of RIF group was only less than a quarter to ATO group (P<0.00001). The rates of other events were similar in the two groups.
Summary and Conclusions: Oral RIF plus ATRA is not inferior to intravenous ATO plus ATRA as treatment of APL. RIF as the first-line treatment of APL stabilized and improved year by year.The security of oral RIF is better than intravenous ATO, and the maintenance treatment costs of RIF is lower than ATO therapy in APL. PML-RARα transcript for the treatment of RIF+ATRA was 100%, suggested RIF+ATRA may be considered as a postremession treatment. It may be considered as a new strategies therapy in APL: oral RIF Replace intravenous ATO.

Acute myeloid leukemia - Clinical 4

P571

LATE RESPONSES AND OVERALL SURVIVAL (OS) FROM LONG TERM FOLLOW UP OF A RANDOMIZED PHASE 2 STUDY OF SGI-110 (GUADECITABINE) 5-DAY REGIMEN IN ELDERLY AML WHO ARE NOT ELIGIBLE FOR INTENSIVE CHEMOTHERAPY

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Background: SGI-110 (guadecitabine) is a novel hypomethylating agent (HMA) given subcutaneously (SC) and provides a prolonged *in vivo* exposure to its active moiety decitabine. We previously reported results from a multicenter randomized dose-response study of guadecitabine given at 2 doses in a 5-day regimen in 51 treatment naïve elderly AML patients not eligible for intensive chemotherapy (Yee *et al.*, 2014). There was no significant differences in overall composite complete response (CRc: CR+CRp+CRi) or safety between the two doses; however 14 patients were still on treatment at the time of the analysis.
Aims: To report on long term follow up including additional late responders and overall survival (OS).
Methods: Previously untreated elderly (≥65y) AML patients who were not eligible to receive intensive chemotherapy were randomized 1:1 to either 60 or 90 mg/m^2/d SC for 5-days in 28-day cycles. Overall composite CR (CRc: CR+CRp+CRi) was the primary endpoint. OS, and Safety were secondary endpoints.
Results: Fifty one patients were treated (24 and 27 on 60 and 90 mg/m2 doses respectively). Patients' characteristics were generally balanced between the 2 doses and represented a poor prognosis population: 73% were ≥75 y old; 47% had poor risk cytogenetics; 45% secondary AML; and 35% poor performance status (PS 2). No differences in either CRc or CR were observed in the previous and current analysis between the 2 treatment doses so data are presented here for the overall patients treated. Two additional patients developed CR with longer follow up (one on each arm) bringing total patients with CR to 19 (37%) and the total patients with CRc to 29 (57%): 19 CR; 7 CRi, and 3 CRp. Median follow up now reached 21.1 months (m) (range 17.2-30.0 m). Median number of cycles administered was 5 (range 1-24). Of the 29 CRc patients, the majority (20 or 69% of responders) occurred after at least 3 cycles and 6 of them (21% of responders) occurred after at least 6 cycles. Two late responders occurred after 8 and 9 cycles respectively. With death events reported in 34/51 patients (67%) the median OS is 10.5 m (95% CI: 5.3-18.1 m). The most common Grade≥3 Adverse Events (AEs) assessed as drug-related by investigators were neutropenia and thrombocytopenia (35% each), febrile neutropenia (20%), leukopenia (18%), and anemia (16%).
Summary and Conclusions: Unlike cytotoxic chemotherapy where responses tend to occur in the first 2 cycles, AML responses with HMA guadecitabine tend to occur after multiple cycles with potential for late responses even after 6 cycles. Clinical activity as demonstrated by 57% CRc including 37% CR and median OS of 10.5 months in this elderly poor prognosis population is promising. A Phase 3 trial in previously untreated AML patients not eligible for intensive chemotherapy has been initiated with 5-day regimen at 60 mg/m^2/d SC.

Reference

Yee K, Daver N, Kropf P, et al: European Hematology Association Meeting, June 12-15, 2104; Milan, Italy. Abstract S647.

P572

PHARMACOKINETIC/PHARMACODYNAMIC EVALUATION OF AG-120, A POTENT INHIBITOR OF THE IDH1 MUTANT PROTEIN, IN A PHASE 1 STUDY OF IDH1-MUTANT ADVANCED HEMATOLOGIC MALIGNANCIES

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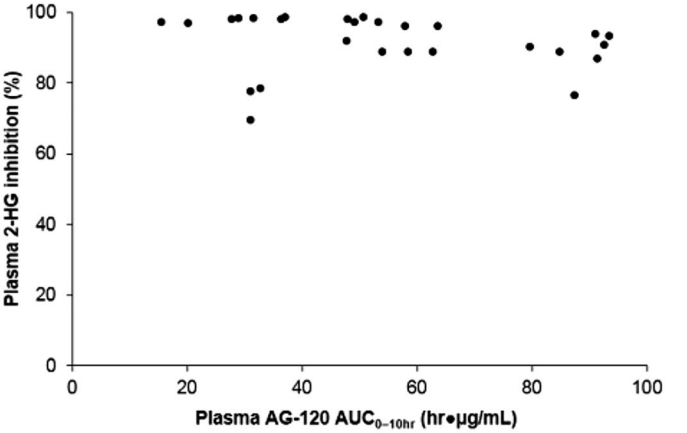
Background: Isocitrate dehydrogenase 1 and 2 (IDH1/2) are critical metabolic enzymes, catalyzing the oxidative decarboxylation of isocitrate to produce alpha-ketoglutarate (α-KG) in the citric acid cycle. Somatic IDH1/2 mutations occur in multiple solid and hematologic tumors, including acute myeloid leukemia. Mutant IDH1/2 proteins possess novel enzymatic activity, catalyzing the reduction of α-KG to produce the oncometabolite, D-2-hydroxyglutarate (2-HG). Accumulation of 2-HG drives multiple oncogenic processes, including impaired cellular differentiation. AG-120 is a first-in-class, oral, potent, reversible, selective inhibitor of the mutated IDH1 protein and has been shown to lower 2-HG levels and restore cellular differentiation in IDH1-mutant primary human blast cells cultured *ex vivo*. We conducted *in vivo* pharmacokinetic/pharmacodynamic (PK/PD) studies in an IDH1-mutant xenograft mouse model to assess the correlation between AG-120 exposure and 2-HG inhibition, and to predict exposure required for efficacy in humans. AG-120 is currently being assessed in a first-in-human, phase 1 study enrolling patients with IDH1-mutant, advanced hematologic malignancies (NCT02074839).
Aims: To compare early PK/PD data from the ongoing, first-in-human, phase 1 study of AG-120 with PK/PD data from *in vivo* and *in vitro* models.
Methods: The AG-120 phase 1, open-label, dose-escalation study includes evaluation of safety, tolerability, maximum tolerated dose (MTD) of AG-120, PK/PD (including 2-HG levels) and clinical activity. Single-agent AG-120 is administered orally once (QD) or twice (BID) daily in continuous 28-day cycles. Informed consent is obtained prior to entry. Patients included in this analysis received doses of 100 mg BID, 300 mg QD, 500 mg QD, and 800 mg QD (N=17). Blood was collected at multiple time points for determination of PK/PD. Concentrations of AG-120 and 2-HG in plasma samples were determined using qualified LC-MS/MS-based methods. Analyses were performed using WinNonLin®. PK/PD relationships for AG-120 were also evaluated *in vivo* in an HT1080 IDH1-R132C-mutant xenograft mouse model following oral administration, and potency was assessed *in vitro* in a number of cell lines carrying IDH1 mutations.
Results: Preliminary analyses demonstrated a favorable PK profile for AG-120 in humans following oral administration. Mean exposure was high at all dose levels tested, and AG-120 displayed a long plasma half-life (>32 hours). Following multiple AG-120 doses, substantial reductions in mean plasma 2-HG concentrations to levels seen in healthy volunteers were observed at all doses (up to 98% 2-HG inhibition). With exposure-response analysis, substantial and constant plasma 2-HG inhibition was achieved from multiple AG-120 100 mg BID doses at the AG-120 $AUC_{0\text{-}10hr}$ value of 15.5 hr•µg/mL (Figure 1). These findings showed good correlation with PK/PD analyses in the HT1080 IDH1-R132C mutant xenograft mouse model. *In vitro* studies showed potent inhibition of 2-HG production with AG-120 treatment in cells expressing IDH1-R132C, -R132H, and -R132S mutations but not in those expressing the IDH2-R140Q mutation, demonstrating selectivity for IDH1 mutations.



2-HG = D-2-hydroxyglutarate; IDH = isocitrate dehydrogenase

Figure 1. AG-120 plasma exposure and 2-HG inhibition correlation in patients with IDH1 mutations.

Summary and Conclusions: AG-120 demonstrated a favorable PK profile in humans following oral administration, with high plasma exposure and a long half-life, supporting QD dosing. AG-120 inhibited plasma 2-HG to within normal levels found in healthy volunteers. Inhibition of 2-HG production by AG-120 translated well from *in vitro* to *in vivo* and from pre-clinical models to humans.

P573

CHARACTERISATION OF TP53 MUTATIONS IN ADULT ACUTE MYELOID LEUKEMIA (AML) PATIENTS: MUTUAL EXCLUSIVITY WITH FLT3 AND NPM MUTATIONS STRONG ASSOCIATION WITH COMPLEX KARYOTYPE AND POOR OUTCOME

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Background: AML is a heterogeneous disease with a variety of structural and numerical chromosomal and genetic alterations that provided important prognostic information capable to guide therapy and predict outcome. The reported *TP53* mutation rate in AML is low (2.1%). By contrast, the incidence of *FLT3* disruption is frequent (20-30%) and is an independent predictor of unfavourable outcome of acquired clinical resistance to *FLT3* inhibitors. *TP53* mutations in AML with a complex karyotype (CK) is higher (69-78%), and patients (pts) with complex karyotype (CK-AML) have generally a poor outcome but few data are available for association between the most common AML associated lesions such as *FLT3, NPM1, DNMT3A, IDH2* and *TP53* mutation or CK.
Aims: To investigate the frequency, the types of mutations, the associated cytogenetic, the molecular abnormalities, the correlation with known molecular alterations (*FLT3, NPM*, etc.) and the prognostic role *TP53* mutations in adult AML pts.
Methods: 236 adult AML pts AML pts with FAB-M0-7, miscellaneous cytogenetic abnormalities and normal karyotype (nK) were examined for *TP53* mutations using several methods, including Sanger sequencing, NGS (20/236) and HiSeq 2000 platform (38/236) and were correlated with cytogenetic analysis. Analysis was focused on coding sequences (RefSeq GRCh37/hg19 NG_017013.2).
Results: By PCR and subsequent Sanger sequencing, mutations of *TP53* were detected in 33 cases (14%). Nine pts revealed 2 mutations. 75.8% of all mutated pts had CK (25/33) by contrast the frequency of mutations was lower in "no CK-AML" pts (24.2%). Overall, between pts with CK, *TP53* mutation frequency is 43.1% (25/58). 38 *TP53* point mutations and 4 *TP53* deletions were found. M237I mutation was found in 4 pts. This mutation was described to severely compromised p53 expression level. The majority of alterations are located in the DNA binding domain (38/42; 90%), 9 of these were located in hot spot sites. Almost all mutations in coding regions were classified by the IARC database (http://p53.iarc.fr/TP53GeneVariations.aspx) as deleterious. WES analysis done in 38 pts (33 *TP53* wt and 5 pts *TP53* mutated) revealed no genes exclusively mutated in the 5 *TP53* mutated pts. 174 and 117 pts were analysed for concomitant presence of *TP53* mutations and *FLT3/NPM1* disruption/mutation revealing a significant relation between pts with *FLT3-ITD* or *NPM1* and *TP53* wild-type (p=0.015 and 0.009 respectively). Of note, alterations of *TP53* were significantly associated with poor outcome in terms of both overall survival (Figure 1A; P <0.0001) and disease free-survival and that *TP53* compare to *FLT3* mutations have worse impact on prognosis (Figure 1B; P <0.0001).

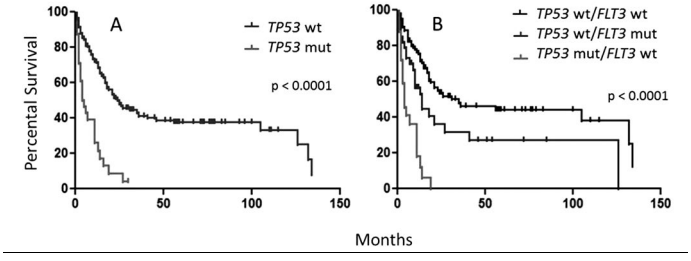

Figure 1. A) Overall survival in AML adult patients considering *TP53* mutational status alone and B) in combination with *FLT3* mutations.

Summary and Conclusions: Our data demonstrated that *TP53* mutations occur in 14% of AML with a higher frequency in the subgroup of CK-AML (p<0.0001-Fischer's exact test) and are mutually exclusive with *FLT3* and/or *NPM1*; they predicted to be deleterious and significantly correlated with worse prognosis and may confer resistance to conventional and innovative therapy. For these reasons, *TP53* mutation screening should be recommended at least in CK-AML pts.
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P574

PHASE 1 TRIAL OF G-CSF, CLADRIBINE, CYTARABINE, AND DOSE-ESCALATED MITOXANTRONE (G-CLAM) IN ADULTS WITH NEWLY DIAGNOSED AML OR HIGH-RISK MDS

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Background: Standard-dose cyarabine with an anthracycline ("7+3") has remained the mainstay of induction chemotherapy for newly diagnosed AML. Recently, some randomized studies have demonstrated improved outcomes with regimens containing high-dose cytarabine, cladribine, or escalated doses of anthracyclines during induction.
Aims: Given these findings and the previously established tolerability and efficacy of G-CLAM in relapsed/refractory AML, we aimed to determine the maximum tolerated dose (MTD) of mitoxantrone within G-CLAM in adults with newly diagnosed AML or high-risk MDS (>10% blasts).
Methods: Eligibility included a treatment-related mortality (TRM) score of ≤6.9 (corresponding to a risk of early death with standard induction chemotherapy of ≤3%) and adequate organ function (LVEF ≥45%, creatinine ≤2.0 mg/dL, bilirubin ≤2.5-times upper limit of normal). Excluded were patients with concomitant illness with expected survival <1 year and those with active, uncontrolled infection. Cohorts of 6-12 patients were assigned to 1 of 4 total dose levels of mitoxantrone (12, 14, 16, or 18 mg/m^2/day on days 1-3, as compared to 10 mg/m^2/day used in standard-dose G-CLAM). Other drug doses were G-CSF 300 or 480 µg/day (for weight <76 kg *vs* ≥76 kg; days 0-5), cladribine 5 mg/m^2/day (days 1-5), and cytarabine 2 g/m^2/day (days 1-5). A second identical course of G-CLAM was given in the case of persistent disease. Dose-limiting toxicity (DLT) was defined as: 1) any grade 3 non-hematologic toxicity lasting >48 hours that resulted in >7 day delay of the subsequent treatment cycle, with the exception of febrile neutropenia or infection; 2) any grade ≥4 non-hematologic toxicity, with the exception of febrile neutropenia or infection or constitutional symptoms, if recovery to grade ≤2 within 14 days.
Results: 31 patients (20M, 11F), median age 58 (range: 27-77) years, median TRM score 2.83 (range: 0.15-5.54) with newly diagnosed AML (n=25), or high-risk MDS (n=6) and cytogenetically favorable (n=1), intermediate (n=23), and adverse (n=6) disease characteristics were enrolled (failed karyotyping: n=1; MRC criteria). One DLT occurred each at dose levels 3 and 4 (respiratory failure in both cases), establishing G-CLAM with mitoxantrone at 18 mg/m^2/day as the MTD in our study. One patient died within 28 days of treatment initiation from thrombocytopenia-related intracranial hemorrhage. Overall, 29/31 patients (93.5%, exact 95% confidence interval [CI]: 78.6-99.2%) achieved a CR (n=24 [77.4%, 95% CI: 58.9-90.4%]), CRp (n=2 [6.5%, 95% CI: 0.8-21.4%]), or CRi (n=3 [9.7%, 95% CI: 2.0-25.8%]) with 1-2 cycles of chemotherapy; one patient at dose levels 1 and 2 each had persistent disease. Only 2 patients required 2 cycles to best response. 26/29 (89.7%, 95% CI: 72.7-97.8%) had no evidence of residual disease by flow cytometry at best response. After multivariable adjustment, the CR rate was similar and overall response rate was favorable as compared to 300 patients treated with 7+3 on the SWOG S0106 trial (OR=1.25, p=0.64, and OR=4.68, p=0.045, respectively). Among responders, median times to an absolute neutrophil count ≥500 and a platelet count of 50,000 were 25 (range: 19-38) and 24 (range: 18-45) days, respectively. Besides infections and neutropenic fever, maculopapular rash, nausea, and hypoxia (fluid overload/infection-related) were the most common grade ≥3 adverse events.
Summary and Conclusions: G-CLAM with mitoxantrone up to 18 mg/m^2/day is feasible, well tolerated, and effective in newly diagnosed AML/high-risk MDS. A phase 2 study has been initiated.

P575

OVERALL SURVIVAL (OS) WITHOUT COMPLETE REMISSION (CR) IN OLDER PATIENTS WITH ACUTE MYELOID LEUKEMIA (AML): AZACITIDINE (AZA) *VS* CONVENTIONAL CARE REGIMENS (CCR) IN THE AZA-AML-001 STUDY

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Background: The international phase 3 AZA-AML-001 study compared treatment (Tx) effects of AZA *vs* CCR on OS in older patients (pts) with AML; AZA prolonged median OS by ~4 months (10.4 *vs* 6.5 months, overall p=0.10) (Dombret, *Haematologica*, 2014:LB2433). AZA is known to improve OS *vs* CCR in pts with higher-risk myelodysplastic syndromes (HR-MDS) who have not achieved CR during Tx (Gore, *Haematologica*, 2013). MDS and AML are biologically and clinically distinct; it is unknown whether OS benefits in the absence of CR shown for AZA in HR-MDS extend to AML.
Aims: This *post hoc* exploratory analysis examined Tx effects on OS in subgroups of pts in AZA-AML-001 who did not attain CR on-study.
Methods: Eligible pts were aged ≥65 years with newly diagnosed AML with >30% bone marrow blasts, and with ECOG PS of 0-2, WBC count ≤15x10^9/L, and intermediate- or poor-risk cytogenetics. Before randomization, pts were preselected to receive 1 of 3 commonly used CCR per investigator choice of most appropriate Tx: intensive chemotherapy (IC; cytarabine IV x7days (d)+an anthracycline IV x3d, with ≤2 subsequent cycles), low-dose cytarabine (LDAC; 20mg SC BID x10d/28d cycle), or best supportive care only. Pts were then randomized to AZA (75 mg/m^2/d SC x7d/28d cycle), or to CCR and received their preselected Tx. CR (IWG 2003) was adjudicated centrally by an Independent Review Committee blinded to Tx assignment. Median OS and 1-year survival

were estimated using Kaplan-Meier methods. OS was compared between AZA and CCR groups by log-rank test stratified by ECOG PS and cytogenetic risk. OS was compared between AZA and LDAC and AZA and IC within preselection groups by unstratified log-rank test. Hazard ratios (HR) and 95% CIs are from a stratified Cox proportional hazards model. Results should be interpreted cautiously, as OS comparisons of pt subgroups defined by post-randomization outcomes may be biased. The current analysis did not control for time-dependency of response or interactions between Tx and response that could influence OS.
Results: Of all pts in AZA-AML-001 (AZA n=241, CCR n=247), 47 (19.5%) in the AZA arm and 54 (21.9%) in the CCR arm attained CR and were excluded from these analyses. Median OS in the remaining pts in the AZA *vs* CCR groups was 6.9 (95%CI: 5.1, 8.9) *vs* 4.2 (95%CI 3.2, 5.1) months, reflecting a 23% reduced risk of death with AZA; HR=0.77 (95%CI 0.62, 0.95), p=0.0171 (Figure 1). Estimated 1-year survival was 33.8% with AZA and 20.4% with CCR (difference 13.4% [95%CI 4.5%, 22.4%]). For pts with no CR who were preselected to receive LDAC, median OS with AZA (n=126) *vs* LDAC (n=120) was 8.1 *vs* 4.2 months, respectively (HR=0.75 [95%CI 0.57, 0.99], p=0.0390), and 1-year survival was 36.8% *vs* 16.4% (difference 20.4% [95%CI 9.4%, 31.3%]). For pts with no CR who were preselected to receive IC, median OS with AZA (n=30) *vs* IC (n=28) was 8.0 *vs* 7.5 months, respectively (HR=0.81 [95%CI 0.46, 1.44], p=0.4765), with 1-year survival of 40.0% *vs* 40.2%.

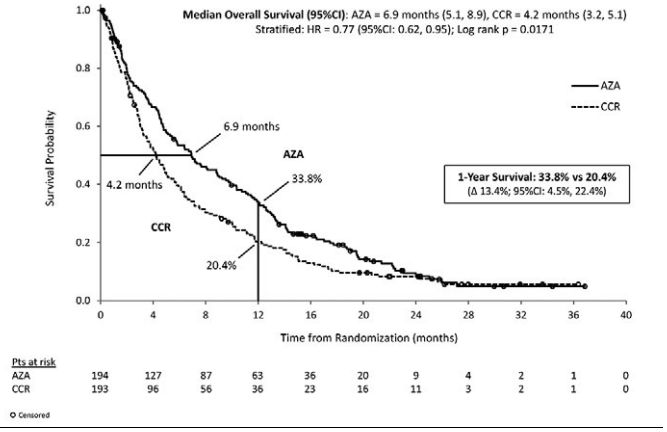

Figure 1. Overall survival for patients who did not achieve CR.

Summary and Conclusions: The relative benefit on OS seen with AZA *vs* CCR for all pts in AZA-AML-001 was maintained in pts who did not attain CR on-study. Similarly, median OS with AZA was almost twice that with LDAC in pts preselected to receive LDAC who did not achieve CR. Clinically meaningful improvements in 1-year survival rates with AZA *vs* CCR (13.4%) and with AZA *vs* LDAC (20.4%) in this analysis are similar to those reported for all AZA-AML-001 pts (12% and 15%, respectively; Dombret, *Haematologica*, 2014). These findings suggest that CR is not necessary to gain a survival benefit with AZA *vs* other commonly used Tx for older pts with AML.

P576

ABSOLUTE QUANTIFICATION OF THE PRETREATMENT PML-RARA MOLECULAR TRANSCRIPT BY DROPLET DIGITAL PCR DEFINES THE RELAPSE RISK IN ACUTE PROMYELOCYTIC LEUKEMIA

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Background: The persistence of resistant leukemic cells after treatment is responsible for relapse in 10-20% of acute promyelocytic leukemia (APL) patients. Until now, the white blood cells count at diagnosis has been considered the most important prognostic factor in APL, able to better identify those patients at higher risk of relapse.
Aims: In this study we performed absolute quantification of the *PML-RARA* transcript by droplet digital polymerase chain reaction (ddPCR) in newly diagnosed APL patients to verify the the prognostic impact of the *PML-RARA* initial molecular burden.
Methods: *PML-RARA* expression analysis was performed by ddPCR in 76 APL patients at diagnosis with Bio-Rad's QX200 system. Primers and probes for *PML-RARA* isoforms were selected according to the standardized protocol for TaqMan based RT-qPCR analysis. After amplification, ddPCR data were analyzed with QuantaSoft analysis software (version 1.7.4). Target concentration in each sample was expressed as *PML-RARA* copies/ng.
Results: ddPCR analysis revealed that the amount of *PML-RARA* transcript at diagnosis in the group of patients who relapsed was higher than in that with continuous complete remission (CCR) (272 *vs* 89.2 *PML-RARA* copies/ng, p=0.0004, respectively). Moreover, considering the arbitrary cut-off of 124 *PML-RARa*/ng (the median value of our APL series), a higher proportion of patients who relapsed (85.7%) had >124 *PML-RARa*/ng compared to the CCR group (39.6%) (odds ratio 0.10; p=0.002). Further parameters (age, sex, WBC count, M3/M3v, bcr transcript type, FLT3 mutation status, CD34 and CD2 expression, relapse risk score) were assessed to verify the presence of different amounts of the fusion gene transcript at diagnosis among these different categories but yielded no statistically significant differences. ROC analysis detected the optimal *PML-RARA* concentration threshold as 209.6 *PML-RARa*/ng (AUC, 0.78; p <0.0001) for discriminating between outcomes (CCR *versus* relapse). Among the 67 APL patients who achieved CR after the induction treatment, those with >209.6 *PML-RARa*/ng had a worse relapse-free survival (RFS) (p=0.0006). At 5-year follow-up, patients with >209.6 *PML-RARa*/ng had a cumulative incidence of relapse of 50.3% whereas 7.5% of the patients with suffered a relapse (p <0.0001). There was no difference in terms of overall survival (OS) between patients with ≤209.6 *PML-RARa*/ng and those with >209.6 *PML-RARa*/ng. It is noteworthy that when the group of patients with early death was excluded from the OS analysis, the difference between the two groups was statistically significant (p=0.02). Cox proportional hazards regression model analysis was performed for RFS identifying the amount of *PML-RARA* before induction treatment as the sole independent prognostic factor for APL relapse (HR 9.26, p=0.0009).
Summary and Conclusions: Our results identified the amount of *PML-RARA* before induction treatment as the sole independent prognostic factor for APL relapse. In our experience the experiments performed with the ddPCR technology were absolutely reproducible, allowing easy measurement of the absolute copies number of the *PML-RARA* transcript, and with high precision. We conclude that the pretreatment *PML-RARA* molecular burden defined by ddPCR could therefore be used to improve risk stratification in order to develop more individualized treatment regimens for high-risk APL patients.

P577

PREVALENCE AND CLINICAL CHARACTERISTICS OF SETBP1 MUTATIONS IN MYELOID DISORDERS

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Background: *SETBP1* mutations have recently been discovered and characterized in myelodsyplastic syndrome (MDS), secondary acute myeloid leukemia (sAML), myelodysplastic/myeloproliferative neoplasms (MDS/MPN), and aplastic anemia. Even though they are infrequent (1-5% in the above mentioned diseases), they are associated with a poor prognosis. The role of *SETBP1* mutations during disease progression for individual patients has rarely been investigated so far.
Aims: To address this subject, we have screened 401 patients with World Health Organization-defined AML (n=48), sAML (n=95), MDS (n=93), classical MPN (n=113), MDS/MPN (n=40), and aplastic anemia (n=12) in an unselected single-center cohort. In addition, we analyzed 103 follow up samples of patients during disease evolution at a median of 10 months (range, 3-65 months) from diagnosis.
Methods: Mutational screening was performed from bone marrow samples or peripheral blood using bidirectional Sanger sequencing of genomic DNA covering the entire SKI homology domain of *SETBP1*.
Results: In total, we found *SETBP1* mutations in 10 patients (2.5%, MDS/MPN, n=6; sAML, n=2; MDS, n=2) who were all included in our clinical follow up. The median age of patients harboring *SETBP1* mutations was 63 years (range, 48-80 years). The majority of patients had a normal karyotype (n=5), chromosomal alterations were: monosomy 7 (n=2), trisomy 8, trisomy 21, loss of chromosome Y (n=1 each). All mutations were located within known somatic hotspots according to the COSMIC database (codons 868, 870, 871, 942). One patient showed a novel 12 base pair deletion starting at codon 867, reaching into the known mutational hotspot. Germline material was available in 5/10 patients and confirmed the somatic origin of mutations. In our clinical follow up, 9 of 10 patients with *SETBP1* mutations had progressive disease and underwent intensive chemotherapy with consecutive allogeneic stem cell transplantation (ASCT, n=3), demethylating treatment with azacytidine as a bridging therapy to ASCT (n=3), palliative chemotherapy with cytarabine (n=2), or best supportive care with transfusion dependency (n=2). One patient with a codon 942 mutation (271993G>GA:942R>R/Q) was diagnosed with MDS RAEB II and had stable disease over 65 months under continuous cytoreductive/demethylating therapy. 2/3 transplanted patients had early relapse after 6 and 2 months, respectively. In the first patient, the *SETBP1*-clone dissolved for 6 months after allotransplant, but re-appeared in relapse. In the second patient, the clone harboring the *SETBP1* mutation persisted through engraftment and was always detectable by Sanger sequencing. One patient has remained in complete remission for 14 months after ASCT.
Summary and Conclusions: Mutated *SETBP1* is associated with rapid disease progression of early relapse in a variety of hematologic diseases, even after ASCT. Since *SETBP1* mutations were never lost during disease progression they may drive disease progression in subgroups of patients and their presence may guide treatment/surveillance decisions in the future.

LB578

PRELIMINARY PHASE II RESULTS OF ARA-C AND IDARUBICIN IN COMBINATION WITH SELECTIVE INHIBITOR OF NUCLEAR EXPORT (SINE) COMPOUND SELINEXOR (KPT-330) IN PATIENTS WITH RELAPSED OR REFRACTORY AML

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Background: Acute myeloid leukemia (AML) is one of the most common leukemia's affecting adults. Although 70-80% of patients achieve a complete remission (CR), patients with AML who fail to achieve a CR after the first cycle of induction therapy and those with relapsed disease have a bleak prognosis. Currently no standard regimen exists for the treatment of patients with relapsed AML and a great clinical need exists for new treatment options. Selinexor, an oral first-in-class Selective Inhibitor of Nuclear Export (SINE) compound, inhibits XPO1 mediated nuclear export to induce cytotoxicity in cells with genomic damage such as tumor cells. *In Vivo* model with selinexor alone or combination with AraC significantly prolonged the survival of leukemic mice from a median survival of 24 days (APL+vehicle) to 39 days, respectively (P <0.0001). Combination therapy prolonged survival (P <0.0001), with some mice being cured of the disease. Phase I clinical study demonstrates encouraging results in AML patient. The role of selinexor as a mono therapy is currently under investigation in phase II.
Aims: This phase II trial explores the efficacy & tolerability of Ara-C and idarubicin in combination with selinexor in patients with relapsed or refractory AML.
Methods: Patients with relapsed/refractory AML are treated with Ara-C (100 mg/m^2, continuous infusion, day 1-7), idarubicin (10 mg/m^2, day 1, 3, 5) every 4 weeks. In the majority of the patients selinexor is administered twice weekly orally starting on day 2 (40 mg/m^2). A small cohort of patients received selinexor after registration and before first induction cycle for correlative studies. The primary endpoint is percentage of patients achieving a complete response or complete remission without normalization of peripheral blood counts (CRi). Other endpoints are partial response rate, percentage of patients undergoing subsequent allogeneic stem cell transplant, early death rate, overall survival (OS), event-free survival and toxicity.
Results: As of 20-April-2015, 18 patients with AML have been enrolled in 3 sites. Informed consent was obtained from all patients. Median age was 56 years. Out of 18 patients, 17 patients received ≥1 induction and 15 patients (9 male, 6 female) were evaluable for efficacy at the time of analysis. In average, patients received 2.4 therapies prior to study start. Overall response rate was 53% (13% of patients achieved CR, 40% of patients achieved CRi). Stable disease was observed in 13% of patients. Sixty-seven percent of patients received or were planned for stem cell transplant or donor lymphocyte infusion. Drug related AEs comprised 61% of all CTC Grade 3/4 and 46% of all CTC Grade 1/2 events. Grade 3/4 drug related adverse events (AEs) include febrile neutropenia (71%), diarrhea (71%), anorexia (57%), thrombocytopenia (43%), leukopenia (43%), anemia (29%) and nausea (29%). Grade 1/2 drug related AEs include: diarrhea (86%), vomiting (71%), nausea (71%), anorexia (43%), constipation (43%), fatigue (29%) and oral mucositis (29%). No drug-related deaths occurred.
Summary and Conclusions: The prognosis of relapsed/refractory AML patients is remarkably poor. Our findings suggest that treatment with Ara-C and idarubicin in combination with selinexor might be a potentially effective strategy for patients with relapsed/refractory AML without unexpected toxicities. Most common AEs were of low CTC grade.

CLL - Biology: Cell-intrinsic defects

P578

NOTCH1 MUTATIONS ARE ASSOCIATED WITH LOW CD20 EXPRESSION LEVELS IN CHRONIC LYMPHOCYTIC LEUKEMIA: EVIDENCE FOR A NOTCH1-MEDIATED EPIGENETIC MECHANISM

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Background: In chronic lymphocytic leukemia (CLL), *NOTCH1* mutations have been associated with clinical resistance to anti-CD20 immunotherapy (Stilgenbauer *et al.* Blood, 2014, Dal Bo *et al.* Ann Hematol, 2014).
Aims: To investigate whether *NOTCH1* mutations could affect CD20 expression in CLL.
Methods: *NOTCH1* mutations were investigated by ARMS PCR, Sanger and next generation sequencing. CD20 expression was evaluated by flow cytometry and QRT-PCR. CLL-like MEC-1 cells were transfected with vectors containing the *NOTCH1* intracellular domain (NICD) carrying either the 7541-7542delCT (NICD-mut) or a stop codon at the beginning of NICD sequence (c.5304G>A, NICD-null).
Results: i) In a cohort of 692 CLL, 87 were *NOTCH1* mutated (*NOTCH1*-mut, 81 cases with the c.7541-7542delCT, 6 with other *NOTCH1* mutations). In 495 CLL, evaluated with a FITC-conjugated anti-CD20, variable CD20 levels were found, with the highest levels in trisomy 12 CLL. *NOTCH1*-mut expressed lower mean fluorescence intensity (MFI) values than *NOTCH1* wild type (*NOTCH1*-wt) cases in both trisomy 12 (20 *NOTCH1*-mut, 69 *NOTCH1*-wt; mean MFI 1,893±196 *vs.*7,051±819; p<0.0001) and non-trisomy 12 (40 *NOTCH1*-mut, 366 *NOTCH1*-wt; mean MFI 1,858±203 *vs* 2,426±112; p=0.017) CLL. Superimposable results were obtained in the remaining 197 CLL, evaluated with a PE-Cy7-anti-CD20, both in trisomy 12 (6 *NOTCH1*-mut, 17 *NOTCH1*-wt; mean MFI 12,926±3,676 *vs* 28,216±5,228; p=0.027) and non-trisomy 12 (21 *NOTCH1*-mut, 153 *NOTCH1*-wt; mean MFI 10,207±1,310 *vs* 15,208±1,578; p=0.017) CLL. Consistently, in 275 CLL (46 *NOTCH1*-mut), transcript levels of *MS4A1*, encoding for CD20, were lower in *NOTCH1*-mut than in *NOTCH1*-wt cases both in trisomy 12 (p=0.006) and non-trisomy 12 (p=0.019) categories. ii) By cell sorting according to CD20 expression in 3 *NOTCH1*-mut CLL, CD20^{low} sorted cells always had a higher *NOTCH1* mutational burden than CD20^{high} cells (*i.e.* 32% *vs* 15%, 38% *vs* 32%, 48% *vs* 39%). iii) CD20 expression was up-regulated in-vitro by gamma-secretase inhibitors (GSI). iv) In keeping with CD20 levels, *NOTCH1*-mut showed lower relative lysis induced by rituximab, in complement-dependent-cytotoxicity assay, than *NOTCH1*-wt cells (6 *NOTCH1*-mut, 7 *NOTCH1*-wt; mean relative lysis 2.5% *vs* 29.4%; p=0.045). v) The stable transfection of the mutated NICD into MEC-1 cells resulted in a strong downregulation of CD20, and, consistently, NICD-mut cells showed lower relative lysis by rituximab (p=0.043). vi) We investigated the interactions of RBPJ, a transcription factor acting either as activator or repressor of NOTCH1 pathway when respectively bound to NICD or hystone deacetylases (HDACs). Compared to controls, NICD-mut cells had RBPJ preferentially complexed to NICD, by co-immunoprecipitation experiments, and showed higher levels of HDACs interacting with the *MS4A1* promoter, by chromatin immunoprecipitation assay. vii) In NICD-transfected cells, treatment with the HDAC inhibitor valproic acid (48 hrs) increased *MS4A1* transcript (NICD-mut, mean increase (MI)=1.7, p=0.001; NICD-null, MI=1.5 p=0.003) and CD20 protein (NICD-mut, MI=1.3, p=0.041; NICD-null, MI=1.4, p=0.029) expression. Similar results were obtained in primary CLL both for *MS4A1* transcript (7 *NOTCH1*-mut, MI=1.5, p=0.05; 6 *NOTCH1*-wt, MI=1.8, p=0.02) and CD20 protein (*NOTCH1*-mut, MI=1.3, p=0.05; *NOTCH1*-wt, MI=1.3, p=0.005).
Summary and Conclusions: *NOTCH1*-mut CLL are characterized by low CD20 levels that may confer resistance to anti-CD20 immunotherapy. The low CD20 expression is due to an epigenetic dysregulation that might be reverted by GSI and/or HDAC inhibitor therapy.

P579

MYD88 L265P SOMATIC MUTATIONS INFLUENCE GENE EXPRESSION AND THERAPEUTIC RESPONSE IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Several driver mutations have recently been identified in CLL, including the MYD88 L265P mutation. MYD88 L265P mutations are highly recurrent in B-cell malignancies such as Waldenstrom's Macroglobulinemia (>90%) and ABC-DLBCL (29%). MYD88 is an adaptor molecule in the Toll-like receptor (TLR) and Interleukin-1 Receptor (IL-1R) pathway. Upon TLR/IL-1R stimulation, MYD88 forms a complex with IL-1R-associated kinase 4 (IRAK4). IRAK4 then phosphorylates IRAK1 leading to its degradation and activation of nuclear factor kappa-B (NF-kB) signaling.
Aims: This study aims to understand the functional consequences of MYD88 L265P mutations in CLL.
Methods: We first profiled the expression of CLL samples with known MYD88 mutation status, using Affymetrix U133 Plus 2.0 arrays. We then used CellTiter-Glo to assess how MYD88 L265P mutations influence response to therapy. Finally, we performed Western blot analysis and ELISA to characterize TLR/IL-1R signaling upon drug treatment.
Results: In our cohort of 160 CLL patients, we identified 10 patients harboring MYD88 L265P mutations (6.25%), all with mutated IGHV status. To determine gene expression changes driven by MYD88 L265P, we compared the expression of these MYD88 L265P samples (n=10) to MYD88 wild-type CLL cells possessing IGHV mutated status (n=76). Using Prediction Analysis for Microarrays (PAM), we identified 28 differentially expressed genes between the two groups. We next examined the expression of these 28 genes across all CLLs (n=150) in relation to clinical features. Using a penalized Cox model, we identified 4 predictive genes, CHL1, UGT2B17, BCAT1, and PDE8A with active coefficients 0.186, 0.174, 0.158, and -0.373, respectively. The composite signature of these 4 genes is strongly associated with overall survival (p=2.6E-09, Cox proportional hazards) and is independent of high-risk clinical features including unmutated IGHV, 17p deletion, and 11q deletion. We next evaluated the response of MYD88 wild-type *versus* L265P CLL cells to pathway inhibition. Inhibition of the B-Cell Receptor pathway with ibrutinib led to modest dose-dependent cell death in both wild-type and MYD88 L265P cells (n=6 per group), with the latter exhibiting slight resistance to ibrutinib (p=0.036). We then tested a highly selective IRAK4 kinase inhibitor, ND-2158 (Nimbus Discovery), to inhibit the TLR/IL-1R pathway downstream of MYD88. CLL cells exhibited dose-dependent cell death upon single agent IRAK4 inhibition, with no difference in sensitivity between MYD88 wild-type and L265P cells. Combination treatment with ibrutinib and ND-2158 resulted in even more marked cell death in both MYD88 wild-type (p=0.0009 *vs* ibrutinib alone; p=0.007 *vs* ND-2158 alone) and L265P CLLs (p=0.003 *vs* ibrutinib alone; p=0.04 *vs* ND-2158 alone). To characterize IRAK4 inhibition in CLL cells, we investigated IRAK1 and IkBa levels by Western blot and evaluated production of the cytokine CCL3 by ELISA, as a measure of TLR-mediated NF-kB signaling. As expected, activation of the TLR pathway via CpG stimulation led to a decrease in IRAK1 levels, increased phospho-IkBa over total IkBa ratio, and increased CCL3 production. Conversely, treatment of cells with ND-2158 inhibited these processes in a dose-dependent manner.
Summary and Conclusions: MYD88 L265P mutations drive expression changes that influence clinical outcome in CLL. IRAK4 inhibition in CLL perturbs TLR signaling downstream of MYD88 leading to inhibition of the NF-kB pathway and resulting in cancer cell death in both MYD88 wild-type and L265P CLLs.

P580

THE GAIN OF THE SHORT ARM OF CHROMOSOME 2 (2P) IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) RESULTS IN XPO1 OVEREXPRESSION AND DRUG RESISTANCE

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Background: CLL is a very heterogeneous disease in terms of response to treatment, with some patients reaching complete and prolonged remissions, while others relapsing early and requiring several lines of treatments. This highly variable course is partly explained by the existence of a heterogenic panel of genetic alterations (mutations, chromosomal abnormalities) that allow the development of drug-resistant aggressive CLL subclones. Therefore, a functional characterization of the cytogenetic alterations associated to CLL drug resistance may provide new means of improving the current therapeutic strategies. We and others have already reported that the gain of 2p (2p+) is recurrent in CLL. However, the candidate gained gene(s) on the 2p remain to be identified.
Previously unpublished data: We have observed that the 2p gain is frequent in previously untreated CLL Binet stages B/C (21/132, 15.9%), and is associated with bad prognostic factors, such as 11q deletion (p=0.0008) and unmutated *IGHV* (p=0.02). Using a SNP-array approach, we have identified a minimally gained region of 1.28Mb on 2p16.1-15. This region included the gene *CRM1*/*XPO1* (Chromosome Region Maintenance 1/Exportin-1), a gene also recurrently mutated in CLL. A qPCR assessment confirmed that *XPO1* was over-expressed in the CLL 2p+ patients (1.4-fold increase compared to CLL 2p-; p=0.02).
Aims: The objective of our work was to identify the potential role of *XPO1* in CLL drug resistance by using the selective XPO1 inhibitor Selinexor (KPT-330, provided by Karyopharm Therapeutics), which is currently in Phase II human clinical trials in hematological and solid cancers.
Methods: We have analyzed 36 CLL 2p+ and we have searched for *XPO1* mutations in 436 CLL samples. CLL drug resistance associated to *XPO1* over-expression/mutation was assessed by measuring the rate of programmed cell death (PCD) on cells from CLL 2p- and wildtype (wt) *XPO1* (n=20), CLL 2p+/*XPO1*wt (n=8) and on CLL *XPO1*mut (n=6). After 24 hours treatment with Fludarabin+Cyclophosphamid+Rituximab (FCR), Ibrutinib, Idelalisib+Rituximab (Ide+R) and Selinexor, cells were stained with Annexin-V and propidium iodide and PCD was assessed by flow cytometry. KPT-301 was used has a negative control.
Results: (i) *XPO1* was found mutated in 23/436 (5.3%) CLL and in 2/30 (6.7%) CLL 2p+; (ii) Selinexor induced PCD in CLL 2p-/*XPO1*wt (35% of PCD). The results were similar in all tested CLL, independently from prognostic factors (del13q, tri12, del11q, del17p, *IGHV* status), while sparing the non leukemic cells from CLL patients or B cells from healthy donors; (iii) CLL *XPO1*mut were significantly resistant to PCD induced by Selinexor (p=0.003). In contrast, the mutations in *XPO1* had no effect in FCR and Ibrutinib PCD induction; (iv) CLL 2p+ cells were resistant to PCD induced by all tested drugs: FCR (p=0.01), Ibrutinib (p=0.003), Ide+R (p=0.004) and Selinexor (p=0.0001).
Summary and Conclusions: Our data show that CLL 2p+ is associated to FCR, Ibrutinib and Ide+R drug resistance. Strikingly, Selinexor, a new XPO1 inhibitor, is unable to induce PCD in CLL 2p+ and/or XPO1mut, which strongly suggests a key role for *XPO1* in the CLL drug resistance associated to the 2p gain. Altogether, our work provide substantial progress in the understanding of the role of *XPO1* in CLL drug resistance and suggests that the assessment of the 2p gain and the mutations in *XPO1* will be considered before to decide a CLL therapy. As 2p gain could be observed in other B malignancies, it is tempting to expand these recommendations before opting for a Selinexor treatment.

P581

A LONGITUDINAL STUDY OF NEXT GENERATION SEQUENCING IN CHRONIC LYMPHOCYTIC LEUKEMIA SHOWS EVIDENCE OF CHEMOTHERAPY-INDUCED MUTAGENESIS AND SNOWBALLING EFFECT DUE TO LOSS OF P53/ATM-MEDIATED DNA REPAIR

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Background: Somatic mutations in multiple genes have been associated with adverse outcome in CLL. However, the causes and interrelationships of such mutations remain unclear.
Aims: To deepen our understanding of the causes and interrelations of the somatic mutations throughout the disease course.
Methods: We analysed CLL samples obtained from 32 patients immediately before (n=10) or after chemotherapy (n=22) for exon mutations in 15 genes (TP53, ATM, NOTCH1, SF3B1, BIRC3, LRP1B, SAMHD1, FBXW7, PCLO, HIST1H1E, XPO1, CHD2, MYD88, POT1 and ZFPM2) using HaloPlex enrichment and Ion Torrent PGM (average depth:2200x, limit of detection:1%).
Results: With SNPs excluded, a total of 60 somatic non-synonymous SNVs &11 indels (VAF:2-98%) from the first 12 genes were identified in 26 of the 32 samples. The most common mutated genes were SF3B1, ATM, TP53, and PCLO found in 11, 10, 9 and 8 cases, respectively. ATM and TP53 mutations did not co-exist in any sample, while most of the SF3B1 (9/11), PCLO (5/8) and other (11/13) gene mutations were found in samples with mutations in either TP53 or ATM. Moreover, both of TP53 and ATM appeared to be the dominant mutant genes in 18 samples, being either the only mutation (in 5 samples) or the one with the highest level of mutant and/or deleted alleles (in 13 multi-gene mutated samples). Strikingly, all but 2 of the 22 samples (91%) from treated patients showed mutations in at least 1 gene compared with only 60% (3/5) in the untreated group (P=0.08). Furthermore, among 15 samples bearing mutations in multiple (2-4) genes, 14 (93%) were from patients who had received chemotherapy, compared with only 1 (7%) in the untreated group (P=0.009). In a longitudinal study, 27 sequential samples before (n=20) or post (n=7) treatment from 22 cases who had somatic mutations already detected were used to study evolution of these mutant clones using the same deep sequencing approach. Including those initially studied, data from a total of 2-3 sequential samples with an average interval of 29

months of each of these 22 cases were compared. Results showed that most (92%) of mutations detected at time of disease progression/drug-resistance existed in samples taken at early stages. But a mutation in ATM, FBXW7, LRP1B and SF3B1 in 5 cases emerged only during disease progression or development of drug resistance. Interestingly, except the one in SF3B1, all other 4 mutations appeared following the expansion of pre-existed either TP53 or ATM mutation. A clear trend of expansion of the mutated subclones was found in majority (94%) of cases. TP53 and ATM were still the most common mutated genes before disease progression or treatment. They were dominant over other mutated genes coexisting in the same clones during clonal evolution. Competition for expansion between subclones bearing mutations in different genes or different mutations in same genes was documented in some cases, suggesting a convergent clonal evolution.
Summary and Conclusions: The observed association between prior treatment and somatic mutations is consistent with the idea that DNA-damaging chemotherapy can be mutagenic *in vivo*. Furthermore, the high frequency of mutations in TP53 and ATM, their association with other mutations and their dominance over these other mutations during the course of CLL progression suggests the existence of a "snowballing effect" in which defective DNA repair due to loss of ATM/p53 function facilitates the acquisition of additional mutations. In addition, this study provided evidence supporting that convergent evolution of CLL clone plays a role in CLL progression.

P582

Abstract withdrawn

P583

TP53 MUTATIONS ARE EARLY EVENTS IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) DISEASE PROGRESSION AND PRECEDE CLONAL EVOLUTION TO COMPLEX KARYOTYPES

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Background: *TP53* abnormalities lead to resistance to purine analogues and are found in over 40% patients with refractory CLL. At diagnosis, no more than 5% of CLL carry 17p deletion and most cases harbour mutations within the other *TP53* allele. Incidence of *TP53* mutations as sole alteration is also approximately 5%, but dependent on the sensitivity of the technique. Complex karyotype (more than 2 abnormalities) has recently been considered as a stronger adverse prognostic factor than presence of *TP53* alteration. However, there are no longitudinal studies examining simultaneously the presence of 17p deletion, *TP53* mutations and karyotype abnormalities in the same patients.
Aims: Assess the sequence of events of clonal evolution through the longitudinal study of a cohort of high risk patients. Determine the impact of minor *TP53* mutated clones by NGS in a routine setting.
Methods: We conducted a retrospective longitudinal study of 33 high risk CLL (defined by either *TP53* alteration at baseline or relapsed/refractory disease). Del17p was detected by FISH and *TP53* mutations by Sanger sequencing of exons 4 to 9 as well as by NGS (Ion Torrent and Illumina). Two to five samples per patient over 4.9 years (1.7-10.7 years) were studied. An excellent correlation between Illumina and Ion Torrent technologies was obtained.
Results: A *TP53* abnormality was found in 55% (17/33) of the patients at any time point of progression. At diagnosis, only 7/17 patients carried a del17p among which 5 harboured a *TP53* mutation on the other allele. In 3 additional cases, *TP53* mutation was detected as sole alteration. The use of NGS technology allowed the detection of a minor *TP53* mutated clone (3.5% to 20% allele frequency) in 4 additional cases. At diagnosis, only 4/17 cases had a complex karyotype, and all of them had a *TP53* mutation. During progression, clonal evolution was found in 11 patients: i) 17p deletion was detected at a later time point in 7 patients, 5 of which had a previous *TP53* mutation, ii) A novel *TP53* mutation was detected in 3 cases and an additional subclonal *TP53* mutation in 4 patients iii) in 8 patients with non-complex karyotype at diagnosis, a complex karyotype was found during progression. All of them had a *TP53* mutation preceding the evolution to a complex karyotype. In this group of *TP53* mutated cases, 12/17 cases harboured a complex karyotype at some point, as compared to only 4/15 cases in the group without *TP53* alteration (among these 2 had del11q)(p=0,01). Regarding treatment, two patients showed clonal evolution while untreated. In 4/6 patients, we observed an expansion of the TP53 mutated clone after fludarabine-based regimen. Interestingly, in all 4 patients receiving ibrutinib, the allelic burden of *TP53* mutation remained the same after 1 year.
Summary and Conclusions: Presence of *TP53* mutation identifies patients with genomic instability. Small mutated clones are present at an early stage of the disease and precede other signs of clonal evolution, most importantly the development of a complex karyotype. 17p deletion is either a later event, or detected later because of a lower sensitivity of the FISH technique. Complex karyotype occurs as clonal evolution event in patients with preexisting *TP53* mutated (sub)clones, and therefore in itself may not be associated with poor prognosis when *TP53* can be assessed by a sensitive technique such as NGS. NGS allows early detection of *TP53* mutated clones, and is achievable on a routine basis with a dedicated sequencing assay. We strongly recommend early and iterated detection of *TP53* mutations in CLL progressive cases.

P584

EXPRESSION OF DNA HYDROXYMETHYLATION-RELATED GENES IS ASSOCIATED WITH PROGNOSIS IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Epigenetics plays a crucial role in cancer physiopathology. DNA hydroxymethylation is catalyzed from methylated DNA by Ten Eleven Translocation (TET) enzymes requiring co-factors produced by Isocitrate Dehydrogenase (IDH) proteins. This modification could be a step in the demethylation process and could thus have an impact on gene expression. TET2 mutations have already been reported in several leukemias. However, little is known about hydroxymethylation in B-chronic lymphocytic leukemia (CLL).
Aims: We intend to characterize the mRNA expression of enzymes (TET1, 2, 3, IDH1 and 2) involved in DNA hydroxymethylation in purified B cells obtained from CLL patients.
Methods: Expression of TET1, 2, 3, IDH1 and 2 mRNA was assessed by qPCR on purified leukemic B-cells from a cohort of 214 CLL patients with a median follow-up of 75 (6-380) months and compared with those of purified peripheral normal B-cells. The influence of CLL microenvironment on TET enzyme expression, was investigated by culture of CLL cells (n=10) in the presence or absence of bone marrow mesenchymal stromal cells (BMSC).
Results: TET1, 3 and IDH2 are underexpressed in leukemic B-cells compared with healthy volunteers B-cells (P=0.0221, 0.0013, <0.0001 respectively) while IDH1 is overexpressed (P=0.0037). Expression of TET2 is similar in both groups. When we stratified patients according to low and high expression, TET2 and IDH1 significantly predict treatment-free survival (TFS): patients with high TET2/IDH expression had a median TFS of 110 months while patients with low expression presented a median TFS of 78 months (P=0.0071/0.0123). Finally, we observed a decreased TET1 expression (P=0.0371) and an increased TET3 (P=0.0273) and IDH2 expression (P=0.0039) in CLL-cells after co-culture with BMSC. Further analysis in 14 CLL patients shows that ZAP70+ patients present higher hydroxymethylated DNA than ZAP70- patients.
Summary and Conclusions: This is the first report that DNA hydroxymethylation enzymes are deregulated in B-CLL compared to normal B cells: 1) TET2 and IDH1 overexpression have a good prognostic value; 2) TET1, TET3 and IDH2 expression is modulated by microenvironment interactions, resulting in CLL cells survival. Our observations suggest that DNA hydroxymethylation plays a major role in CLL physiopathology and support the potential therapeutic benefit of agents targeting epigenetics in CLL patients.

P585

SF3B1 MUTATIONS AND ALTERATIONS OF FOXP1 TRANSCRIPTION IN CHRONIC LYMPHOCYTIC LEUKAEMIA

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Background: Spliceosome mutations have been described in various myeloid and lymphoid malignancies. In chronic lymphocytic leukaemia (CLL), SF3B1 is mutated in a variable proportion of cases, depending on the studies, ranging approximately from 5 to 20% and the presence of these mutations correlates with an adverse prognosis. Recently, a modification in the species of FOXP1 transcripts, consisting in the appearance of a truncated variant, has been described in 3 patients with SF3B1 K700E or N626Ymutation (Quesada Nat Genet 2012).
Aims: In this work, we aimed to study the transcription profile of FOXP1 in a series of CLL patients, and in control samples. In a second step we investigated a validation cohort, and studied the sensitivity of the technique.
Methods: Peripheral blood mononuclear cells (PBMC) were obtained from 10 normal subjects and 60 patients with CLL after informed consent. The expression of the FOXP1 truncated and the full length transcripts was studied by real-time quantitative PCR. SF3B1 mutations were detected by Sanger sequencing of the exons 14, 15, and 16.

Results: We selected 32 patients with at least one adverse prognostic factor (unmutated IGHV, CD38 +, ZAP-70 +), explored SF3B1 by Sanger sequencing and found 4 mutated cases. Then we studied FOXP1 transcription profile in 10 control samples, the 4 SF3B1 mutated samples and 6 additional CLL samples with wild type SF3B1. The FOXP1 truncated transcript was found at a very low level of expression in the control samples contrasting with the high level measured in the SF3B1 mutated samples, finally the expression of FOXP1 truncated variant in unmutated samples was similar to that observed in controls. In a validation cohort, including 28 patients with the same selection criteria and/or the presence of 11q deletion, we performed the FOXP1 study and SF3B1 sequencing. We found an overexpression of the FOXP1 truncated transcript in 8 patients. SF3B1 mutations were identified in 7 of these patients whereas no mutation was detected in samples with low FOXP1 truncated variant expression. Most of the cases with SF3B1 mutations and overexpression of the FOXP1 truncated variant presented an 11q deletion, however the majority of the samples tested with 11q deletion were not SF3B1 mutated. Overall, in the 11 mutated samples, we identified five different mutations of SF3B1 (3 Y623C, 1 R625H, 3 K700E, 1 G740V and 3 G742D). Finally, using dilutions of mutated sample in unmutated sample, we evaluated the sensitivity of the test at approximately 5%.
Summary and Conclusions: In this study, we investigated a cohort of patients and controls to evaluate the impact of various SF3B1 mutations on the splicing of FOXP1 transcripts. A high expression of the FOXP1 truncated variant was observed in any case with SF3B1 mutation. Therefore, FOXP1 transcription profile could become an alternative tool to identify functionally relevant mutations in the SF3B1 gene. Moreover, we described 4 additional mutations of SF3B1 (affecting the 3 exons tested) that impact FOXP1 splicing. Finally, it is of major interest to explore the FOXP1 transcription variants regarding their functions and their involvement in the mechanisms leading to CLL progression.

P586

ADDITIONAL TRISOMIES AMONGST PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA CARRYING TRISOMY 12: THE PARTNER CHROMOSOME MAKES A DIFFERENCE

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Background: Trisomy 12 (+12), the second most frequent recurrent chromosomal aberration in chronic lymphocytic leukemia (CLL), is associated with clinical and biological heterogeneity. We and others previously reported a minor fraction of +12 cases with co-existence of trisomy 19 displaying distinctive clinicobiological characteristics. However the respective cohorts were small, precluding definitive conclusions.
Aims: Detailed characterization of cases with multiple trisomies in a large series of CLL patients carrying +12, aiming to identify distinctive profiles that would assist in further dissecting the heterogeneity of this major cytogenetic subgroup.
Methods: Within a multi-institutional series of 4486 CLL patients with available classic cytogenetic data, we identified 713 cases (16%) with trisomy 12, of whom 87 (12%) harbored multiple trisomies. Sixty-eight of these 87 cases (78%) had co-existing +19 (group A: +12,+19 CLL), while the remaining 19/87 cases (22%) had other co-existing extra chromosome(s) (group B: +12,+other trisomy CLL). The median time from diagnosis to karyotype analysis was 1.5 months (range, 0-194); the great majority of cases with available treatment/survival information were untreated prior to testing (73/78 cases, 94%). FISH data for the Döhner model aberrations was available in 67/87 (77%) cases. Within the same CLL series, we identified 68 cases with isolated +12 and available clinicobiological data, which were used as a control group for the survival analysis (group C).
Results: In group A, 43/68 (63%) cases also harbored +18. Extra structural abnormalities were detected in 12/63 (18%) cases, mostly concerning deletions and/or translocations involving the 13q chromosome in 7/12 (58%) cases. Interestingly, the great majority of cases harboring extra structural abnormalities (10/12, 83%) displayed +12+18+19 highlighting an association between +18 and clonal evolution. In contrast, no structural abnormalities were observed in group B, within which trisomy 3 predominated, being detected in 8/19 (42%) cases, followed by trisomies 18 and 22 in 6 (32%) and 4 (21%) cases respectively Moreover, different clinicobiological profiles were observed between the two groups. In particular, group A displayed (i) higher incidence of M-CLL (42/45, 95% *vs* 6/8, 75%, p=0.045); (ii) younger median age at diagnosis (59 years *vs* 67 years, p=0.007); (iii) ubiquitous surface IgG expression (23/23, 100% *vs* 2/8, 25%, p<0.0001); (iv) higher incidence of del(13q) (27/51, 53% *vs* 0/11, 0%, p=0.0002); and (v) higher incidence of monoclonal gammopathy (23/37, 62% *vs* 2/8, 25%, p=0.05). Group B displayed higher incidence of: (i) clinical/laboratory autoimmunity (5/10, 50% *vs* 2/33, 6% in group A, p=0.0009); and (ii) other malignancies (6/11, 55% *vs* 4/48, 8% in group A, p=0.0002). Furthermore, each group displayed significant differences (p<0.05) when compared to control group C regarding the aforementioned features. Regarding survival analysis, Groups A and B exhibited longer overall survival compared to Group C (median OS: not reached for Groups A and B *vs* 8 years in Group C; log-rank test, group A *vs* C, p=0.08, and group B *vs* C, p=0.036).
Summary and Conclusions: The presence of extra trisomies defines subgroups of cases with distinctive clinicobiological profiles within +12 CLL cases, further highlighting their heterogeneous biological background and clinical outcome.

P587

MUTATIONS IN THE TP53 GENE SHOW FEATURES OF SOMATIC HYPERMUTATION PROCESS WITH PROMINENT DIFFERENCE BETWEEN IGHV MUTATED AND UNMUTATED CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Introducing somatic hypermutations (SHM) into genes coding for heavy and light chains of immunoglobulins (*IGHV*, *IGLV*) is a physiological process indispensable during antibody maturation. SHM introduction is a two-step process, which in the first step involves deamination of cytosine to uracil by an enzyme Activation-Induced (Cytidine) Deaminase (AID). During the second step, uracil and potentially also flanking nucleotides are removed and error-prone DNA polymerases (mainly polymerase eta) are recruited to fill the gap. Off-targeting of AID may result in mutations in non-immunoglobulin genes, including tumor suppressor gene *TP53*.
Aims: We explored the *TP53* mutation patterns with regards to SHM features emphasizing the differences between mutations occurring in *IGHV* unmutated (U-CLL) and *IGHV* mutated (M-CLL) groups of patients.
Methods: In order to reduce the selection bias, we used the set of *TP53* mutations detected using ultra-deep Next Generation Sequencing (NGS) with high sensitivity (0.2%) allowing to consider the minor subclonal mutations with less prominent selective advantage. For NGS analysis, Illumina Miseq platform has been used with average coverage 34788; (range 1674-177021). For variant detection, an in house bioinformatics pipeline was established. Statistical evaluation was performed by shearwater algorithm computing Bayes classifiers based on a betabinomial model. Only point substitutions were taken into account. Altogether, 464 *TP53* mutations in 73 high risk CLL patients were analyzed. 121 mutations were found in M-CLL cases and 343 mutations were detected in U-CLL patients. The high number of mutations should be attributed to the high number of minor subclonal mutations detected mainly in patients in relapse after treatment. In 60% of patients, results from repeated examinations were included (2-4 examinations per patient; each mutation detected in more than one consecutive sample was considered only once). 72.6% of patients received treatment before the first or during consecutive analyses.
Results: U-CLL showed a higher proportion of mutations in C:G pairs comparing to M-CLL (66% *vs* 51% of all mutations; P=0.003). Out of these, G:C>A:T substitutions, the primary result of AID cytidine deamination, were the prevalent events observed in U-CLL but not M-CLL (59.6% *vs* 40.3% of all C:G mutations; P=0.009). Focusing on the AID-targeted sequence motif RGYW/WRCY, no difference between the two subgroups was observed (2.5% *vs* 3.1%; P=ns). However, we found a significant overrepresentation of mutations in GNW motif in U-CLL (14.58% of all mutations *vs* 6.61% in M-CLL; P=0.025). GNW is a strand-biased motif derived from RGYW most frequently targeted by AID in *IGLV* genes. On the other hand, *TP53* mutations detected in M-CLL cases showed the features of targeting by polymerase eta: (i) frequent targeting of A:T pairs (49% *vs* 34% in U-CLL; P=0.003); with prominent

strand bias favoring A over T (13.8 fold *vs* 3.1 fold in U-CLL; P=0.002; (ii) prevalence of mutations in WA/TW motifs (40.5% *vs* 23.91% in U-CLL; P<0.0001) with strand bias favoring WA observed in both groups, but extremely prominent in M-CLL (23.5 fold in M-CLL *vs* 4.37 fold in U-CLL; P=0.014).
Summary and Conclusions: We documented the significantly different patterns of *TP53* mutations in U-CLL *vs* M-CLL. Mutations detected in U-CLL showed the features corresponding to the first step of SHM process: deamination by AID with strand bias possibly attributable to AID activity on the transcribed strand. In contrast, spectra of mutations detected in M-CLL cases suggested the more prominent involvement of polymerase eta during the second SHM step. *Supported by CZ.1.07/2.3.00/30.0009, CZ.1.05/1.1.00/02.0068, NT13519, NT13493, NT11218, MUNI/A/1180/2014, NGS-PTL/2012-2015/no.306242, MSMT-CR (2013-2015, no.7E13008)*

Chronic lymphocytic leukemia - Clinical 2

P588

SAFETY OF IDELALISIB IN B-CELL MALIGNANCIES: INTEGRATED ANALYSIS OF EIGHT CLINICAL TRIALS

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Background: Idelalisib (Zydelig), a first-in-class, selective, oral inhibitor of PI3Kδ, is approved in the US and EU for the treatment of chronic lymphocytic leukemia (CLL) in combination with rituximab and as monotherapy for patients with follicular lymphoma who have received at least two prior systemic therapies.
Aims: To further characterize the safety profile of idelalisib, we integrated adverse event data from 8 clinical trials.
Methods: The analysis included 760 subjects with CLL, indolent non-Hodgkin lymphoma, or other B-cell malignancy who received IDELA alone (doses=50 mg BID to 350 mg BID) or as part of a combination regimen (IDELA doses=100 or 150 mg BID). Most subjects were heavily pre-treated with relapsed disease.
Results: Common adverse events (AEs) are presented in the Table 1, along with important laboratory results. AEs leading to dose modification included transaminase elevations (13%), diarrhea/colitis (11%), and rash (5%); discontinuations due to these AEs were infrequent (3%, 5%, and 2%, respectively); dose interruption allowed successful re-challenge in most patients. Pneumonitis occurred in 2.3% (monotherapy) and 3.9% (combo therapy) of subjects. Grade ≥3 diarrhea/colitis had a later onset (peak incidence of 9% between 6-12 mo.); other AEs occurred most often in the first 6 months and declined thereafter.

Table 1.

	Monotherapy N = 354		Combo therapy N = 406	
N (%)	Any Gr.	≥ Gr. 3	Any Gr.	≥ Gr. 3
Adverse Events				
Diarrhea/colitis	131 (37)	38 (11)	161 (40)	68 (17)
Pyrexia	96 (27)	7 (2)	169 (42)	47 (12)
Nausea	91 (26)	5 (1)	125 (31)	30 (7)
Cough	80 (22)	3 (1)	118 (29)	21(5)
Rash	60 (17)	7 (2)	99 (24)	30 (7)
Chills	49 (14)	0	86 (21)	23 (6)
Pneumonia	47 (13)	40 (11)	74 (18)	56 (14)
Vomiting	53 (15)	5 (1)	60 (15)	18 (4)
Dyspnea	43 (12)	7 (2)	68 (17)	20 (5)
Laboratory Abnormalities				
Neutropenia	162 (46)	83 (23)	234 (58)	151 (37)
Anemia	102 (29)	18 (5)	145 (36)	34 (8)
Thrombocytopenia	94 (27)	37 (11)	143 (35)	50 (12)
Transaminase increase	176 (50)	56 (16)	190 (47)	53 (13)

Summary and Conclusions: As well-characterized in this large dataset, IDELA has an acceptable safety profile.

P589

PHASE I, FIRST-IN-HUMAN TRIAL OF BI 836826 (AN ANTI-CD37 ANTIBODY) IN PATIENTS (PTS) WITH RELAPSED/REFRACTORY (R/R) CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

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Background: BI 836826 is a novel Fc-engineered IgG1 type II antibody targeting CD37, a tetraspanin predominantly expressed on normal and malignant B-cells, with a dual cytotoxic mode of action by directly inducing apoptosis and increasing antibody-dependent cellular-cytotoxicity. *Ex vivo*, BI 836826 treatment results in a significantly greater depletion of CLL cells than rituximab, irrespective of genetic risk. We report preliminary results of an ongoing Phase I, first-in-human, dose-escalation study of BI 836826 in pts with R/R CLL (NCT01296932; 1270.1).
Aims: To investigate the maximum tolerated dose (MTD), safety, pharmacokinetics, and efficacy of BI 836826 monotherapy in pts with R/R CLL.
Methods: Pts with R/R CLL after ≥2 prior therapy lines were eligible if they had adequate organ function, neutrophils ≥1000/µL and platelets ≥25000/µL. Increasing doses of intravenous (IV) BI 836826 (1-400 mg) were administered as a rate-controlled IV infusion in a modified 3+3 design. In Course 1, 10% of the total BI 836826 dose (not exceeding 10 mg) was administered on day 1 with the total dose remaining administered on day 2 of a 14-day course. In subsequent courses, BI 836826 was administered on day 1. The MTD will be determined based on dose-limiting toxicity (DLT; defined as any drug-related non-hematologic adverse event [AE] ≥grade 3, except infusion-related reactions [IRRs]) observed during the first treatment course. All pts provided written informed consent.
Results: To date, 33 pts (mean age 66.2 yrs; 70% male) have been treated with BI 836826 (1 mg [n=3], 3 mg [n=3], 9 mg [n=6], 25 mg [n=6], 50 mg [n=3], 100 mg [n=3], 200 mg [n=6], 400 mg [n=3]). Dose escalation is ongoing. At screening, 12 pts (36.4%) were Binet stage C or Rai III/IV. Pts had received a median of 4 (range 2-10) prior therapy lines. Molecular genetics at baseline were del(17p) and/or TP53 mutation in 63.6%, and del(11q) in 29.0%; 72.7% had unmutated IGHV. Four treatment courses were planned, 18 pts (54.5%) completed 8 courses. Five pts (15.2%) stayed on therapy beyond 8 courses due to sustained clinical benefit. One pt in the 200 mg cohort had a DLT of grade 3 hypophosphatemia. Dose escalation is ongoing and the MTD has not yet been determined. The most frequent AEs were IRRs (66.7%), chills (60.6%), pyrexia (51.5%), anemia (42.4%), thrombocytopenia (42.4%), neutropenia (39.4%), diarrhea (36.4%), and nausea (33.3%). Drug-related AEs included IRRs (66.7%), chills (57.6%), pyrexia (48.5%), neutropenia (36.4%), and thrombocytopenia (27.3%). Serious AEs included pyrexia (9.1%), neutropenia, leukopenia, cardiac failure, and IRRs (all 6.1%). PK exposure increased with increasing doses and short half-lives were observed. Of 26 evaluable pts (*i.e.*, received full intended dose) at ≥9 mg doses, the overall response rate based on investigator assessment was 42.3% (all partial remission); 14 pts (53.8%) had stable disease. Of 23 pts with elevated baseline counts at ≥9 mg doses, a ≥50% lymphocyte reduction was observed in 78.3% and a reduction to <4000/µL was observed in 52.2%, including pts with high-risk genetic features.
Summary and Conclusions: Promising responses have been observed with BI 836826, even at low doses. The safety profile was acceptable with AEs predominantly IRRs and cytopenias which were manageable with routine supportive care.
These data support further clinical evaluation of BI 836826 as a single agent and in combination.

P590

TELOMERE LENGTH AND POT1 MUTATIONS IN CLL: INCIDENCE, ASSOCIATIONS AND CLINICAL IMPACT IN THE COMPLEMENT 1 TRIAL

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Background: *POT1* mutations in CLL have been reported to be functionally relevant by a dominant negative effect, leading to uncapping of the telomeric ends, thereby enabling telomerase to aberrantly elongate the telomeres, causing fusion events and chromosomal aberrations. However, their impact on telomere length and outcome has not been established in a clinical trial cohort.
Aims: To study the impact of *POT1* mutations on telomere length and disease outcome in a clinical trial cohort.
Methods: We assessed telomere length associations with disease characteristics, especially with that of *POT1* mutations in the Complement 1 (OMB110911) trial (1st line chlorambucil (Chl) *vs* ofatumumab-Chl (O-Chl)) in patients considered inappropriate for fludarabine-based therapy. Telomere length was analysed using quantitative PCR from baseline samples of 368 patients (82.3%) with available DNA and this cohort was representative of the full trial population with regard to baseline characteristics. Validation of the technique was done using terminal restriction fragment length analysis (TRF) (R^2=0.859, P<0.001) in an independent control sample set (n=18) and 6 of these samples were included in every batch as controls.
Results: Analysis of telomere length associations was performed by dichotomizing the cohort based on the median telomere length (4.53kb). No significant association of telomere length was found with the clinical characteristics age, sex, Binet stage and ECOG status while short telomeres were significantly associated with high WBC count (P<0.001), high β2-MG (P<0.001), and high CIRS score (P<0.01). As previously reported in a similar trial (CLL8, Jebaraj *et al.*, ASH 2013), short telomeres were significantly associated with other adverse prognostic factors namely, unmutated *IGHV* (P<0.001), 17p- (P<0.04) and 11q- (P<0.001). Also gene mutations in *NOTCH1* (P<0.001), *SF3B1* (P<0.02), *TP53* (P<0.02), *ATM* (P<0.001) and *BIRC3* (P<0.01) were all significantly associated with short telomeres. *POT1* mutations were found in 7.6% (n=28 of 368 cases) cases in Complement1 and the mutations were predicted to be dominant negative in function. *POT1* mutations were not significantly associated with any of the clinical characteristics or genomic aberrations. Interestingly, there was also no significant association of telomere length with *POT1* mutations (4.76 kb *vs* 5.34 kb, P=0.68). At a median follow-up of 31.7 months, there were 245 (67%) events for progression free survival (PFS) and 60 (16.3%) events for overall survival (OS). Incidence of *POT1* mutations (Chl: n=16, O-Chl: n=12, P=0.70) and short telomeres (Chl: n=95, O-Chl: n=89, P=0.83) were balanced in both treatment arms. Analysis of the impact of *POT1* mutations showed no significant association with remission rate, PFS and OS. In contrast, short telomeres were found to be significantly associated with reduced overall response (72.0% *vs* 87.6%, P<0.001), shorter PFS (HR=1.98, P<0.001, Figure 1) and OS (HR=1.88, P=0.02). To evaluate their independent prognostic impact, we performed multivariable analysis by Cox regression including as variables the treatment arms, 11q-, +12q, 17p-, mutation status of *IGHV, TP53*, *NOTCH1, SF3B1, BIRC3, MYD88, ATM, FBXW7, POT1* and telomere length. For PFS, O-Chl (HR 0.43, P<0.001), 17p- (HR 3.01, P=0.001), *NOTCH1* (HR 1.45, P=0.04), *TP53* (HR 1.78, P=0.04 and telomere length (HR 1.71, P=0.001) were identified as independent factors, while for OS, only 17p- had an adverse prognostic impact (HR 3.90, P<0.01).

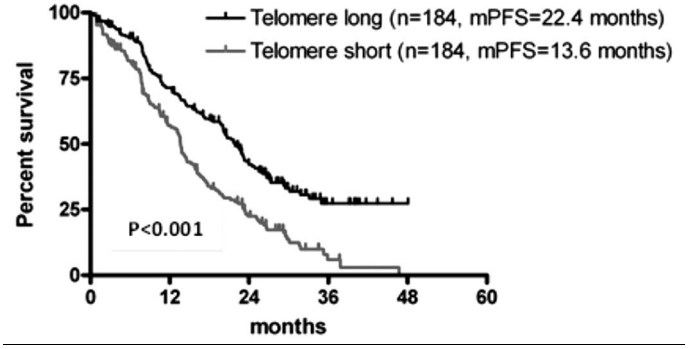

Figure 1. Progression free survival.

Summary and Conclusions: In the Complement1 trial short telomeres were found to be significantly associated with various adverse clinical and biological prognostic disease characteristics and poor outcome, including PFS and OS, but had no association with *POT1* mutations. There was no association between *POT1* mutations and clinical outcome.

P591

PREVALENCE AND CHARACTERISTICS OF CENTRAL NERVOUS SYSTEM (CNS) INVOLVEMENT BY CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

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Background: Clinically significant "extramedullary" involvement by CLL is rare, with only 192 cases reported in literature between 1975 and 2012. Although the CNS has been the most commonly reported site, either as CLL or Richter Transformation (RT), the available information is derived exclusively from case reports of individual patients.
Aims: To determine the prevalence and characteristics of CNS involvement in CLL.
Methods: We used the Mayo Clinic CLL database to identify all patients with CLL/small lymphocytic lymphoma (SLL) who were followed at our center between 07/1997 and 11/2014 and who underwent an MRI of the head or of the spine because of neurological symptoms.

Results: Of 4317 patients with CLL followed at Mayo between 07/1997 and 11/2014, 1115 (26%) had an MRI of the head or spine to evaluate neurologic symptoms. Of these, 47 (1%) had radiological findings indicative of CNS involvement by a lymphoproliferative neoplasm (21 leptomeningeal involvement, 26 parenchymal brain involvement). Of these 47 patients, 24 underwent concomitant lumbar puncture (LP) and tissue biopsy, 5 tissue biopsy only, 13 LP only, and 5 had no tissue obtained. Tissue biopsy revealed CLL infiltration in 11 cases, infiltration by RT in 9 cases (diffuse large B-cell lymphoma [DLBCL] in 8 cases, peripheral T-cell lymphoma in 1 case), other etiologies in 6 cases (2 progressive multifocal leukoencephalopathy, 2 vasculitis, 3 primary brain tumors), and normal brain tissue in 2 cases. Of the 13 patients with MRI abnormalities who underwent a LP only, 4 demonstrated clinically significant CLL involvement. Collectively, 24 (0.5%) of 4317 CLL patients cared for at our center over a 17 year interval developed clinically significant CNS involvement by CLL or RT.
Summary and Conclusions: Although neurologic symptoms are frequent in patients with CLL/SLL and prompt diagnostic MRI imaging in up to 26% of CLL cases, radiographic findings suggestive of CNS involvement by CLL or RT are rare (about 1% of patients). Tissue confirmation with brain biopsy is necessary to confirm the diagnosis of lymphoproliferative neoplasm and the subtype (CLL *vs* DLBCL) in such cases. Clinically significant CNS involvement by CLL or RT is a relatively rare event, observed in 0.5% of CLL patients (~1 of every 200 patients).

P592

MAKING THE RIGHT CHOICE: THE ROLE OF FITNESS STATUS IN THE MANAGEMENT OF FIRST-LINE TREATMENT IN CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS (CLL FITNESS STUDY)

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Background: While fludarabine+cyclophosphamide+rituximab (FCR) has shown a survival advantage in CLL, it cannot be considered the standard of care for all patients, due to prolonged cytopenias and increased susceptibility to infections in particular among the elderly. For these reasons, FCR is the treatment of choice for young fit patients with CLL, while recent studies have demonstrated that elderly patients with comorbidities may benefit from chlorambucil, in association with anti-CD20 antibodies. How these findings translate into everyday care is still to be defined and several factors have been proposed, but none validated, in helping physicians make better decisions.
Aims: We designed an investigator-driven, multicenter, observational, retrospective study based on the review of medical records. The study aimed at defining the most relevant parameters when choosing the first-line treatment in clinical practice and how these parameters associate with treatment tolerance.
Methods: The target population included patients with CLL, requiring first-line treatment at participating centers between Jan 1, 2009 and Dec 31, 2010. Information on global health status, disease parameters, treatment and follow-up was collected.
Results: Four hundred and forty eight patients from 37 Italian sites were evaluated. Eight participating centers treated ≥10 CLL patients per year in the front line setting, 12 centers 5-10 and 14 centers <5. The median age was 66 years, with a male:female ratio of 1.5:1. The most frequent treatment choices -FCR (195) and alkylating agent (AA) as monotherapy (79 patients)- were further analysed. FCR- and AA-treated patients were significantly different in terms of baseline features and, not surprisingly, FCR-treated patients showed younger age, less comorbidities and better ECOG performance status. Investigators reported age as the most relevant parameter in selecting treatment, followed by ECOG PS, comorbidities (based on clinical evaluation) and disease burden. Seventy one percent of FCR-treated patients received the planned number of cycles, but 53% required dose reduction or delay. At multivariate analysis, age was the only factor able to predict tolerance to FCR (p=0.032), with CIRS index showing a trend to significance (p=0.058). In 70% of cases discontinuations and reductions of FCR were related to toxicities. The most frequent AEs included neutropenia (36%), general (17%) and gastrointestinal disorders (14%), infections (11%), thrombocytopenia (8%) and anemia (5%). More than 90% of physicians who chose FCR, after evaluating response and tolerance, would choose again the same first-line therapy.
Summary and Conclusions: Our data suggest that age, together with the comorbidity burden, is widely used for the treatment choice in both FCR and AA-treated patients, remains the best predictor of tolerance to therapy and accurately guides first-line treatment in CLL patients. In the AA-group functional and mental status have also some relevance, while the biologic profile was less significant. The use of FCR was associated with a lower frequency and grade of infections than reported, reasonably due to a higher awareness of the potential toxicity among hematologists. A broader use of infectious prophylaxis as well as a younger median age of the patients receiving FCR in our study as compared to CLL8 most likely accounts for the lower incidence of hematologic toxicities recorded. In the general practice, the use of FCR is becoming safer likely due to an improved a priori selection of the target population and a better management of the side effects.

P593

EFFICACY OF PHOSPHATIDYLINOSITOL-3 KINASE INHIBITORS WITH DIVERSE ISOFORM SELECTIVITY PROFILES FOR INHIBITING THE SURVIVAL OF CHRONIC LYMPHOCYTIC LEUKEMIA CELLS

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Background: Pharmacological inhibition of phosphatiylinositide-3-kinase (PI3K)-mediated signaling holds great promise for treating chronic lymphocytic leukemia (CLL). PI3K inhibitors exhibit various degrees of isoform specificity, which influences their effectiveness on CLL cells. Specifically targeting the p110-δ isoform, which dominates B cell receptor signaling and is preferentially expressed in CLL cells is an attractive treatment option.
Aims: Three structurally related PI3K inhibitors were investigated that target the PI3K-δ isoform for their ability to inhibit the survival of CLL cells *in vitro*. The purely PI3K-δ-selective inhibitor idelalisib was compared to copanlisib (BAY 80-6946) and duvelisib (IPI-145), with isoform target profiles that additionally include PI3K-α or PI3K-γ, respectively. In addition, the direct cell killing capacity of PI3K inhibitors in combination with CD20 antibodies was assessed as well as interference with antibody-dependent cell mediated cytotoxicity.
Methods: Freshly isolated B lymphocytes and PBMC's from CLL patients as well as CLL-derived JVM-3 cells were treated *in vitro* for 48 hours with copanlisib, duvelisib and idelalisib. Annexin/7-ADD negative cells were subsequently determined by flow cytometry. In addition, migration of primary CLL cells towards SDF-1 upon inhibitor treatment was determined as an index of migrated cells in a two-chamber based assay. Survival-inhibition of inhibitor-treated CLL cells in coculture with bone marrow stromal cell line HS-5 was also determined by flow cytometry.as well as combination treatments with CD20 antibodies. Interference of ADCC through inhibitors was assessed by measuring LDH release after 4 hour co-cultivation of JVM-3 cells as target and healthy donor PBMC's as effector cells.
Results: The concentrations leading to halfmaximal reduction of the survival of CLL cells were more than ten-fold lower for copanlisib than for idelalisib and duvelisib. At concentrations reflecting the biological availability of the different inhibitors, high levels of apoptotic response among CLL samples were attained more consistently with copanlisib than with idelalisib. Copanlisib selectively reduced the survival of CLL cells compared to T cells and to B cells from healthy donors. In addition copanlisib and duvelisib impaired the migration of CLL cells towards CXCL12 to a greater extent than equimolar idelalisib. Similarly copan-

lisib and duvelisib reduced the survival of CLL cells in co-cultures with HS-5 cells more strongly than idelalisib. Survival inhibition by copanlisib and idelalisib was enhanced by the monoclonal CD20 antibodies rituximab and obinutuzumab (GA101), while antibody-dependent cellular cytotoxicity mediated by alemtuzumab and peripheral blood mononuclear cells was not substantially impaired by both PI3K inhibitors for the CLL-derived JVM-3 cell line as target cells.
Summary and Conclusions: Among the investigated PI3K-δ-selective inhibitors, the cytotoxicity for CLL cells of copanlisib by far surpassed that of idelalisib and duvelisib. Targeting the α- and δ- p110 isoforms with copanlisib may be therefore a useful strategy for the treatment of CLL and warrants further clinical investigation. Taken together, targeting p110-δ showed promising clinical efficacy for CLL treatment, but could be further improved by PI3K inhibitors showing more potent direct tumor cell killing than idelalisib.

P594

HEALTH RELATED QUALITY OF LIFE AND PATIENT REPORTED OUTCOMES OF OFATUMUMAB PLUS CHLORAMBUCIL *VERSUS* CHLORAMBUCIL MONOTHERAPY IN THE COMPLEMENT1 TRIAL

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Background: The COMPLEMENT1 study has demonstrated a statistically significant improvement in PFS in patients who are treated with ofatumumab plus chlorambucil in the first line setting for CLL over chlorambucil monotherapy and the combination therapy was well tolerated. Given that ofatumumab was added to chlorambucil, it is important to consider the impact of ofatumumab on health related quality of life (HRQoL).
Aims: Given that ofatumumab was added to chlorambucil, it is important to consider the impact of ofatumumab on health related quality of life (HRQoL).
Methods: During the COMPLEMENT1 trial, the QLQ-C30 and the QLQ-CLL16 patient questionnaires were administered in patients during treatment, during the follow up stage and at the time that progression was identified. The primary specified patient reported outcomes were health related quality of life and fatigue from the QLQ-C30 questionnaire.
Results: In HRQoL, there was no significant difference (p=0.67) or clinically relevant difference between the arms at any time point measured during treatment. In the fatigue scale, there was similarly no significant differences noted between the arms (p=0.17) during treatment. This trend of no clinically significant changes in HRQoL and fatigue continued during follow-up (p=0.49 and p=0.10 respectively). A summary of HRQoL scores measured on a scale of 0 to 100 with 100 being the maximum HRQoL can be seen in Figure 1.

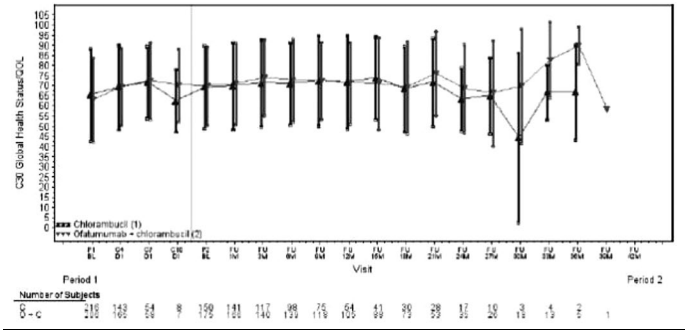

Figure 1.

Summary and Conclusions: These results demonstrate that adding ofatumumab to frontline chlorambucil therapy do not result in any perceptible changes in HRQoL or fatigue, with any adverse events having minimum impact. This is consistent with the adverse event profile of ofatumumab in the COMPLEMENT1 study, which demonstrated high tolerability irrespective of age and fitness.

P595

GENOMIC PROFILE OF CHRONIC LYMPHOCYTIC LEUKEMIA IN KOREA: ETHNIC DIFFERENCE IN RECURRENT MUTATIONS IDENTIFIED BY TARGETED EXOME SEQUENCING

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Background: Chronic lymphocytic leukemia (CLL) is the most common leukemia in western countries, while it is extremely rare in Asian countries. Yet, genetic profiles of CLL have not been reported in Asian.
Aims: Aim of our study is to characterize the genomic profiles of Korean CLL and to find out ethnic differences in somatic mutations with prognostic implications.
Methods: A total of 71 patients with CLL was enrolled (median age at diagnosis 61 year, range 23-81). We performed target exome sequencing for 87 gene panel using next-generation sequencing (n=48). Targeted genes were sequenced by IlluminaHiSeq 2500 and the sequencing reads were analyzed, based on the pipeline of bioinformatics tool. Candidate mutations were confirmed by Sanger sequencing. Among 71 patients, G-banding (n=60) and fluorescent *in situ* hybridization (FISH) for RB deletion, 13q deletion, chromosome 12 enumeration, and TP53 deletion (n=51) were performed.
Results: Overall, 43 out of 48 patients (89.6%) harbored at least one mutation and mean number of mutation per patient was 2.8 (range 0 -9). Average coverage of target regions was >800-fold. Aberrant karyotypes were observed in 28.3% by G-banding and 66.7% by FISH. The targeted exome sequencing analysis revealed 136 substitutions and insertion/deletions. Most recurrent mutation (>10% frequency) was *ATM* (21%) followed by *TP53* (15%), *FAT4* (15%), *LAMB4* (13%), *NFKBIE* (10%), *NOTCH1* (10%) and *SF3B1* (10%). Mutations of *ITPKB, SF3B1, NFKBIE* and *TP53* was associated with adverse prognosis (*P*=0.033, *P*=0.023, *P*=0.016, and *P*=0.023, respectively, by conventional cox regression model, P<0.05). *BRD2* and *LAMB4* mutation was mutually exclusive (r=0.55, P<0.05). Mutation of *NOTCH1, TP53, and SF3B1* showed similar incidence with Caucasian, while mutation of *ATM, BCOR, SAMHD1, KLHL6, ITPKB and EGR2* was higher than Caucasian. Especially, *ATM* mutation showed 2 fold higher incidence (20%) than Caucasian. *MYD88* mutation (4%) showed lower incidence than Caucasian. Novel mutations in Korean CLL was FAT4 (15%) and LAMB4 (15%) mutation, which was not reported in CLL of Caucasian.
Summary and Conclusions: Mutation profile of Korean CLL was different with those of Caucasian, while profile of cytogenetic aberrations was similar to those of Caucasian. Novel mutations discovered in the present study must be validated through large cohort study, which might suggest a clue for an ethnic difference in incidence. Future evaluation of therapeutic strategies should be taken into account for these mutated genes.

P596

CYTOTOXICITY FOR CHRONIC LYMPHOCYTIC LEUKEMIA CELLS OF THE CD37 ANTIBODY BI 836826 IN COMBINATION WITH CHEMOTHERAPEUTIC AGENTS OR PI3K INHIBITORS

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Background: The Fc-engineered CD37 antibody BI 836826 showed promising direct cell killing activity for chronic lymphocytic leukemia (CLL) cells *in vitro* and is currently investigated in early phase clinical trials.
Aims: To explore its potential for combination therapy, its direct cytotoxicity was assessed in combinations with the DNA alkylating agents chlorambucil and bendamustine, the nucleoside analogue fludarabine or the PI3K inhibitors idelalisib and copanlisib.
Methods: Freshly isolated B lymphocytes from CLL patients were treated *in vitro* for 48 hours with fixed concentrations of chemotherapeutic agents (3 and 10 µM) and monoclonal antibodies (mAbs, 10 µg/ml). The percentages of viable cells, which were not stained by annexin V and 7AAD, were subsequently determined by flow cytometry. The effects of binary combinations were calculated as fractional products of the separate single agent effects.
Results: Among the examined mAbs only BI 836826 and alemtuzumab significantly reduced the survival of CLL cells in a set of twelve CLL samples. The mean relative survival of CLL cells after treatment with BI 836826 was approximately 50%. The percentages of CLL samples, in which the relative survival was reduced by more than 5%, after treatment with BI 836826, alemtuzumab, obinutuzumab and rituximab were 83, 70, 25 and 17%, respectively. The three investigated chemotherapeutic agents significantly reduced the survival of CLL cells at a concentration of 10 µM and also of 3 µM in the case of fludarabine. In combinations BI 836826 enhanced the cytotoxicity of chemotherapeutic agents more efficiently than the other tested antibodies including alemtuzumab and obinutuzumab.. By combinations of BI 836826 with 10 µM of the chemotherapeutic agents the median relative survival of CLL cells was reduced to 20%. In a different set of CLL samples BI 836826 enhanced the cytotoxicity of the PI3K inhibitors idelalisib and copanlisib more efficiently than the CD20 antibodies rituximab and obinutuzumab. The mutual enhancement of cytotox-

icity by BI 836826 in combination with PI3K inhibitors was slightly stronger than that with chemotherapeutic agents. The apoptotic response to the combination of BI 836826 with PI3K inhibitors was more evenly distributed among samples than with chemotherapeutic agents.
Summary and Conclusions: In line with its remarkable capacity of inducing apoptosis in CLL cells, combinations of BI 836826 with chemotherapeutic agents and PI3K inhibitors consistently resulted in higher percentages of apoptotic cells than correspondent combinations including the mAbs in current clinical use.

P597

SOLUBLE CALRETICULIN PREDICTS FOR TIME TO FIRST TREATMENT OF PATIENTS WITH EARLY CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Increased circulating levels of soluble calreticulin (sCRT) are found in the sera of patients with autoimmune disease and in lung cancer they correlate with the hystopathologic subtype. However, studies addressing the issue in patients with hematologic malignancies are absent.
Aims: We analyzed the correlation between well-established clinico-biological parameters of prognostic relevance in chronic lymphocytic leukemia (CLL) and serum levels of CRT by evaluating the impact of these variables on the time to first treatment (TFT) in a series of 70 previously untreated CLL patients in Binet stage A.
Methods: For this purpose peripheral venous blood samples collected at the time offirst diagnosis and stored at−70 ◦C were analyzed for the quantitative determination of circulating CRT level using a commercial Human CRT ELISA assay (CUSABIO, Biothech CO, LTD).
Results: Circulating levels of sCRT of CLL Binet stage A patients did not differ (median, 0.55 ng/mL; range, 0.1-2.9) from levels of healthy controls (median, 0.4; range 0.1-1.2)(P=0.09). The analysis restricted to the cohort of patients with CLL revealed that levels of sCRT did not correlate with age (P=0.25), gender (P=0.70), LDH (P=0.90), ß2-microglobulin (P=0.48), CD38 (P=0.41), ZAP-70 (P=0.50), mutational status of IGHV (P=0.65). In contrast, a positive correlation with peripheral blood (PB) lymphocytosis (P=0.04) and Rai substages (P=0.03) was observed. After a median follow-up time of 36 months (range, 1-204 months) 19 out of 70 (27.1%) patients experienced progressive disease requiring therapy according to IWCLL criteria. Receiver operating characteristic (ROC) analysis identified a serum concentration of 0.8 ng/mL as the threshold (area under curve [AUC]=0.67; sensitivity 0.58; specificity 0.78) that best allowed the identification of two subsets of patients with different clinical outcomes with respect to TTFT (Hazard ratio [HR], 4.51; P=0.0002). Finally, in multivariate analysis along with serum level of sCRT (P=0.003), Rai substages (P=0.02) and PB lymphocytosis (P=0.02) entered the Cox model at a significant level.
Summary and Conclusions: In CLL CRT, an antigen highly expressed by stromal cells, may stimulate B-cell antigen receptor (BCR) therefore contributing to the protective effect that stroma exerts on CLL cells. Our results shed a new light on the role of CRT as predictor of disease-progression in early CLL.

LB598

RESULTS OF A PHASE 3 RANDOMIZED CONTROLLED STUDY EVALUATING THE EFFICACY AND SAFETY OF IDELALISIB (IDELA) IN COMBINATION WITH OFATUMUMAB (OFA) FOR PREVIOUSLY TREATED CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

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Background: IDELA is a selective oral PI3Kδ inhibitor approved in combination with rituximab for previously treated patients with CLL.
Aims: This open-label study (NCT01659021) compared the safety and efficacy of IDELA plus OFA *vs* OFA in patients with relapsed or refractory CLL.
Methods: Patients with CLL progressing ≤24 months from last therapy, and who had received ≥2 cycles of a purine analogue or bendamustine, were randomized 2:1 to either Arm A (IDELA 150 mg BID continuously plus OFA, 300 mg IV week 1, then 1 gm IV weekly x 7 and q 4 week x 4) or Arm B (OFA, same as Arm A except 2 gm was substituted for 1 gm dosing). Stratification was performed for relapsed *vs* refractory, del17p and/or TP53 mutation, and *IGHV* mutation. Response and progression were assessed by an independent review committee (IRC) based on clinical data and imaging using modified IWCLL 2008 criteria. The primary endpoint was PFS and alpha-protected secondary endpoints were confirmed ORR, lymph node response (LNR), OS, PFS in patients with del17p and/or TP53 mutation, and CR rate. Results are from the final analysis of the primary endpoint.
Results: Patient characteristics were balanced in the 2 arms: median age 68; Rai II/III/IV 18/13/51%, median number of prior regimens 3, refractory disease 49%, del17p/TP53 mut 40%, *IGHV* unmut 78%. The median exposure to IDELA was 12.3 months (range: 0.2-23.9). Disposition and efficacy are shown in the Table 1. There was a disproportionate dropout rate with an excess of Arm B patients discontinuing prior to PD or death. Efficacy results, demonstrating superiority of the combination, were consistent across risk groups. The median duration of response was 14.9 months and 6.7 months in Arm A and B patients, respectively. The most frequent non-haematologic Gr ≥3 AEs in Arm A patients were diarrhea/colitis (20.2%), pneumonia (12.7%), and febrile neutropenia (11.6%). Grade 3/4 ALT/AST elevation occurred in 12.9% of Arm A patients. Infusion-related reactions of any Grade/Gr ≥3 were reported in 16.8%/ 2.3% and 26.7%/1.2% of patients in Arms A and B, respectively. Deaths in Arm A occurred in 16.8% of patients on-study (until 30 days post-discontinuation) and 6.9% off-study; and in Arm B in 9.3% of patients on-study and 16.3% off-study. TEAEs leading to death were reported in 10.4% (exposure-adjusted rate=0.10/yr) and 7.0% (exposure-adjusted rate=0.18/yr) of patients in Arms A and B, respectively.

Table 1.

	Arm A (IDELA/OFA)	Arm B (OFA)	HR / OR[1]
Pts randomized/dosed	174/173	87/86	
Months on study (range)	13.6 (1.1-24)	5.8 (0-20)	
Reason for study D/C[2]			
PD (%)	34 (19.5)	41 (47.1)	
Death (%)	22 (12.6)	6 (6.9)	
AE / MD decision (%)	21 (12)	19 (21.8)	
Withdrew consent/other (%)	13 (7.5)	15 (17.2)	
Med PFS, mo	16.3	8.0	HR = 0.27, p < 0.0001[3]
ORR, %	75.3[4]	18.4	OR = 15.9, p < 0.0001[3]
LNR, %	93.3	4.9	OR = 487, p < 0.0001[3]
Med OS, mo	20.9	19.4	HR = 0.74, p = 0.27
Med PFS: del17p/TP53mut, mo	13.7	5.8	HR = 0.32, p < 0.0001

[1]odds ratio; [2]per MD; [3]null hypothesis formally rejected; [4]includes 1 CR

Summary and Conclusions: IDELA plus OFA yielded superior PFS, ORR, and LNR compared to OFA in patients with previously treated CLL, including within high-risk subgroups. Safety was manageable with a profile similar to that previously observed with IDELA in CLL trials.

Chronic myeloid leukemia - Clinical 2

P598

INDIVIDUALIZED IMATINIB THERAPY BASED ON [C]MIN LEVELS MONITORING IN NEWLY DIAGNOSED CML PATIENTS RESULTED IN HIGHER MAJOR MOLECULAR RESPONSE RATES AT 12 MONTHS. RESULTS OF THE OPTIM-IMATINIB STUDY

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Background: Imatinib mesylate (Gleevec, IM) remains a standard for chronic phase CML (CP-CML) therapy. The recommended dose is 400 mg/d. Pharmacokinetic studies pointed-out the inter-individual variability in IM exposure. The value of IM dose optimization based on pharmacokinetic parameters is controversial.
Aims: We conducted the first prospective randomized trial to evaluate the efficacy and the feasibility of IM daily dose optimization based on imatinib [C]min (trough level) in newly diagnosed CP-CML patients (OPTIM-imatinib trial, EudraCT 2008-006854-17).
Methods: Patients (pts) diagnosed with CP- CM for less than 3 months, not previously treated or treated by IM 400 mg/d for less than 1 month were eligible and treated with IM 400 mg/d. IM [C]min was determined at day 15 after enrollment, 20 to 24h after intake. Pts with a [C]min <1000 ng/ml were randomized between a dose-increase strategy to improve the [C]min ≥1000 ng/ml (C1) *versus* observation only (C2). Pts with adequate dosage (≥1000 ng/ml) were allocated to the standart arm (C3). All pts were managed according to the ELN 2009 recommendations (amended for ELN 2013 recommendations). IM [C]min levels were assessed monthly for pts in C1 and C2 and every 3 months in C3. The primary end-point was major molecular response (MMR) at 12 months (missing data were analyzed as failures).
Results: 139 pts were enrolled in the OPTIM-imatinib trial from Oct 2010 to Mar 2014. Median age was 64y (25 to 88y), sex ratio (M/F) was 1.4 and Sokal score distribution was 21%, 41% and 38% for high, intermediate and low categories respectively. Six pts were not randomized (3 were intolerant to IM and 3 declined the dosage). In 86 pts (65%) [C]min was below 1000 ng/ml (median 619; 180 to 998). 43 pts were randomized in C1 and 43 in C2. 47 pts were allocated to C3 (median 1328 ng/ml; 1007 to 3294). At 3 months, median [C]min was 838 ng/ml in the adapted C1 arm, 566 and 998 ng/ml in the C2 and C3 arms respectively. These values were 987, 601and 996 ng/ml at 6 months; 1062, 604 and 935 ng/ml at 9 months and 998, 653 and 930 ng/ml at 12 months. The corresponding mean daily doses of IM in C1, C2 and C3 were 540, 395 and 400 mg/d at 3 months; 614, 390 and 397 mg/d at 6 months; 621, 390 and 394 mg/d at 9 months and 628, 394 and 385 mg/d at 12 months. A higher rate of dose reductions was recorded in C3 suggesting that some of these pts may be over dosed. 19 pts (14%) out of the 133 randomized pts were switched from IM to second generation TKIs during the first 12 months based on the investigator decision, 11 for intolerance (4 in C1, 3 in C2 and 4 in C3), 4 for failure and 4 for non-compliance. No SAE was observed in the adapted arm as compare to 6 in C3. In an intent to treat analysis, MMR was achieved in 27 out of 43 pts (63%; 95% CI, 49 to 77) at 12 months in the C1 dose-adaptation arm *versus* 16 out 43 pts (37%; 95% CI, 23 to 51) in the C2 non-adapted arm (p=0,031), as compared to 22 out of 47 pts (47%; 95% CI, 33 to 61) in C3 (p=0.12).
Summary and Conclusions: The dose adaptation strategy was successfully applied in 95% of the pts recruited. Two third of them were considered under dosed based on IM [C]min. The individualized dose adaptation strategy resulted in a significant increase in the proportion of pts achieving MMR at 12 months (63% *versus* 37%), a magnitude similar to the results reported in studies with second generation TKIs first line. These results may influence the choice of first line therapy and may provide a strong rational to optimize the use of IM and IM generic formulations.
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P599

ATTEMPT TO EARLY DISCONTINUE DASATINIB FIRST LINE IN CHRONIC PHASE CML PATIENTS IN EARLY MOLECULAR RESPONSE AND INCLUDED IN THE PROSPECTIVE OPTIM-DASATINIB TRIAL

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Background: The Optim dasatinib trial validated a dose optimization strategy driven by dasatinib residual plasmatic level (EudraCT 2008-006854-17). We reported that dasatinib dose individualization was associated with a lower risk of pleural effusion and produced high levels of deep molecular responses (Rousselot *et al.* EHA2014). Dasatinib discontinuation was included in the trial and we report the first prospective dasatinib STOP study in dasatinib first line patients.
Aims: The aim of the study was to test the value of early discontinuation of dasatinib in early molecular responders.
Methods: First line patients included in the OPTIM-dasatinib trial, in MR4.5 for 2 years and on dasatinib for at least 3 years were eligible. The OPTIM-dasatinib trial started to recruit patients in Jun 2009, therefore no patient was eligible for the discontinuation study until Jun 2012. Loss of major molecular response (MMR) was used as the criteria to resart dasatinib as validated for imatinib treated patients in the A-STIM study.
Results: From Aug 2012 to Nov 2014, 20 patients were offered to discontinue dasatinib in MR4.5. Median age (52y, range 32-70) and Sokal score distribution (low, 45%; intermediate 30%; high 25%) was similar to the whole population. Sex ratio was 1 (10 males; 10 females) as compare to 1.3 in the whole population. Five patients were included in the adapted arm, 2 in the non-adapted arm and 13 in the standard arm. All patients were early molecular responders (BCR-ABLIS ≤10%) at 3 months and all except one were in major molecular response at month 6 (BCR-ABLIS ≤0.1%). Mediane time for dasatinib therapy was 43 months (range 36.3 to 56.5). Median time for the achievement of MR4.5 was 10 months (range 5.6 to 23.4). Median follow-up after discontinuation was 13.2 months. Ten patients (50%) restarted dasatinib after a median of 6.1 months (range 3.3 to 14.5). Median duration of treatment free remission (TFR) was 11.4 months. At 12 months, the proportion of patients in TFR was 41% (95 CI, 17.5 to 65.7) (Figure 1).

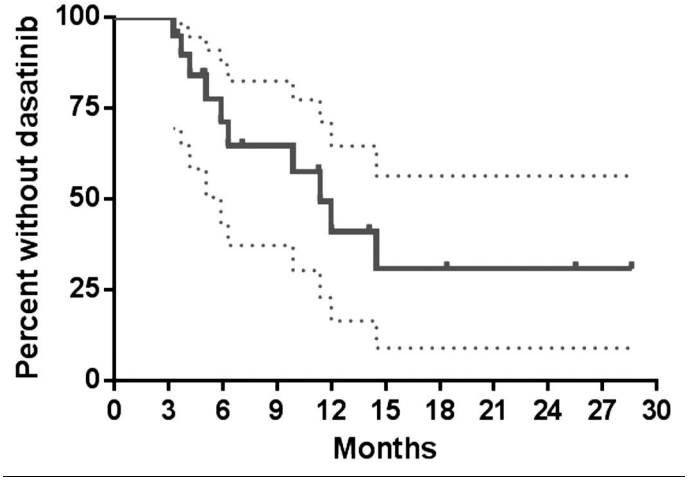

Figure 1. Treatment free remission.

Summary and Conclusions: No published study has described the kinetic of the molecular response before treatment discontinuation. For the first time we report a prospective follow-up of patients included in a prospective clinical trial testing dasatinib first line before discontinuation. Our patients were selected to be early molecular responders and were discontinued after the shortest duration of therapy reported to date. Despite the use of dasatinib and with the limitation of a short follow-up, we failed to improve TFR as compare to previous studies with imatinib first or second line or second generation TKIs second line. Our results suggest that early molecular response is not the path to cure and that duration of therapy is probably the most important parameter to consider before making the decision to discontinue tyrosine kinase inhibitors.

P600

REAL LIFE ANALYSIS OF CML MANAGEMENT DEMONSTRATES THAT SECOND-LINE THERAPY IS FREQUENTLY USED BUT IS PREMATURELY DISCONTINUED FOR INTOLERANCE: REPORT OF THE GROUPE QUÉBÉCOIS DE RECHERCHE EN LMC-NMP

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Background: Since the introduction of imatinib two decades ago, there has been a continual evolution in the management of CML. With the advent of second-generation tyrosine kinase inhibitors (TKI) and better molecular monitoring tools, the decision making for CML patients has become increasingly complex. A variety of treatment guidelines has emerged to help clinicians make decisions based on evidence obtained from clinical trials. However, little is known about the utilization of these guidelines in the real life setting, where patients have more co-morbidities than in clinical trials, and where clinical decision-making may deviate from the strict application of therapeutic guidelines due to diverse factors, such as drug availability, patient choice, and the definition of tolerance and resistance.
Aims: To evaluate in an unbiased, population-based registry the patterns of utilization of CML treatments in the second- and third-line setting in order to assess the discordance between real life data and those expected from clinical trials.
Methods: The CML patient registry of CML-MPN Quebec Research Group (www.gqr-lmc-nmp.ca) prospectively follows 590 patients with CML in 18 oncology centers across the province of Québec. All patients have provided informed consent to participate in the registry. For this interim analysis, 266 patients diagnosed after 2002 and with complete clinical data have been analysed.
Results: The mean age of the cohort was 54 years (range 18-92). The most frequently used front-line TKI included: imatinib (83%), dasatinib (8.6%), and nilotinib (5.6%). In this cohort, a high proportion of patients (44%) needed second-line treatment. The two main causes of first-line discontinuation were resistance (45%) and intolerance (42%). The distribution of the second-line therapy was: dasatinib (43%), nilotinib (29%), stem cell transplantation (8.5%), and other treatments (14%). Thereafter, second-line TKI therapy was discontinued at the following rates: dasatinib was discontinued in 48% of patients after a mean of 9.6 months due mostly to intolerance (48%) and resistance (28%). Similarly, nilotinib was discontinued in 55% of patients after a mean of 15 months, the main cause of discontinuation being intolerance (53%) and resistance (26%). Third-line treatment was instituted in 60 of 100 patients. The most frequent choices were: nilotinib (33%), dasatinib (29%), stem cell transplantation (16%), best supportive care (5%), interferon (2%) or other (11%). Surprisingly, intolerance lead to less discontinuation in the third-line setting (nilotinib: 20%; dasatinib: 10%) and mean time on treatment was longer (nilotinib: 24 months; dasatinib: 25 months).
Summary and Conclusions: Registry-based real life data indicate that intolerance is a predominant trigger for switching therapy in first- and in second-line treatment of CML. This is in contrast with clinical trial data where resistance is the most frequent cause of treatment discontinuation, particularly in second-line. We also observed a paradoxical tolerance benefit for nilotinib and dasatinib when used in third-line. We hypothesize that the increased flexibility associated with real life setting renders physicians more prone to change therapy in the presence of lower grade side effects that would not justify therapy modification in the setting of a clinical trial. Interestingly, in the third-line setting, which is the last resort for most patients, they are more willing to endure their adverse effects and continue on therapy.

P601

CARDIOVASCULAR (CV) AND PULMONARY ADVERSE EVENTS (AES) IN PATIENTS (PTS) TREATED WITH BCR-ABL TYROSINE KINASE INHIBITORS (TKIS): UPDATED ANALYSIS FROM THE FDA ADVERSE EVENT REPORTING SYSTEM (FAERS)

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Background: The publicly available FAERS database includes AEs reported to pharmaceutical companies and voluntary reports submitted from pts and physicians. FAERS can be mined as part of postmarketing surveillance to identify potential safety signals based on exposure of a broader population than clinical trials. A prior report of FAERS through Sept 2012 described CV and pulmonary AE signals for pts treated with dasatinib (DAS), imatinib (IMA), and nilotinib (NIL) (Cortes 2015, *AJH*).
Aims: As FAERS identifies potential signals emerging over time, a reanalysis using the previous criteria was conducted through Dec 2013. Although data may be sparse (potential 'Weber effect') on recently approved bosutinib (BOS), and altered due to potential 'notoriety effect' of FDA suspension of ponatinib (PON), due to interest in emerging safety signals associated with both agents, they were included in the present analysis.
Methods: Data mining using the validated Multi-Item Gamma Poisson Shrinker (MGPS) Bayesian method is an accepted tool to identify drug-event associations in surveillance databases. MGPS provides a robust estimate of disproportionality (reporting frequency of drug-event pair compared to expected frequency) referred to as the Empirical Bayesian Geometric Mean, and the corresponding 90% CI (EB05, EB95). EB05 ≥2 indicates a 95% chance an event is reported at least 2x as frequently as expected. A threshold of EB05 ≥4 (4x expected reporting frequency) was used to identify events more likely to be clinically relevant and attributable to drug. We assessed the frequency of preferred terms in 3 MedDRA system organ classes (SOCs) with safety signals associated with DAS/IMA/NIL (cardiac; respiratory, thoracic and mediastinal; and vascular) in pts ≥18 years (yrs) and for age cohorts: 18-45, 46-64, and ≥65 yrs.
Results: Drug-event associations with an EB05 ≥4 were identified for DAS/IMA/NIL (Table 1). Drug-event pairs that reached EB05 ≥4 for all 3 of these agents included pleural and pericardial effusions. NIL was uniquely associated with EB05 ≥4 for vascular occlusive events. While no events reached EB05 ≥4 for BOS/PON, EB05 ≥2 was noted for pleural effusion with both, and for pericardial effusion, peripheral arterial occlusive disease, and hypertension with PON. In all age cohorts, pleural effusion with DAS/IMA and pericardial effusion with DAS reached EB05 ≥4. Peripheral and cardiac vascular AEs were noted with NIL with EB05 ≥4 in pts ≥46 yrs. Key limitations were the inclusion of terms in only 3 MedDRA SOCs and the reclassification of preferred terms by SOC since the last report; additionally, there was an inability to establish causality or to determine incidence of safety signals, and the possible effect of outside influences on reporting frequency.

Table 1. Drug-event pairs with EB05 ≥4.

TKI	MedDRA PT	EB05 ≥4
Dasatinib	Chylothorax	72.22
Dasatinib	Pleural effusion	30.95
Dasatinib	Pericardial effusion	11.40
Dasatinib	Pulmonary edema	5.45
Dasatinib	Pulmonary hypertension	4.96
Dasatinib	Pleurisy	4.80
Imatinib	Pleural effusion	5.35
Imatinib	Pericardial effusion	4.34
Nilotinib	Peripheral artery stenosis	54.92
Nilotinib	Intermittent claudication	40.55
Nilotinib	Peripheral arterial occlusive disease	32.82
Nilotinib	Arteritis	20.68
Nilotinib	Arterial stenosis	17.17
Nilotinib	Coronary artery stenosis	16.39
Nilotinib	Arterial disorder	14.88
Nilotinib	Femoral artery occlusion	12.27
Nilotinib	Peripheral ischemia	7.79
Nilotinib	Angina pectoris	7.75
Nilotinib	Acute coronary syndrome	5.96
Nilotinib	Acute myocardial infarction	5.59
Nilotinib	Pleural effusion	5.54
Nilotinib	Arteriosclerosis	4.73
Nilotinib	Pericardial effusion	4.69
Nilotinib	Myocardial ischemia	4.28

MedDRA PT, MedDRA preferred term; TKI, tyrosine kinase inhibitor.

Summary and Conclusions: This analysis suggests a strong association of pleural effusion with DAS, with a weaker association with IMA/NIL. An association between NIL and multiple vascular events was identified. Due to limited exposure and potential bias for BOS/PON, no signals reached EB05 ≥4; however, some events reached EB05 ≥2 and warrant monitoring in future analyses with longer follow-up.

P602

LONG-TERM BOSUTINIB IN PATIENTS WITH CHRONIC PHASE CHRONIC MYELOID LEUKEMIA (CML) AFTER PRIOR IMATINIB FAILURE

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Background: Bosutinib (BOS) is a Src/Abl tyrosine kinase inhibitor for adults with Ph+ CML resistant/intolerant to prior therapy.
Aims: We assess long-term efficacy/tolerability of BOS after ≥5 y *vs* ≥2 y follow-up from last enrolled patient (pt).
Methods: Data were from an ongoing phase 1/2 study in chronic phase CML pts on 2nd-line BOS (500 mg/d start dose) after imatinib failure (n=284). Informed consent was obtained from all pts.
Results: 41% of pts remained on BOS at 5 y (54% at 2 y; per protocol, 1 y=48 wk); 60% and 50% had newly attained or maintained baseline major cytogenetic response (MCyR) or complete cytogenetic response (CCyR) (most in ≤2 y). Kaplan-Meier probability of maintaining MCyR or CCyR in responders was similar at 2 y (76%, 79%) and 5 y (71%, 71%); 6 and 7 pts, respectively, lost MCyR and CCyR >2 y. Cumulative incidence of on-treatment transformation to accelerated phase (AP)/blast phase (BP) CML at 5 y was 4%; 55% discontinued without transformation 2 y transformed to AP in y3-5. Kaplan-Meier overall survival was 84% at 5 y (91% at 2 y; 40% censored 37 pts discontinued BOS y3-5 (*vs* 131 ≤2 y), mostly for disease progression (n=11), AE (n=7, y3: coronary artery disease, scleroderma, renal failure; y4: ascites and serositis [same pt], blood creatinine increased, pulmonary hypertension; y5: thrombocytopenia), and unsatisfactory efficacy (n=7). Common newly occurring AEs (in >5 pts) in y3 were cough (n=7), blood creatinine increased (n=7), pyrexia (n=6), and blood creatine phosphokinase increased (n=6); y4: blood creatinine increased (n=6), pleural effusion (n=6); y5, none in >5 pts. Newly occurring AEs of interest (Table 1) were most common in y1-2; vascular AEs >y2 were primarily hypertension (y3, n=5; y4, n=3; y5, n=2). Four on-treatment deaths occurred y3-5, none BOS-related.

Table 1.

Newly Occurring AEs*					
n (%)	Year 1 N=284	Year 2 N=189	Year 3 N=148	Year 4 N=130	Year 5 N=124
Diarrhea	239 (84)	3 (2)	0	1 (1)	0
Cardiac	23 (8)	7 (4)	8 (5)	7 (5)	4 (3)
Vascular	11 (4)	8 (4)	5 (3)	4 (3)	5 (4)
Renal	14 (5)	4 (2)	8 (5)	7 (5)	5 (4)

**Not experienced by same patient previously (denominator=patients on treatment each y; 1 y=52 wk).*

Summary and Conclusions: BOS showed durable efficacy and manageable toxicity; a large proportion of chronic phase CML pts with prior imatinib failure remained successfully treated at 5 y.

P603

IMATINIB DISCONTINUATION IN CHRONIC MYELOID LEUKEMIA: A RETROSPECTIVE ANALYSIS ON PATIENTS IN CLINICAL PRACTICE

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Background: The introduction of Tyrosine Kinase Inhibitors (TKIs) has dramatically changed the prognosis of chronic myeloid leukemia (CML). For patients who maintain deep molecular remission for many years, it is now under discussion the possibility to discontinue TKI therapy. In the last 8 years different studies analyzed the outcome of patients who discontinued imatinib. For most of these studies, the main criteria for discontinuation were the duration of complete molecular response (CMR) and the depth of molecular response, defined at least as MR4.
Aims: To present our experience with imatinib discontinuation in clinical practice.
Methods: We retrospectively analyzed the outcome of patients treated in 6 Divisions of Hematology in Piemonte and Lombardia, who discontinued imatinib. All these patients had discontinued imatinib in CMR defined at least as MR4. After discontinuation patients were monitored. In case of loss of response, therapy was restarted. Only patients with a minimum follow-up of 6 months were included. Statistical methods: We estimated MR4 relapse-free survival and treatment-free remission (TFR) with the Kaplan-Meier method. Prognostic factors for MR4 relapse-free survival were assessed by univariate Cox regression model analysis.
Results: We analyzed a total of 46 patients who discontinued imatinib from August 2003 to October 2014. Median age was 53 year old (46-67). 24 were male, 22 were female; 27 (61%), 13 (30%) and 4 (9%) were low, intermediate and high Sokal score respectively; 43% (20/47) were pre-treated with interferon α (IFN). Reasons for discontinuations were comorbidities for 3 patients, patient request for 26 cases, intolerance for 13 patients, desire of pregnancy for 4 patients. Median time to CMR after the initiation of imatinib was 18 months (10-40). Median duration of CMR was 37 months (31-56) before stop. Median time of treatment with imatinib was 62 months (49-100). At 12 months, estimated MR4 relapse-free survival was 57.7% (95%CI 44.7%>74.2%). After a median follow-up of 37 months (14-77), estimated MR4 relapse-free survival was 42.9% (95%CI 29.8%>61.7%) (Figure 1). Overall, 24% (11/46) of patients lost major molecular remission (MMR). Only 2 patients lost Complete Cytogenetic Remission (CCyR). Median time to loss of MR4 was 4 months (2-7). Most of the patients lost MR4 within the first 7 months (figure 1). 48% (22/46) restarted treatment; median time to restart treatment was 6 months (4-12). 4 patients lost MR4 but never restarted treatment. No patients progressed. We assessed age, sex, Sokal score, previous IFN therapy, duration of imatinib therapy, time to CMR, and CMR duration until discontinuation as potential prognostic factors for MR4 relapse-free survival, but no statistically significant association were found. Patients who had to restart the therapy were treated with imatinib (19/22), nilotinib (2/22), vaccine (1/22). All of them regained at least an MMR (14 patients re-achieved a response ≥MR4). All patients were alive at the last follow up with the exception of one who died of colon cancer.

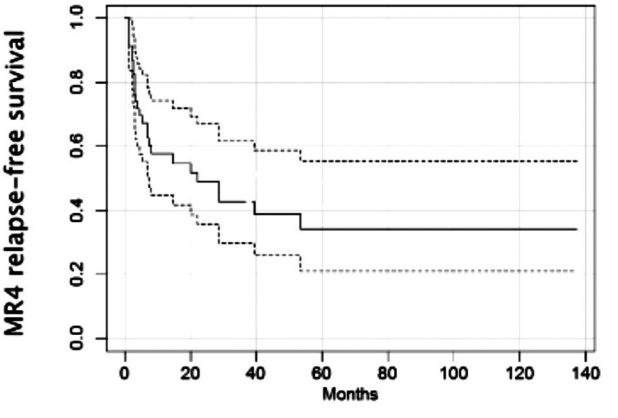

Figure 1. MR4 relapse-free survival.

Summary and Conclusions: Our experience, aligned to the literature, confirms that treatment discontinuation is feasible and safe for CML patients treated with imatinib front-line or after INF. No progressions occurred among our patients, considering that 75% of our population had a follow-up longer than 77 months. A close molecular monitoring is however required. 64% of patients regained a deep response, and may be considered for a second attempt of discontinuation.

P604

VALIDATION OF A NEW PROGNOSTIC SCORE AIMED TO IDENTIFY THREE GROUPS WITH DIFFERENT PROBABILITIES OF DYING OF CHRONIC MYELOID LEUKAEMIA IN 1,120 PATIENTS WITH FIRST-LINE IMATINIB TREATMENT

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Background: A new prognostic score for differentiating cumulative incidence probabilities (CIPs) of dying of chronic myeloid leukemia (CML) was developed in the IN-study section of the European Treatment and Outcome Study (EUTOS) registry. From 2002 to 2006, all patients were enrolled in prospective, controlled clinical trials. They had Philadelphia chromosome-positive chronic-phase (CP) CML and started first-line imatinib-based treatment within half a year after diagnosis. In the OUT-study section of the registry, patients were prospectively registered, but, as the only contrast to the IN-study, not part of clinical trials.
Aims: To compare the performance of the Sokal, the Euro, the EUTOS, and the new score in the OUT-study data
Methods: Survival was counted from start of imatinib treatment and censored at the time of allogeneic stem cell transplantation (SCT) in first CP. Only death after recorded disease progression was regarded as death due to CML. CIPs of dying of CML were estimated, treating death due to any other cause as a competing event. All prognostic scores were calculated at diagnosis of CML. Overall level of significance was 0.05.
Results: Applying the same inclusion criteria as for the IN-study section, 1,120 patients of the OUT-study section were evaluable with regard to all prognostic scores and survival. These patients came from registries in Russia, the Czech Republic, Poland, Spain, and Romania. Median observation time was 5.6 years. Allogeneic SCT in first CP was performed in 29 patients (3%, 7 died). Without prior SCT in first CP, 116 patients died, 6-year CIP was 11% [95%>confidence interval (CI): 9-14%]. In 56 cases (48% of 116), cause of death was CML. Six-year CIP of death due to CML was 5% [95%>CI: 4-7%] and 6% [95%>CI: 5-8%] for death due to other causes. The new prognostic score identified 681 low-risk (61% of 1,120), 302 intermediate- (27%), and 137 high-risk patients (12%). Their 6-year CIPs of dying of CML were 3% [95%>C.I.: 2-5%], 6% [95%>C.I.: 3-9%], and 17% [95%>C.I.: 11-25%]. In the corresponding risk groups of the Sokal score the 6-year CIPs resulted in 3% [n=450, 95%>C.I.: 2-5%], 5% [n=411, 95%>C.I.: 3-8%], and 11% [n=259, 95%>C.I.: 8-16%] and with the Euro score in 4% [n=466, 95%>C.I.: 2-6%], 5% [n=523, 95%>C.I.: 3-7%], and 15% [n=131, 95%>C.I.: 9-23%]. The EUTOS score found a 6-year CIP of 5% [n=971, 95%>C.I.: 3-6%] for its low-risk and of 12% [n=149, 95%>C.I.: 7-18%] for its high-risk group. Each of the 4 prognostic scores provided significantly higher probabilities of dying of CML for the high-risk group as compared with each of the non-high-risk group(s) (all p values <0.005). The new prognostic score was also able to identify significantly different CIPs of dying of CML between the low- and the intermediate-risk group (p=0.04).
Summary and Conclusions: Judging from the CIPs and the distribution of the patients into the risk groups, the new score showed the best prognostic performance also in the out-study patients. Only the new model identified three risk groups with pairwise significantly different CIPs of dying of CML. With similar CIPs, the new score identified a much larger group of low-risk patients than the Sokal and the Euro score. Altogether, validation of the new score in the out-study data was successful. In the 681 patients of the low-risk group (61%), the new score showed an excellent long-term outcome when starting treatment with imatinib. Due to the worse results, for the high-risk patients (12%), an upfront comparison between different tyrosine kinase inhibitors would be highly desirable.

P605

PROGRESSION OF CHRONIC PHASE CHRONIC MYELOID LEUKEMIA TO ADVANCED PHASE ON TKI THERAPY: A POPULATION BASED ANALYSIS FROM THE SWEDISH CML REGISTER

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Background: Chronic myeloid leukemia (CML) chronic phase (CP) patients treated with tyrosine kinase inhibitors (TKIs) today have an excellent prognosis, but those progressing to accelerated phase (AP) or blast crisis (BC) still have a dismal outcome. The primary goal of CML treatment is therefore to prevent disease progression, and with Imatinib treatment progression rates have been reduced to less than 3% per year. By identifying risk factors for progression already at diagnosis, and by early therapeutic intervention in patients showing insufficient treatment response, the progression rates may be further reduced. The Swedish CML register is population-based and contains data on Swedish CML patients at diagnosis and follow up. The estimated coverage is >95% of newly diagnosed CML patients. This gives us an opportunity to characterize CML patients who are diagnosed in CP and progress to AP or BC during TKI treatment in a population based material.
Aims: To characterize CML patients diagnosed in CP and treated with TKIs who progress to AP or BC within 24 months from diagnosis with regard to baseline characteristics, adherence to national guidelines for CML management and to evaluate progression rates and outcome in a population based setting.
Methods: Patients diagnosed in CP and treated with TKIs between 1 Jan 2007 and 31 Dec 2012 were selected from the Swedish CML register database and those who progressed within 24 months from diagnosis were further evaluated through review of patient charts.
Results: In total, 437 CP CML cases, aged 61 (47-72) years, were registered during the study period. Nineteen patients (4.6%), all treated with Imatinib first line, progressed to advanced phases (5 AP, 13 BC, 1 unknown). Six of these progressed within 6 months, seven between months 6-12 and six during the second year from diagnosis. This corresponds to a cumulative probability of progression of 3.1% at 12 months and 4.6% at 24 months. At baseline, 37% of progression patients were EUTOS high risk, as compared to 13% of patients who did not progress (p=0.014). Patients later progressing to AP/BC had a higher percentage of blast cells in peripheral blood at diagnosis (median 2.4 *vs* 1.0%, p<0.001) and lower platelet counts (322 *vs* 429x10^9/l; p=0.022). No difference between progression and non-progression cases were observed with respect to age, WBC, Hb, Sokal and Hasford score. Adherence to national guidelines for monitoring was suboptimal in several progression cases. Thus, at 6 months, 42% of the patients that later progressed to AP/BC were not subject to genetic evaluation by cytogenetics or qRT-PCR. Clonal evolution was noted in 5 patients (28%) as an early sign of progression.
First line treatment of AP/BC consisted of chemotherapy in combination with TKI switch in 6 patients, TKI switch only in 7 patients, TKI dose increase in 2 patients and palliative chemotherapy in 2 patients. Eight BC patients and 1 AP patient underwent allogeneic HSCT. Median survival after transformation to AP/BC was 1.4 years (Figure 1).

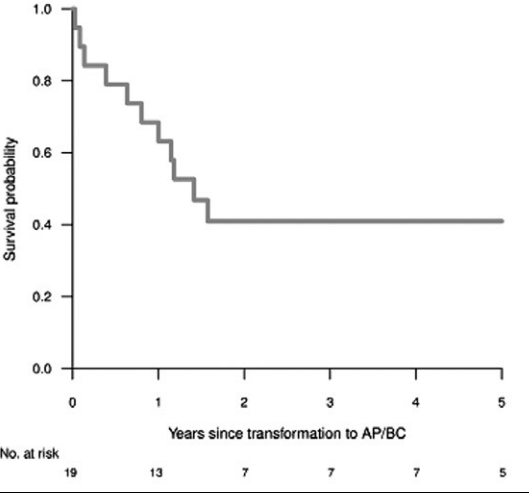

Figure 1.

Summary and Conclusions: The cumulative probability of progression from CML CP to AP or BC at 24 months from diagnosis in this Swedish population based cohort[JR1] [SS2] was 4.6%, which is similar to findings in studies based on more selected patient groups. Median survival for patients progressing to AP/BC was poor. The proportion of patients progressing to AP/BC may be reduced by more stringent monitoring and by earlier therapeutic intervention as prescribed in national and international guidelines.

P606

INTERFERON-ALPHA REVISITED: INDIVIDUALIZED IMMUNE-MODULATION EASED UP SELECTION PRESSURE OF TKI ON BCR-ABL MUTATED CLONES AND IMPROVES MOLECULAR RESPONSE IN HIGH-RISK CML PATIENTS

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Background: CML patients in whom the tyrosine kinase inhibitor (TKI) therapy

(imatinib, dasatinib, nilotinib) fails due to T315I BCR-ABL kinase domain mutation, should be switched to ponatinib or for bone marrow transplantation. However, in individual cases these treatment scenarios cannot be applied. We used treatment with interferon-α (IFN, given solo, sequentially or together with TKI) assuming that TKI-independent mechanism of action may lead to T315I mutant clone repression.

Aims: To study an effect of IFN-based treatment on mutated BCR-ABL transcript level and overall response of CML patients who failed on TKIs. In addition to that to assess the role of the sensitive measurement of BCR-ABL mutant mRNA load dynamics and immune response induced by IFN in individualized treatment.

Methods: Six CML patients who had failed on TKIs due to T315I or other highly resistant mutational profile have been treated with individual IFN-based treatment strategies. Kinase domain mutations in cDNA of BCR-ABL were analyzed in total 54 samples (median 6 samples of each patient, range 5-15) of peripheral blood leukocytes using next-generation deep sequencing (GS Junior, Roche Applied Science). The T315I clone reactivity on lowering imatinib concentration (2.0, 1.0, 0.4 and 0 μM imatinib) was studied *in vitro* on KCL22-R cell line resistant to 4.0 μM imatinib with 45% of BCR-ABL T315I. KCL22-R were incubated in each imatinib concentration for 14 days and checked for mutation. The basic immune-profile was analyzed in 5/6 CML patients using flow-cytometry detecting populations of CD4+, CD8+, regulatory T-cells (Tregs) and NK cells in specific time points (*e.g.* before IFN initiation, during IFN-based treatment).

Results: The switch of TKI to IFN-based treatment resulted in decreasing of mutated BCR-ABL mRNAs to undetectable levels in 4/6 patients. Three of those patients achieved major molecular response (MMR). IFN has been stopped, based on non-mutated BCR-ABL transcripts detection, continuing with solo nilotinib in 1/4 patient who achieved complete cytogenetic remission (CCgR). One case of MMR responding patient on solo IFN treatment showed decreased levels of CD3+ T-lymphocytes and elevated levels of NK cells. Other two patients who achieved MMR after IFN expressed elevated levels of NK cells representing probably an immune activation on dasatinib pre-treatment following with restoring immunological surveillance after application of combined treatment IFN with TKI. In 2/6 cases TKI cessation and IFN introduction did not contribute to the BCR-ABL mutations reduction and response improvement.

No changes in the BCR-ABL T315I transcript levels have been observed after 14 days of incubation of KCL22-R upon easement of selective pressure of imatinib. We assume that longer time of incubation, which is ongoing, would be necessary to follow-up any changes.

Summary and Conclusions: In this work we showed that CML patients with TKI multi-resistant mutation T315I or other highly resistant BCR-ABL mutations may achieve CCgR or MMR thanks to IFN-based individualized treatment. These achievements may relate to immune-mediated anti-leukemic effects in this specific group of patients resulting in BCR-ABL clones repression. The sensitive measurement of mutated BCR-ABL transcript levels augments safety of this individualized treatment strategy.

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P607

PREDICTION OF THE NUMBER OF CML- PATIENTS IN GERMANY: UP TO 20,000 ARE TO BE EXPECTED IN THE NEAR FUTURE

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Background: Since introducing Tyrosine Kinase Inhibitors (TKI) into the treatment of chronic myeloid leukemia (CML) the survival rates have increased remarkably and thus the patient numbers are rising too. It has been estimated that the prevalence of CML rises annually by about 10%, but exact figures are missing. As the treatment costs are considerable, a prediction of the number of patients in the future is of interest for a proper assessment of the economic impact.

Aims: Our first objective was to estimate prevalence and incidence of CML by using routine data from the Bavarian Association of Statutory Health Insurance Physicians (Kassenärztliche Vereinigung Bayerns). Based on these estimates the second objective was to find a model to predict patient numbers in Germany for the future accounting also for the changes in demography.

Methods: Data on ICD-10 codes as well as prescribed medication were available for about 10 million people in the statutory health insurance system in Bavaria for the years 2008 to 2013. Only patients with at least two recordings of CML (ICD C92.1) and a CML-related treatment (TKI, Interferon, Hydroxyurea, Busulfan) were included to avoid misclassification bias.

As survival rates could not be estimated from our data base, corresponding values were adapted from two recent publications, which both reported age-specific relative survival rates (Nennecke *et al.*, Bundesgesundheitsblatt 2014, and Smith *et al.*, BMJ open 2014), as well as current survival rates for the German population, obtained from the German Federal Statistical Office (GFSO). Data on the expected population projection - in twelve different scenarios, structured by birth rate, life expectancy and migration balance - was provided by the GFSO as well.

Results: The mean estimated raw incidence rate was 1.851 for women and 2.249 for men; the age-standardized (Old European Standard Population) rates were 1.300 and 1.768 for women and men. For mid-2009, we estimated the prevalence for men/women (each per 100,000 inhabitants): 0.100/0.285 (age 0-19 years), 1.065/1.904 (20-29), 3.523/3.798 (30-39), 6.778/6.845 (40-49), 7.919/11.151 (50-59), 19.282/21.935 (60-69), 22.877/28.194 (70-79) and 15.419/26.440 (80 and older). Based on the population data described above, we estimated a total number of about 7,000 CML patients in Germany for 2009. The estimations of the number of patients in the future according to the twelve population scenarios combined with the different survival assumptions (and the average of both) are depicted in Figure 1. An increase of the prevalence is expected in every scenario. Differences were due to the chosen survival scenario rather than due to the population scenarios. The number of patients in the most probable average survival scenarios will increase for the next 25 to 35 years.

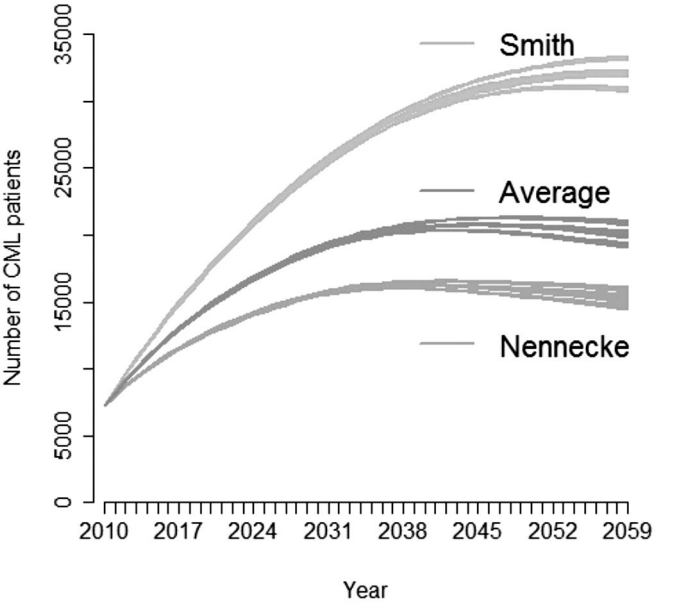

Figure 1.

Summary and Conclusions: The estimated incidence rates presented here are comparatively higher than in recent publications. Using a restrictive definition for case patients we do not think that there is much overestimation.

We expect the number of CML patients to increase further for at least 20 years, probably even longer. The survival estimate by Smith is based on relatively few patients and might thus lead to an overestimation. Vice versa, the estimate by Nennecke is in part based on patients known by death certificate only and might be slightly too pessimistic. From our point of view, the mean survival scenario seems to be the most realistic one, thus in Germany a maximum of about 20,000 CML patients is to be expected.

P608

KILLER IMMUNOGLOBULIN-LIKE RECEPTORS CAN PREDICT TKI TREATMENT-FREE REMISSION IN CHRONIC MYELOID LEUKEMIA PATIENTS

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Background: Discontinuation of Tyrosine Kinase inhibitors (TKIs) in chronic myeloid leukemia (CML) patients is currently a much debated challenge. Several drug-, disease- and patient-related parameters have been suggested to be predictive of durable treatment-free remission (TFR). Despite the well known anti-tumor effect of the innate immune system, few data exist on the role of natural killer (NK) cells and their killer-cell immunoglobin-like receptors (KIRs) in CML. In a previous report we found that a decreased frequency of the KIR2DL2 inhibitory receptor and homozygosity for KIR A haplotype were significantly associated to achievement of undetectable molecular response ($UMR^{4.5}$). A more recent report by other authors found that early disease relapse after TKI discontinuation was associated to both low numbers and impaired function of NK cells.

Aims: The primary aim of this study was to investigate whether NK cells and/or the KIR gene system could provide us with predictive immune-biomarkers of stable TFR in CML. A second endpoint was to evaluate which other patient/disease factors were related to relapse/non relapse after stopping TKI treatment.
Methods: The distribution of KIR and human leucocyte antigen (HLA) gene frequencies, KIR and HLA genotypes and KIR-ligand combinations were investigated in 22 chronic-phase CML patients who discontinued TKI treatment after achieving UMR$^{4.5}$. Age at diagnosis, gender, white blood cell (WBC) and platelet (PLT) counts, Sokal risk, splenomegaly, previous interferon treatment, rapidity in obtaining UMR$^{4.5}$ and overall treatment duration were also included in the binary logistic regression model in which TFR was considered as a dependent variable.
Results: Median age at diagnosis was 68 years (range 37-88) and 16/22 of the patients were males (72.8%); splenomegaly was detected in 13 patients (59%), ranging from 1 to 16 cm below the costal margin. Sokal risk was low in 54.5% of the patients, intermediate in 40,9% and high in 4.6%. Median follow-up was 11.5 years. Six/22 patients (27.3%) had been previously treated with interferon followed in 5 cases by imatinib; 14 patients were treated upfront with imatinib and 2 with nilotinib. All patients achieved UMR$^{4.5}$ after a median of 17 months (range 3-96) and, within the framework of a clinical protocol, discontinued TKI treatment at a median of 72 months from the start of treatment (range 23-105). The 36-month probability of TFR was 58.7%. The majority of relapses occurred within 8 months of interrupting therapy. TFR was significantly higher in patients not carrying the inhibitory KIR2DL2 receptor (81.4% *vs* 36.4%, p=0.039) (Figure 1). There was also a trend toward better TFR in patients homozygous for KIR A haplotype (80% *vs* 41.7%). Younger age, male sex and lower duration of treatment were significantly associated with relapse. All relapsed patients regained UMR$^{4.5}$ after restarting treatment with the same TKI.

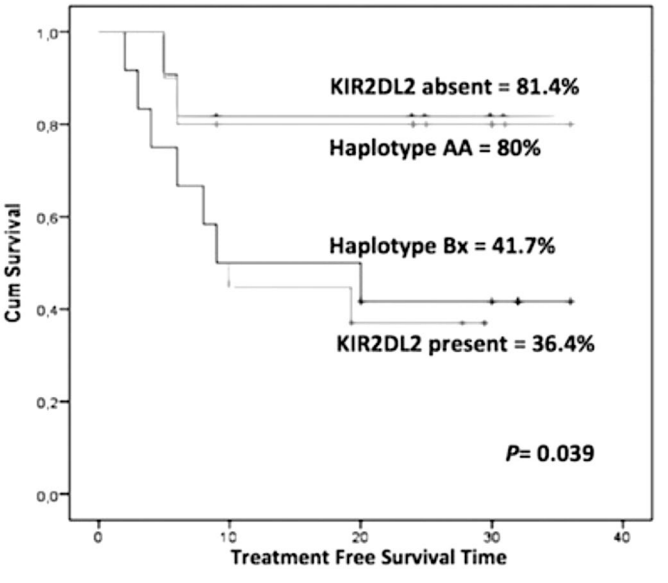

Figure 1.

Summary and Conclusions: Further investigation into innate immunity and the modulation of NK cell function could represent an important step ahead in the management and recovery from CML. Our results show that patients carrying the KIR2DL2 inhibitory receptor are at risk for not maintaining durable TFR. Previous reports show that homozygosity for KIR A haplotype is associated to better immune response with resistance to viral infection and tumor cells. Our findings suggest that this genotype could be a useful predictive marker when deciding whether or not to withdraw treatment in CML patients.

Myelodysplastic syndromes - Biology

P609

AML1-EVI-1 FUSION GENE INHIBITED DIFFERENTIATION AND APOPTOSIS IN ZEBRAFISH MYELOPOIESIS

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Background: *AML1-Evi-1* is a chimeric gene generated by the t(3;21)(q26;q22) translocation, which leads into malignant transformation of hematopoietic stem cells (HSCs) by many mechanisms which have not been thoroughly elucidated.
Aims: We established a stable line of zebrafish expressing the human *AML1-Evi-1* fusion gene under the control of a heat stress-inducible bidirectional promoter, named as Tg(*AE*:HSE:EGFP) line, to study its roles in hematopoiesis and hematologic malignancies.
Methods: Using Wright Giemsa stain detected the blood cell distribution in peripheral blood and kidney. The changes of hematopoietic regulatory factors were examined through RT-PCR and whole mount *in situ* hybridization. Agilent microarray analysis was performed to find the most relevant Kyoto Encyclopedia of Genes and Genomes (KEGG) terms associated with differentially expressed genes (DEGs) between wild type and Tg(*AE*:HSE:EGFP) F2 generation. Furthermore, we investigated the *in vivo* effects of histone deacetylase inhibitor, valproic acid (VPA), on *AML1-Evi-1*-expressing cells.

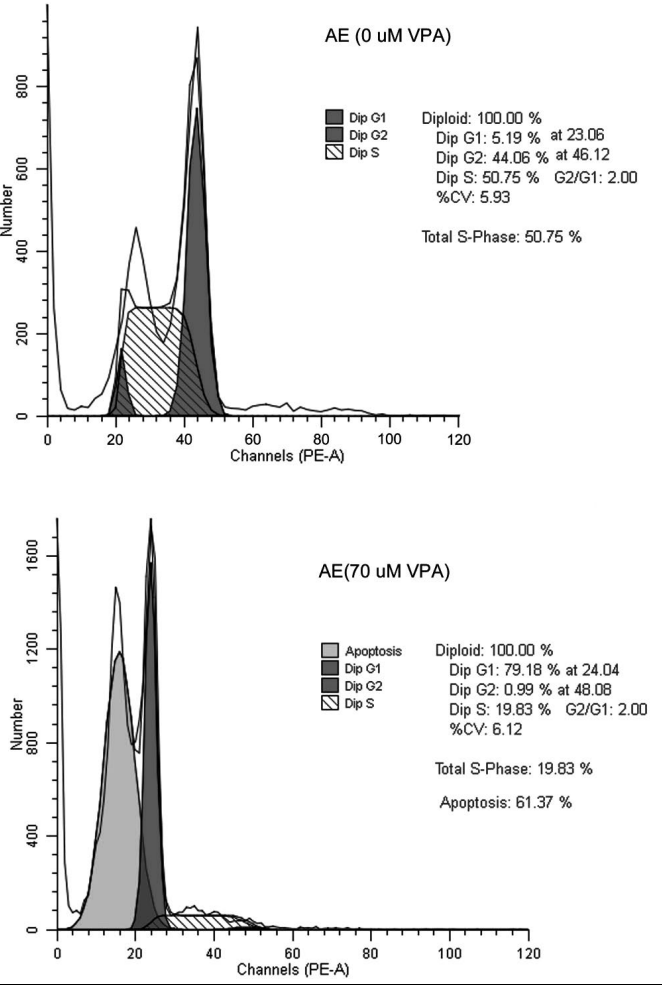

Figure 1.

Results: Three of 146 (2.1%) mosaic F0 zebrafish embryos were identified as the stable positive germline transgenic zebrafish, including 2 males and 1 female. The Transgenic F1 generation were mated to create homozygous Tg(*AE*:HSE:EGFP) line. Immature myeloid cells and hematopoietic blast cells were accumulated in peripheral blood and single cell suspension from kidney in adult Tg(*AE*:HSE:EGFP) zebrafish. *AML1-Evi-1* presented an intensive influence on hematopoietic regulatory factors. Consequently, primitive hematopoiesis was enhanced by upregulation of *gata2* and *scl*, erythropoiesis was downregulated due to the suppression of *gata1*, while early stage of myelopoiesis was flourishing

according to the higher expression of *pu.1* but inhibited along with the lower level of *mpo* expression. These characters closely resemble to the main aspects of human myeloid leukemia. Therefore, this model facilitated dissection of *AML1-Evi-1*-mediated pathomechanism *in vivo*, and enabled high-throughput scale screens to identify the potential therapeutic targets. Microarray analysis demonstrated that *AML1-Evi-1* upregulated Proteasome, Cell cycle, Glycolysis/Gluconeogenesis, Tyrosine metabolism, Drug metabolism, and PPAR pathway. Meanwhile, transforming growth factor β (TGFβ), Jak-STAT, DNA replication, Mismatch repair, p53 pathway, JNK signaling pathway, and Nucleotide excision repair were distinctly suppressed. It's worth noting that histone deacetylase 4 was dramatically decreased. Factors involved in cell proliferation were obviously suppressed after 3-day treated with VPA. Accordingly, higher proportion of G1 arrest and apoptosis were manifested by the propidium iodide staining.
Summary and Conclusions: The phenotypes of Tg(*AE*:HSE:EGFP) fish resemble to those of the human's MDS-RAEB or AML. *AML1-Evi-1* could promote the primitive hematopoietic cell proliferation and apoptosis resistant, inhibit myeloid cells differentiation through synergy of several pathway and factors. We propose VPA to be an attractive choice in the molecular targeting therapy of *AML1-Evi-1*-related leukemia.

P610

THE INFLUENCE OF SF3B1 MUTATIONS ON THE RESPONSIVENESS OF BLOOD CANCERS TO MOLECULAR THERAPEUTICS

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Background: In recent years, high-throughput sequencing has identified mutations in the SF3B1 gene in a variety of blood cancers such as myelodysplastic syndromes (MDS), acute myeloid leukemias (AML) and chronic lymphocytic leukemias (CLL). The majority of mutations reported involved a single nucleotide substitution that alters a lysine codon into a glutamate (K700E). This alteration, along with all other known mutations, resides within the HEAT domains of the protein. This narrow localisation of the substitutions in combination with the absence of nonsense mutations suggests that SF3B1 mutations lead to alteration of function rather than simple haploinsufficiency. Furthermore, their prevalence implies the mutations are relevant to these diseases, if not disease-causing. In terms of clinical utility, identifying therapies which target cells harbouring mutant SF3B1 would be of great benefit, as there is pressing need to improve available therapies for all these diseases; particularly MDS, a disease of the elderly.
Aims: To explore the tractability of SF3B1 mutations by standard and advanced molecular therapies; using isogenic pre-clinical models of myelodysplasia and acute myeloid leukemia harbouring the mutant protein.
Methods: A lentiviral platform was used to introduce wild-type or mutant SF3B1 into cell lines representing the spectrum of MDS>AML, including MDS-L, SKM-1 and OCI/AML-3. The system allows finely tuneable conditional expression of the gene. The effects of variable gene expression, both mutant and wild-type on the susceptibility of these cells to molecular agents was assessed by cytology, proliferation, viability and apoptosis assays. Further genome-editing via CRISPR/Cas9 nucleases is being used to create isogenic models. In addition, other molecular effects, such as reduced DNA damage response were assessed.
Results: The conditional expression system was successful in producing titratable levels of the mutant protein. Cells which over-expressed the mutant protein had reduced proliferation compared to those harbouring only the wild-type gene. Those cells also had higher rates of spontaneous death in culture without further insult. Furthermore, the mutant expressing cells showed altered DNA damage repair after exposure to DNA-damaging chemotherapies, such as Etoposide. In line with this defect, they also demonstrated greater sensitivity to Poly ADP Ribose Polymerase inihibitors (PARPi).
Summary and Conclusions: This study has shown that an altered spliceosome, via SF3B1 mutation, results in a DNA damage repair defect. In terms of clinical applicability, this finding supports the idea that these mutant cells, with resulting DNA repair defects, can be treated with compounds that target DNA repair. One of the newest classes of small molecules in multiple clinical trials, are Poly ADP Ribose Polymerase inhibitors (PARPi), which showed a selective effect on mutant cells. In conclusion, these data suggest that this class of compounds has great potential in the treatment of SF3B1 mutant blood cancers; especially MDS or secondary AML.

P611

POLYMORPHISMS OF DNMTS AND FOLATE/METHIONINE METABOLISM GENES INFLUENCE DNA METHYLATION, GENETIC SUSCEPTIBILITY AND PROGNOSIS OF MYELOID NEOPLASIA PATIENTS

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Background: Global hypomethylation and targeted hypermethylation are considered defining characteristics of human tumors, including myeloid neoplasias (MN). Folate, methionine and vitamin B12 can influence the supply of methyl groups and, therefore, the biochemical pathways of methylation processes. Moreover, single nucleotide polymorphisms in genes that encode DNA methyltransferases (DNMTs) as well as enzymes from folate/methionine metabolism can influence the DNA methylation status, as well as the individual susceptibility to develop MN, namely Acute Myeloblastic Leukemia (AML), Myelodysplastic Syndromes (MDS) and Myeloproliferative Neoplasias (MPN), including Chronic Myelogenous Leukemia (CML).
Aims: In this context, we analyzed the influence of the polymorphisms in *DNMT1*, *DNMT3a*, *DNMT3b*, *RFC1*, *CBS* and *MTRR* genes in DNA methylation status and its role as risk and prognosis factors for myeloid neoplasia.
Methods: This study enrolled 333 patients diagnosed with myeloid neoplasia (80 AML, 106 MDS, 147 MPN, being 77 CML) and 261 healthy controls. The genetic polymorphisms of *DNMT1* (rs759920), *DNMT3a* (rs2289195), *DNMT3b* (rs2424908), *RFC1* (rs1051266), *MTRR* (rs162036), MTHFR (rs1801131, rs1801133), and *CBS* (844ins68) were assessed by RFLP-PCR and Tetra-primer-ARMS-PCR. The localized methylation status was analyzed through *p15*, *p16*, *DAPK,* and *KEAP1* methylation profile by MSP. Global methylation status was assessed by 5-methylcytosine (5mC), 5-hydroxymethylcytosine (5hmC) levels and LINE-1 methylation. The statistical analysis was carried out by variance analysis, $\chi 2$ test, and Fisher exact test ($p<0.05$).
Results: Our results show that *CBS, MTHFR*, *DNMT1,* and *DNMT3b* genes influence the localized and global methylation status in a genotype-dependent manner. Moreover, *DNMT1* AA and *DNMT3b* CT genotypes were protector factors for MDS development [OR 0,49 (CI95% 0,27-0,90), p=0,03; OR 0,50 (CI95% 0,28-0,83), p<0,001]. The *DNMT3a* AG genotype was also a protector factor for AML development [OR 0,16 (CI95% 0,05-0,52), p<0,001]. Besides that, individuals with *DNMT1* GG genotype have an increase risk for develop SMD about 1,78-fold (CI95% 1,03-2,01; p=0,04), while those with the *DNMT3a* GG and *DNMT3b* CC genotypes have an increase risk for develop AML about 6,82-fold (CI95% 1,22-37,95; p=0,03) and 2,01-fold (CI95% 1,07-11,48; p=0,03), respectively. The *RFC1* GG genotype also increases the risk of CML development about 2,65-fold (CI95% 1,53-4,57; p<0,001). Moreover, *DNMT3a* genotype can influence prognosis since MDS patients with *DNMT3a* GG genotype had lower survival (p=0,032). We also observed that allele C from MTHFR polymorphisms (rs1801133) and the AC genotype from the same gene (rs1801131) significantly increase 1,55-fold (IC95%: 1,08-2,23; p=0,031) and 2,01-fold (IC95%: 1,25-3,21; p=0,012) the risk of MDS development, respectively.
Summary and Conclusions: These results suggest that genetic polymorphisms in DNMTs and folate/methionine metabolism genes can influence the DNA methylation status, the susceptibility to develop MN and the prognosis of MDS patients.
This work was supported by CIMAGO (Project 22/09) and R. Alves is supported by the FCT fellowship FRH/BD/51994/2012.

P612

APG101 (SOLUBLE CD95-FC) IMPROVES BFU-E GROWTH IN LOWER RISK MYELODYSPLASTIC SYNDROME WITH COLLAPSED ERYTHROPOIESIS: A PRECLINICAL STUDY

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Background: The production of red blood cells is negatively regulated by caspase-dependent apoptosis of immature precursors mediated by death receptor CD95 when activated by its ligand, CD95L, in the bone marrow. CD95 is overexpressed at $CD34^+$ progenitor cell surface in two thirds of patients with low/int-1 risk myelodysplastic syndromes, and this contributes to anemia. CD95 overexpression may depend on methylation level (1) and on single nucleotide poly-

morphisms (SNP) at -1377 and -670 from ATG in the 5'UTR of *CD95* gene promoter. We have previously reported that resistance to erythropoiesis-stimulating agents (ESA) was significantly associated with CD95 overexpression and poor residual erythropoiesis.
Aims: In a preclinical study, we investigated an alternative therapeutic strategy for anemia in lower risk MDS based on inhibition of CD95-dependent apoptosis of erythroid precursors.
Methods: From a cohort of 250 MDS patients and 30 controls, CD95 and CD95L expression was quantified by flow cytometry, and SNP rs2234767 (-1377G>A) and rs1800682 (-670A>G) were determined by Sanger sequencing. The effect of APG101, a fusion protein consisting of the extracellular domain of human CD95 and Fc fragment of human IgG1, on erythropoiesis was investigated in 20 low/int-1 MDS and 6 control bone marrow samples from patients with another type of anemia in liquid cultures and/or clonogenic assays.
Results: CD95 and CD95L were overexpressed in 146/250 (58%) and in 48/82 (58%) MDS patients, respectively. In 147 samples, we showed that the -1377G allele, to which the transcription factor SP1 can bind, is more frequent in MDS compared to controls and that the -1377G/-670A haplotype is associated with a higher CD95 expression level than the -1377A/-670G ancestral haplotype (P=0.042). CD95L epigenetic regulation is currently under study. In liquid cultures, APG101 increased the number of erythroid progenitors of BFU-E type and the proliferation of erythroblasts by inhibiting apoptosis of immature precursors in 4 MDS samples, but not in 3 controls. In a series of 20 MDS with high CD95 expression level, APG101 improved the growth of bone marrow mononuclear cell (BMMC)-derived BFU-E in 15 MDS patients which erythropoiesis was initially collapsed with ≤15 BFU-E/10^5 BMMC, independently of CD95L level. APG101 had no effect on BFU-E number of control samples. More importantly, APG101 does not increase the expansion of non-erythroid leukemic clusters counted at day 7 of clonogenic assays.
Summary and Conclusions: These results delineate a subgroup of lower risk MDS with high CD95 expression and collapsed erythropoiesis that could benefit from alternative strategy to ESA. This provides with arguments for a phase I/II clinical trial in ESA-resistant lower risk MDS.

P613

AZACITIDINE IMPROVES THE T-CELL REPERTOIRE IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES AND ACUTE MYELOID LEUKEMIA WITH MULTILINEAGE DYSPLASIA

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Background: Patients with myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML) with multilineage dysplasia show several immunological abnormalities. Azacitidine represents a therapeutic option for these disorders and, beside the well known effects on bone marrow precursors, has been demonstrated to potentially influence T-cell polarization.
Aims: The aim of this study is to monitor the kinetic of the T-cell receptor (TCR) repertoire during Azacitidine treatment in order to explore its potential ability, not only to restore the hematopoietic function, but also to reverse the immune derangement typical of these patients
Methods: Our study consisted in a flow cytometric and spectratyping analysis performed on the peripheral blood of 11 patients (5 with MDS and 6 with AML with multilineage dysplasia) and 30 normal controls. Each patient was evaluated at baseline and then every 3 cycles of Azacitidine. The flow cytometry analysis was based on a panel of 24 beta variable (BV) family-specific antibodies. The profile of the third complementarity-determining-region (CDR3) in separated helper and cytotoxic T-cells was then analyzed by spectratyping. After immunomagnetic CD4+/CD8+ cell separation, RNA extraction and reverse transcriptase PCR, CDR3 fragment analysis was performed through capillary electrophoresis.
Results: By flow cytometry, we first demonstrated a variable improvement from baseline to the following evaluations in the frequency of CD3+ (mean 63.00% *vs* 68.24%, p=0.03), CD4+ (40.09% *vs* 42.05%, p=0.04) and CD8+ cells (20.27% *vs* 25.10%, p=0.02), whereas the frequency of CD16+ CD56+ cells was stable (8.50% *vs* 8.45%, p=0.22). Also the frequency of regulatory T-cells (0.53% *vs* 0.45%, p=0.84) and BV expansions was substantially stable in both CD4+ (mean 1.60% *vs* 2.53%, p=0.36) and CD8+ cells (5.20% *vs* 5.60%, p=0.79). Noteworthy, when monitored by spectratyping during their treatment our patients showed significant changes in their CDR3 profiles, which were much more evident in helper T lymphocytes. In fact, the frequency of skewed B*Vs* was significantly decreased from baseline to the following evaluations in the CD4+ subset (mean 81.45% *vs* 70.17%, p=0.004). In more details the mean frequency of skewed subfamilies in CD4+ cells decreased from 81.45% at baseline to 75.91% at 3 months, 80.86% at 6 months, 58% at 9 months, 17% at 12 months and 22% at 18 months. This pattern was even more pronounced in patients responding to Azacitidine (89.60% *vs* 61.47%, p=0.002). Also in the CD8+ subset a slight but statistically significant trend towards a reduction in the frequency of skewed CDR3 profiles (mean 99.27% *vs* 98.74%, p=0.01) was demonstrated. Beside our patients showed a substantially stable frequency of oligoclonal profiles in both CD4+ (mean 3.00% *vs* 2.74%, p=0.47) and CD8+ cells (11.82% *vs* 11.61%, p=0.19).
Summary and Conclusions: Our findings firstly confirmed in our patients an overall derangement of the TCR repertoire. However this pattern seems to gradually improve during Azacitidine treatment, as witnessed by the improvement in T-cell counts observed on flow cytometry but much more by the progressive restoration of the CDR3 diversity detected by spectratyping, especially within the CD4+ subset. Therefore our data suggest that Azacitidine could be potentially able to reverse the immune derangement typical of patients with MDS and AML with multilineage dysplasia.

P614

REPOPULATING PROGENITOR CELLS IN PRIMARY MDS BONE MARROW CELL CULTURE IN SEVERE HYPOXIC CONDITIONS

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Background: Myelodysplastic Syndromes (MDS) are clonal disorders. However, whether the transforming event occurs in a myeloid committed cell or in earlier progenitor (stem cell ?) it is still not ascertained. Evidence have been accumulated in both senses, but certainly, MDS initiating cells must be capable of sufficient repopulating capacity to perpetuate the disease.
Aims: We evaluated the repopulating ability and stem cell potential of hypoxia maintained primary bone marrow (BM) progenitors derived from Myelodysplastic Syndromes (MDS).
Methods: Thirty seven BM samples were obtained at diagnosis from MDS patients (WHO: 10 RA, 12 RCMD, 7 RAEB, 8 AL/post MDS). Mononuclear BM cells were isolated and cultured with TPO, FLT3-L, SCF, IL-3 in severe hypoxic conditions (0,3%O_2, 5%CO_2, 95%N_2), for 10-13 days (LC1). The stem cell potential of these cultures was explored by transferring cells to growth-permissive secondary cultures in normoxia (LC2), in the presence of SCF, G-CSF, IL-6, IL-3, and according to the Culture-Repopulating Ability (CRA) assay methodology, cell proliferation was evaluated daily. Expression of CD34, CD38, CD117, CD133 was determined and when present, the persistence of chromosomal aberrations was analyzed by FISH at various stages of cell culture. In parallel, MDS mononuclear BM cells were intravenously injected in sublethally irradiated NOD-SCID mice before hypoxic culture and after LC1 (1x10^6 cells inoculated per mouse). Mice were sacrificed at day 56 after graft. The presence of CD45+ human cells in the peripheral blood of injected mice was evaluated by flow cytometry analysis every two weeks, and quantified to evaluate the repopulating ability and the different engraftment capacity of cells before hypoxic culture and after hypoxic selection. Mice marrow trephines as well as spleen and liver biopsies were also evaluated morphologically to test the engraftment of human MDS cells.
Results: In all 37 MDS cases studied, cultured cell number decreased of one log or more after 10-13 days of culture in hypoxic conditions. Only 12/37 MDS cases showed a significant repopulating ability at day 17 of LC2, all classified as Low/INT1 IPSS risk category. In IPSS high and INT-2 risk cases, repopulating ability according to CRA method was absent. CD34+ cells were always decreased after hypoxia and did not co-express CD38. We demonstrated the persistence of chromosomal aberrations in CD34pos/CD38neg cells after hypoxic culture in 5 MDS cases (del5q, +8, del20q, -Y, complex karyotype). We observed in sublethally irradiated NOD-SCID mice transplanted with hypoxia cultured cells a significantly higher percentage of CD45+ human cell that in mice transplanted with non hypoxia selected cells, at days 28 after transplantation (5,5% *vs* 0,56%); at the same time, the analysis of cells derived from mice spleen showed a significantly higher percentage of CD45+ human cell (1,83%) in mice transplanted with hypoxia selected cells than mice transplanted with non selected cells (0,16%); by the other hand, the analysis of cells derived from mice bone marrow showed an higher percentage of CD45+ human cells in mice transplanted with non hypoxia selected cells than mice transplanted with selected cells (2,16% *vs* 1,26%).
Summary and Conclusions: Severe hypoxic culture conditions are maintaining a sub-population of MDS cells endowed with increased repopulating capacity *in vitro* and *in vivo* in sublethally irradiated NOD-SCID mice. Most importantly, repopulating ability was observed exclusively in IPSS lower risk MDS cases.

P615

DIAGNOSTIC UTILITY OF MYELOID NUCLEAR DIFFERENTIATION ANTIGEN EXPRESSION AND OTHER FLOW CYTOMETRIC PARAMETERS IN MYELODYSPLASTIC SYNDROME

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Background: Diagnosis of myelodysplastic syndrome (MDS) based on morphology is particularly difficult in low grade MDS (*i.e.* <5% blasts, no ring sideroblasts and karyotypic abnormality). Hence, a more objective method is essential. Currently there are various guidelines for standardization of flow cytometry (FCM) in MDS. However; their widespread applicability may be challenging especially in resource limited settings, due to vast array of markers recommended by these guidelines.

Aims: This study was conducted to assess the utility of expression of MNDA along with quantitative FCM approach using "mini-panel" of antibodies (CD34, CD10, CD19, CD45, CD11b, CD15 and CD56) in MDS diagnosis.
Methods: Bone marrow aspirates (BMA) collected from 52 consecutive patients with suspected diagnosis of MDS were divided into three groups: (1) proven MDS (n=12) based on morphology and/or cytogenetics (2) suspected MDS (n=6), non-contributory morphology and cytogenetics (3) non-MDS (n=34). Sixteen control BMA were also studied. 44 cases (12 MDS, 06 suspected MDS and 26 non MDS)were analyzed for MNDA expression (on granulocytes, blasts, monocytes and lymphocytes) and 52 cases were analyzed for seven quantitative parameters: (1) CD34+ myeloblasts% in nucleated cells (2) CD34+ B-cell progenitor% in all CD34+ cells (3) lymphocyte/myeloblast CD45 mean fluorescence intensity ratio (4) granulocyte/lymphocyte SSC peak channel ratio and the proportion of CD34+ myeloblasts expressing (5) CD15 (6) CD11b and (7) CD56. ROC curves were constructed for each parameter. A score of 1 was given to each of these parameters beyond the cut-off and score ≥3 was considered as FCM positive.
Results: MNDA expression in granulocytes and myeloblasts was significantly lower in MDS cases as compared to controls (p=0.003 and 0.001 respectively). The percentage of MNDA negative granulocytes (mean+SD, 79.9%+13.4%, p<0.001) was higher in MDS cases as compared to non MDS (53.46%+19.6%). Similar differences were also seen in myeloblasts (88.85%+7.3% in MDS *Vs* 60.3%+18.15% in non MDS, p<0.001). Monocytes, however; failed to show any significant difference between these groups (p=0.802). ROC cut-off value for granulocytes had sensitivity and specificity of 75% and 76.92%, respectively and for myeloblasts 91.6% and 88.5%, respectively for discriminating between MDS and non MDS. MNDA expression was significantly reduced in suspected MDS as compared to non MDS cases in both: granulocytes (p=0.032) and myeloblasts (p=0.004). On the basis of ROC cut offs, MNDA expression was altered in either granulocytes or myeloblasts (single abnormality) in five/six cases (83.3%) where four/six cases (66.7%) had dual abnormality (altered in both granulocytes and myeloblasts). However significant difference between the suspected MDS and non MDS was seen in the presence of dual abnormality only (p=0.022) and not in case of single abnormality (p=0.336). In other quantitative FCM parameters assessed, a score of ≥3 successfully distinguished MDS from non-MDS with a sensitivity of 100% and specificity of 95.2%. After incorporating MNDA score into the quantitative FCM scoring (total 8 parameters), score ≥3 successfully discriminated suspected MDS from non MDS cases (p=0.005) and MDS from non MDS (p<0.001) with a sensitivity and specificity of 100% and 95.24%, respectively which is similar to those obtained at a score of ≥3 with quantitative approach (Figure 1 and Figure 2).

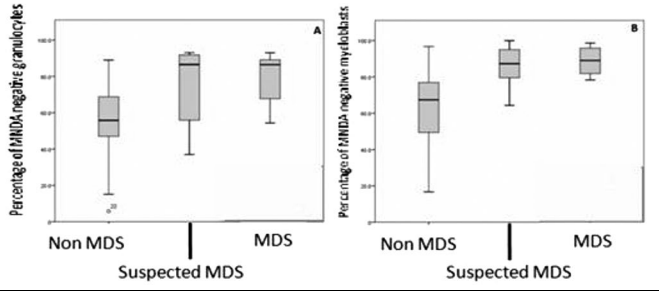

Figure 1. Box and whisker plot showing MNDA expression in different groups of patients. Percentage of MNDA negative granulocytes (A) and percentage of MNDA negative myeloblasts (B) in non MDS, suspected MDS and MDS cases.

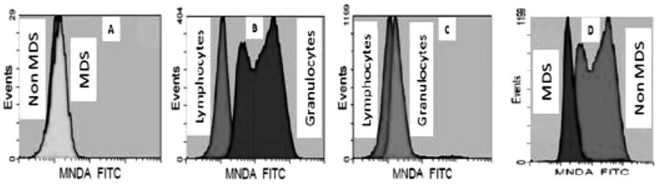

Figure 2. MNDA analysis in non MDS and MDS cases. MNDA expression is displayed for lymphocytes and granulocytes in a histogram. (A) Lymphocytes showing similar MNDA expression in both MDS and non MDS used as internal negative control (B) MNDA expression in granulocytes in a non MDS patient. (C) MNDA expression in granulocytes in a MDS patient.

Summary and Conclusions: Both MNDA analysis and quantitative FCM approach using "mini panel" of antibodies are independently able to discriminate MDS from non MDS and controls and correctly categorize suspected MDS as a distinct group. On merging the two approaches, the sensitivity and specificity of flow cytometric analysis did not improve. More studies validating this in a large cohort of patients are required before any conclusion can be reached regarding superiority of any method.

Myelodysplastic syndromes - Clinical 2

P616

OVERALL SURVIVAL (OS) AND BASELINE DISEASE CHARACTERISTICS IN MDS PATIENTS WITH PRIMARY HMA FAILURE IN A RANDOMIZED, CONTROLLED, PHASE III STUDY OF RIGOSERTIB

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Background: ONTIME was a randomized (2:1) study of rigosertib (RIG) *vs* best supportive care (BSC, including optional low-dose ARA-C) in 299 pts with higher-risk MDS who had relapsed after, failed to respond to, or progressed during treatment with hypomethylating agents (HMAs). For MDS patients who fail HMAs, there are no FDA-approved therapies. Thus an unmet medical need exists for effective second-line therapies. ONTIME showed a significant treatment effect with RIG in the subgroup of patients with "primary HMA failure" (Garcia-Manero *et al.*, Blood 2014).
Aims: To describe differences in overall survival (OS) after primary or secondary HMA failure in 299 patients treated with RIG (N=199) or BSC (N=100) in a Phase III study.
Methods: We evaluated the correlation between baseline disease characteristics and OS in pts with primary HMA failure (RIG N=117; BSC N=52) as ascertained by a centralized, blinded reader.
Results: Pts with primary HMA failure were generally male (69%), age 50-86 years (median 73), at high or very high risk per IPSS-R (80%), with 5-19% bone marrow blast count (92%). A meaningful difference in median OS between RIG and BSC was observed not only in the overall population of pts with primary HMA failure, but also in several subgroups (see Table 1). Overall, adverse events (AEs) were reported in 99% of RIG pts and 88% of BSC pts. The following AEs >=Grade 3 were reported by ≥ 10% of pts: anaemia (16% RIG *vs* 10% BSC), thrombocytopenia (15% *vs* 6%), neutropenia (15% *vs* 8%), febrile neutropenia (13% *vs* 10%), pneumonia (12% each).

Table 1.

Median (months) OS by Baseline Disease Characteristics in Pts with Primary HMA Failure						
	RIG		BSC		Log-rank p-value	Hazard ratio (RIG/BSC) (95% CI)
Characteristic	N	OS	N	OS		
All patients with Primary HMA Failure	117	8.6	52	4.5	0.011	0.63 (0.44-0.90)
IPSS-R category of high or very high	97	8.6	38	4.1	0.0015	0.52 (0.35-0.79)
Bone marrow blast = 5%-19%	93	10.1	36	4.7	0.0079	0.58 (0.39-0.87)
Duration of prior HMA treatment < 9 months	79	8.6	37	4.5	0.0014	0.49 (0.31-0.76)

Summary and Conclusions: Patients with primary HMA treatment failure and certain subgroups identifiable on the basis of baseline disease characteristics randomized to RIG showed an improvement in OS compared to BSC. Such characteristics should be considered in the design of future trials in second-line in primary HMA failure patients.

P617

INFERIOR LONG-TERM OUTCOME OF FRONT-LINE HYPOMETHYLATING AGENT COMPARED TO SUPPORTIVE CARE IN PATIENTS WITH LOWER RISK MDS: PROPENSITY SCORE MATCHED ANALYSIS

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Background: Supportive care (BSC) including hematopoietic cytokines still remains an important component of treatment for lower-risk myelodysplastic syndrome (LR-MDS) including low or intermediate-1 risk by International Prognostic Scoring System (IPSS) even in the era of hypomethylating agents (HMA). The role of front-line HMA for LR-MDS has not been clearly defined yet.
Aims: We evaluated the long-term outcomes of patients with LR-MDS treated with front-line HMA compared to those treated with supportive care.
Methods: The data of 353 patients diagnosed with LR-MDS from Oct 1992 to Jul 2013 were retrospectively evaluated. The prognostic factors affecting overall survival were evaluated within all population. Then, we performed a case-constrol study using 122 patients with propensity score matched (PSM) population.
Results: Initial patient population (n=353) included 110 patients treated with BSC and 243 treated with HMA. Patients characteristics (age, gender, IPSS risk groups, IPSS blast score, IPSS cytopenia score) were similar between two groups, however, ECOG performance status (PS) and IPSS cytogenetic score were biased between two groups. ECOG-PS 2-3 were 32 patients (29.1%) in BSC and 20 (9.3%) in HMA group (p<0.001) and IPSS cytogenetic score ≥0.5 were 27 patients (25.2%) and 33 (14.6%) in HMA group (p=0.032). In the multivariate analysis, ECOG-PS 2-3 (HR 4.586, p<0.001), IPSS blast ≥0.5% (HR 2.549, p<0.001) and front-line HMA therapy (HR 2.019, p=0.006) were unfavorable factors affecting OS. Using PSM population, we performed a case-control study comparing the outcomes of 61 patients in each group who treated with BSC and front-line HMA. Patient characteristics were well balanced between two group. In the multivariate analysis, ECOG-PS 2-3 (HR5.036, p<0.001), IPSS blast ≥0.5% (HR 2.157, p=0.035), and first-line HMA therapy (HR 2.213, p=0.026) were still unfavorable factors for OS. The 5-year OS rate was 62.5±10.8% in BSC group and 41.0±7.4 in HMA group, respectively (p=0.049). Infection was the main cause of death among patients who received HMA ≤4 cycles (61.3%) compared to those who received HMA >4 cycles (22.2%, p=0.051) (Figure 1).

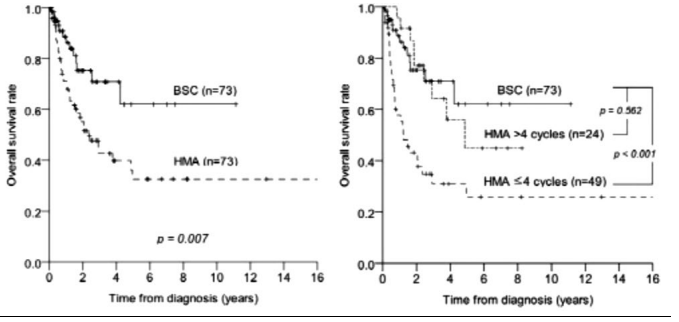

Figure 1.

Summary and Conclusions: Front-line HMA for patient with LR-MDS showed inferior long-term outcomes compared to BSC in PSM population. Early infection-related mortality could compromise the favorable effect of HMA. The effect of HMA on later leukemic transformation and resistance should be elucidated in the future studies.

P618

EFFICACY OF CC-486 (ORAL AZACITIDINE) TREATMENT (TX) IN PATIENTS WITH HIGHER-RISK MYELODYSPLASTIC SYNDROMES (HR-MDS)

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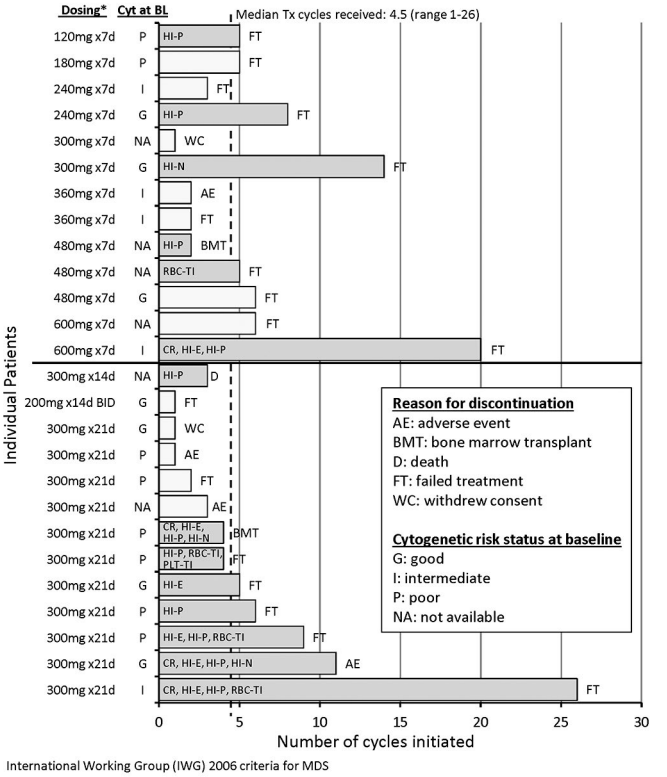
Background: CC-486 has clinical activity in patients (pts) with IPSS-defined lower-risk MDS (Garcia-Manero, ASH 2010, 2012). This *ad hoc* analysis assessed CC-486 efficacy in a small group of pts with HR-MDS (IPSS Intermediate [INT]-2 or High-risk) from 2 phase I/II studies. While all pts had HR-MDS, CC-486 dosing schedules in these studies varied between administration for 7 days (d) per 28d cycle and extended administration (CC-486 x14 or x21d/28d cycle); prognostic disease variables at baseline differed; and, at study entry, some pts were MDS Tx-naïve while others had progressive disease despite prior Tx.
Aims: To explore whether differences in dosing schedules, prognostic disease features, and receipt of prior MDS Tx influenced overall response rate (ORR) with CC-486 in pts with HR-MDS.

Methods: Pts received CC-486 120mg to 600mg QD x7d/28d cycle (7D cohort) or in an extended dosing schedule (ED cohort): 300mg QD x21d/28d (21D); 300mg QD x14d/28d (QD-14D); or 200mg BID x14d/28d (BID-14D). Dosing schedules in the ED cohort were analyzed collectively. Prognostic Risk Index scores for this *ad hoc* analysis were calculated as 1 point each for the following abnormalities at baseline: Hgb <11 g/dL, ANC <1.0x10^9/L, platelets <100x10^9/L, BM blasts ≥5%, and transfusion of ≥4 RBC units or ≥2 platelet units in the 56 days before study entry. ORR (IWG 2006) included complete or partial remission, RBC or platelet transfusion independence, and any hematologic improvement. ORR was evaluated based on dosing cohort (7D or ED), prior MDS Tx (yes/no), and prognostic Risk Index scores (≤3/>3 abnormalities).
Results: In all, 13 pts (50%) comprised the 7D cohort and 13 (50%) comprised the ED cohort (21D n=11, QD-14D n=1, BID-14D n=1). At baseline, most pts had IPSS INT-2 MDS (n=24, 92%), were male (n=19, 73%), and had ECOG PS of 0-1 (n=22, 85%). Pt diagnoses were RAEB-1 (n=11, 42%), RAEB-2 (n=11, 42%), RCMD (n=3, 12%), and RA (n=1, 4%). Overall, 11 pts (42%) had received prior MDS Tx (ESAs, growth factors, thalidomide, lenalidomide, and/or hypomethylating agents [HMAs, n=7]). Median ages in the 7D and ED groups were 69 (range 31-91) and 75 (47-87) years, respectively. Median time since diagnosis was 3.9 months (range 0.7-40) in the 7D group and 5.3 months (0.4-119) in the ED group. Median Hgb, ANC, and platelet counts in the 7D group were 9.1 g/dL, 0.6x10^9/L, and 54x10^9/L, respectively, and in the ED group were 9.0 g/dL, 0.4x10^9/L, and 19x10^9/L. Most pts (n=17, 65%) had prognostic Risk Index scores >3. One pt (7D cohort) was not evaluable for response (no post-baseline data). ORR for all pts was 56% (14/25) (Figure 1), including 5/8 pts with poor-risk cytogenetics. ORR in the 7D and ED cohorts were 46% (6/13) and 67% (8/12), respectively. ORR in pts who had received prior MDS Tx was 40% (4/10, including 2/7 pts who had received prior HMA Tx and responded to CC-486) and in pts who had no prior MDS Tx was 67% (10/15). ORR in pts with prognostic Risk Index scores ≤3 and >3 were 50% (4/8) and 59% (10/17), respectively.



International Working Group (IWG) 2006 criteria for MDS

*All but 1 patient received CC-486 doses once daily (QD)

BL = baseline; CYT = cytogenetics; CR = complete remission; HI = hematologic improvement; HI-P = HI platelet lineage; HI-E = HI erythroid lineage; HI-N = HI neutrophil lineage; RBC = red blood cell; PLT = platelet; TI = transfusion independence

Figure 1. CC-486 in patients with Higher-risk MDS.

Summary and Conclusions: More than one-half of these pts with HR-MDS attained a hematologic response with CC-486. In this small patient group, prolonged exposure to CC-486 resulted in a higher proportion of response. Absence of prior MDS therapy was associated with better response, though prior HMA Tx did not preclude response to CC-486. Baseline prognostic Risk Index score did not influence ORR, suggesting that underlying molecular pathways or genetic factors may have a greater influence on Tx response.

P619

AZACITIDINE (AZA) IN HIGHER RISK MDS PATIENTS WITH CHROMOSOME 7 ABNORMALITIES (ABN 7): RESULTS OF A RETROSPECTIVE STUDY FROM THE GFM AND GESMD REGISTRIES

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Background: Cytogenetic alterations are the most discriminative prognostic variables in MDS, complex karyotype (CK) and Abn7 having the poorest outcome. Data from small series have suggested that non complex Abn 7 are associated with relatively favorable response to AZA in MDS.The paradigm that CR or PR is necessary to prolong OS with AZA treatment has also been challenged.
Aims: We analyzed in a large series of higher risk MDS with Abn 7 treated with AZA the impact of response on survival compared to best supportive care (BSC).
Methods: This study analyzed 235 patients with higher risk (IPSS Int-2 and high) MDS with Abn 7 included in the French and Spanish MDS registries. 115 patients received AZA (75 mg/m^2/d for 7 days [n=92] or 5 days [n=23] every 4 weeks; median number of cycles, 5 [range, 1 - 32]) and 120 received only BSC (control group). The effect of response to AZA (IWG 2006 criteria) on outcomes was evaluated by time-dependent multivariable methods.
Results: Baseline characteristics were similar in both groups (Table 1). 91 AZA-treated patients were evaluable for response. The ORR was 42.8% (n=39), including 16.4% CR and 26.4% SD with HI. Among non-responders, 53.8% had SD without HI and 46.2% progressed. 11 (16%) of 67 RBC transfusion dependent patients became transfusion independent. The ORR was 43% in pts with CK, 35% in pts with non complex and -7 and 50% in pts with non complex and del(7q) (p=0.1 for complex *vs* non complex Abn 7). At last follow up, 61 patients had relapsed or progressed (53%) and 92 (80%) had died. AML-free survival and OS estimates at 1 and 2 years were 62 and 31% and 43% and 16%, respectively. In multivariable time-dependent analyses in the overall series response to AZA, IPSS risk group, and CK had a significant independent impact on OS and AML-free survival. Compared to untreated patients, any type of response to AZA and SD without HI was significantly associated with a reduced risk of mortality and AML progression (Tables 2 and 3).

Table 1. Patients and disease characteristics.

VARIABLE	AZA N=115	SUPPORTIVE CARE N=120	P
Age. mean years	67.4	67.4	
Age. median	69.7	73.8	
Cytogenetic at diagnosis			0.804
Monosomy 7 (-/+ other abn)	26 (22.8%)	29 (24.2%)	
del (7q-) (-/+ other abn)	16 (14%)	22 (18.3%)	
otra (7p-??)	2 (1.8%)	2 (1.7%)	
Complex Karyotype	70 (61.4%)	67 (55.8%)	
Complex Karyotype			0.355
No	44 (38.6%)	54 (45%)	
Yes	70 (61.4%)	66 (55%)	
IPSS			P=0.118
Int-2	52 (45.2%)	67 (55.8%)	
High	63 (54.8%)	53 (44.2%)	

All data at diagnosis regarding supportive care patients and at AZA onset regarding AZA patients

Table 2. Cox OS. Multivariate analysis among R *vs* SD *vs* NR *vs* no treatment.

Variable:	HR	95%IC	p
Age (reference <60)	1.285	0.909-1.818	0.15551
Treatment (reference=no treatment):			
No response	2.199	1.389-3.483	**0.00078**
Response	0.399	0.232-0.687	**0.00092**
SD wo HI	0.514	0.284-0.930	**0.02785**
IPSS (reference=Int-2)			
High	1.542	1.140-2.085	**0.00490**
Karyotype (reference=non complex)			
Complex	1.813	1.327-2.476	**0.00018**

Table 3. Cox LFS. Response *vs* no response *vs* SD *vs* no treatment multivariate analysis.

Variable:	HR	95%IC	p
Age (reference <60)	1.149	0.717-1.844	0.5635
Treatment (reference=no treatment):			
Response	0.196	0.106-0.364	**<0.0001**
No response	9.179	5.247-16.057	**<0.0001**
SD wo HI	0.113	0.05-0.255	**<0.0001**
IPSS (reference=Int-2)			
High	1.972	1.302-2.987	**0.0013**
Karyotype (reference=non complex)			
Complex	2.085	1.336-3.254	**0.0012**

Summary and Conclusions: AZA yields a significant response rate in higher risk MDS with Abn 7. Response to AZA, including SD without HI, translates into a survival benefit.

P620

Abstract withdrawn

P621

TREATMENT OF HIGHER-RISK MYELODYSPLASTIC SYNDROME (MDS) WITH AZACITIDINE: A 4-YEAR POPULATION-BASED ANALYSIS FROM BRITISH COLUMBIA, CANADA

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Background: MDS is a clonal disorder with propensity to transform to AML. Prior to 2010, treatment was limited to supportive care or, in young patients (pts), stem cell transplantation (SCT). Azacitidine (AZA), a hypomethylating agent, produces hematologic improvement (HI) in 50% of higher-risk MDS pts and prolongs overall survival (OAS; Fenaux, 2009). Health Canada approved AZA in October 2009 after which the British Columbia Cancer Agency (BCCA) began funding the drug for MDS pts in the province of BC.
Aims: The research objective was to review patterns of AZA use and to determine its efficacy in a population-based analysis.
Methods: An IRB-approved retrospective review of pts receiving AZA for MDS in BC was performed by obtaining dispensing records from the BCCA pharmacy data base and clinical notes from the Cancer Agency Information System. Final analysis date was August 1, 2014.
Results: Between 01/2010 and 04/2014, 181 pts were treated with AZA in BC; there were 127 males and 54 females with median age 71 years (range 1-90). Diagnosis (WHO 2008) was (oligoblastic) AML (60 pts), RAEB-2 (45 pts), RAEB-1 (24 pts), therapy-related neoplasm (19 pts), CMML (16 pts), RCMD (14 pts) or MDS, unclassifiable (3 pts). IPSS karyotype was available in 136 pts - 34% were good-risk/normal, 40% were intermediate-risk and 26% were poor-risk. Pts were treated at teaching hospitals in Vancouver (VAN, 72 pts), BCCA sites outside Vancouver (BCCA, 44 pts) or one of 14 smaller hospitals (OTHER, 65 pts) of which only 3 sites treated >5 pts. Starting AZA dose was 75 mg/m^2/d in 78%, 50 mg/m^2/d in 9%, 37.5 mg/m^2/d in 6% or other in 7% of pts. Overall, 50% of pts received 75 mg/m^2/d throughout treatment; 36%, 29% and 12% of pts required ≥1, 2 or 3 dose reductions. AZA doses were re-escalated in 20% of pts and 9% of pts were increased to 100 mg/m^2/d. Median number of AZA cycles given was 5 (range 1-42) with 60% of pts receiving ≤5 cycles; 35 pts (19%) received only one cycle and 37 pts (20%) received 2-3 cycles. Pts receiving ≤3 cycles was similar at VAN, BCCA and OTHER (42%, 30% and 45% of pts, respectively). IWG responses were evaluable in 163 pts and were CR (18 pts, 11%), PR (4 pts, 2%) or HI (47 pts, 29%); 14 pts (9%) had stable disease. HI by lineage was 64% erythroid (73% major), 49% platelet (79% major) and 34% neutrophil (85% major). Bone marrow exam follow-up was done infrequently in responding pts making upgrading from HI to PR/CR problematic and determination of cytogenetic response not possible. Responses were independent of cytogenetic classification with overall response (HI+PR+CR) similar in good- (52%) and poor-risk (44%) karyotypes. Reason for stopping AZA could not be determined in 35% of pts; most common identifiable reasons were lack/loss of response (43%), death from complications of MDS/AML (36%), decision to proceed to SCT (10%) and AZA side effects/patient discretion (10%). Median OAS for the entire cohort was only 7 months (range 1-37) with 43 pts (24%) alive at date of analysis. Including only the 109 pts receiving ≥4 cycles, median survival was 13 months.
Summary and Conclusions: This population-based analysis of AZA treatment outcomes in MDS suggests that many pts are not given adequate trials (≥4 cycles) at standard doses. This may explain why, despite IWG response rates

in line with the pivotal randomized trial, median survivals were inferior to expectations. Physician and patient education on the importance of adequate dosing and duration of therapy is ongoing in BC.

P622

ASSOCIATION OF AZACITIDINE AND LENALIDOMIDE (COMBINATION *VS* SEQUENTIAL TREATMENT) IN HIGH-RISK MYELODYSPLASTIC SYNDROMES. UPDATE OF THE RESULTS OF A RANDOMIZED PHASE II MULTICENTER STUDY

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Background: Azacitidine (AZA) is able to induce hematologic responses in 50-60% of patients (pts) with Myelodysplastic Syndromes (MDS) and moreover to prolong survival in higher risk MDS pts. However the duration of therapeutic response to AZA is limited, with a median survival advantage of only 9.5 months. Recently, several studies have evaluated the efficacy and safety of combining, in high-risk MDS pts, AZA with Lenalidomide (LEN), either administered concurrently *(Sekeres, 2010; 2012)*, or sequentially *(Platzbecker, 2013)*, in both cases showing promising results, although in a limited number of pts.
Aims: The aim of this study was to evaluate the efficacy and safety of the combination *vs* the sequential use of AZA and LEN in high-risk MDS pts (IPSS score risk: High or INT-2). Primary endpoint: ORR, defined as the Rate of Complete Remission (CR), Partial Remission (PR), Marrow Complete Remission (mCR), and Hematological Improvement (HI), following the International Working Group (IWG) criteria *(Cheson, 2006)*.
Methods: A randomized, phase II, multicenter, open label study, including pts with MDS (WHO 2008 classification) with International Prognostic Scoring System (IPSS) risk High or Intermediate-2, without previous treatment with AZA or LEN. ARM 1 (combined treatment): AZA: 75 mg/m2/day (days 1-5) I.C.+LEN: 10 mg/day (days 1-21), orally, every 4 weeks. ARM 2 (sequential treatment): AZA: 75 mg/m2/day (days 1-5) I.C.+LEN: 10 mg/day (days 6-21), orally, every 4 weeks. The induction treatment was planned for 8 cycles (32 weeks). For responder patients (CR, PR, mCR, or HI) the same treatment will be continued until disease progression or unacceptable toxicity. A sample size of 44 pts was planned. At the time of this analysis, 44 pts have been enrolled, 22 pts are still on therapy, and 7 of them have not yet completed 6 cycles of treatment.
Results: From March 2013, 44 pts (27 males), with a median age of 72 (48-83 yrs) were enrolled, from 13 hematologic italian Centers. At baseline, WHO diagnosis was: RCMD: 2 pts; RCMD-RS: 1 pt ; RAEB-1: 11 pts; RAEB-2: 30 pts; IPSS risk was: Intermediate-2: 32 pts; High: 9 pts; not determined (N.D.) (because of lack of cytogenetic data): 3 pts. (all with RAEB-2). IPSS cytogenetic risk was: Good: 16 pts; intermediate: 12 pts; Poor: 13 pts. 21 pts were randomly assigned to ARM 1, and 23 pts to ARM 2. At the time of this analysis, 44 pts have been enrolled, 28/44 pts (63.6%) completed ≥6 cycles of treatment, and are evaluable for response. 22/28 pts (78.6%) showed a favourable response to treatment, following the International Working Group (IWG) criteria *(Cheson, 2006)* : 9 pts achieved Complete Remission (CR), 2 pts attained Partial Remission (PR), 2 pts showed Marrow Complete Remission (mCR), and 9 pts obtained Hematological Improvement (HI). Responder pts were: 11/13 (84.6%) in ARM 1 (2 CR; 2 PR; 1 mCR; 6 HI), and 11/15 (73.3%) in ARM 2 (7 CR; 1 mCR; 3 HI), respectively. A grade >2 non hematologic toxicity was observed in 18/43 (41.9%) pts. 21/43 pts (48.8%) had a dose reduction of LEN because of hematologic or non-hematologic toxicity. 11 pts died; 6 pts showed progression to AML.
Summary and Conclusions: Our results, although preliminary, seem to confirm the feasibility of the association of AZA and LEN in high-risk MDS pts. Ongoing analyses are aimed to assess possible relationships between response to therapy and specific alterations affecting inositide signalling molecules, mutational status and microRNA profiling.

P623

CLINICAL IMPACT OF DEL (11Q) IN PATIENTS WITH DE NOVO AND SECONDARY MYELODYSPLASTIC SYNDROMES- A SINGLE INSTITUTION EXPERIENCE

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Background: Del(11q) is an uncommon cytogenetic abnormality (<1%) in patients with myelodysplastic syndromes (MDS), and when associated with disruption of the *MLL* gene portends an inferior survival. With the recent revision of the IPSS, isolated del 11q (non-*MLL*) is considered to be a favorable karyotype.
Aims: To describe the incidence, clinical features and outcomes of 11q deleted MDS and the impact of the *MLL* gene rearrangement.
Methods: After due IRB approval, patients diagnosed with MDS from 2000-2015 at Mayo Clinic Rochester were screened. Consecutive cases of MDS with del (11q) were identified and reviewed. Patients underwent a bone marrow aspirate at diagnosis and all clinical and pathologic data including FISH studies and cytogenetics were retrospectively reviewed. The cohort was stratified into three groups according to the underlying karyotype; isolated del (11q), del (11q) with an additional structural abnormality, and del (11q) with a complex karyotype. Survival analysis was estimated by Kaplan-Meier method and log ranks was used to compare the curves.
Results: A total of 1550 MDS patients were screened and, 23 (1.5%) with del (11)q were identified. Eleven patients (47%) were males. Four (17%) patients had secondary MDS, while the remainder had de novo disease. The median age at diagnosis was 70 years. Median presenting features included the following; absolute neutrophil count (ANC) of $1.8x10^9$, hemoglobin (Hb) of 8.8 g/dl, platelets (Plt) $75x10^9$, and bone marrow (BM) blasts of 3%. The median follow up was 12 months and over all 17 deaths (74%) and 2 (%) leukemic transformations were documented. The median OS for *MLL* rearranged *versus* non rearranged patients was 16 *vs* 24 months, respectively (p=0.49). Patients were then grouped based on additional cytogenetic abnormalities. Group 1: Six pts had del (11q) as a sole abnormality, one of who had a *MLL* rearrangement. IPSS-R stratification score included 4 with low risk and 2 with intermediate risk. Median overall survival (OS) was 30 months (*P*=0.0094) comparing survival among the three groups and diminished to 28 months with *MLL* rearrangement (*P*=0.0015). Group 2: Nine pts had del (11q) and an additional structural abnormality, with only one of these demonstrating the MLL rearrangement. None of the additional karyotypic abnormalities were considered poor prognosis based on IPSS-R. As per the IPSS-R score, there were 5 intermediate risk and 3 low, 1 missing data. Median OS was 24 months *vs* 9 months without and with *MLL* rearrangement, respectively (*P*=0.0015) comparing survival based on *MLL* rearrangement among the three groups. Median OS of the group was 21 months. Group3: Eight patients with del (11q) with a complex karyotype, of which three demonstrated the MLL rearrangement (2 with therapy related MDS). Notably there were no patients with a monosomal karyotype. As per the IPSS-R score, there were 6 very high risk and 2 high risk. Median survival of the group was 7 months. Median OS again diminished with *MLL* rearrangement at 5.6 *vs* 10 months, respectively (*P*=0.0015) (Figure 1).

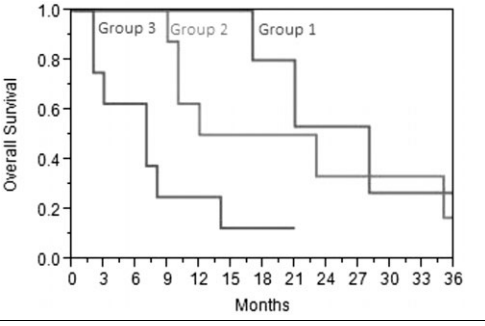

Figure 1.

Summary and Conclusions: Del (11q) is a rare karyotype in MDS with an incidence of <2%, 25% with del (11q) have associated *MLL* gene rearrangements. Although non-*MLL* rearranged isolated del (11q) is classified as a very good cytogenetic aberration, the observed OS in our group was inferior to IPSS-R predicted survivals. In subgroup analysis *MLL* gene rearrangements independently impacted OS.

P624

A PROGNOSTIC MODEL FOR PREDICTING SURVIVAL AFTER STOPPING HYPOMETHYLATING AGENT IN MYELODYSPLASTIC SYNDROME

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Background: Hypomethylating agent (HMA) is widely used for the treatment of myelodysplastic syndrome (MDS) not only for hematopoietic cell transplantation (HCT) ineligible patients but also HCT eligible patients for a bridging therapy. However survival after HMA stop is very dismal although more intensive therapy like HCT can expect longer survival. However, we don't know which patient can survive longer even after stopping HMA is still unknown.
Aims: We wanted to know which clinical factors will be important for predicting prognosis after HMA failure. Here we present a prognostic model for predicting survival after stopping HMA in MDS.
Methods: Data were retrospectively collected. All MDS patients who had received HMA were selected. For the analysis of prognostic model after stopping HMA, we excluded patients with on-going HMA therapy, patients who stopped HMA for planned HCT or patients who died during HMA therapy.
Results: We retrospectively collected 236 MDS patient who had received HMA. We excluded on going HMA therapy, stopping HMA for planned HCT or stopping HMA by death. Therefore total 187 patients were selected for this analysis. Median survival after HMA stop was 11.4 (95% CI, 9.369-13.432) months. HCT or supportive care had longer survival than chemotherapy (p=0.024) as a salvage therapy after HMA stop. Many clinical factors were validated as prognostic factors for predicting survival; gender (p=0.568), age<70y (p=0.034), ECOG 0 or 1 (p=0.065), prior cancer or multiple comorbidity (p=0.017), initial IPSS-R very low/low/intermeidate (p=0.001), time to HMA start >4 weeks (p=0.067), azacitidine as HMA (p=0.127), HMA treatment duration <3M (p=0.346), SD/HI as best response to HMA (p=0.003), CR/PR/SD as final response to HMA (p<0.001), HMA stop by progression/loss of response (p=0.002). Multivariate analysis revealed that age<70 (HR 1.766, 95% CI 1.156-2.698; p=0.008), no prior cancer or multiple comorbidity (HR 2.340, 95% CI 1.127-4.859; p=0.023) or CR/PR/SD final response (HR 2.225, 95% CI 1.480-3.344; p<0.001) were prognostic factors. Median survival for favorable (no risk factor; n=55), intermediate (1 risk factor; n=97) or poor risk group (2 or more risk factors; n=34) were 30.653 (95% CI 17.786-43.520), 10.349 (95% CI, 8.267-12.431) or 5.322 (95% CI, 2.757-7.888) months, respectively (p<0.001; Figure 1).

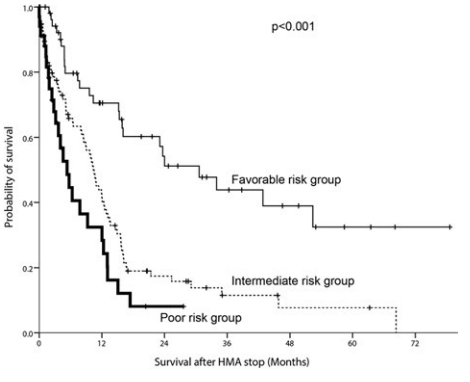

Figure 1.

Summary and Conclusions: Our data showed that survival after stopping HMA was dismal, but some prognostic factors can predict longer survival among them even with supportive care.

P625

CORRELATION OF OVERALL SURVIVAL (OS) WITH BONE MARROW BLAST (BMBL) RESPONSE IN PATIENTS (PTS) WITH MYELODYSPLASTIC SYNDROMES (MDS)

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Background: BMBL% is the prognostic variable with the greatest impact on outcome in MDS patients at diagnosis and subsequent time points. Current composite response criteria (IWG, Cheson 2006) do not consistently correlate with OS. Treatment impact of BMBL as an independent response criterion has not been adequately evaluated.
Aims: To evaluate the correlation between OS and BMBL in pts with higher-risk (HR) MDS.
Methods: We accessed 4 datasets encompassing 7 studies with 887 pts and evaluated the correlation of BMBL response/stabilization at 4- and 12-week timepoints with OS. The 4 studies were: ONTIME, a Phase III randomized study of 2nd-line rigosertib (RIG) *vs* best supportive care (BSC) (N=299; Silverman, ASH 2014); 4 Phase I/II studies of RIG in pts with MDS/AML (N=39; Silverman, Hematol Oncol 2014); AZA-001, a Phase III study of azacitidine (AZA) *vs* 3 conventional care regimens (N=358; Fenaux, Lancet Oncol 2009; Gore, Haematologica 2013); Cancer & Leukemia Group B (CALGB) Study 9221, a Phase II, randomized trial of 1st-line AZA *vs* BSC (N=191; Silverman, J Clin Oncol 2002). Change in blasts was defined similarly in each of the 4 studies: BM complete response is BMBL ≤5%; BM partial response is ≥50% decrease from baseline, but BMBL still >5%; stable disease is <50% decrease or increase from baseline.
Results: In ONTIME, landmark time-dependent analyses showed correlation of BMBL response/stabilization with OS at 4 wks (P=0.011) and 12 wks (P<0.001). In the 4 Phase I/II studies, BMBL response/stabilization at 4-8 weeks was associated with a quadrupling of median OS (P<0.001). In Study AZA-001, time-dependent analysis of BMBL stabilization was associated with a significantly reduced risk of death in both treatment cohorts (P<0.001) compared to progression. In Study 9221, landmark analysis of BMBL response/stabilization showed a 6-fold improvement in OS (P<0.001).
Summary and Conclusions: These studies, spanning more than a decade with different therapeutic agents and settings, demonstrate a consistent positive correlation between BMBL response and OS in pts with HR-MDS, including pts on supportive care. This suggests that use of reduction/ stabilization in BMBL can serve as a new early response parameter, as an intermediate clinical endpoint for evaluation of new agents, and as a biomarker for disease progression in HR-MDS itself.

BMF syndromes incl. PNH - Clinical

P626

THE INTERIM ANALYSIS OF PROSPECTIVE OBSERVATIONAL STUDY WITH PNH-TYPE CELLS IN JAPANESE PATIENTS WITH BONE MARROW FAILURE (THE OPTIMA STUDY)

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Background: Paroxysmal nocturnal hemoglobinuria (PNH) is a disease deriving from an acquired mutation of the phosphatidylinositol glycan class A (*PIGA*) gene in the hematopoietic stem cells. In some cases of aplastic anemia (AA) or low-risk types of myelodysplastic syndromes (MDS), it is known that PNH-type cells can be often detected through the high-resolution flow cytometry-based method. Because these patient groups are reported to have a good response to immunosuppressive therapies as opposed to the other patient groups lacking PNH-type cells, detection of these cells is potentially useful in determining a treatment plan for the cases of bone marrow failure syndromes. To confirm this preliminary information, a large cohort study is needed.
Aims: In order to determine the prevalence and significance of PNH-type cells in bone marrow failure patients, we conducted a nationwide multi-center prospective 0bservational investigation, the OPTIMA study (Observation of GPI-anchored protein-deficient cells in Japanese patients with bone marrow syndrome and in those suspected of having PNH).
Methods: A nationwide multi-center prospective observational study (OPTIMA) was started in July 2011 to determine the prevalence of patients with bone marrow failure syndromes who carried PNH-type cells and to clarify the clinical significance of the presence and quantitative changes of these cells with regard to the clinical features. Each of the 6 laboratories in different universities was assigned as a regional analyzing center. The percentage of PNH-type cells was measured by the high-resolution flow cytometry-based method, originally established in Kanazawa University. At the six individual laboratories, cross validations were conducted to minimize the inter-laboratory variations in the detection sensitivities, cutoff values, etc. For the detection of PNH-type granulocytes, the liquid FLAER method (≥0.003%) was used, and cocktail method (≥0.005%) with CD55 and CD59 antibodies was used for PNH-type erythrocyte detection.
Results: As of July 2014, flow cytometry data have been collected from 1739 patients and are included in this interim analysis. Of these patients, 67 (3.9%) were diagnosed with PNH, 546 (31.4%) with AA, 475 (27.3%) with MDS, and 651 (37.4%) with bone marrow failure (BMF) of unknown etiology. Overall, 172 (9%) patients had ≥1% of both PNH-type erythrocytes and granulocytes: 66 (98.5%) patients with PNH; 66 (12.1%) with AA; 22 (4.6%) with MDS; and 18 (2.8%) with BMF of unknown etiology. In total, 607 (34.9%) patients had ≥0.005% PNH-type erythrocytes and ≥0.003% PNH-type granulocytes. These consisted of the followings: all 67 (100%) patients with PNH; 295 (54.0%) with AA; 85 (17.9%) with MDS; and 160 (24.6%) with BMF of unknown origin. No patients with MDS-RAEB-1 or -2 had PNH-type cells. Lactate dehydrogenase (LDH) levels ≥1.5 × upper limit of normal range were seen in 4% patients with 0.005-1% PNH-type erythrocytes, 37% patients with 1-10% PNH-type erythrocytes, and 97.4% patients with ≥10% PNH-type erythrocytes.Periodic blind validation tests revealed that inter-laboratory differences in absolute measurements of PNH-type cells were always within 0.02%.
Summary and Conclusions: A high-resolution flow cytometry-based method that enables the detection of very low percentages of PNH-type cells was successfully transferred to 6 laboratories across Japan. Our results demonstrated that the proportion of patients identified as having small percentages of PNH-type cells differed depending on diagnosis (PNH, AA, MDS, or unknown BMF) and that elevated LDH levels (>1.5xupper limits of normal range) were more frequently associated with higher percentages of PNH-type erythrocytes. PNH-type cells didn't detected in patients with MDS-RAEB-1 or -2. Our findings suggest that the high-resolution method is helpful as a diagnostic tool in BMF syndromes, including AA, MDS, and PNH, and may prove useful in understanding the pathophysiology of these disorders.

P627

HYPOMEGAKARYOCYTIC THROMBOCYTOPENIA (HMT): AN IMMUNE-MEDIATED BONE MARROW FAILURE CHARACTERIZED BY AN INCREASED NUMBER OF PNH-PHENOTYPE CELLS AND PLASMA THROMBOPOIETIN LEVELS

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Background: Chronic thrombocytopenia is one of the most common abnormalities that are seen at hematology clinics. Although immune thrombocytopenic purpura (ITP) is the most frequent diagnosis of chronic thrombocytopenia without anemia and leukocytopenia, there are a considerable number of cases exhibiting megakaryocytic hypoplasia which do not meet the diagnostic criteria for ITP, aplastic anemia (AA), myelodysplastic syndrome (MDS), or paroxysmal nocturnal hemoglobinuria (PNH). Patients with hypomegakaryocytic thrombocytopenia (HMT) are usually followed without therapy when their thrombocytopenia is mild, *i.e.*, $50x10^9$/L. However, some patients may progress to aplastic anemia (AA) after a long observation period, which is often refractory to immunosuppressive therapy (IST). It is therefore important to elucidate the pathophysiology of patients with HMT at the time of diagnosis, even when their thrombocytopenia is not severe. We previously conducted a retrospective analysis of 29 patients with HMT and found that 55% patients possessed an increased number of glycosylphosphatidylinositol (GPI) anchored protein (GPI-AP)-deficient blood cells (PNH-type cells), which are often detected in patients with AA and are associated with a better response to IST than patients not possessing PNH-type cells. Ten of such 16 PNH(+) patients received cyclosporine (CsA) monotherapy and 60% (6/10) responded to the therapy (unpublished observation). Prospective studies may help to clarify the pathophysiology and prognosis of HMT.
Aims: To further characterize the clinical findings of HMT, we began a multi-center prospective observational study in 2009 (UMIN-CTR 000002729).
Methods: The inclusion criteria were WBC $>3x10^9$/L, Hb >10 g/dL and Plt $<100×10^9$/L with a decreased number of megakaryocytes. The patients who met the diagnostic criteria for well-established hematologic diseases including ITP, AA, MDS and PNH were excluded. PNH-type cells were detected using a high-sensitivity flow cytometry assay at the time of diagnosis and annually thereafter. The cutoff values of the assay were >0.003% for granulocytes and 0.005% for erythrocytes. The plasma thrombopoietin (TPO) levels were determined at the time of enrollment using an ELISA. The therapeutic plans were left to the discretion of each institution. The data were collected in January 2015 for this interim analysis.
Results: From August 2010 to March 2014, 25 HMT cases (male/female, 14/11) were enrolled. The median of age was 65 years (range, 25-79). The mean of the complete blood cell counts was as follows: white blood count (WBC) 4.1 (2.77-8.50)$x10^9$/L; hemoglobin (Hb) 11.9 (10.0-14.8) g/dl, and platelet (Plt) 40.0 (1.0-95.0)$x10^9$/L. PNH-type cells were detected in seven of the 25 cases (28%) and the median clone size of the PNH-type cells in PNH(+) patients was 0.073%, (range, 0.015% - 20.5%) in granulocytes and 0.069% (range, 0.0% - 3.8%) in erythrocytes. The TPO level ranged from 0.4 - 42.8 fmol/ml (median, 8.4 fmol/ml). Eleven (44%) patients showed TPO levels greater than 9.1 fmol/ml, which is the lowest level observed in our patients with typical AA (Seiki, *et al.* Haematologica, 2013). The patients with high TPO levels (TPO^{high} patients) showed significantly lower Hb levels (11.0 *vs* 12.4, $P<0.03$) and a higher prevalence of increased PNH-type cell numbers (64% *vs* 0%, $P<0.001$) than patients with low TPO levels (TPO^{low} patients). A total of six patients were treated with CsA or prednisolone (PSL) while the other 19 patients left untreated; four TPO^{high} patients, including three PNH(+) and one PNH(-) patient, were treated with CsA and all three PNH(+) patients responded well to the therapy. Two TPO^{low} patients treated with PSL improved. Among the 19 untreated patients, the hematological parameters of 11 patients remained unchanged during the median follow-up period of 30 months. On the other hand, thrombocytopenia progressed in one TPO^{low} patient and four of seven (57%) TPO^{high} patients, which include two PNH(+) patients. Notably, two TPO^{high} PNH(+) untreated patients progressed to moderate AA in 15 and 12 months after the diagnosis of HMT. One TPO^{low} patient developed acute myeloid leukemia in 18 months and one TPO^{low} patient demonstrated spontaneous resolution of the thrombocytopenia (Figure 1).

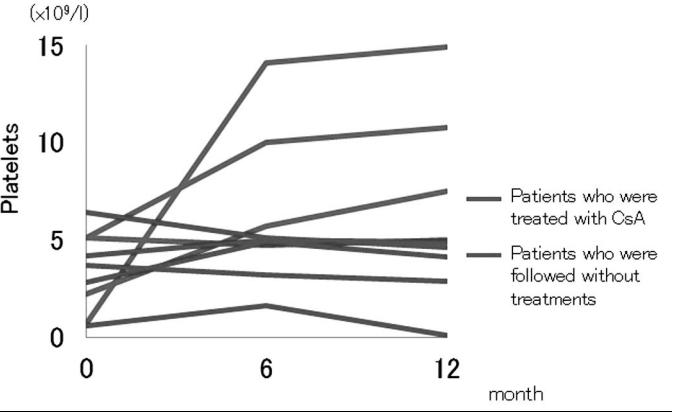

Figure 1. Changes of the platelet count in TPO^{high} patients with hypomegakaryocytic thombocytopenia (HMT).

Summary and Conclusions: This prospective analysis confirmed our previous

observation that approximately 50% of all patients with HMT have an immune pathophysiology similar to AA. Early treatment with CsA may therefore improve the prognosis of HMT patients who are generally followed up without therapy until their thrombocytopenia progresses. A long-term follow-up is needed to clarify the influence of CsA therapy on the natural course of HMT.

P628

OUTCOME OF STEM CELL TRANSPLANTATION IN PATIENTS WITH SHWACHMAN- DIAMOND SYNDROME WITH OR WITHOUT SECONDARY MDS/AML: AN ANALYSIS OF THE GERMAN SHWACHMAN-DIAMOND SYNDROME COHORT BY THE SCNIR- EUROPE

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Background: Shwachman-Diamond Syndrome (SDS) is a rare autosomal recessive disorder characterized by exocrine pancreatic insufficiency, bone marrow failure and a varying degree of congenital abnormalities including the skeleton, liver, heart and the immune system as well as psychomotor retardation. It has been documented that SDS patients have an increased risk to develop myelodysplasic syndrome (MDS), pancytopenia and leukemia, particularly acute myeloid leukemia (AML). So far, stem cell transplantation is the only therapeutic option for SDS patients with secondary bone marrow failure or leukemia.
Aims: The aim of this analysis is to evaluate the outcome on SCT in patients with Shwachman-Diamond Syndrome.
Methods: In the SCNIR we have collected long term data on 41 German patients with a clinical diagnosis of Shwachman-Diamond Syndrome. In 37 patients SDS diagnosis was confirmed by detection of compound heterozygous SBDS gene mutations. Here we report on the outcome of stem cell transplantation (SCT) in 9 patients with SDS in Germany, which were performed between 1999 and 2014. Reasons for SCT were MDS (1 patient), secondary leukemia (2 patients), pancytopenia (4 patients), cytogenetic abnormality (deletion 20q) (1 patient) and severe lung infections (1 patient).
Results: Patients' characteristics are as follows: Gender (4 female, 5 male), age at SCT (1.5 years - 30 years), median follow-up time post- SCT (52 months), G-CSF treatment prior to SCT (n=3), stem cell source (MUD: n=6, MSD: n=2, haploidentical: n=1). The majority of patients received a Fludarabine/Treosulfan or Fludarbine/Melphalan based conditioning regimen in combination with ATG or Campath. 2 patients received a second SCT due to graft failure. Outcome: 3 patients received a SCT due to secondary hematologic malignancy: AML (n=2) and MDS (n=1). Both patients with AML have died. Patient 1 (AML) received a MUD SCT without anti-leukemic therapy and 37% blasts in the bone marrow. The preparative regimen consisted of Busulfan, Melphalan, VP-16 and ATG. After initial engraftment, the patient relapsed on day +101 and died 1 month later due to disease progression. Patient 2 (AML) received AML- induction therapy with Ara-C and Daunorubicin. He went into prolonged aplasia and received a haploidentical SCT from this mother after a conditioning regimen with Fludarabine, Thiotepa, ATG and total lymphoid irradiation. He died due to severe bacterial infection on day+11. Patient 3 (MDS with complex karyotype) is alive and well 107 months after SCT. The patient received a MUD SCT after a preparative regimen of Fludarabine, Treosulfan, Melphalan and Campath. Patient 1 and 3 developed bacterial sepsis and acute Graft *versus* Host Disease (GvHD) within the first 100 days after SCT (grade I and II). 6 patients received a SCT (MUD: n=4, MSD: n=2) for other reasons: pancytopenia (n=4), deletion 20q (n=1) and severe lung infections (n=1). Complications within 100 days post SCT included acute GvHD grade II (n=2), bacterial sepsis (n=2), hepatic candidiasis (n=1) and primary (n=1) and secondary (n=1) graft failure with successful second SCT. 1 patient (pancytopenia) died due to idiopathic pneumonia syndrome on day+80 after he had received a cord blood transplant (conditioning regimen: Fludarabine, Treosulfan, Melphalan, ATG). The other 5 patients are alive 8-106 months after SCT.
Summary and Conclusions: In summary, out of 9 patients with SDS who underwent SCT, 6 have survived documenting that SCT is the treatment of choice in patients who developed leukemia or pancytopenia.

P629

PEARSON SYNDROME: MULTISYSTEM MITOCHONDRIAL DISORDER WITH BONE MARROW FAILURE

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Background: Pearson syndrome (PS) was originally reported as a fatal disease in infancy characterized by sideroblastic anemia with vacuolization of marrow precursors and exocrine pancreas dysfunction. In addition, involvement of multiple other organs and subsequent development of Kearns-Sayre Syndrome (KSS) have also been reported. Both PS and KSS are associated with single mitochondrial DNA (mtDNA) deletions. There are few systematic studies on PS because of its rarity.
Aims: To characterize the clinical and hematological presentations and courses of patients with PS.
Methods: We retrospectively reviewed clinical and laboratory data of 20 patients (12 M/ 8 F) with PS diagnosed between 1987 and 2012 in Germany.
Results: The median hemoglobin level was 5.9 g/dl (2.2-9.8 g/dl) and the mean corpuscular volume of red cells (MCV) was elevated or normal in 12 and 14 patients, respectively (unknown in 4). Some patients had normal neutrophil and platelet counts at diagnosis (n=5/6), but all of them developed bi- or pancytopenia later. The hemoglobin F level was elevated for age in 8 of 9 patients examined. Bone marrow was normo- and hypocellular in 13 and 7 patients, respectively. All patients had vacuolization of erythroid and myeloid precursors, but ringed sideroblasts are only observed in 13 of 19 patients examined. Serum lactate was elevated in 14 of 15 patients studied. The diagnosis of PS could be confirmed by the detection of mtDNA deletion in all patients. The median age at the time of the last follow-up was 39 months (6 months to 14 years). Hematological recovery was observed in most patients with a longer follow-up time. Ten patients became transfusion independent at a median age of 27 months (11 to 67 months), while one patient was still transfusion dependent when he died at the age of 8 years. Various symptoms and organ dysfunctions developed during clinical courses, including failure to thrive/short stature (n=8, median age of presentation: 26 months (m)), liver dysfunction (n=2, 21 m), renal tubulopathy/Fanconi syndrome (n=5, 32 m), pancreas insufficiency (n=6, 13 m), cardiac disease (n=3, 45 m), diabetes (n=1, 19 m), other endocrine dysfunctions (n=4, 63 m), hearing loss (n=1, 115 m), ophthalmoplegia (n=1, 19 m), retinitis pigmentosa (n=1, 92 m), cataract (n=1, 39 m), muscle hypotonia (n=4, 31 m), ataxia (n=2, 54 m) and encephalopathy (n=1, 74 m). Seven patients died of acute metabolic acidosis with other complications at the median age of 49 months (14-101 months). The longest survivor (death at 14 years of age) suffered from KSS-like disease.
Summary and Conclusions: These findings suggest that PS is a multisystem mitochondrial disorder in infancy with bone marrow failure as the main presenting feature. Because patients can initially present with only anemia, clinicians should consider PS as a differential diagnosis of hypoproliferative anemia in early childhood. Although hematological improvement can be expected in long survivors, patients have highly heterogeneous clinical courses with varied extents of multi-organ involvement. Therefore, intensive monitoring and managing of multisystem complications are crucial.

P630

PEARSON SYNDROME: A RETROSPECTIVE COHORT STUDY FROM THE MARROW FAILURE STUDY GROUP OF THE AIEOP (ASSOCIAZIONE ITALIANA EMATO-ONCOLOGIA PEDIATRICA)

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Background: Pearson Syndrome is a multisystem disorder caused by mutation of mitochondrial DNA (mtDNA). It typically consists of hypoplastic macrocytic anemia with vacuolated marrow precursors, lactic acidosis and exocrine pancreatic dysfunction. Additional manifestations may include myopathy, neurologic symptoms, skin lesions and renal tubular acidosis.
Aims: To provide clinical, biochemical and hematological insights through the analysis of a reasonably sized cohort of patients.
Methods: A retrospective analysis via dedicated Case Report Form including more than 160 items sent to all 55 A.I.E.O.P. (Associazione Italiana di Ematologia ed Oncologia Pediatrica) Centers. Information was collected on all presumed PS patients diagnosed in Italy in the period 1993-2014.
Results: this series, with 11 enrolled patients, is one of the largest ever reported. The principal characteristics of the patients are shown in Tables 1 and 2.

The median age at diagnosis was 299 days. At onset, among the 11 patients, pancytopenia was present in 5, anemia associated with leucopenia and/or neutropenia in 5, isolated anemia in 1; the median value of Hgb was 5.7 gr/dL, the lowest value of platelets was 72,000/μL and neutropenia (present in 10 children) was severe (Absolute Neutrophil Count (ANC) <0.5x10^9/L) in 1, moderate (ANC 0.5-1x0^9/L) in 8, and mild (ANC of 1.3xx10^9/L) in another. HbF was assessed in 5 patients outside the neonatal period and it was found elevated (>2%) in all of them. BM examination was performed in all patients: a precursor vacuolization associated with reduced cellularity was found in 6 children, isolated vacuoles in 2, mild dyserythropoiesis in 1, and hypocellularity in 2. One patient evolved, after allogenic Bone Marrow Transplantation, into acute myeloid leukemia. No solid tumor was observed. Exocrine pancreatic deficiency and neurologic symptoms were observed respectively in 3 and 8 children. A hypertrophic cardiomyopathy was present in 4 out of 10 evaluated patients. Serum lactate was dosed in all patients and found elevated in all but one. A plasmatic amino acid analysis was performed in 10 children: in 9/10 alanine was high. Urine organic acids were analyzed in 9 patients: in 7/9 an increased excretion of lactate and fumaric acid was noted; interestingly an increase of malic acid excretion was also found in 4/9 patients. All patients were transfused with packed red blood cells (PRBC), 6 with platelets and 2 with plasma. A spontaneous improvement of the Hgb values was noted in 8 of the 9 evaluable patients. Therapy with EPO was attempted, without any efficacy, in 3 children. Granulocyte-colony stimulating factor (GCSF) was administered in 3 patients with proven efficacy in one of them. After a median FUP of 5.7 years 8/11 patients died (two at less than 6 months of life), 1 was lost at FUP at 45 months of age, and only 2/11 patients are alive (at the age of 2.9 and 6.6 years respectively). In 3 cases sepsis was the cause of the death.

Table 1. Clinics and laboratory.

Sex	bp del.	Hepatomegaly	Splenomegaly	Ins. Ex. Pancreas	IDDM	Growth Impair.	VWT	Neurol. Sympt.	Kearn/Sayre	Eye Problems	↑ serum Lactate	↑ Serum Alanine
1/M	5000	Yes	No	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes
2/M	6720	No	No	No	No	Yes	No	No	No	No	Yes	Yes
3/F	4000	Yes	No	No	No	No	NK	No	No	Yes	Yes	ND
4/F	5000	Yes	No	Yes	No	Yes	Yes	Yes	No	No	Yes	Yes
5/M	5000	No	No	No	No	Yes	No	Yes	No	Yes	No	No
6/M	5000	No	No	No	No	Yes	Yes	Yes	No	NK	Yes	Yes
7/M	3300	Yes	Yes	No	No	Yes	No	No	No	Yes	Yes	Yes
8/M	5000	Yes	No	No	No	No	Yes	Yes	No	Yes	Yes	Yes
9/F	5000	Yes	No	No	Yes	No	No	Yes	Yes	No	Yes	Yes
10/F	8000	Yes	Yes	No	No	Yes	No	Yes	Yes	Yes	Yes	Yes
11/F	5000	Yes	Yes	Yes	No	No	Yes	No	No	NK	Yes	Yes

IDDM: type 1 insulin-dependent diabetes mellitus. VWT: ventricular wall thickness. NK: not known. ND: not determined.

Table 2. Clinics and laboratory.

Pt	↑ urine Lactate	↑ urine Fumarate	↑ urine Malic	Fetal Hgb	EPO	Reticulocytes > 60.000/μl	BM	Transfusion Independent	Severe Infections	D/A (years)	Cause of death
1	Yes	Yes	No	ND	ND	Yes	Vacuoles AND ↓ cellularity	Yes	Yes	D (6.4)	Severe acidosis
2	Yes	Yes	Yes	ND	↑	Yes	Vacuoles AND ↓ cellularity	Yes	No	A (2,9)	/
3	No	No	No	ND	↑	Yes	Dyserythropoiesis	Yes	No	Lost at FUP (3,7)	/
4	Yes	Yes	Yes	↑	↑	Yes	Vacuoles	No	Yes	D (5,7)	Renal failure
5	ND	ND	ND	↑	↑	Yes	Vacuoles AND ↓ cellularity	Yes	Yes	A (6,6)	/
6	Yes	Yes	Yes	ND	ND	Yes	Vacuoles AND ↓ cellularity	NP	No	D (0.33)	NK
7	ND	ND	ND	ND	↑	Yes	Vacuoles	Yes	Yes	D (8,0)	Sepsis
8	Yes	Yes	No	ND	↑	No	↓ cellularity	NP	Yes	D (0,53)	Sepsis
9	No	No	?	↑	ND	Yes	Vacuoles AND ↓ cellularity	Yes	Yes	D (10,42)	AML
10	Yes	Yes	Yes	↑	ND	Yes	Vacuoles AND ↓ cellularity	Yes	Yes	D (10,44)	Renal failure
11	Yes	Yes	No	↑	ND	Yes	↓ cellularity	Yes	Yes	D (3,9)	Sepsis

Pt: patient. NK: not known. ND: not determined. D/A: dead/alive. EPO: Erythropoietin. BM: bone marrow. FUP: Follow-up. AML: Acute Myeloid Leukemia.

Summary and Conclusions: PS is a rare disease. Our report enables to estimate the likely incidence of PS in our country as 1/million newborns. Furthermore it shows for the first time that an increased level of serum alanine along with increased excretion of fumaric acid and hypertrophic cardiomyopathy, even though never described in previous reports, are a common finding in PS patients. Finally it seems that transfusion independency is highly probable in case of survival after the first 2-3 years of life.

P631

SCREENING OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA CLONES BY MULTICOLOR FLOW CYTOMETRY USING FLUORESCENT AEROLYSIN (FLAER) - A SINGLE CENTRE STUDY OF 212 PATIENTS

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Background: Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired clonal stem cell disorder with varied clinical manifestations, consequent to deficiency of membrane-bound glycosylphosphatidylinositol (GPI) anchored protein. It is known to be associated with other hematological conditions like aplastic anemia (AA) and myelodysplastic syndrome. In the last few years fluorescent aerolysin (FLAER) based flow cytometry analysis has become the gold standard for screening of these abnormal PNH clones.
Aims: FLAER based flow cytometric screening for PNH clones in patients presenting with pancytopenia, features of hemolysis or thrombophilia; and to correlate the clone size with the clinical and laboratory parameters.
Methods: A total of 212 patients were screened between January 2014 to February 2015 using FLAER based flow cytometry protocols. Stain -Lyse -Wash technique was used. Neutrophils and monocytes were analyzed using a four color antibody panel which included FLAER/CD24/CD15/CD45 and FLAER/CD33/CD14/CD45 respectively. For red blood cell analysis a combination of CD235/CD59 was used. A minimum of 5000 polymorphs and 3000 monocytes were analyzed. The patients were divided into three groups based on clone size, small (<10%), medium (11-49%) and large (≥50%). A correlation between the clone size along with clinical and laboratory parameters was performed.
Results: The indication for screening included pancytopenia (n=172), evidence of hemolysis (n=25) and as a part of thrombophilia workup (n=15). A total of 53/212 patients tested positive of which 45/172, 8/25 and 0/15 were for the above mentioned indications respectively. Amongst the patients who underwent a bone marrow examination at our centre, 43 showed features of aplastic anemia whereas 10 patients showed variably cellular marrow with erythroid hyperplasia. Flow cytometric examination showed large clone sizes in 47% (25/53), medium in 19% (10/53) and a small clone size in 34% (18/53) patients respectively. The patients with features of hemolysis showed larger clone sizes (large clone-6 and medium clone -2) as compared to pancytopenic/AA group. 3 of the 16 (18.8%) patients from the pancytopenia/AA group, who were on antothymocyte globulin therapy, showed a clonal PNH evolution with large clone sizes. On comparing the clone size with other laboratory parameters, larger clone size was found to be directly and significantly correlated with a higher S. LDH levels, S. creatinine and reticulocyte count.
Summary and Conclusions: Most common indication of the present study was pancytopenia. FLAER appeared to be a sensitive technique for detection of PNH clone in these patients. The clone size in patients presenting with hemolytic features was higher as compared to those with pancytoepnia/AA. A fair proportion of patients on ATG therapy showed clonal PNH evolution.

P632

LONG TERM OUTCOME OF IMMUNOSUPPRESSIVE THERAPY IN A LARGE COHORT OF 1430 PATIENTS OF APLASTIC ANEMIA FROM A SINGLE TERTIARY CARE CENTRE IN INDIA

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Background: Acquired aplastic anemia (AA) is a T-cell mediated suppression of hematopoietic stem cells. Treatment options available in these patients as an alternative to allogenic stem cell transplantation include weak androgens (Stanozolol, Danazol) or immunosuppressive therapy [Cyclosporine(CsA) and/or Antithymocyte Globulin(ATG)]. There is paucity of studies showing superior response rates for ATG+CsA as compared to other conventional treatments. ATG is limited by its high cost.
Aims: To analyse and compare the response of ATG+CsA to other treatment options in AA using the standard response criteria.
Methods: Consecutive patients of AA (n=1430) were enrolled in this retrospective study. Patients were randomly allocated to different treatment protocols. Response was measured as time to attainment of CR or PR. Response was also assessed for relapse and clonal evolution with respect to treatment groups.
Results: Patients with AA (mean age 22±16.9 years, 70% males) were followed up for median of 4 months (range 0.5 to 132 months) of which 5% patients died. Anemia (97%) was the major symptom followed by bleeding (70%) fever (47%) and jaundice in 4.5% patients. In 5% patients the stress cytogenetics was positive. Very severe (VSAA): Severe (SAA): Non-Severe (NSAA) were 15%: 76%: 9%, respectively. Treatment given to patients included; Stanazolol-37.5%, CsA-8.5%, Stanazolol+CsA-35%, ATG+CsA-15.5% and others-3.5%. The median time to PR and CR was 4 and 7 months respectively. There was a significant difference in the overall response (OR) rates (CR+PR) of ATG+CsA with respect to other treatment protocols (59% *vs* 30%; p<0.001). The OR rates were also significantly different with respect to severity of AA *i.e.* (45%.*vs* 10%; p<0.001) in VSAA, (61%*vs* 32%; p<0.001) in SAA but not in NSAA. (77% *vs* 48%; p=0.07). The rates of CR and PR were also significantly superior in the ATG+CsA group as compared to others (34% *vs* 15%; p<0.001) and (25% *vs* 15%; p<0.001) respectively. The relapse even though non-significant was noted more frequently in the ATG+CsA group as compared to others (3.2% *vs* 1.2%; p=0.07). The rate of clonal evolution to acute leukemia was almost similar in both the groups (0.8% *vs* 0.5%; p=0.64).
Summary and Conclusions: Although matched sibling allogenic bone marrow transplant is the gold standard treatment for aplastic anemia, but immunosuppressive therapy with ATG+CsA is a good alternative superior to other conventional treatments. However, alternative immunosuppressive therapy can still be considered for patients with non-severe aplastic anemia.

P633

COMBINED IMMUNOSUPPRESSIVE THERAPY IN PATIENTS WITH APLASTIC ANEMIA WITH REPEATED COURSES OF ATG

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Background: According to published data, current immunosuppressive therapy (IST) proved to be effective in 70-90% of patients with AA. However, not all the questions are solved, particularly, treatment of patients with AA refractory to IST without HLA-compatible donor.
Aims: The purpose of the study is optimization of treatment algorithm of patients with AA
Methods: A total of 86 patients with AA were included in the study: 51 males and 35 females at median age 23 years (range 17-65 years). The median period from AA diagnosis to IST was 4 months (range 1-25 months). Severe forms of AA (SAA) were detected in 66 patients, non-severe (NSAA) in 20 pts. PNH-clone was detected using flow cytometry before treatment and monitored during of IST. The combined IST consisted of Antithymocyte Globulin (ATG 20 mg/kgx5 days in 81pts, rabbit ATG 3.75 mg/kgx5 days in 5 pts) and Cyclosporin A (CsA) during 2 years (at least one year after achieving of remission).
Results: IST with several courses of ATG and long-term treatment of CsA was effective in 73 from 86 pts (84.9%): 54 (81.8%) from 66 pts with SAA and 19 (95%) from 20 pts with NSAA. After one course of ATG+CsA positive response was received in 53 (61.6%) from 86 pts and after two courses of ATG in 16 (18.6[LE1]%) . Three courses of ATG were conducted in 5 patients with SAA, 3 of them achieved partial remission. After 3 months from the beginning of IST positive response was achieved in 52.8% and after 6 months in 83, 4% pts with AA. Overall survival and 7 years event-free survival were 89% (95% CI: 83-96%) and 93% (95% CI: 88-97%), respectively. PNH clone was detected in 20 (60.6%) from 33 pts before IST: granulocyte PNH clone did not changed in 16 from 20 pts during period of observation, in 3 patients decreased more than in 5 times and in 1 cases clone was eliminated. Response to IST attained in 17 (85%) from 20 PNH^+ pts and only 8(61%) from 13 PNH^- pts.
Summary and Conclusions: Thus, IST with repeated courses of ATG and long-term treatment of CsA provided a positive response and long-term survival in the most of patients with AA in the study, including SAA. The second course of ATG significantly improves results of the IST, and the optimal period of time to second course in patients with no answer to the first ATG course should be 3- 6 months from the start of treatment no longer. Patients with no response to two courses of ATG could be assigned to a group of patients with refractory AA. These patients require further intensive therapy and alternative methods of treatment (thrombopoietin receptor agonist, alemtuzumab, chelation therapy). As well the results of our research support that PNH+ pts has a better response to the IST than PNH-.

P634

PRELIMINARY DATA ON L-LEUCINE THERAPY OF DBA PEDIATRIC PATIENTS

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Background: The first line of therapy for patients with Diamond-Blackfan anemia (DBA) is oral corticosteroids. Initially respond only 80% of patients, less than a half can keep it for extended periods. The majority of DBA patients received regular blood transfusions. Animal models showed efficacy of L-leucine, a translation enhancer, in the DBA and 5q- syndrome, which has the same altered ribosome function as the DBA. It was observed that treatment with L-leucine improves hemoglobin level and induce transfusion independence in patients with DBA and 5q- syndrome.
Aims: To evaluate the overall safety and efficacy of L-leucine therapy transfusion dependent DBA patients.
Methods: After approval by Local Ethics Committee and obtained signed informed consent form six children (aged 2 - 8 years, 3 girls and 3 boys) were enrolled in the study. The diagnosis of DBA in all patients was established according to the criteria of the DBA Working Group of the European Society for Paediatric Haematology and Immunology. L-leucine (700 mg/m2) was given orally 3 times daily during 9 months. The criteria of efficacy of L-leucine therapy were haemoglobin (Hb) levels, reticulocytes count (Retic) and soluble transferring receptor (sTFR).The safety was monitored by blood chemistry parameters, urine analysis, amount and severity of adverse events. Response criteria were: complete response - Hb >90 g/L and transfusion-independence; partial response - Hb <90 g/L and increased Retic >1% and any increase in transfusion interval from baseline; no response - no change in transfusion requirements and no significant change in Hb or Retic.
Results: Baseline Retic range in all cases was 0.1-0.5%; transfusions - every 3 weeks. Complete response was obtained in 2 (30%) cases (both girls 2 years old). One of these patients has mutation in gene*RPL11* ex. 2 c.65delT (p.Cys21Ser_fs*33) hetero. In the other case the whole genome sequencing is in the progress. No response - in 4 cases; their genotype: *RPS19*ex. 2 c.3G>A (Met1Ile) hetero; *RPL5*ex. 3 c.187 C>T (Gln63*);*RPS19* ex. 2 c.31 C>T (Gln11*) hetero. Increasing growth rate was noticed in all cases: twice-higher compare to the same period of time before the study. No one showed any kind of adverse events. The blood chemistry parameters were within reference range in all cases.
Summary and Conclusions: The L-leucine therapy might be perspective for some DBA patients.

P635

GENETIC VARIANTS OF COMPLEMENT C5 AND POLYMORPHISMS OF COMPLEMENT C3 IN CHINESE PATIENTS WITH PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

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Background: Paroxysmal nocturnal hemoglobinuria (PNH) is a clonal hematopoietic stem cell disorder, which is characterised by intravascular hemolysis, an abnormal tendency of thrombosis, and various degrees of bone marrow failure. Eculizumab suppresses the terminal complement system, by inhibiting the cleavage of C5,with subsequent decrease of cytolysis of PNH erythrocytes . 3.2% of the Japanese PNH population did not respond to eculizumab,due to a single missense C5 heterozygous mutation, c.2654G→A. The prevalence of this mutation was similar to that of healthy Japanese population (3.5%) and was also identified in the Han Chinese population(1/120). Mutant C5 did not bound to and was not blocked by eculizumab. Meanwhile, eculizumab preserves upstream components that culminate in C3b-mediated extravascular hemolysis, which results in the permanent transfusion requirements. British researchers found that eculizumab-treated patients who had homozygote C3S/C3S allele had a significantly higher degree of C3 loading on PNH. But the PNH complement C3 alleles screening was conducted mainly in Europe, not in Asia.
Aims: We intend to study genetic variants of complement C5 and polymorphisms of complement C3 in Chinese patients with PNH in order to determine its potential impact on Eculizumab efficacy.
Methods: 220 patients with PNH (diagnosed by CD55/CD59, Flaer analysis) and 259 normal controls were included. DNA was extracted from peripheral blood mononuclear cells . Complement C5 exon 21 PCR products and C3 genotypes were analyzed by Sanger method, in order to search the gene mutation of complement C5 gene (rs56040400),and to analyses the genetic polymorphisms of C3 (rs2230199).
Results: For patients with PNH, there were 118 males and 102 females, with a medium age of 39 years(4-75 years), mean LDH 1078 (IU/L), mean Hb 82 (g/L). 152 patients were classic PNH, 68 patients were aplastic anemia with PNH clone, five patients had thrombosis. In normal control group, there were 138 male and 121 females with a medium age of 35 years(8-72 years). In both the PNH patients and normal control subjects,we found no a single missense C5 heterozygous mutation. When we analysed C3 genetic polymorphisms,they were all homozygous SS allele, no F allele was found.
Summary and Conclusions: In the small sample of Chinese PNH patients and normal subjects,we found no C5 exon gene mutations that had been reported before, and only SS polymorphism was seen in C3 polymorphismsin analysis. These data suggest that Chinese patients will probably not affected by the C5 mutation when exposed to Eculizumab, but may undergo C3 mediated extravascular hemolysis.

Multiple myeloma - Biology 2

P636

EFFECT OF IMID COMPOUNDS ON CD38 EXPRESSION ON MULTIPLE MYELOMA CELLS: MOR202, A HUMAN CD38 ANTIBODY IN COMBINATION WITH POMALIDOMIDE

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Background: MOR202 (MOR03087), a human CD38 antibody currently under evaluation in a phase I/IIa trial, mediates antibody-dependent cell-mediated cytotoxicity/phagocytosis (ADCC/ADCP) of multiple myeloma patient-derived cells with high potency (EC_{50} ~200 pM).
Aims: To evaluate *in vitro* the ability of the IMiD compounds lenalidomide and pomalidomide, both of which are approved for multiple myeloma, to modulate CD38 expression and enhance the cytotoxicity of MOR202.
Methods: CD38 expression +/- lenalidomide and +/- pomalidomide on multiple myeloma cell lines was analyzed by flow cytometry. The antitumor activity of pomalidomide combined with MOR202 was evaluated *in vitro*; analyses included the induction of direct cytotoxicity on multiple myeloma cells and the activation of immune effector cells. On a functional level, the combinatorial effects of MOR202 with pomalidomide were assessed in ADCC assays. Different incubation schemes were used to separate the effect of pomalidomide on target and effector cells, as well as in the evaluation of the combined effects. The observed combination effects were analyzed for synergistic potential using the fractional product concept.
Results: Pomalidomide and lenalidomide mediated a substantial CD38 upregulation on multiple myeloma cell lines. Pomalidomide as a single agent demonstrated activation of effector cells with high potency (EC_{50} ~150 nM), and demonstrated cytotoxic effects on multiple myeloma cell lines. Additionally, pomalidomide dose-dependently induced an up to 3-fold CD38 upregulation (EC_{50} ~20 nM) on CD38-expressing multiple myeloma cell lines. Pomalidomide-mediated effects were time-dependent, with the most pronounced effects after 72 h incubation. Combining MOR202 with pomalidomide led to a synergistic enhancement of cytotoxic activity. The synergic benefit ranged 1.2-3.1-fold above theoretical additivity depending on the cell line used, and was most prominent in the case of strong CD38 upregulation.
Summary and Conclusions: Upregulation of CD38 was mediated by both lenalidomide and pomalidomide and may represent a class effect of IMiD compounds. The cytotoxic activity of MOR202 on multiple myeloma cells was enhanced by pomalidomide via multiple mechanisms: direct cytotoxicity, CD38 upregulation and activation of effector cells. These results provide a mechanistic rationale for the combination of MOR202 with IMiD compounds and warrant further clinical evaluation.

P637

CENTROSOME ASSOCIATED GENES PATTERN FOR RISK SUBSTRATIFICATION IN MULTIPLE MYELOMA

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Background: Multiple myeloma (MM) is a lymphoproliferative disease characterized by the clonal expansion of neoplastic plasma cells within the bone marrow. The genome of the malignant plasma cells is extremely unstable characterized by a complex combination of structure and numerical abnormalities. Our previous study defined centrosome associated genes pattern with impact in myeloma pathogenesis. Revealed molecular signature is related to OS as well as to clinical parameters and ISS staging (Kryukov *et al.*, 2013).
Aims: The objective of our study was to create and validate risk stratification model based on previously described centrosome associated genes pattern in MM.
Methods: One hundred and fifty patients were evaluated for this study. The patients' baseline characteristics were as follows: males/females 79/71, median age of 67 years (range 38-90 years). Newly diagnosed (90/150) and relapsed (60/150) patients were included in this study; most of them had advanced stage of MM (DS II/III n=110; ISS II/III n=144). Interphase FISH with cytoplasmic immunoglobulin light chain staining (clg FISH) and gene expression profiling (GEP) were performed on CD138+ plasma cells separated by MACS. Training and validation cohort includes 72 and 78 pts, resp.
Results: Using multinomial logistic regression 12 candidate genes (*BUB1, BUB1B|PAK6, RAD51, PLK1, BRCA1, CENPA, BARD1, AURKA, MAD2L1, CENPH, XRCC2* and *CDC25C|FAM53C*) for patients, risk stratification were defined. Based on expression of these genes, patients can be stratified into three groups - "High-", "Mediate-" and "Low expressed". The overall survival of patients in "High" and "Medium" subgroups was significantly worse than in patients in "Low" subgroup for both training and validation cohorts and was in concordance with previously published results (Kryukov *et al.* 2013). To characterize the prognostic significance of centrosome associated genes pattern (CAGP), multivariate Cox proportional hazards survival model was used. Significantly worse prognosis was found for united "High & Medium" group (HR=2.182; 3-year OS=28.3%) compared to "Low" group (3-year OS=63.8%, p<0.01). Besides CAGP, the following parameters were used for multivariate Cox proportional hazards survival model: ISS stadium, β2-microglobulin, del 17p13, t(4;14), amp 1q21. The variables in the multivariate model were the only variables, which remained statistically significant when potential predictors were combined together as well as CAGP, which was forced into the model. Among all subsequently tested combinations of predictors, the best results in risk of death assessment were obtained for CAGP combined with del 17p13 (p<0.001). It is worth to mention that both prognostic factors were independent. United "High & Medium" CAGP subgroup as well as TP53 deletion had significantly higher risk of death assessment (HR=3.189 and HR=3.699 resp. p<0.005). Survival characteristics for different risk groups are presented in the Table 1.

Table 1. Survival characteristics for different risk groups (validation cohort, n=69).

Stratification group	Survival Median	3-years Overall Survival
Low Risk CAGP	47.2 months	69.2%
High Risk CAGP	22.5 months	28.3%
TP53-	33.3 months	46.1%
TP53+	5.9 months	14.3%
Low Risk CAGP/TP53-	47.2 months	69.2%
Low Risk CAGP/TP53+	5.9 months	25.0%
High Risk CAGP/TP53-	22.8 months	24.7%
High Risk CAGP/TP53+	0.7 months	0.0%

"Low Risk CAGP" group includes patients with "Low expressed" centrosome associated gene pattern. "High Risk CAGP" group includes patients with united "High & Medium expressed" centrosome associated gene pattern.
"TP53+" group includes patients with deletion 17p13; "TP53-" group includes patients without deletion 17p13 (positivity cutoff >20%).

Summary and Conclusions: We have created a new GEP-based model for classification of every patient into one of three prognostic subgroups, which can be easily used in routine practice. This approach can be used independently as well as in combination with other factors. Best results in risk of death assessment are obtained when the new stratification model is used in combination with detection of TP53 loss analyzed by FISH. Thus, the new model can be used for substratification of prognosis in patients with TP53 loss. These findings need to be confirmed on larger cohort with longer follow-up.
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P638

BONE MARROW MESENCHYMAL STROMAL CELLS FROM HEALTHY DONORS SECRETE EXOSOMES THAT INHIBIT *IN VIVO* TUMOR GROWTH AND ANGIOGENESIS IN MULTIPLE MYELOMA

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Background: The bone marrow microenvironment plays a key role in the growth and survival of multiple myeloma (MM) cells. MM cells interact with mesenchymal stromal cells (MSC) in the bone marrow, resulting in cell proliferation and angiogenesis. The bone marrow derived MSC is one of the most promising source of cell therapy. Resently, MSC-derived exosomes have been found to be efficacious in animal models for the treatment of non-neoplastic disease, such as cardiovescular disease and liver injury, because MSC-derived exosomes carry and transfer their cargo, which promote therapeutic response.
Aims: We sought to determine the anti-tumor effect of MSC-derived exosomes by nude mouse heterotransplant model, aiming to establish the exosome-based theraputic approch using MSC-derived exosomal miRNAs.
Methods: Human MSCs obtained from bone marrow of healthy donors (BM-MSC; age 19 to 72) were purchased from Lonza. BM samples were obtained

from MM patients (age 62 to 77) in accordance with the Declaration of Helsinki and using protocols approved by the research Ethics Committee of Tokyo Medical University (IRB No. 2648), and MSCs derived form MM patients (MM-MSCs) were isolated by the classical adhesion method. Hypoxia-resistant myeloma cell line (RPMI8226-HR) established in our laboratory were cultured at 1% O_2. Exosome secreted from MSCs (MSC-exosome) were isolated from conditioned medium using a Total Exosome Isolation Reagent (Invitrogen). The size of MSC-exosomes was confirmed using a NanoSight LM10. To examine whether MSC-exosomes could be transferred into recipient cells, RPMI8226-HR cells or human endothelial cells, were cultured with fluorescent dye-labeled MSC-exosomes. Exosomal miRNA profiling was done using a TaqMan low-density array (ABI), and Student's t-test was used to determine statistical significance for comparisons between MM patient and healthy donor groups using R software. In Matrigel plug assay, BALB/c nude mice were injected subcutaneously with 400 µL Matrigel containing myeloma cell lines with or without MSC-exosomes. After 3 weeks, the Matrigel plugs were harvested, and stained with anti-CD31 antibody to analyze vessel density.
Results: BM-MSCs displayed long spindle shape, and there was no morphological difference between the BM-MSCs from non-aged donors (19 and 21 years) and that from aged donors (68 and 72 years). In contrast, MM-MSCs displayed the flat cellular morphology. The nanoparticle size distribution of MSC-exosomes was approximately 50 nm, and there were no significant differences in size and amount of exosomes in BM-MSCs and MM-MSCs. We found a diferential exosomal miRNA expression profile between BM-MSCs and MM-MSCs. Then we could subdivide miRNAs as follows: (1) miRNAs upregulated in BM-MSCs, (2) miRNAs upregulated in MM-MSCs. We also found the transport of these exosomal miRNAs derived from BM-MSCs into either myeloma cell or human endothelial cell via exosomes using *in vitro* model. MM-MSC-exosomes enhanced angiogenesis and cell proliferation of MM cells in Matrigel plug containing RPMI8226-HR cells. Of note is that BM-MSC-exosomes significantly reduced the density of CD31 positive endothelial cells and the remarkable decrease of myeloma cells in Matrigel plug, indicating the anti-tumor effect of BM-MSC-exosomes. The growth inhibitory effect was more prominent in BM-MSC-exosomes from non-aged donors.
Summary and Conclusions: Our results suggest that the BM-MSC-exosomes is able to transfer the miRNAs, which have the ability to inhibit tumor growth and angiogenesis in MM. The present study provide a theraputic potential of exosomes derived BM-MSCs in MM.

P639

ANTITUMORAL ACTIVITY OF AMILORIDE IN MULTIPLE MYELOMA THROUGH APOPTOSIS INDUCTION, REGARDLESS OF TP53 MUTATIONAL STATUS

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Background: Multiple myeloma (MM) remains incurable despite the novel agents with different mechanisms of action. Therefore, the investigation of the potential antimyeloma activity in other therapeutic agents is stillneeded. The antihypertensive agent, amiloride, has been demonstrated to inhibit tumor cell proliferationin different experimental models. Here we explore the activity of amiloride in myeloma cells.
Aims: To investigate the antimyeloma effect of amiloride in myeloma cell lines and primary samples and to identify the potential mechanisms involved.
Methods: Seven human myeloma cell lines (NCI-H929, JJN3, KMS12-BM, KMS12-PE, U266, MM1S and RPMI-8226) and 9 bone marrow (BM) samples from MM patients were used for the experiments. Cell viability, apoptosis and cell cycle analyses were carried out by CellTiter-Glo assay, annexin V staining and PI/RNASE solution following manufacturer's instructions, respectively. Caspases 3/7, 8 and 9 activities were evaluated by luminescent Caspase-Glo®Assays and the measuring of mitochondrial membrane potential ($\Delta\Psi_m$) by flow cytometry analyses. The gene expression was quantified by Taqman assay qRT-PCR. Synergy with bortezomib and dexamethasonewas calculated with the "CalcuSyn" software program.
Results: To test the antiproliferative effect of amiloride, myeloma cells were treated with increasing concentrations of the compound (0.1mM-1mM) for 24, 48 and 72 hours. Amiloride displayed potent antimyeloma activity in the panel of 7 cell lines. A substantial induction of apoptotic cell death (greater than 40% at 24 h and 60% at 48 h) and cell cycle blockade (a 12% increase in G_0/G1, and a 10% and 5% decrease in the G_2/M and S phases of cell cycle, respectively) was observed in all MM cell lines. The apoptosis induced by amiloride was not related to the TP53 mutational status, since amiloride was still able to induce cell death through a p53-independent pathway in p53 mutated cell lines, such as U-266, KMS12-BM and JJN3. Moreover, this drug caused a notable decrease in mitochondrial membrane potential ($\Delta\Psi_m$) and an activation of caspases 3/7, 8 and 9. Mechanistic studies showed that amiloride□triggered apoptosis was associated with the overexpression of BAX, BAK1, BBC3, TNFRSF10B, FAS, CDKN1B and CDKN1A, even in those MM cell lines in which p53 gene was mutated. Further, we evaluated the potential synergism of amiloride with other antimyeloma agents. H929 and JJN3 cell lines were treated for 48 h with combinations of suboptimal doses of amiloride, and bortezomib and dexamethasone, in double combinations. Amiloride was synergistic with bortezomib in NCI-H929 and JJN3 cell lines (CI=0.80 and 0.89, respectively). The combination indexes for dexamethasone were in the additive range. Finally, the antiproliferative/cytotoxic effect of amiloride in BM cell subpopulations obtained from 9 patients with MM was assessed. *In vitro* studies confirmed the antimyeloma efficacy of amiloride in patient samples and showed a clearly lower cytotoxicity in the remaining cell subpopulations compared to tumor plasma cells.
Summary and Conclusions: Our results demonstrate a potent and selective antimyeloma effect of amiloride by p53-dependent and independent pathways. The present data support the investigation of amiloride as a treatment option for MM patients, either alone or in combination.
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P640

BORTEZOMIB-RESISTANCE OF BONE MARROW FIBROBLASTS IN MULTIPLE MYELOMA IS CLOSELY CONNECTED TO ACTIVATION OF AUTOPHAGY AS SURVIVAL MACHINERY

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Background: Cancer-associated fibroblasts (CAFs) are a relevant cell type within the stroma of many tumors entailed with high malignancy grade, progression, and poor prognosis. In multiple myeloma (MM), CAFs give a noteworthy contribution in terms of growth, proliferation, and survival of tumor cells[1].
Aims: Since drug resistance of MM cells depends on tumor microenvironment, we analyzed the mechanism which involves CAFs in bortezomib-resistance of MM cells.
Methods: Proteomic, phospho-proteomic, western blot, immunofluorescence and flow cytometry studies were performed to demonstrate the effect of CAFs on the bortezomib-induced apoptosis of MM cells.
Results: CAFs purified from bone marrow of bortezomib-resistant patients were *in vitro* insensitive to the bortezomib treatment, and protected MM cells as RPMI8266 cell line and CD138+ plasma cells from bortezomib-induced apoptosis. The effect was related to the ability of bortezomib-resistant CAFs treated with bortezomib to secrete high levels of prosurvival cytokines/growth factors, namely insulin-like growth factor 1 (IGF-1), interleukin-6 (IL-6), interleukin 8 (IL-8), and transforming growth factor beta (TGFβ), compared to CAFs purified from 1st diagnosed patients. Proteomic and phospho-proteomic studies suggested that resistance of CAFs to bortezomib was related to a cellular stress condition. Flow cytometry and immunofluorescence studies showed that bortezomib-resistant CAFs had higher production of reactive oxygen species (ROS) compared to CAFs purified from 1st diagnosed patients. When treated with bortezomib cells increased the ROS expression and activated the autophagy pathway. Indeed, the bortezomib treatment induced an increase of microtubule-associated protein light chain 3 (LC3)-II with a reduction of p62 proteins, two autophagic markers, and inhibited the phosphorylation status of mTor, the major autophagic pathway. Finally we demonstrated that TGFβ, which acts in autocrine way, activated prosurvival autophagy and induced bortezomib resistance.
Summary and Conclusions: Our results highlight that CAFs from bortezomib resistant patients are insensitive *in vitro* to the bortezomib treatment and protect MM cells from bortezomib-induced apoptosis. The *in vitro* resistance of bortezomib-resistant CAFs to drug treatment is closely connected to activation of autophagy as prosurvival machinery. Accordingly, we speculate that targeting CAFs in MM patients may be envisaged as a cell-based therapeutic strategy.

Reference

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P641

POLYMORPHISMS WITHIN THE CEREBLON AND BETA-CATENIN ENCODING GENES IN PATIENTS WITH MULTIPLE MYELOMA

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Background: The recent studies have suggested that cereblon (CRBN) is essential for the anti-myeloma (MM) activity of immunomodulatory drugs (IMiDs), such as thalidomide and lenalidomide and that dysregulation of Wnt/β-catenin pathway may be one of the possible reasons of lenalidomide resistance.
Aims: This prompted us to analyze the effect of the polymorphisms within the genes coding for cereblon (*CRBN* (rs121918368, C>T)) and β-catenin (*CTNNB1* (rs4135385, A>G; rs4533622, A>C).
Methods: MM patients (n=144) and healthy individuals (n=126) were genotyped using the Light SNiP assays.
Results: The *CTNNB1* (rs4135385) *AA* homozygosity was more frequent among patients with better response to CTD - cyclophosphamide, thalidomide, dexamethasone (8/11 *vs* 42/72, p-=0.047) and were at lower risk of disease progression after thalidomide treatment (22/31 *vs* 8/18, p=0.078). Patients carrying the *CTNNB1* (rs4533622) *AA* genotype were better responders to the first line therapy with thalidomide containing regiments (p<0.05). No significant association was observed between the effect of lenalidomide therapy and polymorphisms studied. However, the occurrence of neutropenia during lenalidomide therapy was more frequent among the *CTNNB1* (rs4135385) *AA* carriers (p=0.019) while the *CTNNB1* (rs4533622) *AA* homozygosity characterized patients with high grade (3-4) neutropenia (p=0.044). No association was found for the *CRBN* polymorphism.
Summary and Conclusions: The response to chemotherapy in patients with multiple myeloma is associated with the *CTNNB1* polymorphism.

P642

A NEW NEXT GENERATION SEQUENCING STRATEGY TO EVALUATE GENETIC ABNORMALITIES IN MULTIPLE MYELOMA

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Background: Approximately 60% of multiple myeloma (MM) patients harbor a translocation predominantly involving the *IGH* locus at 14q32. *IGH* translocations are a primary event and determine the prognostic outcome of a patient. These events are routinely assessed by FISH, which can easily detect the *IGH* translocation, although the partner chromosome frequently remains unknown. Next Generation Sequencing (NGS) techniques may overcome this limitation with the additional advantage of identifying the precise localization of the breakpoints. This would allow the subsequent design of patient specific PCRs for monitoring the aberrant chromosomal translocation over disease evolution. Moreover, this methodology may provide other relevant information, such as the identification of the clonal V(D)J rearrangements, IgH isotypes or somatic gene mutations.
Aims: To design a NGS-based strategy in order to investigate the landscape of relevant chromosomal abnormalities and gene lesions in a series of MM patients.
Methods: A custom pull down panel to identify *IGH* translocations, V(D)J rearrangements and the most common somatic mutations in myeloma patients (involving *NRAS*, *KRAS*, *HRAS*, *TP53*, *MYC* and *BRAF* genes) was designed. Samples from 48 newly diagnosed MM patients, enrolled in GEM2005 clinical trial, were included in the study. DNA was extracted from bone marrow CD138+ plasma cells separated by autoMACS®. Regions of interest of the aforementioned genes were captured with the NimbleGen strategy (Roche NimbleGen, Inc., Madison, WI). Massive parallel sequencing was performed in a GS FLX+ System with a mean of 350 bp paired-end read length and a mean depth of more than 23.000X per sample (>95% coverage). Results were correlated with FISH, molecular and clinical data of the patients.
Results: In a preliminary analysis (with ≥40 bp overlap depth and ≥90% overlap identity), we could confirm the presence of 9/17 *IGH* translocations that had been detected by FISH (5 t(11;14), 2 t(4;14), 1 t(14;16) and 1 t with unknown partner). Breakpoints in *IGH* were located in μ switch and enhancer regions (67%) and in *IGHJ* region (33%). One unknown partner chromosome turned out to be chromosome 12q24, and the breakpoint was located between *SCARB1* and *UBC* genes. Interestingly, this patient was the only one in this series that had an associated amyloidosis. We also detected 6 potential additional translocations that will be further analyzed for confirmation. Regarding IgH subclass, we could observe that the splice process occurred between μ switch (S) region and $S\gamma_1$ (in 50% cases), $S\gamma_2$ (20%), $S\gamma_3$ (10%), $S\gamma_4$ (10%) and $S\alpha_1$ (10%). This preliminary analysis also allowed us to identify the V(D)J rearrangement in 18 patients (38%). Finally, mutation analysis revealed the presence of missense protein-coding alterations in 19/48 (40%) patients, distributed as follows: 7/48 (15%) *NRAS*, 7/48 (15%) *KRAS*, 7/48 (15%) MYC, 2/48 (4%) *TP53*, 0/48 (0%) *HRAS* and 0/48 (0%) *BRAF*.
Summary and Conclusions: Our capture-based NGS study allows the simultaneous identification of *IGH* translocations, V(D)J rearrangements, IgH isotype switching and somatic mutations in myeloma patients. The precise location of the breakpoints can be used as a target for minimal residual disease detection. Lastly, it is also interesting the discovery of novel translocations, as it is the case of the t(12;14) translocation that we have found here. Nevertheless, this study still requires further optimization and standardization that will involve both biological and bioinformatics evaluation.
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P643

HUMAN MYELOMA CELL ENGRAFTMENT IN NEXT GENERATION HUMANIZED MICE EXPRESSING HUMAN INTERLEUKIN 6

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Background: Multiple myeloma (MM) remains a non-curable disease for most patients, and novel therapeutic options need to be evaluated. The clonal heterogeneity and plasticity of human MM disease emphasizes the need to use primary human MM cell populations that reflect this in preclinical murine MM models. However, the engraftment of primary human MM cells in immunocompromised mice is poor, likely due to the fact that murine hematopoetic growth factors and in particular Interleukin-6 (IL-6), which is important for intramedullary MM growth, share only little homology with their human counterparts. This poor engraftment of human MM cells severely limits the usefulness of xenograft MM models for the assessment of novel therapies in the preclinical setting *in vivo*.
Aims: To explore whether expression of human IL-6 in immunocompromised mice would allow efficient engraftment and systemic bone marrow growth of human MM cells in immunocompromised mice, providing the basis for an improved MM *in vivo* model.
Methods: $2x10^6$ human RPMI8226 and INA-6 (an IL6-dependent human MM cell line) cells were injected into the femur of adult humanSIRAPα transgenic/humanIL6 and humanTPO-knockin Rag2-/- gamma chain-/- mice (TPO-IL6-SIRPα mice). Mice were sacrificed 38-54 days after injection and analyzed by histology and immunohistochemistry, FACS and Mikro-CT. Paraproteinemia was detected by ELISA for human light chains.
Results: 5/5 RPMI8226-injected mice developed a measurable paraproteinemia and showed infiltration by myeloma cells also at distant bone marrow sites (sternum), as detected by histology. 3/5 mice developed a soft tissue tumor in the parafemural tissue. Two mice showed a massive infiltration and systemic bone marrow spread to vertebral column, tibia, contralateral femur, hip and humerus with dense infiltration of the marrow cavity and often locally destructive growth with myeloma cells invading the surrounding tissue. Both mice developed paraplegia due to infiltration of the spinal cord at day 54. Mikro-CT revealed osteolytic lesions and generalized loss of trabecular structure, resembling human MM. Hepatic involvement was noticed in both animals by histology. One animal showed small foci of myeloma cells in the perirenal soft tissue. Ongoing experiments likewise demonstrate engraftment of the IL6 dependent cell line INA-6. Further experiments using human primary MM cells are ongoing and will be presented at the meeting.
Summary and Conclusions: Our data suggest that human TPO-IL6-SIRPαRag2-/-γc-/- mice considerably support the systemic growth of human myeloma cells in the bone marrow. TPO-IL6-SIRPα mice may therefore provide the basis for a next generation xenograft MM model that allows studying primary human cells from the intramedullary phase of MM *in vivo*.

P644

OVEREXPRESSION OF SALIVARY-TYPE AMYLASE REDUCES THE SENSITIVITY TO BORTEZOMIB IN MULTIPLE MYELOMA CELLS

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Background: Despite recent progress in treatments for multiple myeloma, a complete cure remains elusive. Amylase-producing myeloma, mainly reported in East Asian and European countries, exhibits refractoriness to chemotherapy including bortezomib with a dismal prognosis. To overcome the obstacle, it is critical to elucidate the mechanisms by which amylase-producing myeloma cells survive after chemotherapy.
Aims: The aim of this work was to elucidate the mechanisms by which ectopic

overexpression and production of amylase contributes to the chemoresistance in myeloma.
Methods: We generated a human myeloma cell line in which the transfected *AMY1* gene was stably expressed. RPMI-8226 was lentivirally transduced to express salivary type amylase (8226/AMY1) or lacZ (8226/mock) as mock control. Cells were treated with anti-myeloma drugs (dexamethasone, bortezomib and lenalidomide). Cell viability and growth of treated cells were examined by the MTS assay. Apoptosis was quantitated by flow cytometry using annexin V - FITC staining. Gene expression changes associated with *amylase* expression were analyzed by microarray gene expression profiling. Cells were subcutaneously injected to mice (n=3 mice, each group), and DMSO or bortezomib (0.3 mg/kg) was administered twice a week for *in vivo* study. The tumor volumes of mice were observed every 3 to 4 days.
Results: Cell proliferation after 48 hours exposure to dexamethasone (40 mM), bortezomib (2 nM), and lenalidomide (1 mM) were significantly high in 8226/AMY1 compared with mock *in vitro* (Figure 1). The degree of apoptosis was significantly reduced in 8226/AMY1 after exposure to bortezomib and lenalidomide, but it was similar when treated with dexamethasone. *In vivo*, tumor growth of 8226/AMY1 markedly increased compared with mock, but did not show significance (Figure 2A). The ratio of tumor size treated with bortezomib to that with DMSO on days 46 was significantly high in 8226/AMY1 compared with mock (p=0.017) (Figure 2A), indicating that bortezomib was less effective in 8226/AMY1 compared with mock *in vivo*. Gene expression analysis by microarray identified 5 genes differentially up-regulated in 8226/AMY1 including the *TCL1A* gene, which codes T-cell leukemia/lymphoma 1 oncoprotein to function as a coactivator of the cell survival kinase AKT, as well as 7 differentially down-regulated.

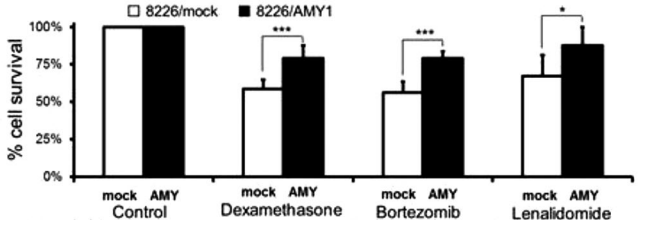

Figure 1. 8226/AMY1 showed the reduced sensitivity to anti-myeloma drugs *in vitro*.

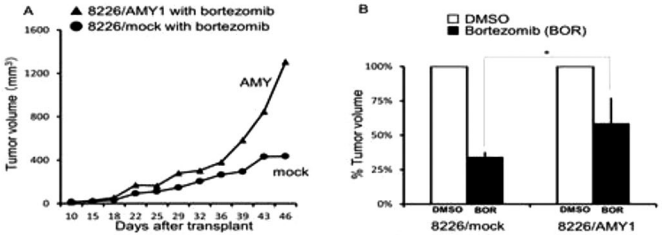

Figure 2. 8226/AMY1 showed the reduced sensitivity to bertezomib *in vivo*.

Summary and Conclusions: We demonstrated that 8226/AMY1 cells had reduced susceptibility to dexamethasone, bortezomib and lenalidomide *in vitro* partly through inhibition of apoptosis induced by these reagents. 8226/AMY1 also showed a weakened anti-tumor effect of bortezomib in exnografted mice. Up-regulation of *TCL1* may influence the anti-myeloma drug resistance by the mechanism of promotion of Akt-induced cell survival and proliferation in 8226/AMY1. Our data provide clues for elucidating the molecular pathology and developing treatment approaches for not only amylase-producing myeloma but relapsed and refractory myeloma.

Multiple myeloma - Clinical 3

P645

A SYSTEMATIC LITERATURE REVIEW AND NETWORK META-ANALYSIS OF TREATMENTS FOR PATIENTS WITH TRANSPLANT-INELIGIBLE NEWLY DIAGNOSED MULTIPLE MYELOMA

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Background: Commonly recommended standards of care for patients (pts) with transplant-ineligible newly diagnosed multiple myeloma (NDMM) include melphalan and prednisone (MP) combined with thalidomide (MPT) or bortezomib (VMP). The randomized, controlled phase 3 FIRST trial demonstrated that continuous treatment with lenalidomide and low-dose dexamethasone (Rd continuous) significantly improved progression-free survival (PFS, the primary study endpoint) and overall survival *vs* MPT in pts with NDMM (Benboubker, *N Engl J Med,* 2014). In the absence of a head-to-head comparison, an indirect comparison of Tx across separate trials can be performed. However, cross-trial comparisons may be biased by differences in pt populations, sensitivity to modeling assumptions, and differences in the definitions of outcome measures.
Aims: A mixed-Tx comparison (MTC), a type of network meta-analysis (NMA), was used to evaluate the relative effect of Rd compared with VMP on overall survival (OS) and PFS.
Methods: A systematic literature review was conducted in Embase, PubMed, and CENTRAL using keywords for MM combined with Tx of interest: lenalidomide, thalidomide, bortezomib, interferon, and bendamustine as monotherapy, combination Tx, and MP combination Tx. The search was then narrowed to randomized trials of clinical efficacy in Tx-naive pts published between 1988 and September 2014. To avoid potential biases, quality assessments for each study were conducted using key questions derived from the Cochrane Handbook for Systematic Reviews of Interventions (Higgins, *BMJ,* 2011). The assessments were validated by independent investigators. Study comparability and Tx relevance were assessed to compare VMP with Rd using MPT as a common comparator. To reduce bias and better reflect the real-world situation, the analysis network was further limited to studies evaluating MPT on a fixed-dose schedule (per the thalidomide summary of product characteristics and current clinical practice). Random- and fixed-effects Bayesian MTC meta-analyses comparing Tx hazard ratios (HRs) for OS and PFS were conducted. The comparison described herein was conducted for research purposes and does not represent a validated hypothesis.

Table 1. Extracted data from studies in the primary analysis network.

Study Information				OS	PFS
Author	Study Group	Tx	Tx	HR (95% CI) *P* Value	HR (95% CI) *P* Value
Facon 2007	IFM 99/06	MPT (n=125)	MP (n=196)	0.59 (0.46-0.81) 0.0006	0.51 (0.39-0.66) < 0.0001
Hulin 2009	IFM 01/01	MPT (n=133)	MP (n=116)	0.68 (0.48-0.96) 0.28	0.61 (0.46-0.82) 0.001
San-Miguel 2013	VISTA	VMP (n=344)	MP (n=338)	0.695 (0.508-0.84) 0.001	0.558 (0.43-0.72) 0.008
Benboubker 2013	MM-020 (FIRST)	Rd (n=535)	MPT (n=547)	0.78 (0.64-0.96) 0.02	0.72 (0.61-0.85) < 0.001
Sacchi 2011	NR	MPT (n=64)	MP (n=54)	0.52 (0.28-0.97) 0.07	0.57 (0.35-0.94) 0.02

Results: The network was composed of 5 trials (IFM 0101, IFM 99/06, Sacchi 2011, VISTA, and FIRST) evaluating Rd continuous, VMP, MP, and MPT (Table 1). An analysis of OS using fixed-effects NMA models documented a significantly lower risk of death with Rd compared with VMP (HR=0.69 [95% credible interval

(CrI), 0.48-0.98]), MPT (HR=0.78 [95% CrI, 0.64-0.95]), and MP (HR=0.48 [95% CrI, 0.36-0.64]). A significantly lower risk of progression or death was detected from a PFS analysis of Rd compared with MPT (HR=0.71 [95% CrI, 0.61-0.85]) or MP (HR=0.40 [95% CrI, 0.31-0.51]). A numerical trend in PFS in favor of Rd was observed when compared with VMP (HR=0.72 [95% CrI, 0.51-1.02]).
Summary and Conclusions: A significant advantage in OS was associated with Rd continuous compared with other first-line Tx (VMP, MPT, and MP). Pts treated with Rd continuous had a lower risk of progression than those treated with MPT and MP. Conclusions are focused on efficacy and do not consider any potential differences in safety.

P646

UPDATED RESULTS FROM A MULTICENTER, OPEN-LABEL, DOSE-ESCALATION PHASE 1B/2 STUDY OF SINGLE-AGENT OPROZOMIB (OPZ) IN PATIENTS (PTS) WITH HEMATOLOGIC MALIGNANCIES, INCLUDING MULTIPLE MYELOMA (MM)

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Background: Preliminary findings from an open-label, phase 1b/2 study (NCT01416428) investigating OPZ in pts with hematologic malignancies (including MM) were presented previously (Vij *et al.*, Blood 2014;124: abstract 34). In the phase 1b portion of the study, the maximum tolerated dose (MTD) of OPZ administered once-daily on days 1, 2, 8, and 9 of a 14-day cycle (2/7 schedule) or on days 1-5 of a 14-day cycle (5/14 schedule) was 300 mg/day (mg/d) and 240 mg/d, respectively. In addition, results from 27 pts who were enrolled in the phase 2 portion of the study on the 5/14 schedule at the MTD (240 mg/d) were presented previously (Vij *et al.*, Blood 2014;124: abstract 34).
Aims: We present updated data from the phase 2 portion of the study (NCT01416428).
Methods: The study is enrolling adult pts with hematologic malignancies who have relapsed after receiving ≥1 line of therapy. The primary objective of the phase 2 portion of the study is to determine the best overall response rate (≥partial response). Given the encouraging anti-MM efficacy of OPZ, in order to improve the gastrointestinal tolerability of OPZ, the study protocol was amended so that pts on both the 2/7 and 5/14 schedules could receive a reduced dose of OPZ during cycle 1 (150 and 240 mg/d, respectively), followed by a higher dose of OPZ during cycles ≥2, if tolerated (180 and 300 mg/d, respectively). Enrollment of pts with MM is continuing in the phase 2 portion of the study in the 2/7 schedule (240/300 mg/d) and 5/14 schedule (150/180 mg/d); the target enrollment is 94 pts with MM. Preliminary safety results are presented for pts with MM who enrolled in the phase 2 portion of the study following modification of the treatment schedules. All pts provided informed consent.

Table 1. Common AEs of interest.

	Phase 2: 2/7 schedule (240/300 mg/d) (n=10)		Phase 2 5/14 schedule (150/180 mg/d) (n=9)	
	All Gr n (%)	Gr 3-4 n (%)	All Gr n (%)	Gr 3-4 n (%)
Any AE	10 (100)	7 (70)	9 (100)	6 (67)
Hematologic AEs				
Neutropenia	1 (10)	1 (10)	2 (22)	0 (0)
Thrombocytopenia	1 (10)	0 (0)	1 (11)	0 (0)
Anemia	0 (0)	0 (0)	1 (11)	1 (11)
Non-hematologic AEs				
Diarrhea	7 (70)	1 (10)	9 (100)	0 (0)
Nausea	6 (60)	1 (10)	9 (100)	0 (0)
Vomiting	3 (30)	0 (0)	3 (33)	0 (0)
Increased blood creatinine	1 (10)	0 (0)	2 (22)	0 (0)
Constipation	0 (0)	0 (0)	2 (22)	0 (0)
Upper respiratory tract infection	0 (0)	0 (0)	2 (22)	0 (0)

Results: As of November 3, 2014, 19 pts with MM were enrolled in the phase 2 portion of the study on the modified treatment schedule, including 10 pts who enrolled on the 2/7 schedule (240/300 mg/d) and 9 pts who enrolled on the 5/14 schedule (150/180 mg/d). Preliminary median treatment duration was 5.6 weeks (range, 0.1-11.3) and 6.7 weeks (range, 3.0-16.7), respectively. Median patient age was 69 years (range, 51-77) and 60 years (52-75), respectively. Response data from the 2/7 schedule (240/300 mg/d) and 5/14 schedule (150/180 mg/d) were not available due to limited treatment exposure. Common adverse events (AEs) of interest are shown in the Table 1. No grade 4 AEs occurred in the two schedules. Two pts (20%) in the 2/7 schedule (240/300 mg/d) and 0 pts in the 5/14 schedule (150/180 mg/d) discontinued treatment due to an AE. Two pts (20%) in the 2/7 schedule (240/300 mg/d) and 1 patient (11%) in the 5/14 schedule (150/180 mg/d) had ≥1 dose reduction due to an AE.
Summary and Conclusions: The gastrointestinal tolerability profile of OPZ improved at dosing levels below the MTD in the 2/7 schedule (240/300 mg/d) and 5/14 schedule (150/180 mg/d). Common grade ≥3 AEs in both treatment schedules included diarrhea, nausea, and vomiting. Updated results from the phase 2 portion of the study will be presented at the meeting.

P647

MULTIPLE MYELOMA MANAGEMENT: PRACTICE PATTERNS ACROSS EUROPE

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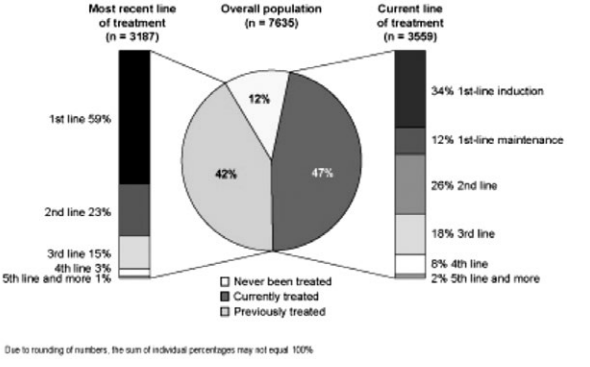
Background: The paradigm of multiple myeloma (MM) therapy has changed markedly over the last 2 decades and continues to evolve, with varying practice patterns across Europe. However, data describing patient management in clinical practice are limited.
Aims: This cross-sectional study estimated the size of each line of treatment for symptomatic MM patients in a real-world setting in Belgium, France, Germany, Italy, Spain, Switzerland and the UK in 2014.
Methods: Data were collected using chart reviews by oncologists/haematologists who manage ≥10 patients with MM per month and were personally responsible for initiating treatments in MM. Data included patient characteristics and current treatment for all patients with symptomatic MM seen during a 2-4-week observation period (depending on number of patients seen during this time) regardless of time since diagnosis, disease stage and current treatment strategy.

Figure 1. Treatment statuses of patients with symptomatic multiple myeloma.



Results: In total, 435 physicians collected data for 7635 patients. Physicians were haematologists/onco-haematologists (68%) or oncologists (32%). Most patients (62%) were ≥65 years and median time since diagnosis was 33 months. Overall, 23% of patients had an International Staging System (ISS) score of I, 37% a score of II and 37% a score of III at diagnosis. Patient characteristics were similar across countries, except for Spain, where fewer patients had ISS III MM (24%). Figure 1 shows treatment statuses of patients. Nearly half (47%) of patients were on active treatment, most of whom were on 1st line therapy. For patients on treatment at the time of the study, bortezomib-based regimens were the most commonly used at 1st line (48% of patients), except in the UK where thalidomide was more frequently used (56%). At 2nd line, lenalidomide was most commonly used (59%), except in the UK, where bortezomib was mainly used. Lenalidomide was also the most common agent overall at 3rd line (51%). Even though only recently reimbursed, the use of pomalidomide was prominent in 4th and 5th line regimens (32% and 43%, respectively). Bendamustine was seldom used at 1st or 2nd line therapy; in later lines, this agent was more commonly used in Germany, Spain and France than in other countries. Overall, 19% of patients currently receiving 1st line treatment had received a stem cell transplant (SCT; this snapshot does not include patients for whom SCT was planned in 1st line); 70% of patients receiving 5th line or later treatment had previously received an SCT. Main reasons for discontinuing previous antitumour therapy were remission and/or disease stabilised (61%) and completed planned cycles (38%). Main reasons for never treating patients were lack of

symptoms (62%) and waiting for biochemical progression (45%). However, in Germany, patient refusal was a frequent reason (19%) and in Switzerland poor life expectancy was selected for 27% of patients.
Summary and Conclusions: Half of patients seen were on active therapy. However, data suggest that many do not receive subsequent treatment lines. Those who reached later lines were more likely to have had a previous SCT. Although treatment practices were broadly similar across countries, there were some notable differences in the agents used. These real-world data provide useful information for designing clinical trials and for health economic evaluations of new and existing agents.

P648

MULTIPLE INFUSIONS OF AUTOLOGOUS ACTIVATED AND EXPANDED NATURAL KILLER CELLS: A NEW THERAPEUTIC OPTION FOR MULTIPLE MYELOMA

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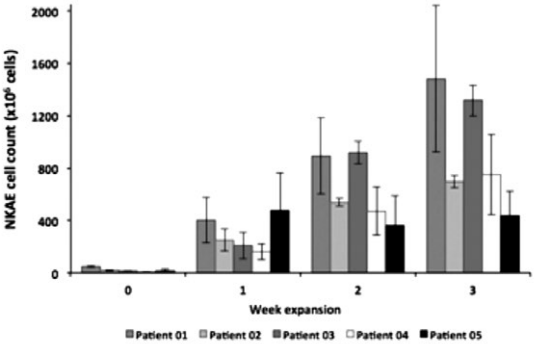
Background: Multiple myeloma (MM) remains an incurable disease. Natural killer (NK) cell infusions could be a novel treatment strategy. NK cells exert cytotoxicity against MM cells. Moreover, it has been suggested that enhanced NK cell cytotoxicity might be part of the mechanism of action of effective anti-myeloma drugs such as lenalidomide or bortezomib. By co-culture with the genetically modified cell line K562-mb15-41BBL it is possible to expand *ex vivo* large numbers of activated NK cells from MM patients.
Aims: We are conducting a phase I clinical trial to evaluate feasibility, safety and tolerability of K562-mb15-41BBL-expanded autologous NK cells (termed "NKAEs") infused in MM patients in combination with anti-myeloma drugs (EudraCT 2012-000514-11). Patients eligible for this trial have MM and persistent or progressing disease.
Methods: Five MM patients on 2nd or later relapse have been enrolled in this phase I clinical trial to date. To activate and expand NK cell, peripheral blood mononuclear cell (PBMCs) were co-cultured with K562-mb15-41BBL cells and 100 IU/ml IL-2. We collected 200 ml of peripheral blood (PB) from patients every cycle (n=4) to produce autologous NKAEs under GMP conditions and cells were harvested on day 14 and 21 for infusions. Four cycles of pharmacological treatment with 2 infusions of $7.5x10^6$ autologous NKAEs/kg on day 1 and 8 of each cycle were performed. NKAEs purity was analyzed by multiparametric flow cytometry. NK cells presence in PB was also assessed by PB smear examination before and after each infusion. C-Myc, telomerase and BCR-ABL expression were determined by real time-PCR.

A)



B)

Patients	Situation Before Inclusion	Complete Treatment	Response	Response Duration	Hematologic Toxicity
01	Biological Progression	YES	Partial Response	13 Months	NO
02	Biological Progression	YES	Stable Disease	9 Months	GII
03*	Very Good Partial Response	NO (2 CYCLES)	Not evaluable (NE)	4 Months	NO
04	Biological Progression	YES	Stable Disease	4 Months	NO
05	Biological Progression	YES	Stable Disease	4 Months	NO

Figure 1. A) Results of NKAE expansion procedures (n=17) from patients included on EudraCT 2012-000514-11 clinical trial. Results are shown as mean ± standard deviation of 4 independent expansion procedures from each patient. B) Summary of EudraCT 2012-000514-11 clinical trial.

Results: Of the 5 patients enrolled, 3 received lenalidomide-based treatment and 2 bortezomib-based treatment. Patients received a total of 34 NKAEs infusions. We have not observed any serious toxicity attributable to NKAE infusion. One patient had grade II neutropenia, which did not require dose adjustment. The 5 MM patients enrolled had 23% (±10%) NK cells of PBMCs. We collected $21x10^6$ ($\pm17x10^6$) NK cells from PB. After 1 week NKAEs number increased x17 fold, at 2nd week fold was x33. We collected $550x10^6$ ($\pm50x10^6$) NKAEs from culture for the first infusion. At 3rd week NKAEs number reached $992x10^6$ and 92% (±7%) purity of NKAEs (Figure 1A). The expression of c-Myc and telomerase were not altered in NKAE end products. The expression of BCR-ABL disappeared from cultures after the first week, and was undetectable in PB after NKAE therapy. PB smear examination showed an increase ofx2.5 fold of activated circulating lymphocytes: $7x10^5$ lymphocytes/L before infusion, reaching $1.75x10^6$ lymphocytes/L after infusion. Patient 01 achieved a partial response and maintained it for 13 months after NKAEs infusion. Patient 02 started NKAEs infusion while in relapse and, achieved stable disease, which was maintained for 9 months before disease progression. Of note, bone marrow infiltration by MM plasma cells decreased at least 50% at the end of NKAE treatment in these two patients. Patient 03 had disease progression 2 months after stopping treatment due to unrelated toxicity. Patients 04 and 05 recently finished NKAEs treatment and achieved disease stabilization 4 months after the first NKAE infusion (Figure 1B).
Summary and Conclusions: Clinical-grade NKAEs can be obtained from MM patients undergoing treatment, and multiple infusions of NKAEs are feasible without serious toxicities. NKAEs showed clinical anti-myeloma activity, with one heavily treated patient achieving durable partial response. These results and clinical observations warrant further development of NKAEs infusion as a treatment modality for refractory MM.

P649

MULTIPLE MYELOMA MANAGEMENT: OUTCOMES IN REAL-WORLD PRACTICE

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Background: Advances in therapy and management have improved survival for patients with multiple myeloma (MM), many of whom live to receive several lines of therapy. With increasing numbers of available therapies, it is timely to examine real-world treatment practices and the course of patient journeys.
Aims: To investigate patient characteristics, treatment durations and outcomes, treatment-free intervals (TFI) and symptom burden across the treatment pathway in Belgium, France, Germany, Italy, Spain, Switzerland and the UK.
Methods: Data were obtained from chart reviews by physicians managing patients with MM, who completed retrospective forms on patient characteristics and treatment from diagnosis for up to 14 patients seen in the previous 3 months, with a quota for patients who had completed specific treatment lines. Patients were included in reverse chronological order.
Results: In total, 435 physicians retrospectively reviewed 4997 patient charts. Median time from diagnosis in patients at 1st line was 19 months, at 2nd line 44 months and at ≥3rd line 65 months. Profiles of patients diagnosed in the last 12 months were similar across countries; common presentations were bone pain (61%), anaemia (39%), vertebral fracture (21%), renal dysfunction (20%) and hypercalcemia (19%). Almost all patients (95%) received ≥1 line of therapy, 61% received ≥2, 38% ≥3, 15% ≥4 and 1% ≥5. Of the 38% of patients eligible for stem cell transplantation (SCT), 95% received SCT at 1st and/or 2nd line; proportions of patients receiving SCT were similar across countries. Median duration of 1st line therapy was 6 months, followed by a median TFI of 10 months. TFI was longer in patients who received SCT (16 months [n=444] *vs* 7 months in those who did not [n=935]). Time to progression (TTP) decreased with later lines of therapy, from median 18 months at 1st line to 5 months at 4th line. Depth of response, as assessed by treating physician, decreased with each additional line of therapy: 74% of patients achieved at least very good partial response (≥VGPR) in 1st line, while only 11% achieved ≥VGPR at 5th line. Better response levels were associated with longer TTP; in 1st line, median TTP for patients in complete response (CR) was 30 months, VGPR, 21 months and PR, 13 months. At the end of each treatment line, patients who were not planned to receive further treatment were significantly (p <0.05*)* more likely to have had a worse response to treatment, a poor overall condition, more adverse events (AEs), shorter life expectancy and to have received suboptimal dosing owing to comorbidities or treatment-related AEs compared to patients who were planned to or those who received a further line of therapy. The most

common AEs at 1st line were neuropathy (45% of patients, all grades), anaemia (23%), neutropenia (22%) and thrombocytopenia (15%). AEs affected optimal treatment dosing in 23% of patients at 1st line, increasing to 45% at 3rd line.
Summary and Conclusions: Information on real-world patient outcomes may be valuable to physicians when assessing treatment options and discussing these options with patients. The data suggest that while over half of patients receive 2nd line therapy, only 15% receive ≥4 lines. Diminishing benefits with each line of therapy, and increasing incidence of AEs leading to suboptimal treatment dosing, highlight the challenges when making treatment decisions across the pathway. The data also indicate which patients are most likely to discontinue therapy, which may help physicians to plan MM treatment strategies.

P650

EUROPEAN POST-APPROVAL SAFETY STUDY (PASS) OF RELAPSED/ REFRACTORY MULTIPLE MYELOMA (RRMM): VTE INCIDENCE, RISK FACTORS, AND USE OF ANTITHROMBOTIC PROPHYLAXIS IN MM PATIENTS TREATED WITH LENALIDOMIDE

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Background: EU PASS is an observational, non-interventional post-authorization study designed to investigate the safety of lenalidomide (LEN) and other agents in the treatment (Tx) of RRMM patients (pts). Venous thromboembolic events (VTEs) are recognized as possible adverse events (AEs) associated with the administration of LEN+dexamethasone (DEX). Larocca (*Blood*, 2012) reported that the incidence of grade 3/4 deep vein thrombosis (DVT) and pulmonary embolism (PE) during the first 6 mos of Tx was similar in selected newly diagnosed MM pts treated with LEN+low-dose DEX randomized to receive either acetylsalicylic acid (ASA) 100 mg/day (2.3%) or low-molecular-weight heparin (LMWH) enoxaparin 40 mg/day (1.2%; *P*=0.452). In the observational MELISSE study of pts treated with IMiD immunomodulatory drugs in 52 IFM centers, 443 pts received thromboprophylaxis at therapy initiation: 307 pts (59%) received ASA, 88 pts (17%) received LMWH, and 81 pts (15.5%) did not receive VTE prophylaxis (Leleu, *Thromb Haemost.* 2013). Through the first year of follow-up, the cumulative incidence of VTEs was 6% (n=31). Overall, VTEs occurred irrespective of whether pts were on ASA or LMWH prophylaxis (*P*=0.62).
Aims: To describe the incidence of VTE (DVT/PE) among RRMM pts enrolled in the PASS, to identify risk factors for VTE, and to compare the incidence of VTE in pts receiving different types of anti-thrombotic prophylaxis and no prophylaxis.
Methods: RRMM pts who had received ≥1 prior Tx were enrolled at the investigator's discretion into a LEN or background cohort (other regimens); this analysis focused only on pts who were treated in the LEN cohort. Thromboprophylactic medication was administered per local standard practice. Baseline risk factors for VTE were extracted. Incidence rates of VTE were calculated for events that occurred during the first 6 mos of follow-up based on pt-mos of exposure to single agents (ie, LMWH, ASA) and pt-mos of no exposure.
Results: A total of 2164 pts received LEN therapy for RRMM. Pts in the LEN cohort had a median age of 68 yrs (range, 25-95 yrs), and 54% were male. The proportion of pts prescribed low-dose DEX (≤160 mg) was 74.2%. Among 1529 pts with new or continuing thromboprophylaxis at baseline, 36.5% received ASA, 24.9% received LMWH, 7.1% received other anti-platelet medications, and 5% received warfarin. Over the entire follow-up interval, 123 pts (5.7%) experienced a VTE. Only 2 pts experienced a VTE while receiving warfarin therapy. Baseline risk factors associated with VTE were previous history of VTE (odds ratio [OR]=1.71; 95% CI, 1.06-2.77), age (OR=2.10; 95% CI, 1.36-3.25), and body mass index (OR=1.47; 95% CI, 0.98-2.21). Considering only single thromboprophylaxis agent use during the first 6 mos of IMiD therapy, the incidence of VTE was highest among pts not receiving prophylaxis during the month the VTE occurred: 1.64 per 100 pt-mos. VTE incidence rates were similar among pts treated with either ASA (0.686 per 100 pt-mos) or LMWH (0.636 per 100 pt-mos). In either case, a significant protective effect was noted for use of either ASA (relative risk [RR]=0.42, *P*=0.003) or LMWH (RR=0.39; *P*=0.004).
Summary and Conclusions: The incidence of VTE among RRMM pts in the PASS (5.7%) was similar to that previously reported in another large cohort study (MELISSE). Identified VTE risk factors were similar to those previously identified (age, prior VTE, BMI). Similar to previously reported results comparing ASA and LMWH, equivalent reductions in VTE risk were identified for pts receiving thromboprophylaxis with either ASA or LMWH.

P651

MANAGEMENT OF ADVERSE EVENTS IN THE STRATUS TRIAL, A PHASE 3B STUDY EVALUATING SAFETY AND EFFICACY OF POMALIDOMIDE+ LOW-DOSE DEXAMETHASONE IN REFRACTORY OR RELAPSED AND REFRACTORY MULTIPLE MYELOMA

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Background: To allow for full-dose treatment (Tx) and maximize therapeutic benefit, management of adverse events (AEs) in patients (pts) with relapsed/refractory multiple myeloma (RRMM) must be optimized. Pts with RRMM who have been exposed to multiple prior Tx may present with comorbidities and be susceptible to frequent AEs. In the pivotal MM-003 study of pomalidomide+low-dose dexamethasone (POM+LoDEX) in pts with heavily pre-treated RRMM, most pts did not have dose reductions and there were few discontinuations due to AEs (Delforge, EHA 2013). Frequent grade (Gr) 3-4 AEs associated with POM+LoDEX were neutropenia, anemia, thrombocytopenia, and infections.
Aims: This sub-analysis examined the safety profile and management of AEs for pts enrolled in the STRATUS trial.
Methods: Pts with refractory or relapsed and refractory disease (progressive disease [PD] within 60 days of last prior Tx) who failed prior lenalidomide and bortezomib Tx and had adequate prior alkylator therapy were included. Key exclusion criteria were: absolute neutrophil count <800/μL, platelet count <75,000 or <30,000/μL (for pts with <50% or ≥50% of bone marrow nucleated cells as plasma cells, respectively), hemoglobin <8 g/dL, creatinine clearance <45 mL/min, and peripheral neuropathy (PN) ≥Gr 2. POM 4 mg was administered D1-21 of a 28-day cycle in combination with LoDEX 40 mg/day for pts aged ≤75 yrs or 20 mg for pts aged >75 yrs on D1, 8, 15, 22 until PD or unacceptable toxicity. Pts required mandatory thromboprophylaxis. AEs were graded according to the National Cancer Institute Common Terminology Criteria for AEs v 4.0. Pts were followed for subsequent anti-myeloma Tx, OS, and second primary malignancy until 5 yrs post enrollment. The primary endpoint of this sub-analysis was safety.

Table 1.

	POM + LoDEX (n = 599)
Dose management	
Median average dose, mg POM (range)	4.0 (1.87, 4.00)
≥ 1 dose reduction for any AE (%)	18
Dose reduction for neutropenia (%)	4.7
Dose reduction for febrile neutropenia (%)	1.0
Patients with specified AEs (any grade) who received supportive care (%)	
Infection (n = 357)	
Granulocyte colony-stimulating factor	53.2
Anti-infective	88.8
Neutropenia (n = 341)	
Granulocyte colony-stimulating factor	73.6
Anemia (n = 287)	
Red blood cell transfusion	57.1
Thrombocytopenia (n = 204)	
Platelet transfusion	32.4
Median time to AE onset, days (range)	
Infections/Infestations (n = 357)	38.0 (1.0-397.0)
Neutropenia (n = 341)	19.0 (1.0-369.0)
Anemia (n = 287)	21.0 (1.0-436.0)
Thrombocyopenia (n = 204)	15.0 (1.0-498.0)

AE, adverse event; LoDEX, low-dose dexamethasone; POM, pomalidomide.

Results: 604 pts have been enrolled as of Sept 15, 2014; 599 pts were evaluable for safety. Median follow-up was 9.3 mos. The most frequent Gr 3-4 hematologic AEs were neutropenia (42%), anemia (29%), and thrombocytopenia (22%); 5% of pts experienced Gr 3-4 febrile neutropenia. The most frequent Gr 3-4 non-hematologic AEs included infections (30%) and fatigue (5%); 11% of pts experienced Gr 3-4 pneumonia. Gr 3-4 venous thromboembolic events (VTEs) occurred in 1% of pts and 4% had at least 1 VTE of any grade. PN of any grade occurred in 15% of pts, with 1% of pts experiencing Gr 3-4 PN. Management of AEs was primarily via dose reductions and/or supportive care (Table 1). 18% and 58% of pts had POM dose reductions or interruptions, respectively due to AEs. The most common AEs leading to dose reductions or dose interruptions, respectively were neutropenia (4.7% and 19.4%), thrombocytopenia (3.3% and 9.7%), and pneumonia (1.5% and 7.3%). Discontinuation of POM due to AEs was infrequent (5%)

and the most frequently occurring AEs leading to POM discontinuation included pneumonia (0.7%), thrombocytopenia (0.7%), anemia (0.3%), and neutropenia (0.3%). Updated data will be presented at the congress.
Summary and Conclusions: This is the largest study conducted to date with POM+LoDEX in a heavily pre-treated RRMM pt population. POM+LoDEX was generally well tolerated with an acceptable safety profile that was consistent with the pivotal trials. There were few discontinuations due to AEs and most AEs could be managed with dose reductions and/or supportive care.

P652

SINGLE CENTER EXPERIENCE WITH EARLY *VERSUS* LATE AUTOLOGOUS STEM CELL TRANSPLANTATION IN MULTIPLE MYELOMA

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Background: In the last couple of years a heated debate developed between supporters of first line autologous stem cell transplant (ASCT) in every multiple myeloma (MM) patients whenever it is feasible and those who think that with the arrival of novel agents (thalidomide, bortezomib and lenalidomide) we can get comparable results without the toxicity of ASCT which we can therefore delay and spare for relapse. Randomized studies from the nineties showed conflicting results regarding the progression free survival (PFS) and overall survival (OS) benefit associated with high dose treatment, and trials exploring this question in the era of novel agents are currently running. These are expected to be reported within the next years, but until then our strategies should mainly be based on retrospective comparisons.
Aims: We analyzed the outcome of 548 myeloma patients transplanted in our centre over 18 years.
Methods: Transplants performed within 12 months from the start of the first chemotherapy were counted as early, others as late. PFS was calculated from the day of ASCT and OS from the first chemotherapy.
Results: The median time from first treatment to ASCT was 9.8 (3.4-192) months in the whole cohort, 7.3 (3.4-11.9) in the early and 16.9 (12-192) in the late ASCT subgroups. The overall response rate post-ASCT was higher in the early group with significantly more CRs (58.1 *vs* 46.8%, p=0.016). The pre-ASCT results were superior with novel agents both in the early and late cohorts, but following the ASCT these differences disappeared. The PFS was significantly longer in the early than the late group [30.2 (26.1-34.3) *vs* 23.3 (16.8-29.8) months; p=0.036], but there was no OS advantage [95.5 (83.8-107.2) *vs* 100.4 (80.7-120.1); p=0.211] (Figure 1). The results were similar in subgroups treated with novel agents [PFS in early *vs* late 31 (25.8-36.2) months *vs* 22.2 (14.9-29.4); p=0.105; OS 84.3 (70.9-97.7) *vs* 122.4 (90.5-154.3) respectively; p=0.101] and without [PFS 27.8 (21.1-34.5) *vs* 27.8 (18.9-36.7) in early *vs* late; p=0.138; OS 97.2 (84-110.4) *vs* 99.1 (93.4-104.8) respectively; p=0.772] however within the subgroups the differences were not significant.

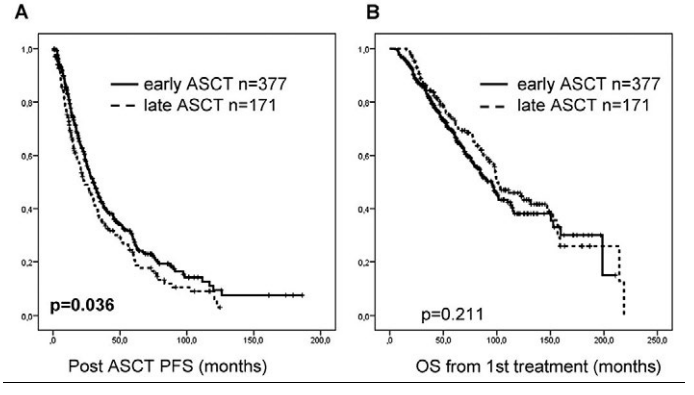

Figure 1.

Summary and Conclusions: Our findings confirmed the results of prior retrospective studies: early ASCTs resulted in more CRs and VGPRs, and a significant post-ASCT PFS benefit, but no OS difference. However one has to be careful with the interpretation of retrospective studies. Even with novel agents there is a significant proportion of patients unsalvageable at relapse. For others transplant becomes unfeasible due to deterioration of performance status or comorbidity. As these patients have never had ASCT they remain unaccounted for in any retrospective ASCT data sets. Another worry is that ASCT may be less effective later in the disease course due to clonal drift resulting in increased chemoresistance. Only prospective randomized studies can answer these questions.

P653

OPROZOMIB (OPZ) AND DEXAMETHASONE (DEX) IN PATIENTS (PTS) WITH RELAPSED AND/OR REFRACTORY MULTIPLE MYELOMA (RRMM): UPDATED RESULTS FROM DOSE ESCALATION IN A PHASE 1B/2, MULTICENTER, OPEN-LABEL STUDY

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Background: The oral proteasome inhibitor OPZ has shown promising antitumor activity in pts with hematologic malignancies, including multiple myeloma (MM; Vij, *Blood* 2014;121:abstract 34).
Aims: We present updated results from an ongoing, single-arm, phase 1b/2 study (NCT01832727) that is evaluating the safety and tolerability of OPZ with DEX in pts with RRMM.
Methods: Pts with RRMM who have received 1-5 prior lines of therapy (≥1 regimen including lenalidomide and/or bortezomib) are eligible. Pts are receiving OPZ tablets (PO) on days 1, 2, 8, and 9 (2/7 schedule) or on days 1-5 (5/14 schedule) of a 14-day cycle. All pts are receiving DEX (20 mg PO) on days 1, 2, 8, and 9. Treatment is being administered until pt withdrawal, disease progression, or unacceptable toxicity. The starting OPZ dose was 210 mg on both schedules. OPZ doses are being escalated in 30-mg increments using standard 3+3 dose escalation. The primary objectives of the phase 1b study are to determine the maximum tolerated dose (MTD), recommend the phase 2 dose (RP2D) of OPZ with DEX, and to evaluate safety and tolerability. Response is being assessed by IMWG criteria, with minimal response (MR) and near complete response defined by modified EBMT criteria. Safety is being assessed by CTCAE, v4.03. All pts provided informed consent.
Results: As of January 19, 2015, 22 and 19 pts were enrolled on the 2/7 and 5/14 schedules, respectively. Preliminary data are available for a total of 35 pts enrolled as of November 3, 2014 (2/7 schedule, n=19; 5/14 schedule, n=16), 31 of whom were evaluable for response (2/7 schedule, n=17; 5/14 schedule, n=14). The median age of pts was 63 years (2/7 schedule) and 63.5 years (5/14 schedule). Pts received a median of 3 (range, 1-5) prior regimens in the 2/7 schedule and 2 (range, 1-5) prior regimens in the 5/14 schedule. Preliminary median OPZ treatment duration was 15 weeks in the 2/7 schedule (range, 1.3-51.3 weeks) and 5.7 weeks in the 5/14 schedule (range, 0.7-24.7 weeks). No dose-limiting toxicities (DLTs) occurred in the 2/7 schedule. In the 5/14 schedule, 3 DLTs occurred (210-mg/day dosing level: grade 2 subarachnoid hemorrhage; grade 3 transaminitis; and grade 4 thrombocytopenia); the MTD was 180 mg/day. The MTD was not reached in the 2/7 schedule up to a dosing level of 300 mg/day. In both schedules combined, the most common adverse events (AEs) of any grade were diarrhea, nausea, and fatigue; the most common grade ≥3 AEs were anemia, diarrhea, and thrombocytopenia (Table 1). Two grade 5 AEs of sepsis occurred: 1 in the 2/7 schedule (240 mg/day) and 1 in the 5/14 schedule (210 mg/day). Three pts on the 2/7 schedule and 9 pts on the 5/14 schedule discontinued treatment due to an AE. Five pts in each schedule had their OPZ dose reduced due to an AE. On the 2/7 schedule, the overall response rate (ORR; ≥partial response [PR]) was 35.3% and the clinical benefit rate (CBR; ≥MR) was 41.2% (6 pts had a PR, 1 had an MR, and 8 had stable disease [SD]). On the 5/14 schedule, the ORR was 7.1% and the CBR was 35.7% (1 pt had a PR, 4 had an MR, and 6 had SD).

Table 1. AEs occurring in ≥25% of pts or grade ≥3 AEs occurring in ≥2 pts.

	2/7 schedule n=19		5/14 schedule n=16	
AE, n (%)	Any grade	Grade ≥3	Any grade	Grade ≥3
Hematologic AEs				
Anemia	8 (42)	5 (26)	5 (31)	2 (13)
Thrombocytopenia	7 (37)	4 (21)	4 (25)	1 (6)
Nonhematologic AEs				
Diarrhea	17 (89)	3 (16)	14 (88)	4 (25)
Nausea	17 (89)	0 (0)	14 (88)	4 (25)
Fatigue	11 (58)	0 (0)	10 (63)	2 (13)
Vomiting	8 (42)	0 (0)	10 (63)	2 (13)
Decreased appetite	4 (21)	0 (0)	6 (38)	0 (0)
Constipation	5 (26)	0 (0)	3 (19)	0 (0)
Headache	6 (32)	0 (0)	2 (13)	0 (0)
Dyspepsia	3 (16)	0 (0)	4 (25)	0 (0)
Hyperglycemia	5 (26)	1 (5)	2 (13)	0 (0)
Hypocalcemia	2 (11)	0 (0)	4 (25)	0 (0)
Hypertension	2 (11)	1 (5)	4 (25)	1 (6)
Increased blood creatinine	2 (11)	0 (0)	4 (25)	1 (6)
Decreased platelet count	1 (5)	0 (0)	4 (25)	3 (19)
Upper abdominal pain	0 (0)	0 (0)	4 (25)	1 (6)
Pneumonia	1 (5)	1 (5)	3 (19)	3 (19)
Decreased white blood cell count	2 (11)	0 (0)	2 (13)	2 (13)

Summary and Conclusions: In the 5/14 schedule, the MTD was 180 mg/day; the MTD was not reached in the 2/7 schedule. Preliminary results suggest that OPZ with DEX has promising antitumor activity in pts with RRMM receiving the 2/7 schedule; this schedule has been selected as the recommended phase 2 schedule. Dose escalation will continue in the 2/7 schedule until the MTD or RP2D is determined. Updated data (including pharmacokinetics) for both schedules will be presented at the meeting.

P654

PROGNOSTIC IMPACT OF IMMUNOPHENOTYPIC RESPONSE AND NORMALIZATION OF SERUM FREE LIGHT CHAIN AMONG PATIENTS WITH MULTIPLE MYELOMA

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Background: With the development of novel therapeutic agents, more than 30% of patients with multiple myeloma (MM) achieve complete response (CR) as defined by the International Myeloma Working Group (IMWG) criteria. However, most of the patients that achieve CR ultimately die due to relapse, suggesting the presence of minimal residual disease (MRD) in these patients. Multicolor flow-cytometry (FCM) allows the detection of < 10^{-4} clonal plasma cells (PCs) in normal bone marrow and has been used for detecting MRD after treatment. FCM-defined immunophenotypic response (iR, clonal PCs<10^{-4}) has emerged as a more relevant prognostic factor in patients with MM. However, the relevance of the prognostic impact of IR and the normalization of serum free light chain (sFLC) ratio remain unclear.
Aims: To investigate the impact of FCM-defined CR (iR), conventional immunofixation negative CR (CR), and CR plus FLC κ/λ normal CR (sCR) on the prognosis of patients with MM who obtained more than very good partial response (VGPR) at Kameda Medical Center, Kamogawa, Japan.
Methods: Among the 265 patients treated at our hospital between April 2004 and February 2015, 102 patients that achieved more than VGPR (70 CR, 32 VGPR) were included in this study. The study population consisted of 64 males and 38 females with a median age of 69 years. All patients received at least one course of novel agent-containing therapeutic regimen. Treatment response was assessed using the IMWG criteria. Best response to treatment during the course of disease was evaluated by simultaneous analysis by immunofixation electrophoresis (IFE), sFLC measurements, and FCM analysis of bone marrow PCs. FCM defined MRD was evaluated by single tube 6-color FCM using CD45, CD38 gating strategy, with combination with CD19. iR was defined as less than 10^{-4} neoplastic PCs in bone marrow samples determined by FCM. Overall survival (OS) and disease free survival (DFS); OS was defined from the day of diagnosis to death from any cause, with censoring performed the date of last contact. DFS was calculated from the date of achieve CR, sCR, or iR to relapse from the respective response date. Survivals were analyzed by the Kaplan-Meier method, and differences between curves were calculated by two-sided log-rank test. Subjects were classified into four categories; MFC positive or negative and κ-λ ratio normal or abnormal, and PFS and OS were compared between groups.
Results: Among 102 patients who achieved more than VGPR, iR, sCR, CR and VGPR was obtained 43 (42%), 80 (78%), 70 (69%) and 32 (31%) of patients, respectively. Normalization of FLC ratio with CR and VGPR was observed in 61 (87%) and 19 (59%) of patients, respectively. iR was achieved in 33 (49%) of the CR patients, in 8 (25%) of the VGPR patients, and in 39 (90.7%) of the iR patients who attained normal κ/λ. The patients with iR and normal FLC showed significantly longer DFS compared to those without iR, although OS was not different between patients with or without iR. However, these survival differences were not translated to the OS among the groups due to less. On multivariate Cox regression analysis, only achievement of iR emerged as an independent prognostic factor for DFS (Figure 1).

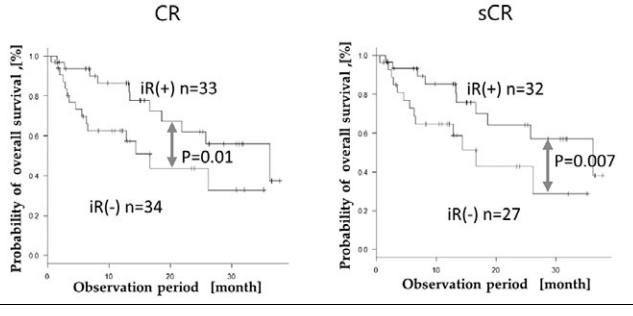

Figure 1. Impact of iR on DFS in patients with CR and sCR.

Summary and Conclusions: Although both achievement of CR, normalization of sFLC κ/λ, and iR appeared to confer on longer DFS and OS, obtaining iR seems to have greater implications for longer survival compared to CR or FLC κ/λ normalization. MFC is a rapid, affordable, and easy performable method for measurement of MRD and iR could be considered as a goal of treatment for patients with MM.

Multiple myeloma - Clinical 4

P655

A PRIOR CANCER DIAGNOSIS IS NOT A RISK FACTOR FOR THE DEVELOPEMENT OF SUBSEQUENT CANCERS IN MULTIPLE MYELOMA PATIENTS

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Background: Patients with multiple myeloma (MM) have been shown to have an increased risk of subsequently developing certain types of cancers, such as acute myeloid leukemia, myelodysplastic syndromes, gastrointestinal and non-melanoma skin cancer. With improved survival in MM patients the incidence of subsequent cancers will increase in the future. Also we have previously shown that the development of a subsequent cancer is associated with a decreased life expectancy. Therefore it is of great importance to identify potential risk factors. Treatment-related factors have been considered to be the main contributing factor to the observed risk increase, but research regarding other risk factors is limited. Prior studies have found genetic instability to be associated with carcinogenesis. In this study we hypothesize that prior cancer is a proxy for genetic instability that could be a potential risk factor for subsequent cancer development in MM patients.
Aims: To investigate whether any form of cancer diagnosis prior to MM diagnosis is an independent risk factor for developing a subsequent cancer after the MM diagnosis.
Methods: All patients diagnosed with MM in Sweden from January 1st, 1958 to December 31st, 2011 were identified from the Swedish Cancer Registry (SCR). All prior and subsequent cancer diagnoses were identified through cross-linkage with the SCR. Patients with a cancer diagnosis after MM diagnosis were classified as cases. For each case, 1-3 controls without a cancer diagnosis after MM diagnosis, matched for age, gender, and date of MM diagnosis, were selected from the cohort. The controls had to be alive when the corresponding case developed subsequent cancer. The type of prior and subsequent cancer was classified according to the International Classification of Disease version 7. Prior cancer diagnosis was compared between cases and controls. Subgroup analysis was performed for each subsequent cancer subtype according to appropriate organ system. Conditional logistic regression was used for the statistical analysis in R.
Results: Results: A total of 26,627 patients were diagnosed with MM during the study period. Of these, 1,645 (6.2%) developed subsequent cancer (cases) and 4,334 MM patients were chosen as matched controls. MM patients with a prior cancer diagnosis did not have a statistically significant increased risk of developing a subsequent cancer compared to MM patients without a prior cancer (odds ratio (OR) 1.19; 95% confidence interval (CI) 0.97-1.46). In a subgroup analysis, prior cancer in MM patients was not associated with increased risk of developing the following cancers: breast (OR=1.21; 95% CI 0.52-2.77), ear, nose and throat (OR=0.90; 95% CI 0.09-8.68), endocrine (OR=0.94; 95% CI 0.18-5.01), female reproductive (OR=0.91; 95% CI 0.26-3.15), hematological (OR=0.84; 95% CI 0.43-1.66), gastrointestinal (OR=0.89; 95% CI 0.56-1.43), kidney and urinary tract (OR=1.86; 95% CI 0.92-3.75), male reproductive (OR=0.58; 95% CI 0.31-1.12), melanoma (OR=1.35; 95% CI 0.52-3.47), nervous system (OR=0.72; 95% CI 0.15-3.38), non-melanoma skin cancer (OR=1.52; 95% CI 0.98-2.36), and respiratory (OR=2.02; 95% CI 0.84-4.86).
Summary and Conclusions: In this large case-control study we report that a prior cancer diagnosis is not associated with increased risk of developing a subsequent cancer in MM patients. Our findings are of major clinical relevance and suggest that the best available therapy should not be withheld from MM patients due to concern for subsequent cancer, irrespective of a prior cancer diagnoses. Further research is needed to shed light on the risk factors for subsequent cancers in MM patients.

P656

MELPHALAN IN COMBINATION WITH BORTEZOMIB AND DEXAMETHASONE (BMDEX) PRODUCES MORE DURABLE RESPONSES THAN CYCLOPHOSPHAMIDE (CYBORD): A MATCHED CASE-CONTROL STUDY

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Background: At present, the majority of patients with AL amyloidosis are treated with combinations of alkylating agents, bortezomib, and dexamethasone. High response rates and short times to response have been reported with these regimens. Cyclophosphamide is used more commonly (CyBorD), but in some centers melphalan is also prescribed (BMDex). At our center both regimens are used, and cyclophosphamide is preferred in patients with potentially reversible contraindications to stem cell transplant and in subjects with renal failure.
Aims: In the present case-control study, we compared the outcome of patients treated with CyBorD and BMDex.

Methods: Eighty-seven newly-diagnosed consecutive patients treated with BMDex were matched with 87 controls who received CyBorD. Matched variables were cardiac stage (with stage III patients having NT-proBNP >8500 ng/L included in a separate stage, IIIb), revised Mayo stage, renal stage, systolic blood pressure <100 mmHg, and bortezomib (1.3 mg/m^2 in all patients) and dexamethasone (40 mg in 26% of patients) dosages.
Results: Cardiac stage was I, II, IIIa, and IIIb in 14%, 42%, 22%, and 22% of patients, respectively. Renal stage was I, II, and III in 53%, 30%, and 27% of cases, respectively. There was no difference in plasma cell infiltrate (median 13% *vs* 12%, P=0.190) and dFLC (median 199 mg/L *vs* 212 mg/L, P=0.953) between the BMDex and CyBorD groups. Patients treated with BMDex were older (median 69 *vs* 60 years, P<0.001). Severe adverse events were observed in 22% of patients treated with BMDex and in 13% of those receiving CyBorD (P=0.108). Most common were cytopenia (6% *vs* 1%, P=0.054) and fluid retention (9% *vs* 8%, P=0.787). Hematologic response rate was 69% in the BMDex group and 55% in the CyBorD group (P=0.061), and CR/VGPR was reached in 55% of patients in the BMDex arm and in 39% in the CyBorD arm (P=0.033). Differences in cardiac (16% *vs* 17%) and renal (29% *vs* 25%) response rates were not significant. Projected overall survivals at 3 years in cardiac stages I, II, IIIa, and IIIb were 100% *vs* 100%, 72% *vs* 51% (P=0.059), 42% *vs* 47% (P=0.605), 12% *vs* 12% (P=0.352) in the BMDex and CyBorD arms, respectively. In the overall population, time to second-line therapy was longer with BMDex (74% *vs* 40% of responses maintained at 3 years, P=0.050). This was due to a longer duration of CR/VGPR (89% *vs* 57% maintained at 3 years, P=0.019), while duration of PR was similar in the two arms (median 9 *vs* 13 months, P=0.093). However, relapsing patients were efficiently rescued, and there was no difference in overall survival of patients achieving CR/VGPR with BMDex and CyBorD (83% *vs* 82% at 3 years).
Summary and Conclusions: Profound reductions in the concentration of the amyloidogenic light chain can be obtained with both BMDex and CyBorD. However, responses are more durable in patients exposed to melphalan, and BMDex may be preferred when there is no need to preserve stem cells.

P657

EVALUATION OF CURRENT CLINICAL MODELS FOR RISK OF PROGRESSION FROM SMOLDERING MULTIPLE MYELOMA TO MULTIPLE MYELOMA IN 287 PATIENTS FOLLOWED IN THE CZECH REPUBLIC

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Background: Two risk stratification models predict the progression from smoldering multiple myeloma (SMM) to multiple myeloma (MM). i) the Mayo Clinic model uses percentage of bone marrow plasma cells (BMPCs) and serum monoclonal protein (M-protein) and free light chain (FLC) ratio, ii) the PETHEMA model uses immunoparesis and the percentage of abnormal PCs (aPCs) by flow cytometry.
Aims: The primary end point was to estimate the cumulative risk of MM occurring during the follow-up of our cohort. The secondary end points were: to validate known clinical models and to establish a new risk model by the Czech Myeloma Group (CMG model) with better prediction of ultra high-risk SMM group.
Methods: Data for this study were obtained from the Registry of Monoclonal Gammopathies (RMG) acquired from hematologic centers of the Czech Republic. In total, 361patients with SMM were enrolled in the RMG study from May 2007 to June 2013. In total, 79.5% (287/361) of patients were analyzed.
Results: 287 SMM patients were followed with median 2.4 years. MM was developed in 51.9% (149/287) of patients. The risk of progression was 16%, 31.2%, 54.8% and 73.4% at 1, 2, 5 and 10 years after diagnosis, respectively. The key predictors factors of progression were as follows: serum (iFLC/uFLC) ratio >30 (HR 2.4 [1.4-4.1], p<0.001), BMPCs ≥15% (HR 2.1 [1.5-3.0]; p<0.001), immunoparesis (HR 2.0 [1.3-2.9]; p<0.001), M - protein concentration ≥2.3 g/dL (HR 2.00 [1.4-2.7]; p<0.001), beta2 microglobulin ≥2.0 mg/l (HR 1.8 [1.2-2.7]; p=0.001), and thrombocyte count ≤250x10^9/l (HR 1.7 [1.1-2.4]; p=0.005). Distribution of SMM patients according to risk groups based on the Mayo Clinic model (Dispenzieri 2008) confirmed predictive power of this model based on low and intermediate- risk group, but did not confirm high-risk group. The high-risk group was represented only by 4 SMM patients. At 2 years, no-risk group had 20.2% risk of progression compared to 23.2%, 45.4% and 25% in groups with 1, 2 or 3 risk factors, respectively (p=0.014) (Figure 1). SMM group with 1, 2 and 3 risk factors in comparison to the reference group had HR (1.14 [0.54- 2.41]; p=0.732, HR 2.58 [1.25- 5.35]; p=0.011, HR 2.18 [0.47-10.03]; p=0.317, n=146), retrospectively. Immunoparesis and ≥95% aPC was used to validate the PETHEMA model (Perez-Persona 2007). The rates of progression at 2 years were 5.9%, 20% and 41.4% for groups with 0, 1 or 2 risk factors, respectively (p=0.058) (Figure 1). SMM group with 1 and 2 risk factors in comparison to the reference group had HR (1.51 [0.40-5.69]; p=0.546, HR 3.31 [0.78-14.06]; p=0.104, n=62), retrospectively. Based on the 3 parameters with independent predictive value in the univariate analysis (immunoparesis, serum M-protein quantity ≥2.3 g/dL and iFLC/uFLC >30) we proposed a new CMG model. The risk of progression from SMM to MM at 2 years was 18.5%, 20.9%, 41.9% and 78.7% if 0, 1, 2 or 3 risk factors are present (p<0.001) with HR of 1.4 [0.7-2.9]; p=0.28, 2.5 [1.2-5.0]; p=0.008, 6.7 [3.0-15.2]; p<0.001, n=139), respectively (Figure 1).

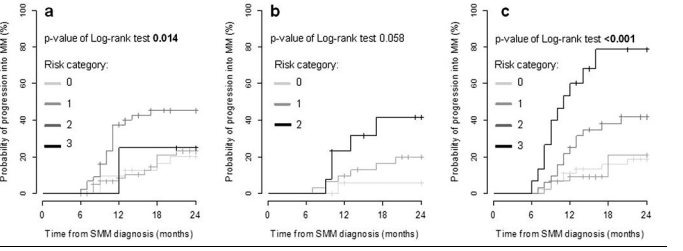

Figure 1. Comparison of 2 year risk of progression of CMG risk model (c) with the existing models developed by Mayo clinic group (a) and PETHEMA group (b).

Summary and Conclusions: We confirmed the validity of previously considered clinical models by the Mayo Clinic group (except high-risk group) and the PETHEMA group. New CMG model for the risk of progression from SMM to MM was established. Better identification of ultra high-risk group with prediction of 79% risk of progression to MM within two years based on easily accessible clinical parameters is advantage.
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P658

VENETOCLAX (ABT-199/GDC-0199) MONOTHERAPY FOR RELAPSED/REFRACTORY MULTIPLE MYELOMA: PHASE I SAFETY AND EFFICACY

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Background: The anti-apoptotic protein BCL-2 has been implicated in mediating the survival of multiple myeloma (MM) cells. Venetoclax is a potent, selective, orally bioavailable small-molecule BCL-2 inhibitor. Venetoclax induces cell death in MM cell lines and primary samples *in vitro*, especially in cells which express a high ratio of *BCL2* to *MCL1*, such as those with t(11;14). The current Phase 1 study evaluates safety and efficacy of venetoclax in patients with relapsed/refractory MM.
Aims: Primary objectives are to evaluate safety, pharmacokinetics (PK), and recommended Phase 2 dose (RPTD); other objectives include preliminary efficacy and impact of chromosomal abnormalities.
Methods: In dose-escalation cohorts, venetoclax was given orally daily at 300, 600, 900, or 1200 mg after a 2-week dose ramp-up. Patients were monitored for tumor lysis syndrome.
Results: As of 12/19/2014, there were 28 patients with median age 65 (12/16 female/male); 9 were ISS stage I, 11 stage II, and 6 stage III. Median (range) prior therapies: 6 (1-13). 23 received prior bortezomib-containing regimens (15 were refractory), 26 lenalidomide (12 were refractory), and 13 autologous

hematopoietic stem cell transplant. 10 patients were t(11;14)-positive. Adverse events (AE; all grades) occurring in ≥20% patients: diarrhea (32%), nausea (32%), neutropenia (21%), and fatigue (21%). Grade 3/4 AEs (≥10%): thrombocytopenia (18%), anemia (14%), and neutropenia (14%). 7 patients had serious AEs, with 1 (epigastric pain) possibly related to venetoclax. 17 patients have discontinued: 14 due to disease progression (PD), 2 for AEs (worsening shortness of breath, hypokalemia), and 1 withdrew consent; 11 are still receiving therapy. 2 deaths occurred (both due to PD). 2 dose-limiting toxicities were seen at 600 mg (cohort was expanded): epigastric pain, nausea with abdominal pain. No patient had tumor lysis syndrome. Preliminary PK (n=11; 300 and 600 mg): mean C_{max} and AUC_{24} were approximately dose-proportional with high intra-dose variability. 21 of 28 patients were evaluable for preliminary efficacy (Table 1).

Table 1. Best response by t(11;14) status.

n (%)	t(11;14)-positive (n=7)	t(11;14)-negative (n=14)
Complete response (CR)	1 (14)	0
Partial response (PR)	1 (14)	0
Minimal response (MR)	1 (14)	0
Stable disease (SD)	2 (29)	9 (64)
Disease progression (PD)	1 (14)	2 (14)
Discontinued	1 (14)	3 (21)
Overall response rate (CR+ PR)	2 (29)	0
Median (range) time on study	5.1 (1.2–8.6)	1.9 (0.4–6.8)

Summary and Conclusions: Venetoclax monotherapy was well tolerated in heavily-pretreated relapsed/refractory MM. Responses (including CR) and longer time on study were observed in t(11;14)-positive patients. RPTD was achieved; this study is now enrolling in the safety expansion cohort at 1200 mg (with 2-week ramp-up).

P659

IMPACT OF FISH ABNORMALITIES ON RISK OF PROGRESSION IN PATIENTS WITH MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE

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Background: Genetic abnormalities can be detected in the clonal plasma cells in nearly all patients with myeloma, and remains the most important prognostic factor in this disease. These abnormalities can be seen in the plasma cells at all stages of the disease spectrum from MGUS to SMM to active MM. In SMM, various abnormalities have been shown to increase the risk of progression to MM. The relevance of these findings in MGUS is unknown.
Aims: The study was performed to define the impact of common FISH abnormalities on risk of progression to multiple myeloma.
Methods: We reviewed the records of 10,244 patients with a diagnosis of MGUS, and identified 382 in whom a FISH test was performed at some time during the course of MGUS, but prior to any progression to SMM or MM. Impact of various FISH abnormalities on the risk of progression to SMM or MM was analyzed.
Results: The median age at diagnosis was 62 years; 57% were male. The estimated median follow up from MGUS diagnosis was 33 months for the entire cohort, 20 patients (5%) had progressed at the time of analysis (5 SMM, 15 MM). FISH demonstrated at least one abnormality in 207 (54%) patients, was normal in 58 (15%) and had insufficient plasma cells in 117 (31%). Among the 265 patients with a productive FISH, del13q was seen in 73 (28%), del17p in 6 (2%), any chromosome 14 translocation in 89 (34%), other chromosome 14 abnormality in 48 (18%), and trisomies of one or more chromosomes in 85 (32%). Considering only the 207 patients with an abnormal FISH, the distribution of the abnormalities above were 35%, 3%, 43%, 23% and 41%, respectively. Presence of t(11;14), del17p and any trisomy were all associated with increased risk of progression, while presence of any of the high-risk translocation (t(4;14, 14;16, or 14;20) did not affect the risk of progression. Overall, trisomy, t(11;14) or del17p were present in 146 (38%) of the patients and was associated with a 6 fold increased risk of progression (95% CI; 2, 38). We repeated the analysis excluding 7 patients who had progression to SMM or MM within 1 year of FISH, and the results were similar with an increased risk of progression in the presence of one of these three abnormalities.
Summary and Conclusions: The spectrum of abnormalities in MGUS appear similar to those in MM, except for presence of abnormalities in only a little over half of the patients (compared to 97% in MM), likely a reflection of low plasma cell burden. Interestingly, the FISH abnormalities associated with a better outcome in patients with MM appear to increase the risk of disease progression in MGUS. This raises the intriguing hypothesis that certain abnormalities are associated with a faster transition to MM state but remain alive longer in the MM phase, thus suggesting that the improved outcomes seen with these abnormalities reflect more of a lead-time bias in diagnosis of symptomatic disease.

P660

POST RELAPSE OUTCOMES IN YOUNG PATIENTS WITH MULTIPLE MYELOMA IN THE ERA OF NOVEL AGENTS

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Background: About 10-15% of patients with multiple myeloma (MM) are ≤50 years of age at diagnosis. Age is known to impact survival outcome for patients with MM. Young patients tend to have significantly longer overall survival (OS) compared to their older counterparts. Our previous accounts have suggested that in contradistinction to the observations in the elderly, young patients have not demonstrated incremental survival improvement over the past decade. Herein, we attempt to determine the disease course in young patients following first relapse in the novel agent era, and compare outcomes with a matched cohort of elderly patients (≥65 years of age) with relapsed MM.
Aims: To evaluate post relapse outcomes in young (≤50 years of age at diagnosis) MM patients, diagnosed between 01/01/2000-12/31/2011.
Methods: Of 242 consecutive young patients with active MM, diagnosed between 2000-2011 and evaluated within 90 days of their diagnosis of MM at Mayo Clinic, Rochester,149 (61.5%) had a documented first relapse prior to our data cut-off date (12/31/2014). Patients were assigned to 2 year groups based on the timing of the diagnosis: Group A (2000-2005; n=79) and Group B (2006-2011; n=70). The outcomes from diagnosis were compared to a reference cohort of elderly patients (≥65y of age), matched (1:1) by the timing of diagnosis, using the Kaplan-Meier method and log-rank test. Additionally, post relapse outcomes were compared to a similar reference cohort of elderly MM patients that had a documented relapse.
Results: The median OS from diagnosis for young MM patients (n=242) was 100.5 months [CI: 91- NR (not reached)]. The median estimated follow-up of the entire cohort was 85 months from diagnosis. The median time to first progression was 33 months (CI 28-38) in 207 young patients for whom the complete progression-related data were available. In Group A, young patients had a superior OS from diagnosis [median 100 months (CI 81-NR) *vs* 38 months (CI 25-49) in elderly; p<0.0001)] and from first relapse [median OS 59 months (CI 38-75) *vs* 22 months (CI 15-33) in elderly; p<0.0001] (Figure 1A). In Group B, OS from diagnosis was better in the young [median NR (CI 76-NR) *vs* 54 months (CI 37-71); p<0.0001]. However, OS from first relapse in the young patient cohort in Group B was not different from that of the reference cohort of elderly patients [median 43 months (CI 26-NR) *vs* 37 months (CI 20-54), respectively; p=0.25] (Figure 1B). Similar observations were made for disease specific survival in the 2 groups (data not shown). Therapy-related information stratified by the year groups is provided in Table 1. While the use of novel agents at relapse increased in the elderly (p<0.0001) as well as the young patients (p=0.03) in the more recent period (2006-2011), a greater proportional increase is evident in the older patient cohort.

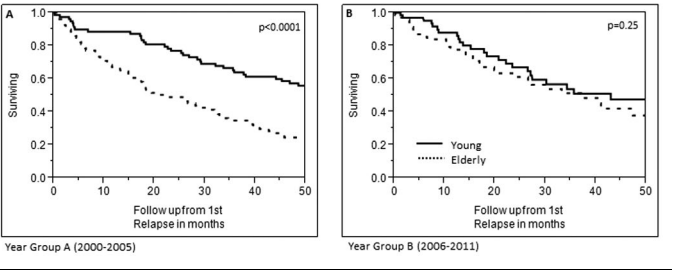

Figure 1. Survival outcomes in young *vs* elderly from first relapse.

Table 1. Therapies utilized at first relapse.

Therapy	Young		Elderly	
	Group A (%)	Group B (%)	Group A (%)	Group B (%)
I) Novel Agent-based	74	88	49	83
Immunomodulatory drug (IMiD)	57	32	39	39
Proteasome inhibitor (PI)	16	35	5	35
IMiD+PI combination	1	21	5	9
II) Conventional agents-based	26	12	51	17

Summary and Conclusions: Although overall survival from diagnosis is superior for young patients with MM compared to the elderly, outcomes from first relapse for the 2 age groups are similar in recent period (2006-2011). While this observation may be attributed to improved survival in the elderly at relapse,

likely related to increased use of novel agents at relapse, our results also underscore that disease course for young patients is not different than that of the elderly after first relapse, indicating that our current approaches for the young patients with MM require substantial improvement.

P661

SKY92 GEP, IFISH AND ISS COMPARISONS FOR RISK STRATIFICATION IN MULTIPLE MYELOMA

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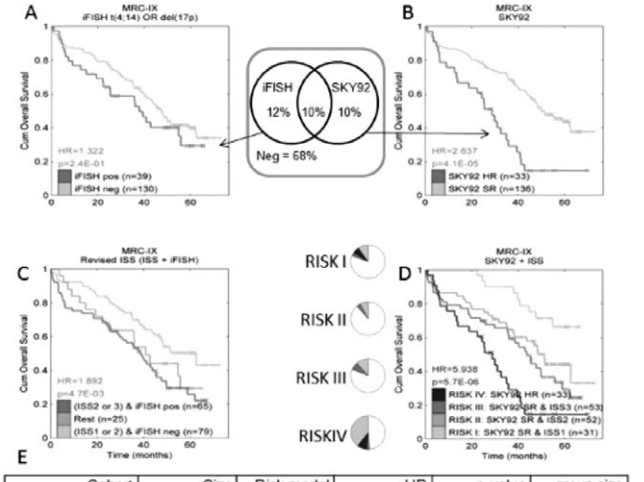
Background: Prognostic biomarkers are valuable for risk assessment in clinical settings in Multiple Myeloma which is a heterogeneous disease with variable outcome. Markers that have extensively and consistently been related to prognosis are t(4;14), del(17), ISS, and GEP signatures. Ideally a prognostic marker is robust across datasets, identifies a relevant fraction of patients and captures as much of the risk as possible. We evaluated four risk models for their clinical performance in two large datasets, HOVON65/GMMG-HD4 and MRC-IX. We compare HR, p-value, proportion of high risk cases and concordance between high risk models. Importantly, we show how individual patients may or may not be high risk depending on the model used.
Aims: To provide insight in the prognostic value of four different high risk models A) iFISH t(4;14) and/or del(17); B) SKY92 [1]; C) iFISH+ISS [2] and D) SKY92+ISS [3] in 2 different datasets.
Methods: 230 cases from HOVON-65/GMMG-HD4 (GSE19784) and 169 from MRC-IX (GSE15695) having GEP, iFISH, ISS data were analyzed. Kaplan Meier analyses were used to calculate Cox proportional hazard ratios and p-values. Venn diagrams visualize the overlap between the high risk models. Note that HOVON65/GMMG-HD4 data is the trainingset for EMC92/SKY92 [3]
Results: iFISH high risk (A) and SKY92 high risk (B) were compared for hazard ratio (p-value), proportion and overlap in two clinical cohorts. All hazard ratios except for iFISH (A) in MRC-IX were significant with p<0.05. The largest HR was observed for SKY92+ISS (D) for the highest risk category in comparison to the lowest risk category of SKY92, namely HR=13,6 in HOVON-65/GMMG-HD4 (Figure 1D) and HR=5,9 in MRC-IX (Figure 1). The two cohorts HOVON65 and MRC-IX have similar overlap of patients positive for iFISH, SKY92 or both (14/12/12% and 12/10/10% respectively) suggesting the robustness of these categories.



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Cohort	Size	Risk model	HR	p-value	group size
HOVON-65/GMMG-HD4	N=230	A	2.505	**4.6E-05**	27%
		B	4.674	**6.4E-12**	25%
		C	2.989	**6.6E-05**	27%
		D	13.557	**2.4E-10**	25%
MRC-IX	N=169	A	1.322	2.4E-01	23%
		B	2.637	**4.1E-05**	20%
		C	1.892	**4.7E-03**	38%
		D	5.938	**5.7E-06**	20%

Figure 1. Shows four prognostic models applied to the (n=169) MRC-IX dataset. OS Kaplan Meier analyses were performed using risk model A, B, C or D (see aim). The overlap between high risk cases in model A and B is given in the Venn diagram. The pie charts visualize - for model D - the enrichment of iFISH t(4;14) (light grey) and del(17) (dark grey), or both (black) in each of the four strata. E) table with hazard ratios between for the extreme strata in each model.

Summary and Conclusions: SKY92 (model B) is a better prognostic marker than iFISH (model A) or FISH+ISS (model C) with twofold higher HR. Besides, addition of ISS to SKY92 (model D) is relatively easy to perform and is powerful for the identification of a group of patients with favorable prognosis as judged by the median OS which is not reached at 60 months in both cohorts.

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P662

MARKERS OF DISEASE EVOLUTION IN SMOLDERING MULTIPLE MYELOMA (SMM)

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Background: SMM is an asymptomatic plasma cell dyscrasia that fulfills the diagnostic criteria of MM, without end organ damage symptoms. Ten percent of the patients are at risk of progression to symptomatic MM of which fewer will evolve within the first 2 years. For these latest patients, it is wondered whether they should be treated if they could be accurately recognized. Biomarkers proposed for that purpose are increased serum free light chains (FLC) or ratio (FLCR), plasma cell BM infiltration above 60% (that already categorizes patients as symptomatic MM), at least one bone lytic lesion by sensitive imaging techniques, etc.
Aims: To investigate prognostic markers of disease evolution in SMM, including FLC/FLCR levels, BM infiltration, skeletal findings, serum syndecan levels and other classical MM biomarkers.
Methods: We studied 147 SMM patients, diagnosed and followed -up in our department. Median age was 69 years (31-86) while 42% were males. Seventy-two percent, 21% and 7% were Durie-Salmon staged 1, 2 and 3 respectively, while 63%, 18% and 19% were ISS staged I, II and III respectively. MM type was IgG in 72%, IgA in 24% and light-chain in 3% of the population, while 2 patients (1%) were biclonal. Soluble syndecan-1 was measured by ELISA in 77 frozen samples collected at diagnosis. Serum FLCs were estimated by nephelometry (FREELITE, The Binding Site Ltd., Birmingham, UK). Serum FLC ratio (FLCR) κ/λ or λ/κ was calculated with the uninvolved LC as denominator. Statistical analysis was performed with SPSS v21.0. Survival was calculated with Log-rank test and depicted with Kaplan-Meier method.
Results: During a median follow-up time of 64 months, 50 patients (36%) evolved to MM. Median time to evolution was 15,5 months Factors highly statistically related to the probability of evolution were LC type MM (p<0,001), IgM <25mg/dl (p<0,001), the presence of at least 1 osteolytic lesion but also of diffuse osteoporosis (p=0,004), BM infiltration >60% and also BM infiltration 30-59% as compared to 10-29% (all p<0,001), FLCR>100 (7 pts, p<0,001), FLCR>8 (48 pts, p<0,001), involved FLC >100 mg/L (46 pts, p<0,001), IgA or IgG >30g/L (29 pts p<0,002) and soluble syndecan-1>120pg/ml (20/77 pts, p=0,001). However none of these factors alone could produce an 80% probability of evolution within two years. This was only possible by combining factors in models, thus patients with at least two factors of FLCR>100, soluble syndecan-1>120pg/ml and IgM <25mg/dl (N-10), had a 90% probability of disease evolution within two years.
Summary and Conclusions: Serum FLC/FLCR, skeletal status, immunoparesis, BM infiltration, soluble syndecan-1 serum levels are all important prognostic factors of disease evolution in SMM patients. The best models to recognize high-risk SMM should still be found and carefully validated in retrospective series because both the administration of an unneeded treatment and the delayed therapy could be harmful.

P663

EFFICIENCY AND TOXICITY OF CYCLOPHOSPHAMIDE BASED STEM CELL MOBILIZATION IN RELAPSED MULTIPLE MYELOMA PATIENTS: OBSERVATIONS FROM THE ONGOING PHASE III TRIAL RELAPSE

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Background: High dose chemotherapy (HD) with subsequent autologous stem cell transplantation (ASCT) is the standard treatment for newly diagnosed multiple myeloma (NDMM) patients and has recently been shown to be effective in the relapsed multiple myeloma (RMM) setting in a prospective phase III trial.[1] Stem cell mobilization in NDMM patients is a safe procedure associated with a low rate of failure to collect sufficient numbers of stem cells after stimulation with chemotherapy and G-CSF. However, the number of preceding chemotherapy cycles and exposure to melphalan have been identified as factors negatively correlated with mobilization success.[2]
Aims: We report data on the efficiency and toxicity of cyclophosphamide based stem cell mobilization in RMM patients from the ongoing phase III trial ReLApsE.
Methods: ReLApsE (EudraCT 2009-013856-61, ISRCTN 16345835) is a randomized, open, multicenter phase III trial of continued lenalidomide/dexamethasone *vs* lenalidomide/dexamethasone induction followed by HD, ASCT and lenalidomide maintenance in a planned study population of 282 MM patients up to 75 years of age in their 1st to 3rd relapse. Patients without available stem cell transplants from earlier apheresis undergo stem cell mobilization and apheresis in both trial arms after three cycles of lenalidomide/dexamethasone to allow for HD and ASCT either as part of the ReLApsE trial or as a subsequent treatment. Mobilization consists of high dose cyclophosphamide (HD-CY; 2*2 $g*m^{-2}$) followed by G-CSF (filgrastim 10 $\mu g*kg^{-1}*d^{-1}$ or lenograstim 300 $\mu g*m^{-2}*d^{-1}$) from day 5 until the last day of apheresis. In patients who fail to harvest sufficient numbers of stem cells, the administration of plerixafor is recommended.
Results: Until February 2015, 186 patients were randomized. Thirty patients underwent stem cell mobilization of which 29 received HD-CY plus G-CSF. Two patients died from HD-CY associated complications (sepsis) and one patient was withheld from stem cell apheresis due to a coinciding myocardial infarction. Of the 26 patients who underwent stem cell apheresis after HD-CY plus G-CSF, 9 patients (34.6%) succeeded and 17 patients (65,4%) failed to collect sufficient numbers of stem cells (≥$2*10^6$ $CD34^+$ cells*kg bw^{-1}). Additional application of plerixafor was initiated in 16 patients and enabled successful apheresis in 13 patients (81.3%).
Summary and Conclusions: Stem cell mobilization with HD-CY plus G-CSF in RMM allows for sufficient stem cell collection in only 35% of patients undergoing apheresis and is associated with marked toxicity. However, with addition of plerixafor as a rescue strategy following HD-CY plus G-CSF, successful stem cell collection is feasible in 85% of RMM patients undergoing apheresis. Our data underline the importance of collection of additional stem cell transplants during first-line treatment, as at this time-point stem cell mobilization is far more efficient and less toxic. If mobilization is performed in the relapsed setting, HD-CY administration and subsequent surveillance should be performed in an inpatient setting to enable early detection of infections and treatment of any severe adverse events.

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P664

HEAVY LIGHT CHAIN RATIO NORMALIZATION ALLOWS IDENTIFICATION OF ELECTROPHORETIC NON-COMPLETE RESPONSE PATIENTS WITH IMPROVED OUTCOMES: A LONG TERM FOLLOW UP UPDATE FOR BMT CTN 0102 CORRELATIVE STUDY

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Background: The prognostic value of pre-transplant heavy light chain (HLC) and free light chain (FLC) response over conventional electrophoretic response in the post-transplant setting is not well understood.
Aims: We correlated FLC and HLC assays with conventional electrophoretic response criteria to study their prognostic impact over 6 years of follow-up among 497 patients enrolled in the BMT CTN0102 trial of tandem autologous (autoHCT) *versus* tandem autoHCT-allogeneic (alloHCT) transplantation.
Methods: Pre-transplant serum samples prior to first autoHCT were analyzed centrally for FLC (Freelite™) and HLC (Hevylite™). Corresponding disease status (IMWG uniform response criteria) was determined by independent data review committee. An HLC-CR was defined as normalization of ratios across all 3 measured HLC pairs or the normalization of clonal isotype with normal ratios of uninvolved pairs. The demographics of the 497 patients with HLC and FLC pre-transplant samples were concordant with the main study (Krishnan *et al.* Lancet Oncol. Dec 2011; 12(13): 1195).
Results: 56 patients in conventional CR were also in HLC-CR. Comparing HLC-CR *vs* conventional CR, sensitivity was 100%, specificity 39%, PPV and NPV values 17% and 100% respectively. Of 211 pts in a conventional response better than or equal to very good partial response (>VGPR), 188 were in HLC-CR (Sensitivity=89%). Comparing HLC-CR *vs* conventional >VGPR: specificity was 52%, PPV 58% and NPV 87%. FLC ratio normalization (FLC-CR) *vs* conventional >VGPR disease state had a sensitivity of 47%, specificity 81% and PPV and NPV of 64% and 67% respectively. Adjusted multivariate models including baseline conventional response, myeloma stage and study arm were used to compare the prognostic utility of HLC-CR and FLC-CR. HLC-CR was an independent predictor of superior PFS (p-0.016), freedom from relapse (p-0.035) and survival (p-0.012) (Figure 1) while FLC-CR was not. A pre-transplant HLC-CR but not FLC-CR was associated with improved outcomes for patients not in electrophoretic CR/VGPR.

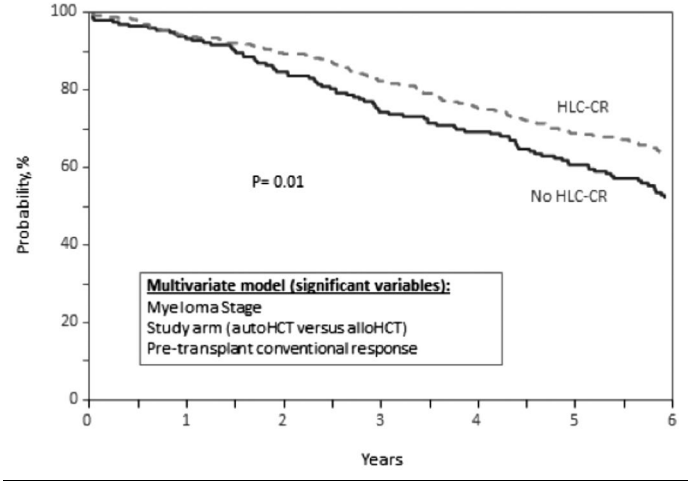

Figure 1. Adjusted survival by HLC remission status.

Summary and Conclusions: We conclude that 1) HLC normalization, but not FLC normalization, is additionally informative about patients not in electrophoretic CR/VGPR and 2) achievement of pre-transplant HLC-CR is a powerful predictor of improved post-transplant outcomes regardless of pre-transplant conventional response.

Myeloproliferative neoplasms - Clinical 2

P665

CLINICAL IMPACT OF BONE MARROW MORPHOLOGY FOR THE DIAGNOSIS OF ESSENTIAL THROMBOCYTHEMIA: COMPARISON BETWEEN THE BRITISH STANDARDS (BCSH) AND THE WHO CRITERIA

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Background: Accurate diagnosis of essential thrombocythemia (ET) persists to be a controversial and challenging issue and is usually performed by applying either the 2014 British Committee for Standards in Haematology (BCSH) guidelines or the 2008 World Health Organization (WHO) criteria. The critical point is that according to the WHO classification bone marrow morphology (BM) is recognized as a major feature, while the BCSH guidelines are more focused on an exclusion of the other subtypes of myeloproliferative neoplasia (MPN) and thus do allow ET diagnosis without BM examination. However, prefibrotic/early stages of primary myelofibrosis (prePMF) may also present with thrombocytosis, while failing to meet the diagnostic signs and symptoms characterizing overt PMF. Since clinical outcome in prePMF patients is considerably worse than in ET patients accurate diagnosis is pivotal to provide adequate management in these cases. Moreover, a small fraction of polycythemia vera (PV) patients may present with low hemoglobin and hematocrit levels, but a platelet count in keeping with BCSH- and WHO-defined ET, thus mimicking phenotypically ET at onset.
Aims: The aim of this study was to investigate the prognostic relevance of BM morphology for ET diagnosis as underscored by the WHO classification *versus* the first set of the BCSH criteria that do not include BM evaluation.
Methods: A clinic-pathological database including 350 MPN patients was created by clinicians and hematopathologists from a major Austrian center. Treatment-naive BM biopsies were centrally re-reviewed at a multiheaded microscope by two of the authors, who were completely blinded to initial data (except for age and gender). Follow-up data including clinical course and mutation status were analyzed after the completion of the histopathology review. For purpose of this study we selected two cohorts of ET patients presenting with a sustained platelet count >450x10^9/L: (1) 164 ET patients in accordance with the diagnostic requirements of the BCSH (A1-A3) that included no BM biopsy examination. (2) 122 patients in strict agreement with the diagnostic guidelines of the WHO including BM biopsy evaluation as major criterion. Both cohorts were compared and analyzed regarding their presenting clinico-pathological data, mutation status and follow-up. Diagnosis of post-ET myelofibrosis or progression into overt myelofibrosis with myeloid metaplasia was made according to well-known criteria.

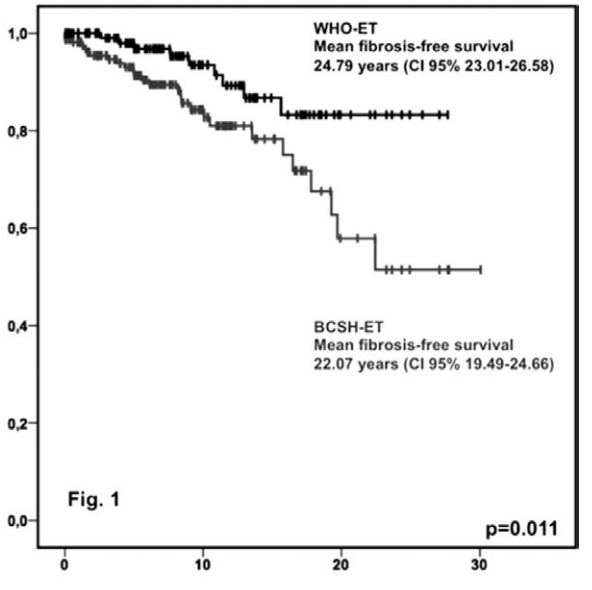

Figure 1.

Results: Following the BCSH criteria, 164 patients were diagnosed as ET. When reclassifying those cases according to the WHO criteria including BM morphology examination, 56 (34.1%) patients were diagnosed as prePMF and 17 (10.4%) as PV. Comparison of clinical features of BCSH-ET and WHO-ET revealed significant differences in white blood cell counts, LDH levels, fibrosis grades in the BM biopsy and in progression to overt myelofibrosis. Fibrosis-free survival was significantly shorter in BCSH-ETs (Figure 1). When reclassifying the BCSH cohort according to WHO criteria, overall survival and progression free survival were significantly shorter in WHO-confirmed prePMF.
Summary and Conclusions: Our results highlight the central role of BM biopsy in the accurate diagnosis and differentiation of ET and prePMF. The differences in clinical features and outcome underscore the importance of an accurate initial diagnosis.

P666

MYELOPROLIFERATIVE NEOPLASMS AND INFECTIONS; A POPULATION-BASED STUDY ON 9,665 PATIENTS WITH MYELOPROLIFERATIVE NEOPLASMS DIAGNOSED IN SWEDEN 1987-2009

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Background: Patients with myeloproliferative neoplasms (MPNs) are at an elevated risk of thrombosis and their life expectancy is shorter compared to the normal population. Survival has improved during recent years due more effective disease management and new targeted treatments. There is however limited information on morbidity and mortality due to infections in patients with MPNs.
Aims: To assess the risk of infections in MPN patients and matched controls in a population-based setting in Sweden.
Methods: All patients with MPNs reported to the Swedish Cancer Register and/or registered in the national Inpatient Register from 1987 to 2009 were included in the study. For each MPN patient, four controls matched for age, sex, and county of residence, were randomly selected from the Swedish Total Population Register. End of follow-up was December 31st 2010. Information on type and date of infection was obtained from the Swedish Patient Registry which captures information on all individual patient-based discharge diagnoses from inpatient (since 1987) and outpatient care (since 2001) as well as from the Cause of Death Register where all dates and causes of death are recorded. The risk of infection was analyzed using Cox regression where death was considered a competing risk. Separate analyses were performed for the overall risk of infection as well as subsets of bacterial and viral infections. Results are presented as hazard ratios (HRs) and 95% confidence intervals (CIs). In addition, the cumulative incidence of infection and death from any cause in MPN patients diagnosed during different calendar periods are illustrated by cumulative incidence curves.
Results: In total, 9,655 MPN patients and 38,660 matched controls were identified. There was a statistically significant two-fold risk elevation for infections (HR=1.9; 95% CI 1.8-2.0, p<0.0001) in MPN patients compared to controls. The risks of bacterial and viral infections were significantly increased (HR=1.8; 1.6-1.9, p<0.0001 and HR=3.0; 2.7-3.3, p<0.0001, respectively). The HRs of sepsis was 3.5 (3.2-4.0), pneumonia 2.2, (2.0-2.3), urinary tract infection 1.5 (1.2-1.6) compared to matched controls, all statistically significant. In addition, there was an increased risk of viral hepatitis with a HR of 3.8 (2.8-5.2, p<0.0001) in relation to controls. A significantly elevated risk of infections was observed in patients of all MPN subtypes compared to matched controls.
The risk elevation remained stable over time when MPN patients diagnosed during different calendar periods were analyzed separately (Figure 1). The overall risk of infection were HR 2.1 (1.9-2.4), 2.1 (1.9-2.3), 1.9 (1.8-2.1), and 2.2 (1.9-2.5) in MPN patients diagnosed during the years 1987-1992, 1993-1998, 1999-2004, and 2005-2009, respectively, compared to matched controls.

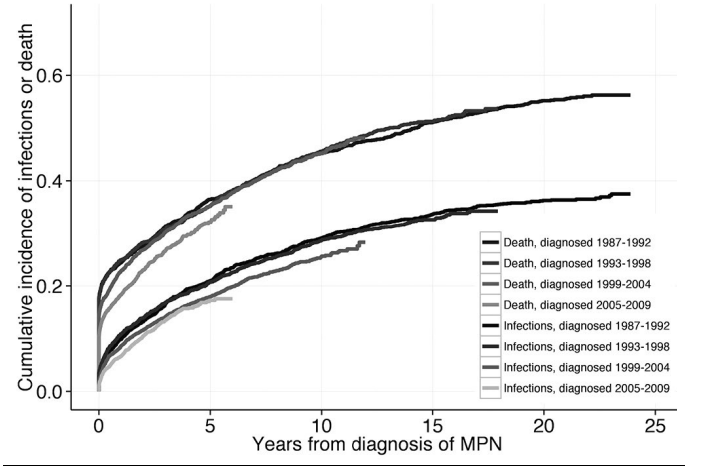

Figure 1.

Summary and Conclusions: MPN patients are at an increased risk of bacterial and viral infections compared to matched controls. The findings are particularly relevant since the JAK2 inhibitors have been shown to further increase the risk of opportunistic infections and reactivation of viral infections. Taken together, our results indicate that the risk of infection and possible immunosuppressive effects need to be considered when choosing optimal treatment in MPN. In addition, further studies are needed to identify subsets of patients that may benefit from infectious prophylaxis.

P667

UNFAVORABLE KARYOTYPE AND MOLECULAR NEGATIVITY SIGNIFICANTLY INFLUENCE THE INFECTIOUS RISK IN MYELOFIBROSIS: EVALUATION ON 426 PATIENTS

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Background: Infectious complications represent one of the main causes of morbidity and mortality in patients (pts) with Primary Myelofibrosis (PMF), Post-Essential Thrombocythemia and post-Polycythemia Vera MF (PET/PPV-MF). An increased infectious risk has been reported in pts treated with ruxolitinib (RUX), a JAK1/2 inhibitor with potent immune-suppressive properties. However, very few data are available on outcome and risk factors of this potentially fatal complication.
Aims: To evaluate risk factors for severe infections in a large cohort of MF patients.
Methods: Clinical and laboratory data of pts with MF were retrospectively collected from the database of 3 Italian Hematology Centers in University Hospitals. Severe infections were defined as grade 3/4 according to the CTCAE. The study was approved by the Ethic Committee of each participating Centers.
Results: Between 1980 and Aug 2014, 426 pts with PMF (319 pts, 75%), or PET-MF (13%) or PPV-MF were diagnosed and followed for a median follow-up of 4 yr (0.5-30.1). Baseline characteristics were (median): age, 67 y (range, 26-87); ≥65 y, 55%; male, 57%; hemoglobin (Hb), 12 g/dL (4-17.9); Hb <10 g/dL, 24%; PLT, 372×10^9/L (15-2513); PLT <100×10^9/L, 8.0%; spleen enlargement, 69% (spleen length ≥5cm: 32.4%); constitutional symptoms, 15%. International Prognostic Score System (IPSS) was low (11%), intermediate-1 (intm-1, 21%), intermediate-2 (intm-2, 43%), high (24%). Molecular analysis was performed on 253 pts (59%) and was positive in 84% (*JAK2*V617F), 14% (*CALR*), 3% (*MPL*W515K/L); 6 pts (2%) were triple negative. Karyotype was abnormal in 34 (16.6%) out of 204 evaluable pts (unfavorable in 10 pts; 5%). Overall, 81 pts (19%) experienced 112 grade 3-4 infectious events, for an incidence rate of 3.3% pt-y. The cumulative incidence was 16%, 26.8% and 43.6% at 5, 10 and 20 y, respectively (Figure 1). Infections were: bacterial (101 events, 90.2%; pneumonia: 54 cases, 53.4%); VZV reactivations (7 events, 6.2%), nodal TBC (2 events, 1.8%), fungal lung infections (2 events, 1.8%). Infectious complications represented the causes of death in 9 (8%) out of 126 deceased pt. Among baseline features, age≥65 y (p=0.004), leukocyte ≥25x10^9/l (p=0.02), spleen length>5 cm (p=0.03), high/intm-2 IPSS (p=0.01), unfavorable karyotype (p<0.001) and JAK2/CALR/MPL negativity (p=0.002) significantly correlated with higher infectious risk; in multivariate analysis, unfavorable karyotype and molecular negativity confirmed their negative impact (p=0.008 and p=0.03, respectively). Overall, 81 pts at intm-2/high IPSS risk were treated with RUX for a median time of 16 mos (1-45). Infection-free survival at 5 y was significantly worse in RUX-treated pts (66%) compared to non RUX-treated intm-2/high risk pts (86%, p=0.01). Among RUX-treated pts, only age ≥65 y correlated with an increased risk of infectious complications (p=0.015).

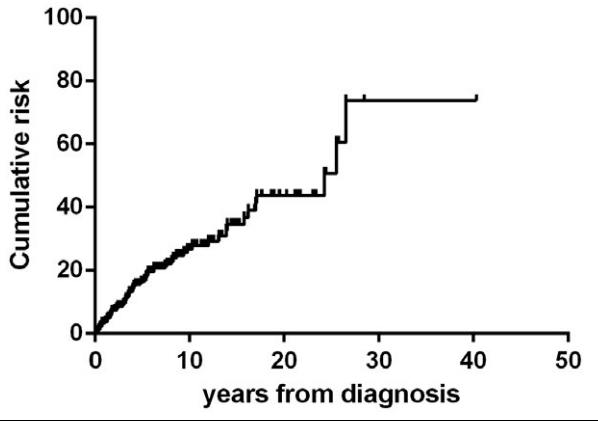

Figure 1.

Summary and Conclusions: This large study confirms severe infections as frequent events involving 19% of MF pts and representing the ultimate cause of death in 8% of the cases. Molecular/cytogenetic data identify a subset of patients at higher infectious risk. In RUX-treated pts, a higher incidence of infections was observed; in this particular subpopulation, only older age represented a significant risk factor for severe infectious complications.

P668

PRIMARY MYELOFIBROSIS, POST-ET AND POST-PV MYELOFIBROSIS HAVE DISTINCT CLINICAL PROFILES AND SYMPTOMATIC BURDENS: AN ANALYSIS BY THE MPN QUALITY OF LIFE INTERNATIONAL STUDY GROUP (MPN-QOL ISG)

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Background: Myelofibrosis (MF) is a myeloproliferative neoplasm (MPN) that may arise as primary (PMF) or result from progression of polycythemia vera (PPV-MF) or essential thrombocythemia (PET-MF). Independent of subtype, MF patients share a debilitating symptom profile and high mortality rate. Historically, all MF patients have been treated similarly without impact of subtype. Recent data suggests perhaps a different natural history amongst subtypes of MF (Passamonti *et al.*, Blood 2014).
Aims: We sought to evaluate impact of MF subtype on MPN total symptom burden and symptom profiles.
Methods: Data was collected among an international cohort of patients with MF including PMF, ETMF and PVMF as reported previously (Emanuel *et al.*, JCO 2013). Subjects completed the Brief Fatigue Inventory (BFI) and Myeloproliferative Neoplasm Symptom Assessment Form (MPN-SAF) instruments. Items were scored on a 0 (absent) to 10 (worst imaginable) scale. MPN-SAF TSS items included fatigue, concentration, early satiety, inactivity, night sweats, itching, bone pain, abdominal discomfort, weight-loss, and fever. For individuals completing at least 6 of the 10 MPN-SAF TSS items, the survey was scored by multiplying the average score across items by 10 to achieve a 0 to 100 scaled score. Demographics, clinical variables and symptom scores were compared between myelofibrosis groups using ANOVA F-tests or chi-squared tests.
Results: *Patient Population*. A total of 835 MF subjects (PMF=488; PET-MF=192; PPV-MF=155) were prospectively enrolled and administered the MPN-SAF and BFI. Most patients were of expected age for MPNs (mean 60.9 years) with a mean disease duration of 8.7 years. Splenomegaly (68.9%) and laboratory abnormalities (62.4%) including anemia (51.4%) and thrombocytopenia (31.3%) were common. Prior hemorrhage (8.7%), phlebotomy use (12.9%) and prior thrombosis (13.2%) were infrequent. *Clinical Features by MF Subtype*. MF subgroups differed by mean age (p<.001) and MPN duration (p<0.001). PMF patients had significantly higher rates of anemia (p=0.005), thrombocytopenia (p=0.006) and laboratory abnormalities (p=0.03) with the lowest mean hemoglobin (p=0.0053) and platelet counts (p<0.001). PMF patients also had the lowest rates of prior thrombosis (p<0.001) and prior hemorrhage (p<0.001). As expected, PPV-MF patients demonstrated the highest rate of current/previous phlebotomies (p<0.001) along with the highest rates of enlarged spleens (p=0.007). *MF Symptom Burden by Disease Subtype*. Mean Total Symptom Scores were similar between PMF (27.2), PET-MF (26.4) and PPV-MF (26.4; p=0.83; Figure 1), however individual symptom patterns differed

by MF subtype. PMF patients had the most frequent and symptomatic fevers (21%, p=0.03; TSS 0.6, p=0.049) and weight loss (45%, p=0.04; TSS 2.1, p=0.007). Inactivity was least common (69%, p=0.01) in PET-MF whereas pruritus was most common amongst PPV-MF patients (60%, p=0.02). No significant differences were noted for items of worst fatigue, early satiety, abdominal discomfort, inactivity, concentration, itching or bone pain (all p>.05) between MF subtypes.

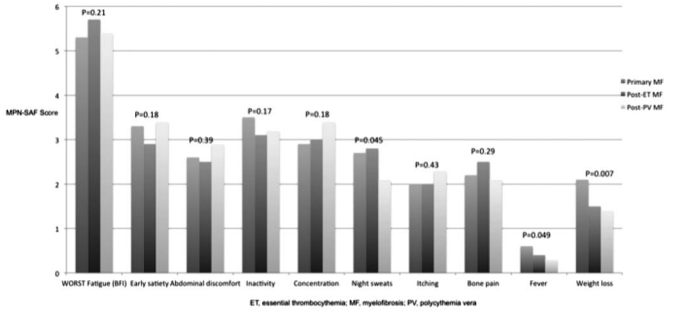

Figure 1. MPN-SAF TSS for individual items by myelofibrosis subtype.

Summary and Conclusions: The results of this study confirm recent reports that that PMF, PET-MF and PPV-MF exhibit unique clinical and symptomatic profiles. PMF patients demonstrate more significant constitutional symptoms. No difference in scores for these items was noted between PET-MF and PPV-MF, contrary to previous findings. Further research is needed to determine if this finding is of clinical relevance in the determination of therapy.

P669

IMPACT OF CALR AND ASXL1 MUTATIONS ON SURVIVAL AND DISEASE COMPLICATIONS IN ESSENTIAL THROMBOCYTHEMIA AND PREFIBROTIC PRIMARY MYELOFIBROSIS STRICTLY DIAGNOSED ACCORDING TO THE WHO-CLASSIFICATION

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Background: In 2013, the landscape of myeloproliferative neoplasms (MPN) was altered dramatically by the identification of somatic mutations in the calreticulin (CALR) gene. With this discovery molecular information, in addition to the JAK2V617F mutation, can be implemented as an affirmative variable for discrimination of MPNs from reactive myeloid proliferations for the first time. Previously, the clinical course of essential thrombocythemia (ET) or primary myelofibrosis (PMF) in patients carrying the CALR mutation has been reported to be more indolent than in JAK2 positive patients and was associated with increased survival.
Aims: Our aim was to investigate the different impacts of CALR mutations on the prognosis and clinical outcome in prefibrotic PMF (prePMF) and WHO-classified ET (WHO-ET). Additionally, the same investigations were made concerning ASXL1 (exon12) mutations, with ASXL1 being one of the most common bystander mutated genes in MPNs.
Methods: In a cohort of 348 patients with the clinical diagnosis of either WHO-ET or PMF, mutational analysis for CALR was available. For study entry, the following eligibility criteria had to be fulfilled: a) availability of mutation analysis for JAK2, MPL and CALR; b) availability of representative, treatment-naive bone marrow biopsy; c) availability of a histological and clinical consensus on the diagnosis; d) complete long-term documentation of clinical data and outcome.
Results: Consenting clinico-pathological findings were consistent with 115 cases of WHO-ET and 85 patients with prePMF. In contrast to the most recent publications, the present study revealed a different CALR mutation frequency in ET (Table 1). This discrepancy in the frequencies of CALR positivity in our ET cohort may be due to our strict adherence to the WHO criteria for diagnosis of ET. Regarding prePMF, we observed CALR mutations in 39% of the patients. 92% of the JAK2/MPL wt subgroup carried the CALR mutation, with JAK2, MPL and CALR wt being observed in only 3% of prePMF. The most striking differences between WHO-ET and prePMF were seen in the comparison of the overall survival (Figure 1A, 1B). While the CALR mutation did not have any beneficial influence on survival in WHO-ET, it was associated with a superior overall survival in prePMF. Such a difference was not seen in the time to transformation into overt MF, and there was only a slightly shorter time to progression to fibrosis in CALR wt prePMFs. There was a trend showing that CALR mutated prePMF patients have shorter thrombosis-free survival compared to CALR wt prePMF patients. No impact of the CALR mutations on thrombosis-free survival in WHO-ET was observed. ASXL1 (exon12) mutations revealed no impact on overall survival (Figure 1C).

Table 1.

	Total cohort (N=200)	WHO-ET (N=115)	prePMF (N=85)	P
Age at diagnosis, years				0,04
Median (range)	58,85 (19-88)	56,4 (19-84)	60,7 (27-88)	
Sex				0,587
Male/female	78/122	43/72	35/50	
Observation period, years				0,066
Median (range)	8 (0-30)	8,5 (0-28)	7,4 (0-30)	
CALR positive				0,001
No. (%)	54 (27)	21 (18,3)	33 (38,8)	
ASXL1(ex12) positive	(N=190)	(N=108)	(N=82)	0,0868
No. (%)	17 (8,9)	13 (12)	4 (4,9)	

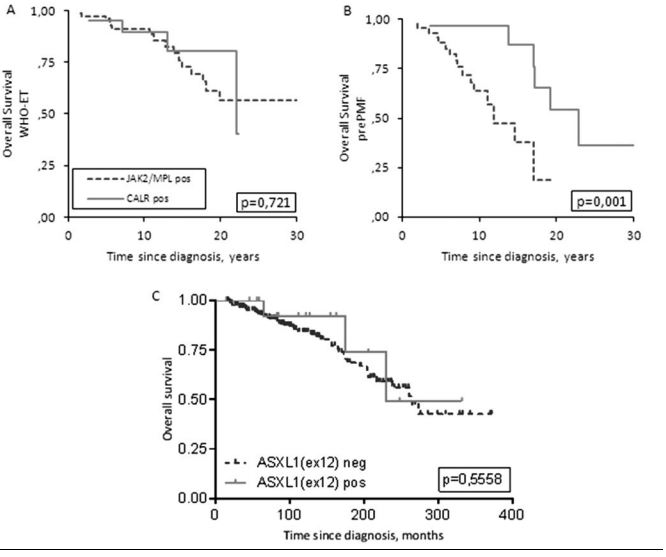

Figure 1. Kaplan-Meier analyses for A) CARL analyzed WHO-ET, B) CARL analyzed prePMF and C) ASXL1(ex12) analysed WHO-ET and prePMF.

Summary and Conclusions: The present data confirm that WHO-ET and prePMF are biologically different sub-entities of MPNs. In prePMF, almost all patients are now associated with known disease-causing mutations. Our data support the classical approach in the diagnosis of thrombocytosis, using BM histology to differentiate WHO-ET from prePMF and more accurately estimate the outcome of the disease.

P670

RUXOLITINIB PROVIDES CONSISTENT HEMATOCRIT CONTROL IN PATIENTS WITH POLYCYTHEMIA VERA (PV) RESISTANT TO OR INTOLERANT OF HYDROXYUREA

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Background: Ruxolitinib (RUX) is a JAK1/JAK2 inhibitor that has shown superiority over best available therapy (BAT) in patients (pts) with PV who had an inadequate response to or unacceptable side effects from hydroxyurea (HU). In RESPONSE, a higher proportion of RUX-treated pts achieved hematocrit (HCT) control without phlebotomy (PBT) than BAT-treated pts (60% *vs* 20%). Pts with inconsistent HCT control may be at a higher risk for thromboembolic events than those who have HCT aggressively targeted to ≤45%.

Aims: Because HCT values can fluctuate over time, and given the importance of a target HCT of ≤45% in PV, we performed an analysis to determine the percentage of time patients maintained HCT control on treatment (ie, HCT ≤45%) in each study arm.
Methods: RESPONSE is a randomized (1:1) open-label phase 3 study to evaluate the efficacy and safety of RUX *vs* BAT in pts with PV who are resistant to or intolerant of HU by modified ELN criteria. Pts with splenomegaly (≥450 cm^3 by MRI) who required PBT for HCT control received RUX 10 mg bid or single-agent BAT. The primary endpoint was the proportion of pts who achieved both HCT control without PBT from wk 8 to 32 (with ≤1 PBT from randomization to wk 8) and a ≥35% reduction from baseline in spleen volume at wk 32, as assessed by MRI. The primary analysis occurred when all pts reached wk 48 or discontinued. In this preplanned analysis at wk 80 data cutoff, the total percentage of time pts had HCT >45% during randomized treatment was estimated. A linear relationship between HCT and time was assumed for any consecutive 2 assessments with no PBT in between. If any PBT was done between 2 assessments and HCT was ≤45% at later assessment, HCT was assumed to be ≤45% immediately after the PBT, and the time patients had HCT >45% was not calculated between the 2 time points; however, if HCT after the PBT was >45%, the time between the 2 time points was assumed to be with HCT >45%.
Results: Pts were randomized to RUX (n=110) or BAT (n=112). Baseline characteristics were well balanced between arms (median HCT, 43.3% *vs* 44.0%; pts with HCT >45%, 25.5% *vs* 22.3%). At data cutoff, 82.7% of pts randomized to RUX remained on treatment and no pts remained on BAT (median exposure, 111 and 34 wk). In the RUX arm, mean HCT decreased continuously from wk 2 to 12 and then remained relatively stable thereafter. A larger proportion of pts in the RUX arm achieved HCT ≤45% than pts in the BAT arm (Figure 1). For pts who had uncontrolled HCT (ie, >45%) at baseline despite PBT, mean HCT in the RUX arm declined to <45% and was lower than that in the BAT arm over the course of treatment. The mean change from baseline in HCT ranged from −3.12% to −4.36% in the RUX arm (from wk 12 to 80) and from +0.06% and +1.03% in the BAT arm (from wk 12 to 32). Over the duration of treatment, pts in the BAT arm spent 12 times more in percentage of time with a HCT >45% than pts in the RUX arm (median, 39.1% [Q1-Q3, 12.9-65.9] *vs* 3.0% [Q1-Q3, 0.0-16.7]). Pts who achieved HCT control without PBT from wk 8 to 32 with RUX (n=66) had an 89% probability (95% CI, 0.78-0.95) of maintaining the response for 80 wk from the initial response. From wk 8 to 32, more BAT pts required PBT (≥1 PBT: 62% *vs* 20%; ≥3 PBT: 20% *vs* 3%); 90% of RUX pts did not have a PBT from wk 32 to 80.

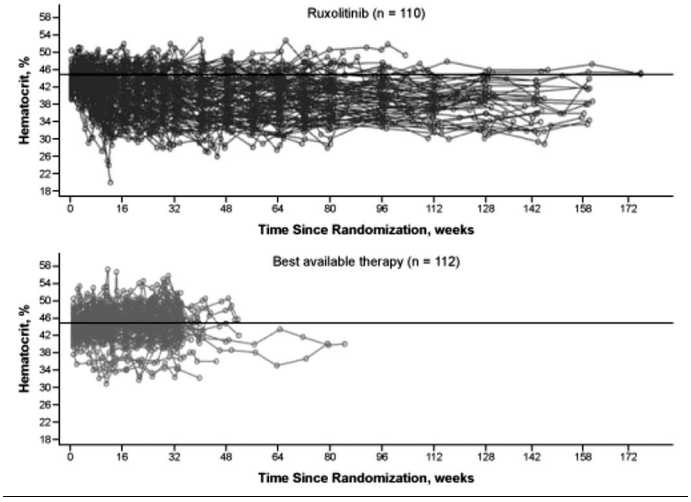

Figure 1. Plot of hematocrit over time for individual patients.

Summary and Conclusions: In RESPONSE, pts who received RUX experienced more consistent control of HCT to ≤45% than those who received BAT. Sustained HCT ≤45% is a key treatment goal for reducing the risk of thromboembolic events in PV, as supported by results of the CytoPV trial.

P671

PHASE 2 STUDY OF A NOVEL CONTROLLED-RELEASE FORMULATION OF ANAGRELIDE (GALE-401) IN SUBJECTS WITH MYELOPROLIFERATIVE NEOPLASM (MPN)-RELATED THROMBOCYTOSIS

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Background: Despite advances in the diagnosis and management of MPNs, treatment of essential thrombocythemia (ET) has remained largely unchanged since the introduction of anagrelide in the US (1997) and Europe (2004). Anagrelide reduces platelet production by inhibition of megakaryocyte hypermaturation, and also inhibits cyclic AMP phosphodiesterase III. Common drug related adverse events (AEs; *e.g.*, headache, palpitations, fluid retention, etc.) might be due to this latter mechanism. As the marketed anagrelide product is an immediate release (IR) formulation with peak plasma levels that may exceed that needed for platelet reduction, an alternate formulation that modifies this pharmacokinetic (PK) profile may improve patient tolerability and treatment outcomes. This led to the development of a controlled-release (CR) formulation of anagrelide (GALE-401). To-date, 98 healthy subjects were enrolled among 5 Phase 1 clinical trials of anagrelide CR at doses ranging from 0.2 to 0.6 mg once or twice daily for up to 42 days. Anagrelide CR induced significant reductions in platelet counts that were not distinguishable from IR, despite C_{max} and AUC_{0-T} values that were 29% and 55% of IR. These trials supported the initiation of a Phase 2 study in MPN-related thrombocytosis, preliminary results for which are reported here.
Aims: The primary objective of the study was to estimate the overall platelet response rate (ORR). Secondary objectives included safety, tolerability, and pharmacokinetics (PK).
Methods: This is an open-label, single-arm, Phase 2 trial. Eligible subjects include those with chronic myelogenous leukemia (CML), polycythemia vera (PV), primary myelofibrosis (PMF) or ET, platelet count ≥600 K/μL, and not previously refractory to anagrelide. Anagrelide CR was given at a starting dose of 0.5 mg twice daily and individually titrated to maintain a target platelet count of 150 - 400 K/μL. Platelet response is defined as complete response (CR, ≤400 K/μL) or partial response (PR, ≤600 K/μL or ≥50% reduction from baseline), and maintenance of the reduction for at least 4 weeks. Safety evaluations include routine physical and laboratory evaluations. The PK profile is assessed at the starting dose, weekly sampling during dose titration, and at the final titrated dose level.
Results: Eighteen (18) subjects who provided written informed consent were enrolled, median age 65 yrs (range 40-80), 10 females/8 males, 13 ET, 4 PV, and 1 PV/ET. Prior treatments for thrombocytosis included hydroxyurea (n=8), anagrelide (8), and none (7). Mean platelet count decreased rapidly to <600 K/μL at 4 weeks, at which time the mean dose was 1.8 mg/day. To-date, the confirmed overall response rate is 67% (12/18) with 4 CR and 8 PR. A total of 151 AEs were reported, of which 69 were study drug related including headache (8 subjects); palpitations (4); abdominal pain, AST increased, and nausea (3 each); and diarrhea, fatigue, hypotension and tachycardia (2 each). Most related AEs were Grade 1-2 in severity. Study drug related serious AEs were reported for 2 subjects and included Grade 3 headache and Grade 3-4 cholestatic jaundice/increased creatinine. Six subjects discontinued treatment for reasons that included study drug related AE (2), subject request (2), investigator decision (1) and other (1). Plasma PK analyses are ongoing.
Summary and Conclusions: Anagrelide CR induced rapid platelet count reductions at daily doses and ORR as anticipated with the IR formulation. AEs were mostly Grade 1-2 in severity and consistent with anagrelide's known side effects, however additional dosing experience in the current trial or comparative controlled studies may be needed to further elucidate the safety profile. Study treatment is ongoing and 6-month data are being collected for analysis and presentation.

P672

SAFETY OF RUXOLITINIB IN PATIENTS WITH POLYCYTHEMIA VERA: RESULTS FROM THE CLINICAL TRIAL PROGRAM

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Background: Polycythemia vera (PV) is a myeloproliferative neoplasm that is characterized primarily by erythrocytosis and an increased risk of thrombosis. To control blood counts and manage PV-related symptoms, some patients may require chronic therapy. The most common drug treatment is hydroxyurea (HU); however, some patients may develop unacceptable side effects or have an inadequate response to HU.
Aims: To evaluate the long-term safety of the JAK1/JAK2 inhibitor ruxolitinib (Rux) in the PV clinical trial program.
Methods: This analysis of Rux safety included data from 3 clinical trials: (1) a phase 2, single-arm, open-label study in patients who were resistant to or intolerant of HU (ClinicalTrials.gov identifier, NCT00726232); (2) RESPONSE, an ongoing, randomized, open-label phase 3 clinical trial (NCT01243944) com-

paring Rux with best available therapy (BAT) in patients who are resistant to or intolerant of HU; and (3) RELIEF, a randomized, blinded, phase 3b comparative trial (NCT01632904) of Rux *vs* HU in patients generally well controlled on HU but reporting symptoms. Data were pooled for Rux-treated patients from the 2 studies with HU-resistant/intolerant patients (phase 2 study and RESPONSE), including patients in RESPONSE who crossed over to Rux following BAT treatment.

Results: Overall, the HU-resistant/intolerant group (n=241) included 457 patient-years of exposure (median Rux exposure [range], 19.5 months [0.3-66.7 months]). The most common adverse events for patients treated with Rux who were HU-resistant/intolerant included diarrhea (8.7 per 100 patient-years of exposure), headache (8.5), and pruritus (7.4); most events were grade 1 or 2 (Table 1). Grade 3 or 4 thrombocytopenia and anemia each occurred in 3.7% of patients over the duration of follow-up. The rate of herpes zoster was 5.0 per 100 patient-years. Nonmelanoma skin cancer occurred at a rate of 2.8 per 100 patient-years (*vs* 2.7 per 100 patient-years in RESPONSE patients randomized to BAT during BAT treatment). There were 7 transformations to myelofibrosis during Rux treatment; 3 of these occurred after crossover from BAT. There were 2 transformations to acute myeloid leukemia (AML); 1 occurring after crossover. These rates of transformation to myelofibrosis and AML were consistent with those published for similar patient populations with PV (Fiannzi. *Blood* 2005;105:2664-70; Passamonti. *Am J Med* 2004;117:755-61; Alvarez-Larran. *Blood* 2012;119:1363-9). The thromboembolic event rate was 2.2 per 100 patient-years (*vs* 8.2 per 100 patient-years in RESPONSE patients randomized to BAT during BAT treatment). In the RELIEF trial, median exposure was 21.6 weeks in the Rux arm (n=54). Adverse events that occurred during the blinded phase were generally consistent with those observed in studies of patients who were HU-resistant/intolerant.

Table 1. Nonhematologic adverse events in ruxolitinib-treated patients who were resistant to or intolerant of HU.

	Patients With HU-Resistance/Intolerance* (n=241)	
	All Grades	Grade 3 or 4
Adverse Event,† n (rate per 100 patient-years of exposure)		
Diarrhea	40 (8.7)	0
Headache	39 (8.5)	2 (0.4)
Pruritus	34 (7.4)	1 (0.2)
Fatigue	32 (7.0)	2 (0.4)
Dizziness	31 (6.8)	0
Cough	30 (6.6)	0
Dyspnea	28 (6.1)	3 (0.7)
Weight increased	28 (6.1)	0
Constipation	26 (5.7)	1 (0.2)
Abdominal pain	25 (5.5)	2 (0.4)
Back pain	25 (5.5)	2 (0.4)
Pyrexia	25 (5.5)	2 (0.4)
Muscle spasms	25 (5.5)	1 (0.2)
Arthralgia	25 (5.5)	0
Herpes zoster	23 (5.0)	3 (0.7)
Nasopharyngitis	23 (5.0)	0

*Patients with HU-resistance/intolerance from (1) the phase 2 trial and (2) RESPONSE, including patients randomized to Rux and those who crossed over to Rux from BAT
†Nonhematologic adverse events with rates ≥5 per 100 patient-years

Summary and Conclusions: This long-term follow-up of Rux experience in the PV clinical trial program provides evidence that supports the safety and tolerability of Rux in patients who have an inadequate response to or are intolerant of HU.

Myeloproliferative neoplasms - Clinical 3

P673

EFFECT OF RUXOLITINIB ON MARKERS OF IRON DEFICIENCY: AN ANALYSIS OF THE RESPONSE TRIAL

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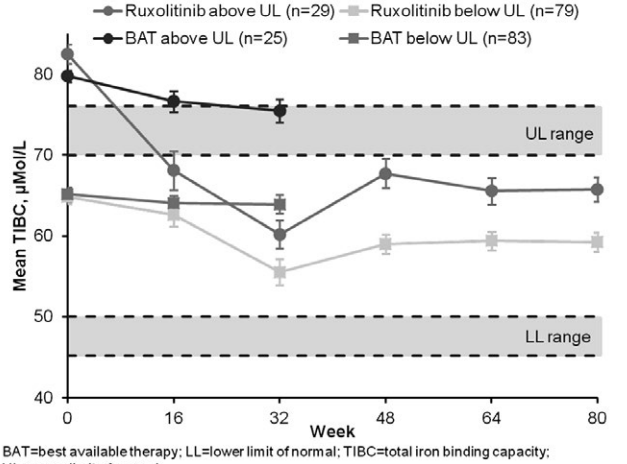
Background: Patients with polycythemia vera (PV) often rely on phlebotomy to control their hematocrit. Frequent phlebotomies may result in a state of iron deficiency, which can be associated with various complications, such as restless leg syndrome and cognitive dysfunction. The RESPONSE trial demonstrated that ruxolitinib lowers hematocrit and reduces the need for phlebotomy in patients with PV who have had an inadequate response to or are intolerant of hydroxyurea (HU).
Aims: A subanalysis of the RESPONSE trial data was conducted to evaluate the effect of ruxolitinib on markers of iron deficiency.
Methods: Total iron binding capacity (TIBC), ferritin, mean corpuscular volume (MCV), and serum iron were Total iron binding capacity (TIBC), ferritin, mean corpuscular volume (MCV), and serum iron were measured at baseline and over time in patients randomized to ruxolitinib and best available therapy (BAT); in the BAT group, these measures were assessed before and after crossover to ruxolitinib. Transferrin iron saturation (TS) was calculated based on observed iron and TIBC. Patients within each arm were stratified into 2 groups: patients with baseline values within the normal range and patients with baseline values outside of the normal range; this was determined for each patient based on the normal ranges for each of the study sites. Values outside of the normal ranges indicative of iron deficiency included TIBC above the upper limit of normal; and ferritin, MCV, serum iron, and TS below the lower limits of normal.

Table 1. Values for markers of Iron deficiency at baseline and week 32 in patients with evidence of iron deficiency at baseline.

Mean(SD)	Ruxolitinib (randomized)		BAT (randomized)	
	Baseline	Week 32	Baseline	Week 32
Ferritin, pMol/L	19.7 (8.0)	134.1 (99.2)	21.7 (7.2)	43.2 (64.0)
Serum Iron, µMoL/L	3.7 (1.2)	16.4 (7.5)	3.9 (1.1)	6.4 (5.4)
MCV, FL	70.3 (6.5)	81.1 (8.9)	70.6 (6.6)	73.1 (10.6)
TS,%	6.00 (2.9)	31.1 (14.9)	6.6 (3.4)	10.6 (9.6)
TIBC, Mol/L	82.5 (6.3)	60.2 (4.2)	79.8 (3.6)	75.5 (6.2)

Increases in ferritin, iron, MCV, and TS=improvement; decreases in TIBC=improvement.



BAT=best available therapy; LL=lower limit of normal; TIBC=total iron binding capacity; UL=upper limit of normal.
*Highest/lowest UL and LL based on cut offs across study sites. In patients with TIBC above the UL at baseline, mean TIBC improved (lowered) during ruxolitinib therapy to the normal range. Patient sample sizes in the legend represent the number of patients at baseline.

Figure 1. Mean (SE) total iron binding capacity over time for patients with baseline values above the upper limit and below the upper limit of normal*.

Results: The majority of patients (in both study arms) had laboratory values outside of normal limits at baseline: TIBC, n=54 (24.3%); ferritin n=141 (63.5%);

MCV, n=137 (61.7%); serum iron, n=181 (81.5%); MCV+iron, n=127 (57.2%); TS, n=197 (88.7%). Among patients with evidence of iron deficiency at baseline, use of iron supplementation was infrequent (<20% of patients) and balanced between treatment arms. Changes in TIBC over time are shown in Figure 1 and Table 1. In patients with elevated TIBC at baseline, mean TIBC improved (ie, lowered) to levels within the normal range during ruxolitinib treatment (in patients randomized to ruxolitinib and after crossover from BAT), but remained above the normal limit during BAT treatment. Consistent with these findings, ferritin, MCV, serum iron, and TS improved (ie, increased) in patients with low baseline levels during ruxolitinib therapy, but remained outside of the normal limits during BAT treatment (Table 1). Similar patterns were also observed when evaluating these markers by gender. Mean values for patients with normal iron status at baseline remained within normal ranges during ruxolitinib therapy.
Summary and Conclusions: In patients with PV who have had an inadequate response to or are intolerant of HU, normalization of 5 markers of iron deficiency was observed with ruxolitinib therapy, while in the BAT arm, there was little or no change in these parameters.

P674

RUXOLITINIB REDUCES JAK2P.V617F ALLELE BURDEN IN PATIENTS WITH MYELOFIBROSIS

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Background: The *JAK2* c.1849G>T (p.V617F) mutation leads to constitutive activation of JAK2 and contributes to dysregulated JAK signaling in myeloproliferative neoplasms. COMFORT-I, a phase 3 study of ruxolitinib *versus* placebo in patients with intermediate-2 or high-risk myelofibrosis (MF), demonstrated rapid and durable reductions in splenomegaly and symptom improvement with ruxolitinib *versus* placebo.
Aims: This analysis was a long-term (maximum of 4 years) follow-up of COMFORT-I to determine if ruxolitinib, an oral JAK1/JAK2 inhibitor, continued to reduce allele burden in patients with the *JAK2*p.V617F mutation.
Methods: *JAK2*p.V617F-positive patients (n=236) were identified from the COMFORT-I dataset. Presence of this mutation was not an eligibility requirement of the trial; but for patients having baseline data demonstrating a *JAK2*p.V617F mutation, allele burden was assessed by genomic DNA isolation from peripheral blood at baseline and weeks 24, 48, 120, 144, 168, and 216. Allele burden was defined as the percentage of mutant allele relative to the total (wild-type+mutant). Complete molecular remission (CMR), partial molecular remission (PMR), and relapse of molecular remission were defined based on the IWGd spleen response (≥ 35% reduction from baseline to week 24) were also assessed with respect to *JAK2*p.V617F mutation.
Results: On average, allele burden decreased in ruxolitinib-treated patients and increased during placebo treatment. The mean/median (range) maximal change in the ruxolitinib randomized arm was −27%/−16% (−100%, 36%), and in patients who crossed over from placebo to ruxolitinib was −19%/−11% (−100%, 12%). Of the 28 patients (12%) achieving a > 50% decrease in allele burden, 20 achieved a PMR and 6 achieved a CMR, with median times to response of 22.2 and 27.5 months, respectively. Allele burden reductions were independent of baseline allele burden; patients with high and low allele burdens both had significant reductions. Patients were grouped into tertiles of maximal percentage allele burden reduction from baseline, with mean/median changes within tertile 1: −62.6%/−63.4%; tertile 2: −15.3%/−14.8%; and tertile 3: −0.5%/−2.2%. Patients with the largest decreases (tertile 1) had the shortest mean/median times from diagnosis at baseline (tertile 1: 36.8/15.2 mo; tertile 2: 52.5/21.7 mo; tertile 3: 44.8/22.1 mo). Comparison of clinical responses across tertile groups indicated that patients with greater allele burden decreases had a larger percent reductions in spleen volume (mean/median changes at week 24 for tertile 1: −41.2%/−45.2%; tertile 2: −38.3%/−37.1%; tertile 3: −23.4%/−22.7%) as well as a greater proportion of patients with a ≥ 35% decrease in spleen volume at week 24 (tertile 1: 63.9%; tertile 2: 55.6%; tertile 3: 31.4%). Even patients with smaller decreases in *JAK2*p.V617F allele burden (31.4% of 35 patients in tertile 3), as well as patients who were *JAK2*p.V617F-negative at baseline (27.5%), had spleen responses.
Summary and Conclusions: Patients treated with ruxolitinib continued to show reductions in allele burden independent of baseline allele burden with ongoing treatment up to 4 years. *JAK2*p.V617F reductions correlated with reductions in spleen volume; however, ruxolitinib is associated with marked spleen volume reductions regardless of mutation status. Patients with shorter disease duration had greater allele burden reductions, suggesting a potential benefit for earlier treatment.

P675

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

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Background: RUX is a potent JAK1/JAK2 inhibitor that has demonstrated reductions in splenomegaly and MF-related symptoms and improved survival in patients (pts) with intermediate (Int)-2- and high-risk MF in the 2 phase 3 COMFORT studies. JUMP (JAK Inhibitor Ruxolitinib in MF Patients) is a phase 3b, expanded-access trial in countries with no access to RUX outside a clinical trial and includes pts with Int-1 MF. As of December, >2200 pts have enrolled in 25 countries.
Aims: To assess the safety and efficacy of RUX in a cohort of pts with IPSS Int-1 MF (N=163).
Methods: Pts with MF classified as high risk, Int-2 risk, or Int-1 risk with a palpable (≥5 cm) spleen were eligible. Starting doses were based on baseline platelet (PLT) counts (5 mg bid [≥50 to <100×10^9/L], 15 mg bid [100 to 200×10^9/L], or 20 mg bid [>200×10^9/L]) and were titrated during treatment. The primary endpoint was safety and tolerability of RUX. Additional analyses included changes in palpable spleen length and symptoms using the FACT-Lymphoma Total Score (FACT-Lym TS). The final analysis will be performed after all pts have completed 24 mo of treatment or ended due to commercial availability.

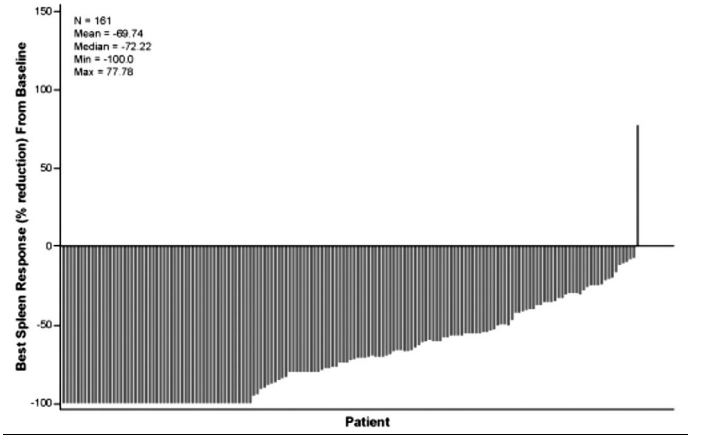

Figure 1. Best percentage reduction from baseline in spleen length by week 72.

Summary and Conclusions: JUMP is the largest study to date of pts with MF treated with RUX and includes the largest cohort of pts with Int-1-risk MF, a risk group not included in the COMFORT studies. Pts with Int-1-risk MF achieved spleen size reductions and symptom improvement consistent with those seen in the overall JUMP population. The safety and efficacy of RUX in Int-1 pts in JUMP is consistent with that in the phase 3 COMFORT studies.

P676

INTERIM ANALYSIS OF A PHASE II PILOT TRIAL OF RUXOLITINIB COMBINED WITH DANAZOL FOR PATIENTS WITH MYELOFIBROSIS SUFFERING FROM ANEMIA

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Background: Approximately 75% of myelofibrosis (MF) patients develop anemia during evolution of the disease process predicting decreased survival. Previous studies exploring the effect of danazol in the treatment of anemia in MF demonstrate responses in anemia of 30-55%. In prior randomized, controlled studies, ruxolitinib demonstrated improvements in splenomegaly, symptom burden, and even survival yet improvements in cytopenias were uncommon.
Aims: We designed a phase II multicenter pilot study to evaluate the efficacy and tolerability of combination therapy with ruxolitinib and danazol in MF patients with anemia.
Methods: This is a pre-planned interim analysis of a Simon optimum two-stage phase II trial, minimum of 10 and a maximum of 27 patients. Participants received

ruxolitinib 10mg (plat >75x10x9) BID or 5mg (plat <75x10x9) BID with tapered danazol up to 200mg orally TID. Dose escalation was allowed after completion of 28 days for lack of response or for disease progression. Patients without progression were continued for 6 cycles at 56 days each. Treatment modifications were based on adverse events (AE) including thrombocytopenia, leukopenia, and elevation in creatinine and transaminases. Treatment responses were evaluated every cycle by the IWG-MRT response criteria (Blood 2006). Patient reported outcome questionnaires (MPN-SAF and EORTC QLQ-C30) were administered at baseline, prior to treatment cycles, and at study discontinuation.

Results: *Patients:* Ten of the 14 evaluable patients enrolled (median age 70.5, range 57-78, M:F ratio 4:1) are included in this analysis. Eight patients had primary MF, 1 post essential thrombocytosis MF, and 1 post polycythemia vera MF. Jak2 V617F mutation was positive in 30%. All were DIPSS Int-2 or higher and 40% received an erythrocyte transfusion in the last month. Med baseline hemoglobin was 9.0 g/dL (range 8.3-12.4), med platelet level 172x10 (9)/L (range 56 - 346). Most (90%) had received prior therapy. *Tolerability:* Seven patients completed treatment, with med duration of treatment received of 45 days (range 24-287). Treatment discontinuation was due to progression of disease in 2 patients, unrelated AE in 1, stem cell transplant in 1, unrelated death in 1, comorbidity in 1, and decline of therapy in 1. Hematologic Grade 3 or >AE included anemia (60%), neutropenia (20%), and leukopenia/thrombocytopenia (10%). Non-hematologic Grade 3 or >AE included electrolyte abnormalities (20%), edema (10%), infection (10%), and intracranial hemorrhage (10%). *Efficacy:* Overall, treatment response per IWG-MRT response criteria included stable disease in 60%, clinical improvement in 30% (all spleen responses), and progressive disease in 10%. The median change in hemoglobin and platelet count from baseline was 0.60 g/dL (range -0.3-1.8) and 35.5x10(9)/L (range -143 - 140) respectively, however 7/10 (70%) had improvement at some point during treatment in level of anemia and/or thrombocytopenia. Among patients with a baseline and at least one post-baseline MPN-SAF TSS, 30% of patients had at least 50% improvement from baseline. Responses might have been confounded due to impact of prior therapies.

Summary and Conclusions: On interim analysis, an improvement in anemia and/or thrombocytopenia was observed in some treated patients however; the clinical improvement in 30% of patients treated was limited to spleen response. Combination therapy was well tolerated with no major incremental toxicity attributable to the addition of danazol. Maturation of data is needed to fully evaluate efficacy of ruxolitinib and danazol combination therapy for the treatment of MF associated anemia.

P677

BONE MARROW FIBROSIS BY WHO GRADE AND QUANTITATIVE IMAGE ANALYSIS IS REDUCED BY PRM-151 IN PATIENTS WITH MYELOFIBROSIS AND ASSOCIATED WITH IMPROVED BONE MARROW MORPHOLOGY AND INCREASED PLATELET COUNTS

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Background: PRM-151 (PRM) is a recombinant form of Pentraxin-2, an endogenous human protein that acts at sites of tissue damage, inducing macrophage differentiation to prevent and reverse fibrosis. 27 patients (pts) with Primary Myelofibrosis (MF), post-Polycythemia Vera MF, or post-Essential Thrombocythemia MF and ≥Grade 2 MF received PRM-151 10 mg/kg IV QW (n=8) or Q4W (n=7), or ruxolitinib plus PRM-151 10 mg/kg IV QW (n=6) or Q4W (n=6).

Aims: This study investigates the reduction of BM (BM) fibrosis by quantitative image analysis and WHO MF grading, and the changes in BM fibrosis and histotopography in pts treated with PRM-151.

Methods: Morphologic analysis was performed on serial BM specimens from 25 pts: 4 at baseline (BL) and 12 weeks, 9 at BL, 12 and 24 weeks, 3 at BL, 12, 24, and 36 weeks, and 8 at BL, 12, 24, 36, and 48 weeks. Two hematopathologists blinded to treatment assessed reticulin grade, collagen quantity, osteosclerosis quantity, cellularity, megakaryocytic morphology and topography, myeloid to erythroid (M:E) ratio and presence of erythroid islands. Pts were divided into fibrosis responders by morphologic analysis (FR: ≥1 grade decrease or stable grade 1 at the last follow-up) and non-responders (FNR: worsened grade or persistent grades 2 or 3 at the last follow-up), and platelet (PLT) responders (PR: ≥12 week doubling or normalization in PLT in pts with baseline PLT count <100x10^9/L and/or PLT transfusion independence in pts requiring ≥2 PLT transfusions in prior 12 weeks) and PLT non-responders (PNR). Computer assisted image analysis (CIA) was performed on whole slide scans from serial BM specimens from 26 pts at the same timepoints as the morphologic analysis (1 additional pt at BL and 12 weeks) for objective quantification of overall fibrosis level and osteosclerosis of all post-treatment samples compared to baseline samples. Area occupied with bone trabeculae (% of total core biopsy) and reticulin fibers (% of hematopoietic areas excluding the fat) were calculated. Values of bone and reticulin fibers were compared across serial sections of each patient to determine the trend.

Results: At baseline 2 patients had WHO MF-1, 9 pts - MF-2 and 14 pts - MF-3. There were 9 FR and 16 FNR; the baseline fibrosis grades in the FR group were 1 pts - MF-1, 3 pts - MF-2, and 5 pts - MF-3. No differences in BM morphology between FR and FNR or between PR and PNR groups were seen at the baseline. The FR group had reduced collagen quantity (p=.007) and a trend toward reduced osteosclerosis (p=0.1) at the last timepoint compared to the FNR group. CIA revealed statistically significant decreased fibrosis in all treated samples (p=0.003) but no significant difference in 12 weeks samples (p=0.28) in treated patients compared to baseline (Figure 1). FR pts showed a trend for fewer paratrabecular megakaryocytes (44% *vs* 81%, p=0.08). There were 8 PR and 17 NPR. PR was associated with normalization of the M:E ratio (p=0.03) and borderline association with fibrosis reduction (p=0.09).

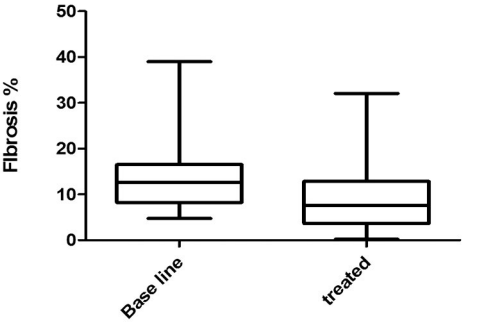

Figure 1. Fibrosis reduction by quantitative image analysis at baseline and post-baseline (p=.0031).

Summary and Conclusions: Reduction in BM fibrosis and improved PLT counts in pts treated with PRM-151 appear to be associated with reduction in collagen and osteosclerosis, improvements in megakaryocytic topography, and normalization of the M:E ratio. Post treatment fibrosis reduction is confirmed by CIA analysis and may be a useful adjunct to conventional morphologic-based grading, as it may more accurately assess fibrosis heterogeneity following therapy. Studies of intra- and inter-patient differences between CIA and morphologic analyses are in progress.

P678

THE ITALIAN MASTOCYTOSIS REGISTRY: THE FIRST FIVE YEARS

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Background: Mastocytosis is a rare disease characterized by abnormal proliferation and accumulation of mast cells (MC) in several organs and tissues (skin, bone marrow, liver, gastrointestinal tract, lymphnodes). WHO classification includes mastocytosis into cutaneous mastocytosis (CM) and systemic mastocytosis (SM) that is subdivided in indolent (ISM), "smoldering" (SSM),

systemic mastocytosis-associated hematologic non-mast disease (SM-AHN-MD), aggressive SM (ASM), mast cell leukemia (MCL), solitary mastocytoma. Difference in incidence of CM and SM correlates with age at onset. While the diagnosis of SM requires the presence of multifocal dense mast cell infiltrates in one or multiple extra-cutaneous organs (mostly bone marrow, due to the origin of MCs), mastocytosis encompasses a wide range of clinical entities, extremely heterogeneous for clinical course and prognosis. Due to its heterogeneity, mastocytosis is a multidisciplinary disease. Concerns remain about the possible misdiagnosis or lack of identification of different subtypes of mastocytosis due to different diagnostic procedures between various specialties. For example, in pediatric cases, invasive procedures are not mandatory considering the good prognosis.

Aims: The Italian Mastocytosis Registry was set up in 2009 to collect data of mastocytosis patients at a multidisciplinary and national level.

Methods: Patient data are being routinely collected after written informed consent in 18 Italian centers. An on-line database (www.registroitalianomastocitosi.it) has been created for data collection. Patients' features at onset of disease according to age, type of mastocytosis and specialty are shown in Table 1.

Table 1.

Table 1. Patients' features at onset according to age, type of mastocytosis and specialty.

	Type of MCD	Age (yrs)	Hematology center		Other multidisciplinary center	
			n	%	n	%
Age	CM	≤11	2/71	3	175/313	56
		12-19	2/71	3	12/313	4
		≥20	67/71	94	126/313	40
	SM	≤11	0/73	0	2/70	3
		12-19	0/73	0	3/70	4
		≥20	73/73	100	65/70	93
Cutaneous biopsy	CM	≤11	-	-	12/63	19
		12-19	-	-	2/3	67
		≥20	7/15	47	30/41	73
	SM	≤11	-	-	1/2	50
		12-19	-	-	1/2	50
		≥20	6/19	32	14/17	82
Bone marrow biopsy performed	CM	≤11	-	-	-	-
		12-19	-	-	0/8	0.0
		≥20	5/10	50	1/4	25
	SM	≤11	-	-	-	-
		12-19	-	-	1/1	100
		≥20	23/27	85	14/15	93
Positive bone marrow biopsy	CM	≤11	-	-	0/8	0
		12-19	-	-	-	-
		≥20	3/10	30	1/4	25
	SM	≤11	-	-	-	-
		12-19	-	-	1/1	100
		≥20	19/27	70	13/15	87
			n	Median (range)	n	Median (range)
Tryptase (µg/l) (normal value: <11.4)	CM	≤11	0	-	22	6 (2;195)
		12-19	0	-	2	430 (114;746)
		≥20	9	35 (3;176)	31	30 (4;248)
	SM	≤11	0	-	2	27 (4;49)
		12-19	0	-	2	430 (114;746)
		≥20	9	53 (22;176)	12	39 (11;248)

MCD: mast cell disease; CM: cutaneous mastocytosis; SM: systemic mastocytosis

Table 2.

Table 2. Type of therapy according to age, type of mastocytosis and type of specialty center.

	Type of MCD	Age (yrs)	Hematology center		Other multidisciplinary center	
			n	%	n	%
Phototherapy	CM	≤11	-	-	3/91	3
		12-19	2/2	100	0/4	0
		≥20	3/32	9	27/93	29
	SM	≤11	-	-	0/5	0
		12-19	-	-	0/2	0
		≥20	4/49	8	8/71	11
Interferon alfa	CM	≤11	-	-	0/91	0
		12-19	0/2	0	0/4	0
		≥20	2/32	6	2/93	2
	SM	≤11	-	-	0/5	0
		12-19	-	-	0/2	0
		≥20	5/49	10	2/71	3
Anti HH1, Anti HH2 therapy	CM	≤11	-	-	64/91	70
		12-19	0/2	0	4/4	100
		≥20	23/32	72	64/93	69
	SM	≤11	-	-	5/5	100
		12-19	-	-	2/2	100
		≥20	32/49	65	50/71	70
Corticosteroids	CM	≤11	-	-	29/91	32
		12-19	0/2	0	0/4	0
		≥20	0/32	0	7/93	8
	SM	≤11	-	-	0/5	0
		12-19	-	-	0/2	0
		≥20	5/49	10	9/71	13
Cladribine (2CdA)	CM	≤11	-	-	0/91	0
		12-19	0/2	0	0/4	0
		≥20	0/22	0	0/93	0
	SM	≤11	-	-	0/5	0
		12-19	-	-	0/2	0
		≥20	1/49	2	0/71	0
Imatinib/Dasatinib/ Nilotinib	CM	≤11	-	-	0/91	0
		12-19	0/2	0	1/4	25
		≥20	2/32	6	3/93	3
	SM	≤11	-	-	0/5	0
		12-19	-	-	1/2	50
		≥20	7/49	14	3/71	4
			n	Median (range)	n	Median (range)
Time from diagnosis to first-line therapy (months)	CM	≤11	-	-	94	3.4 (-84;519)
		12-19	3	61 (54;31)	5	154 (2;419)
		≥20	37	16 (-8.2;181)	101	62 (-3.8;315)
	SM	≤11	-	-	5	463 (422;519)
		12-19	-	-	2	321 (222;419)
		≥20	52	14 (-49;99)	74	22 (-48;315)

MCD: mast cell disease; CM: cutaneous mastocytosis; SM: systemic mastocytosis.

Results: Data on 431 patients have been collected from 5 hematology centers (97 patients) and 11 multidisciplinary centers (334 patients). Clinical evaluations were available at onset of disease in 134 patients. At diagnosis, 87% of these 134 patients presented systemic symptoms; 89 (66%) presented cutaneous symptoms (CM: 35/63 (56%) ≤11 yrs, 3/3 (100%) 12-19 yrs, 46/56 (82%) ≥20 yrs; SM: 2/2 (100%) ≤11 yrs, 2/2 (100%) 12-19 yrs, 25/36 (69%) ≥20 yrs). 83 (62%) patients were asymptomatic at onset. Of these, 28 (34%) presented symptoms in a median time of 15 months (range 3-271). SM was reported in 143 (33%) cases: 2 at ≤11 yrs, 3 at 12-19 yrs, 138 at ≥20 yrs. Of these, 65 (45%) had progressed from a CM: 0 (0%) at ≤11 yrs, 1 (2%) at 12-19 yrs, 64 (98%) at ≥20 yrs. First diagnosis was made <18 years of age in 183 (42%) patients. Table 2 shows therapeutic options (total exceeds 100% because therapy combination is allowed). 94 of 431 patients were evaluated for both bone marrow percentage of mast cell infiltrate and tryptase serum level and there was a significantly positive correlation between the two (Spearman rank correlation=0.81; p=0.005). Number of bone marrow biopsies performed was compared between hematology and other multidisciplinary centers and no statistical difference was observed (p=0.345). We also compared the number of positive bone marrow biopsies between the two types of centers; again, there was no statistical difference (p=0.248).

Summary and Conclusions: The on-line database is a useful tool for data collection at a national level. In spite of the complexities of the multidisciplinary context, our study confirms a homogeneous approach with a high level of knowledge among Centers in terms of diagnostic algorithm and treatment, in keeping with the recent guidelines

P679

HIGH RATE OF DISCORDANCY REGARDING DIAGNOSIS AND SUBCLASSIFICATION OF SYSTEMIC MASTOCYTOSIS ON BONE MARROW BIOPSIES BETWEEN INITIAL LOCAL AND CENTRALIZED REFERENCE HEMATOPATHOLOGY

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Background: Advanced systemic mastocytosis (SM) is associated with a poor prognosis. The median overall survival is approximately 0.5, 2 and 3.5 years in patients (pts.) with mast cell leukemia (MCL), SM and associated non-mast cell lineage hematologic neoplasm (SM-AHNMD) and aggressive SM (ASM), respectively. AHNMD is usually a myeloid neoplasm such as chronic myelomonocytic leukemia (CMML), myelodysplastic/myeloproliferative neoplasm unclassified (MDS/MPNu) or chronic eosinophilic leukemia (CEL). A thorough histologic and immunohistochemical examination, *e.g.* tryptase, CD117 (KIT) and CD25, of the bone marrow (BM) is required for appropriate diagnosis and subclassification including markers for characterization of AHNMD, *e.g.* CD34 for blasts or CD14 for monocytes. ASM is diagnosed through the presence of C-findings, *e.g.* cytopenias, liver function abnormalities, splenomegaly, ascites, hypoalbuminemia, weight loss, or osteolyses. The *KIT* D816V mutation is present in >80% of SM pts. and plays a pivotal role in the pathogenesis of SM.

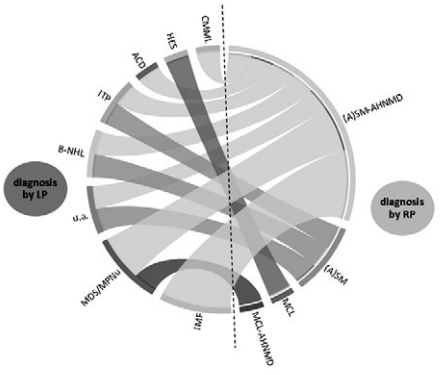
Aims: We sought to retrospectively evaluate the concordance of diagnosis and subclassification of SM between two consecutively performed BM trephine biopsies.

Methods: Within the ´German Registry on Disorders of Eosinophils and Mast Cells´, we retrospectively analyzed 65 pts. (median age 64 years, range 40-85; male 65%) who had two consecutive diagnostic BM trephine biopsies (median time between biopsies 4 months, range 0-71). The initial biopsy was evaluated by a local pathologist (LP) while *KIT* D816V$^+$ SM was diagnosed in the subsequent biopsy in all 65 pts. by reference pathologists (RP) of the 'European Competence Network on Mastocytosis (ECNM)'.

Results: According to the WHO classification, final diagnoses by RP were SM or aggressive SM [(A)SM (n=27)], (A)SM-AHNMD (n=34), MCL (n=3) and MCL-AHNMD (n=1). In peripheral blood, the serum tryptase level (normal value <11.4 ng/ml) was elevated in 59/59 (median 132 ng/ml, range 12-1690) pts. and *KIT* D816V was detectable in 57/60 (95%, median allele burden 26%, range 1-76) pts. in 15/65 (23%) pts., the initial diagnoses by LP were inconsistent and included primary myelofibrosis (n=3), MDS/MPNu (n=3), indolent B-cell lymphoma (n=2), autoimmune thrombocytopenia (n=2), CMML (n=1), hypereosinophilic syndrome (n=1), anemia of chronic disease (n=1) or without pathological findings (n=2). Twelve of those 15 (80%) pts. with discordant diagnosis had (A)SM-AHNMD with AHNMD being subclassified as MDS/MPNu, n=6; CMML, n=3; MDS, n=2; CEL, n=1) (Figure 1). Immunohistochemical markers for qualitative and quantitative assessment of MCs, *e.g.* CD117 (KIT) or CD25, were only used in 43/65 (66%) and 24/65 (37%) cases, respectively. In 50 concordantly diagnosed patients (median time between biopsies 6 months, range 1-8), the extent of MC infiltration was quantified by LP in only 34/50 (68%, median 20%, range 5-90) pts., subclassification of SM was not consistent because AHNMD was not diagnosed in 9/50 (18%) pts. and *KIT* D816V mutation anaylsis was suggested or performed in only 13/50 (26%) correctly diagnosed SM pts.

Summary and Conclusions: Adequate diagnosis and subclassification of SM warrants an evaluation of BM histology/immunohistochemistry by reference hematopathologists in combination with molecular genetics (*KIT* D816V) and clinical parameters. A more frequent evaluation of serum tryptase would help

to prevent misdiagnosis.



Circos diagram: pairwise linking between diagnosis assigned by local pathologist (LP, left) vs. reference pathologist (RP, right). The width of the ribbon/column correlates to the relative frequency of individual diagnosis. Systemic mastocytosis (SM) or aggressive SM, (A)SM; (A)SM with associated clonal hematologic non-mast cell lineage disease, (A)SM-AHNMD; mast cell leukemia, MCL; idiopathic myelofibrosis, IMF; myelodysplastic/myeloproliferative neoplasm unclassified, MDS/MPNu; autoimmune thrombocytopenia, ITP; B-cell non-Hodgkin lymphoma, B-NHL; chronic myelomonocytic leukemia, CMML; hypereosinophilic syndrome, HES; anemia of chronic disease, ACD; without pathological findings, u.a.

Figure 1.

P680

CLINICAL AND MOLECULAR CHARACTERISTICS OF PATIENTS WITH SYSTEMIC MASTOCYTOSIS AND ASSOCIATED ACUTE MYELOID LEUKAEMIA

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Background: In systemic mastocytosis (SM), the most aggressive clinical course has been reported from aggressive SM (ASM) and mast cell leukaemia (MCL) with median survival times of 2-3 years and less than 6 months, respectively. A significant proportion of patients with indolent SM (ISM), ASM or MCL may present with an associated haematological non-mast cell lineage disease (AHNMD) which most commonly represents chronic myelomonocytic leukaemia (CMML), myelodysplastic/myeloproliferative neoplasm unclassified (MDS/MPN-U), myeloproliferative neoplasm unclassified (MPN-U) or chronic eosinophilic leukaemia (CEL). Very rarely, an associated acute myeloid leukaemia [(A)SM/MCL-AML] is diagnosed which can be either *de novo* or secondary, *e.g.* evolving from CMML, MDS/MPN-U, MPN-U or CEL. A *KIT* D816V mutation is present in >90% of patients with advanced SM and plays a pivotal role in its pathogenesis. However, recent data have suggested that additional mutations, *e.g.* in *TET2*, *SRSF2*, *ASXL1* or *RUNX1* etc., are detectable at variable frequencies in >80-90% of patients with (A)SM/MCL-AHNMD (Schwaab *et al.*, Blood 2013).

Aims: To analyse the clinical presentation, genetic profile, treatment and prognosis of (A)SM/MCL-AML.

Methods: Retrospective analysis of 11 patients (male, n=6; female, n=5) with SM-AML recruited within the "German Registry on Disorders of Eosinophils and Mast Cells".

Results: SM was diagnosed prior to AML (n=8), concomitantly with AML (n=1) or due to lack of durable remission following intensive chemotherapy of AML (n=2).The median age at diagnosis of SM and AML was 55 years (range 26-78) and 62 years (range 27-75), respectively. The subtype of SM was ASM (n=9) or ISM (n=2) while AML was *de novo* (n=6) or secondary (CMML, n=3; MPN-U, n=2, MDS/MPN-U, n=1). At time of diagnosis of AML, cytogenetic analysis of 6 patients with available material/data revealed a complex karyotype (n=1), monosomy 7 (n=1), trisomy 8 (n=1) or a normal karyotype (n=3). The KIT D816V mutation was identified in 10 of 11 (91%) patients and additional mutations in 8 of 8 (100%) patients with available material. The number of additional mutations in individual patients was 1 (n=3), 2 (n=2) and >2 (n=3). The most frequently identified mutations included *RUNX1* (n=4), *ASXL1* (n=3), *SRSF2* (n=3), and *TET2* (n=2). Ten patients received intensive chemotherapy according to disparate AML study protocols (*e.g.* daunorubicin/ara-C or mitoxantrone/high-dose ara-C). Seven patients achieved a complete remission. Two patients died as consequence of treatment or disease-related complications 2 and 12 months after start of chemotherapy. Six patients received an allogeneic stem cell transplantation (ASCT) immediately after chemotherapy (n=1), in first remission (n=3) or following relapse (n=2) after remission. Four patients died a median of 13 months (range 1-36) after ASCT while 2 patients are alive 9 and 29 months, respectively, after ASCT. Of the non-transplanted patients (n=5), 4 patients died within the first 13 months following diagnosis of AML while only one patient is alive 70 months after diagnosis and conventional treatment of AML. The single patient without intensive chemotherapy died 4 months after diagnosis of AML. Overall, median survival after diagnosis of SM was 3 years (range 0.7-16.6) and 13 months (range 2-74) after diagnosis of AML, respectively.

Summary and Conclusions: SM-AML is a rare entity with a very poor prognosis. Diagnosis of SM may be missed when presenting simultaneously with AML. The high frequency of additional mutations suggests a pathogenetic role for the aggressive clinical course and poor prognosis. Long-term survival is more likely in patients achieving complete remission following intensive chemotherapy with or without subsequent ASCT.

Indolent Non-Hodgkin lymphoma - Clinical

P681

VALIDATION OF THE SIMPLIFIED PROGNOSTIC SCORE FOR SMZL OF THE SMZLSG (SMZL STUDY GROUP) IN A SERIES OF PATIENTS TREATED WITH RITUXIMAB MONOTHERAPY, AND LONG TERM OUTCOME OF RITUXIMAB RESPONDERS

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Background: Rituximab monotherapy is a highly effective therapy for SMZL. However, there is a subset of patients not responding to rituximab with compromised survival. Several prognostic factors have been proposed without reproducible results.
Aims: To assess the long term outcome of rituximab treated patients and validate the reproducibility of the simplified risk stratification score of the SMZLSG in a series of SMZL patients treated homogeneously with rituximab monotherapy as a first line therapy.
Methods: The study population included 76 patients who had received rituximab as 1st line therapy for SMZL between 2003 and 2014. The prognostic system is based on the on the combination of 4 factors: haemoglobin levels <9.5 g/dL, platelet counts <80×10^9/L, elevated LDH and the presence of extrahilar lymphadenopathy (outside the splenic and hepatic hilum). All variables were considered to have the same statistical weight and were assigned the same value of one point each. The prognostic score was calculated as the sum of the 4 variables (0 or 1; range 0-4). Three risk groups were identified: low (A) for patients with 0 points, intermediate (B) for patients with 1 or 2 points and a high risk group (C) for patients with 3 or 4 points. The Kaplan-Meier method was used to estimate survival and the log-rank test to compare survival curves.

Table 1. Main characteristics, stratification and outcome of our patients.

Characteristics	At Diagnosis	At Treatment Initiation
	# (%)	
Male Sex	33/76 (43)	33/76 (43)
Median Age, years (Range)	65 (41-91)	65 (41-91)
Hb <9,5 G / dl	14/76 (18)	16/76 (21)
PLTS <80 × 10^3 / µl	6/76 (8)	9/76 (12)
LDH> N	23/74 (31)	28/74 (38)
Extrahilar Lymphadenopathy	20/75 (27)	21/75 (28)
Group A	34/73 (47)	27 (37)
Group B	36/73 (49)	42 (58)
Group C	3/73 (4)	4 (5)
7-Year PFS (%)		
Group A 83		
Group B 48		
Group C 50		

Results: Among 76 patients with SMZL included in the present study, 73 had complete data for all four variables and were therefore analyzed for the prognostic system (Table 1). The median age was 65 years (45-95). The main clinical characteristics and the stratification of our patients in the three 3 groups are presented in table. Among the 76 SMZL patients, 3 did not respond to or progressed during rituximab treatment, for an overall response rate of 96%. At 1, 2, 3, 4, 5, and 7 years, PFS was 95%, 88%, 83%, 74%, 69%, and 69% respectively. At the time of this analysis 6 deaths were recorded, 3 due to the disease and 3 urelated. The 7-year overall survival and disease-specific survival were 87% and 91% respectively. According to the simplified prognostic score the stratification of our patients in the three risk groups at treatment initiation were as follows: 27/73 (37%) were classified into group A, 42/73 (58%) into group B and 4/73 (5%) into group C. 7-year PFS were 83% for group A, 48% for group B and 50% for group C (p=0.051). Difference in 7-year PFS was more prominent when groups B and C were analyzed together (7-year PFS for group B+C was 49% *vs* 83% group A, p=0.02). In order to evaluate long term outcome of rituximab responders, 24 patients who had received rituximab and remained in remission for ≥5 years were analyzed. The 5-year PFS was 96%. At a further median time of 24.5 months (range, 4-72) beyond 5 years, 22 patients still remain in remission, while two relapsed at 6 and 62 months, respectively. The subsequent 5-year PFS (10 years post-treatment) was 96%.
Summary and Conclusions: This study validates the applicability of the SMZLSG prognostic system regarding PFS, in a series of 73 SMZL patients homogeneously treated with Rituximab monotherapy. The long-term disease-specific survival exceeded 90%. This study confirms the efficacy of Rituximab as first line treatment in SMZL and demonstrates the potential for a long-term disease control, beyond the initial 5 years, in a substantial patient subgroup.

P682

INCIDENCE OF TRANSFORMATION IN FOLLICULAR LYMPHOMA: MULTICENTRE RETROSPECTIVE ANALYSIS OF THE SPANISH GROUP OF LYMPHOMA AND AUTOLOGOUS STEM-CELL TRANSPLANTATION (GELTAMO)

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Background: Follicular lymphoma (FL) may, over time, transform into an aggressive lymphoma, usually diffuse large B-cell lymphoma (DLBCL). Transformed follicular lymphomas (tFL) have a worse prognosis due to poorer response to treatment than primary DLBCL. The incidence of transformation is estimated in ~3% per year, although it varies largely between different studies (24%>70% overall). These differences are mainly due to different criteria to define tFL, to lack of evidence of tFL by biopsy, absence of clonality studies discarding secondary *de novo* NHL, studies performed in the pre-Rituximab era, or different follow-up times among studies. With all this pitfalls, the actual incidence of transformation remains an open question.
Aims: The aim of the present study is to analyse the incidence and prognostic impact of transformation in patients with FL in a large retrospective series of the Spanish group of Lymphomas (GELTAMO).
Methods: A total of 1096 patients (grade I, II, and IIIa) from 8 Spanish centres diagnosed of FL between 2000 and 2010 were included in the study. Data were obtained from the database of centres willing to participate in this study. True tFL (FL to DLBCL) were recorded. Composite FL+DLBCL, discordant tFL (FL in bone marrow and DLBCL in adenopathy or *viceversa*), and downgrading tFL (DLBCL at diagnosis and relapse of FL) were excluded from the preliminary analysis. This study was approved by the Salamanca University Hospital Ethic Committee.
Results: Seventy-one patients (median follow up of 6 years) were transformed to DLBCL (6,5%). Cumulative incidence of transformation at 5, 10, and 15 years was of 5%, 8%, and 14%, respectively. Median time to transformation was 30 months, ranged 3-150. Considering survival from diagnosis of FL, tFL patients had a shorter OS than non-transformed (20% *vs* 68%, p<0,0001). Most of the tFL patients (92%) have previously received treatment for FL, 56% of them with Rituximab. Median number of treatment lines before transformation was 2 (1-6). Patients receiving Rituximab as first line therapy showed decreased time to transformation at 15 years (10% *vs* 19%, p=0.025). Other factors influencing risk of tFL included no response to first line therapy, age at diagnosis >60 years, FLIPI, and Ann Arbor stage. Consolidation therapy with autologous transplant for tFL showed an increase in OS (67% *vs* 8%, p=0.001). However, these results should be confirmed in prospective studies.
Summary and Conclusions: High risk FLIPI, Ann Arbor stage III-IV, age >60 years, not use of Rituximab in first line therapy, and response to first line have shown to predispose to a higher risk of transformation. Autologous transplantation could have a benefit in terms of OS in transformed patients. Effort should be made in order to clarify criteria for transformation, and biological studies on tumoral samples at diagnosis and at transformation will help to determine pathogenesis of transformation in helping to design clinical trials with new molecules based on molecular characteristics of transformation.

P683

PROSPECTIVELY DEFINED TRIAL- (N=13) AND PATIENT- (N=3837) LEVEL ANALYSIS OF COMPLETE RESPONSE RATES AS SURROGATE END-POINTS FOR PROGRESSION-FREE SURVIVAL (PFS) IN FIRST-LINE FOLLICULAR LYMPHOMA (FL)

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Background: Current first-line treatments for FL provide prolonged disease control (median PFS ~7 years). Thus, studies evaluating first-line treatment options based on the accepted regulatory endpoint of PFS require extended patient follow-up, increasing the challenges associated with clinical development of novel treatments by prolonging the evaluation process.
Aims: The Follicular Lymphoma Analysis of Surrogacy Hypothesis (FLASH) group conducted a prospectively planned meta-analysis with individual patient data (IPD) to evaluate whether the treatment effect on clinical endpoints at early landmarks, for example, complete response rate(s) at 24 (CR24) or 30 months (CR30), could accurately predict treatment effect on PFS.
Methods: Randomized controlled trials testing first-line treatments in FL, published after 1990, with available IPD were eligible. Association between CR rates and PFS was assessed at both the trial and patient level. Treatment effects on PFS and binary CR endpoints were quantified by hazard ratios (HRs) and odd ratios (ORs), respectively. The trial-level correlation of CR rate with PFS was evaluated using linear regression (R^2_{WLS}) and copula bivariate (R^2_{Copula}) models. Prespecified criteria for establishing surrogacy required either R^2_{WLS} or R^2_{Copula} ≥0.80 with a lower bound of the 95% confidence interval (CI) >0.60, and neither <0.70. The patient-level association between CR status (at 24 and 30 months) and PFS was assessed using a stratified Cox model with a landmark approach and by the global OR using a bivariate copula model. The minimum treatment effect on CR needed to predict a significant PFS difference was calculated.
Results: Of 13 eligible randomized first-line trials, 8 explored induction and 5 explored maintenance regimens; IPD was available for 3837 patients (Table 1). Nine trials included an experimental arm exploring a rituximab-based regimen. The prespecified threshold for CR30 surrogacy was met: R^2_{WLS} was 0.88 (95% CI, 0.77-0.96) and R^2_{Copula} was 0.86 (95% CI, 0.72-1.00), demonstrating that treatment effect on CR30 predicted effect on PFS in previously untreated FL. A minimum 10% improvement in CR30 over a control rate of 50% predicted a significant improvement in PFS. Multiple sensitivity and IPD surrogacy analyses supported the robustness of the primary CR30 analysis. Treatment effects on CR24 also met prespecified surrogacy criteria for R^2_{WLS} 0.84 (95% CI, 0.63-0.95), but not for R^2_{Copula}; CR24 analyses were influenced by one outlier study.

Table 1. Correlation of CR30 and CR24 with progression-free survival.

		Trial-Level Surrogacy		Patient-Level Surrogacy (Copula)
	N of Trials (N of pts)	R^2_{WLS}[a] (95% CI)	R^2_{Copula}[a] (95% CI)	Global OR[b] (95% CI)
CR30				
Overall	13 (3837)	0.88 (0.77-0.96)	0.86 (0.72-1.00)	11.84 (10.03-13.65)
With/Without rituximab				
Rituximab trial	9 (2851)	0.85 (0.62-0.97)	0.80 (0.56-1.00)	11.08 (9.13-13.03)
Non-rituximab trial	4 (986)	0.91 (0.05-1.00)	0.96 (0.90-1.00)	14.40 (9.96-18.84)
Treatment setting				
Induction trial	8 (2207)	0.89 (0.75-0.98)	0.89 (0.74-1.00)	10.34 (8.27-12.41)
Maintenance trial	5 (1630)	0.93 (0.84-1.00)	0.89 (0.71-1.00)	14.14 (10.82-17.46)
CR24				
Overall	11 (2728)	0.84 (0.63-0.95)	0.67 (0.35-0.99)	8.27 (6.82-9.71)

Note: Some studies were excluded because of high proportion of missing CR data.
Abbreviations: CI, confidence interval; CR, complete response; CR30, complete response rate at 30 mo; CR24, complete response rate at 24 mo; OR odds ratio; pts, patients.
[a]R^2 values range from 0 (no association) to 1 (perfect prediction).
[b]**Global odds ratio** represents the OR for progression-free survival status beyond a particular time point comparing patiehts with CR versus non-CR.

Summary and Conclusions: This large IPD meta-analysis of chemo/immunotherapy trials demonstrates that treatment effect on CR30 is a robust predictor of the treatment effect on PFS in first-line FL; the correlation of CR24 and PFS treatment effects is promising but requires further confirmation. The use of the CR30 endpoint may expedite the evaluation of, and thus improve the speed of access to, promising novel therapeutics for this patient population.

P684

MYD-88 L265P IN A SERIES OF CD5(-) CLONAL B-CELL LYMPHOCYTOSIS OF MARGINAL ZONE ORIGIN (CD5- CBL)

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Background: CD5- CBL displays features consistent with marginal zone origin. This entity may be associated with monoclonal gammopathy, frequently of the IgM type. Therefore differential diagnosis from Waldenstrom macroglobulinemia (WM) /lymphoplasmacytic lymphoma (LPL) is mandatory. The recently identified gain of function L265P MYD 88 mutation is considered a sensitive and specific marker of WM and might be a valuable tool to differentiate the two entities.
Aims: To determine the presence of L265P MYD-88 mutation in a well characterized series of CD5- CBL pts along with an extended analysis of the morphologic, clinical, immunophenotypic and histologic features in order to identify whether some of these cases may be in fact WM/LPL.
Methods: We retrospectively evaluated 40 patients with CD5- CBL presenting in the blood and/or in the bone marrow (BM), without evidence of any other disease localization or signs of chronic and/or active inflammation, autoimmunity, organomegaly or cytopenia. The identification of MYD 88 L265P mutation was performed in blood/BM mononuclear cells with allele specific PCR. For the purpose of the present analysis, patients were divided in 3 groups: Group 1 included patients with a mutated MYD 88. Group 2 consisted of MYD 88 wild type patients with paraproteinemia, whereas patients with wild type MYD 88 but without paraproteinemia were classified as group 3.

Table 1. Patients' characteristics according to the presence of MYD-88 L265P mutation.

	PATIENTS' CATEGORY		
	GROUP 1	GROUP 2	GROUP 3
Characteristics	MYD-88(+) N=8	MYD-88(-) with MG* N=12	MYD-88(-) without MG N=20
Male sex	5 (62%)	4 (33%)	8 (40%)
Median age (range) [years]	71 (51-79)	66 (46-80)	69 (54-83)
Median lymphocyte count x10^9/L (range)	3.85 (2.4-11.25)	3.51 (1.0-7.8)	5.07 (1.4-29.2)
Lymphocytosis (%)	3/8 (38)	3/12 (25)	11 (55)
Blood lymph morphology # (%)			
Small	6 (100)^	11 (100)^^	20 (100)
Villous	2 (33)	4 (36)	17 (86)
Monocytoid	3 (50)	2 (18)	10 (50)
LPL	0	1 (9)	0
Paraprotein	8 (100%)	12 (100%)	
IgM	5	6	-
IgG	2	6	-
IgM and IgG	1		
Paraprotein levels mg/dl median (range)			-
IgM	947 (358-1472)	608 (114-1420)	
IgG	1120 (995-3420)	908 (643-1541)	
Immunophenotype # (%)			
CD23 (+)	2/5 (40)	5/10 (50)	7/20 (35)
CD25(+)	3/4 (75)	1/6 (17)	2/17 (12)
CD11c (+)	0	3/10 (30)	7/18 (39)
CD38 (+)	2/4 (50)	0	0/20
% BM infiltration (range)	40 (15-60)	17 (15-80)	27 (12-70)
BM Pattern of infiltration # (%)			
Mixed	6 (75)	6 (50)	7 (35)
Interstitial	2 (25)	4 (33)	3 (15)
Intrasinusoidal	3 (37)	4 (33)	9 (45)
Nodular	6 (75)	4 (33)	3 (15)
Diffuse	0	1 (8)	0
Plasmacytic differentiation #(%)	6 (75)	6 (50)	3 (15)
Immunohistochemistry			
DBA-44	0/3	5/6	8/11
sIgM	6/6	1/1	7/8
cIgM	5/6	2/2	6/7
sIgD	4/6		2/3
sIgG	2/6		

**MG*=monoclonal gamopathy

^2 pts did not have circulating clonal B-cells

^^1 pt did not have circulating clonal B-cells

Results: All patients were asymptomatic at diagnosis presenting predominantly with lymphocytosis, or more rarely with a paraproteinemia. Thirty-six patients

had lymphocytosis, while circulating clonal B-cells were found in 93%. Blood lymphoid population consisted mainly of small lymphocytes, admixed with villous lymphocytes and monocytoid cells in various proportions. Paraproteinemia was evident in 20 cases. BM was invariably infiltrated with features of plasmacytic differentiation in 15 cases, 12 of which were associated with the presence of paraproteinemia. The L265P MYD-88 mutation was present in 8 cases (20%). The laboratory, morphologic, immunophenotypic and histologic characteristics of the three groups are shown in the Table 1. All MYD-88 (+) cases (group 1) had paraproteinemia (5 IgM, 2 IgG and 1 IgM plus IgG) and 6 of them also displayed plasmacytic differentiation in the BM. Regarding group 2 patients (N=12), 6 had an IgM paraprotein and 6 plasmacytic differentiation in the BM. Among the 20 group 3 patients, only 3 showed plasmacytic differentiation in the BM. Thus, MYD-88 mutated cases had more frequently IgM paraproteinemia, higher percentage of BM infiltration and plasmacytic differentiation, and CD38 expression. At a median follow-up time of 41 months, 1 patient progressed to SMZL and 4 developed cytopenias due to extensive BM infiltration. The limited number of patients in each group and the relative short follow-up time prevent any correlation between the MYD-88 and outcome.

Summary and Conclusions: Almost 2/3 (12/20) of pts with monoclonal gamopathy were MYD-88 (-) and therefore these pts are still classified as CD5- CBL. Further on our study shows for the first time, that 20% of CD5- CBL cases harbor the L265P MYD 88 mutation - a marker that is WM specific. The L265P MYD mutated subgroup displayed more often plasmacytic differentiation, CD38 expression, and a paraprotein of the IgM type. Whether these cases represent true WM or borderline cases between these two entities remains to be clarified.

P685

EFFICACY AND SAFETY OF R-COMP REGIMEN IN NON HODGKIN LYMPHOMA PATIENTS WITH CARDIOVASCULAR COMORBIDITIES

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Background: Anthracyclines-based regimens remain the gold standard for the treatment of Lymphomas, although the associated cardiac toxicity may limit their use, especially in frail and elderly patients.

Aims: The aim of this study was to evaluate the efficacy and tollerability of RCOMP inpatients with poor-risk lymphoma and cardiovascular comorbidity

Methods: From October 2008 to January 2015 we treated 44 newly diagnosed patients with poor-risk lymphoma and cardiovascular comorbidity using the R-COMP regimen (rituximab, cyclophosphamide, non-pegylated liposome-encapsulated doxorubicin, vincristine and prednisone)

Results: Median age was 74 years (range:46-83 yrs ; 45% ≥75 years). As for histology, 22/44 (50%) were Diffuse Large B-cell Lymphoma; 8/44 (18%) Mantle-cell Lymphoma; 5/44 (11%) Follicular Lymphoma; 3/44 (7%) Peripheral B-cell Lymphoma; 4/44 (9%) Nodal Marginal Zone Lymphoma; 2/44 (4%) other B-cell indolent Lymphoma. IPI score was Intermediate-High/High in 61,3% of the patients, 75% had an advanced Ann-Arbor stage and 43% presented at diagnosis with extranodal involvement. The median age adjusted Charlons comorbidity index was 6 (range: 3 to 9). Cardiovascular risk factors were considered: hypertension (30/44pts: 68%), 14/44 pts (31,6%) had a history or recent acute myocardial infarction and 4/44 (10%) suffered of Atrial fibrillation. Treatment was well tolerated and toxicities were limited grade III/IV cytopenia. Complete remission was achieved in 30/44 (68%) and partial response in 6/44 (14%). Progressive disease was present in 8/44 (18%). After a median follow up of 18 months, the median OS (Overall Survival) was not reached and 4/36(11%) responders relapsed. Cardiac toxicity was observed in one patients who died for pulmonary edema, while two patients developed arrhythmias.

Summary and Conclusions: This study confirm the efficacy and tolerability of R-COMP regimen in elderly patients with cardiovascular comorbidities.

P686

THE EFFECTIVENESS OF ANTIVIRAL THERAPY IN PATIENTS WITH HEPATITIS C ASSOCIATED INDOLENT LYMPHOMA (IL+C)

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Background: B-cell indolent non-Hodgkin's lymphoma is typical manifestation of chronic extrahepatic hepatitis C. The incidence of HCV infection in patients with B-cell indolent non-Hodgkin's lymphomas is approximately 15%.

Aims: In our study included 93 patients with indolent lymphoma and hepatitis C markers (IL+C) and control group of 146 patients with indolent lymphomas without markers of hepatitis C (IL-C). In patients with indolent lymphoma associated with hepatitis c proteins of virus expression on tumor cells in 82% patients.

Methods: In 93 patients with IL+C 43 patients received antiviral therapy as the first treatment. 50 patients with IL+C received polychemotherapy therapy as first line of therapy. All patients with indolent lymphomas without markers of hepatitis C received only chemotherapy.

Results: Antiviral therapy(AVT) in patients with IL+C received complete remission(CR)-77%, partial remission(PR) - 11%, stabilization-4%, 8% progression. All patients, in whom antiviral therapy was not effective(stabilization+progression), virus proteins of hepatitis C on tumor cells was not detected. On chemotherapy in patients with IL+C CR received in 64%, PR-23%, stabilization-9%, 4%>progression. All patients with progression on chemotherapy on tumor cells was detected proteins of hepatitis C virus. On chemotherapy in patients in control group with IL-C received CR in 53%, PR-31%, the stabilization-5%, 11%>progression. The median relapse-free survival(RFS) in patients with IL+C on AVT was 36mth. The median RFS in patients with IL+C on chemotherapy was 19 months. The median RFS in patients with IL-C on chemotherapy was 33 months. 37 patients with relapse IL+C after chemotherapy was conducted AVT. CR was received in 81% of patients, PR was achieved in 11% and stabilization/progression+8% of patients. The median RFS in patients with IL+C recurrence after chemotherapy for AVT was 31 months.

Summary and Conclusions: The effectiveness of AVT was significantly higher than chemotherapy in patients with IL+C. Median disease-free for patients with IL+C significantly more on antiviral therapy. AVT should be the first line therapy in patients with the IL+C.

P687

COMPARISON OF SUBCUTANEOUS AND INTRAVENOUS RITUXIMAB IN THE MAINTENANCE SETTING: UPDATED SAFETY RESULTS OF THE PHASE III SABRINA STUDY IN PATIENTS WITH FOLLICULAR LYMPHOMA

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Background: Subcutaneous rituximab (R^{SC}) offers improved patient (pt) convenience and healthcare resource savings *versus* intravenous rituximab (R^{IV}). The phase III SABRINA study (NCT01200758) investigated induction R^{SC} or R^{IV} in combination with chemotherapy followed by maintenance R^{SC} or R^{IV} in pts with follicular lymphoma (FL). With a median follow-up time of 14 months, the R^{SC} and R^{IV} arms had similar response rates and safety profiles, without any new clinically relevant safety findings reported for R^{SC}. However, longer follow up is needed to define the safety of R^{SC} *versus* R^{IV} in the maintenance setting.

Aims: We present an updated safety analysis of R^{SC} and R^{IV} for the maintenance phase of SABRINA (median follow-up time 26 months).

Methods: Pts with treatment-naïve CD20+ grade 1-3a FL (n=410) were randomised to receive R^{SC} or R^{IV} (n=205/arm), stratified by FL International Prognostic Index score, chemotherapy regimen and region. All pts received R^{IV} 375 mg/m^2 in cycle 1; for cycles 2-8, pts received R^{SC} 1400 mg or R^{IV} 375 mg/m^2 every 3 weeks. Pts received ≤8 cycles of CHOP (cyclophosphamide, doxorubicin, vincristine and prednisone) or 8 cycles of CVP (cyclophosphamide, vincristine and prednisone). During maintenance, pts received R^{SC} or R^{IV} every 8 weeks. Non-serious AEs were reported for 28 days following the last dose of rituximab. Serious AEs (SAEs) were recorded for 1 year post-treatment or until the start of new anti-lymphoma treatment. SAEs considered possibly related to study treatment were recorded indefinitely.

Results: In total, 407 pts received ≥1 dose of rituximab (safety population). Six R^{SC} pts discontinued treatment after cycle 1 (R^{IV} in both arms) and were included in the R^{IV} safety population (R^{SC} n=197 [81 male, 116 female]; R^{IV} n=210 [109 male, 101 female]). In total, 86% of pts started maintenance (R^{SC} n=172, 87%; R^{IV} n=178, 85%). During maintenance, the most common AEs were of the system organ class (SOC) infections and infestations (R^{SC} 41%; R^{IV} 42%); most were grade 1/2 upper respiratory tract infections, urinary tract infections or nasopharyngitis. Grade ≥3 infections were reported in 8% (R^{SC}) and 3% (R^{IV}) of pts. Other common AEs (≥15% pts) belonged to the SOCs general disorders and administration site conditions (R^{SC} 28%; R^{IV} 19%; difference mainly driven by grade 1 injection site erythema [R^{SC} 8%; R^{IV} 0%]); gastrointestinal disorders (R^{SC} 24%; R^{IV} 19%); musculoskeletal and connective tissue disorders (R^{SC} 23%; R^{IV} 21%); respiratory, thoracic and mediastinal disorders (R^{SC} 18%; R^{IV} 17%); blood and lymphatic system disorders (17% each; most were grade ≥3 [10% of pts/arm]); and skin and subcutaneous tissue disorders (R^{SC} 16%; R^{IV} 15%). SAEs reported during maintenance (≥2% of pts) were: infections and infestations (R^{SC} 8%; R^{IV} 2%); gas-

trointestinal disorders (R^{SC} 1%; R^{IV} 4%); musculoskeletal and connective tissue disorders (R^{SC} 2%; R^{IV} 1%); blood and lymphatic system disorders (R^{SC} 1%; R^{IV} 2%); and neoplasms (R^{SC} <1%; R^{IV} 2%). In both arms, grade ≥3 AEs were more common in females (R^{SC} 27%; R^{IV} 29%) than in males (R^{SC} 18%; R^{IV} 19%) during maintenance. In total, 5% (R^{SC}) and 3% (R^{IV}) of pts discontinued maintenance due to AEs. At data cut off, 27 pts (R^{SC} n=11, 6%; R^{IV} n=16, 8%) had died.

Summary and Conclusions: Maintenance R^{SC} and R^{IV} were well tolerated in treatment-naïve pts with FL, with no new safety signals noted for R^{SC}. Availability of R^{SC} administration over approximately 6 minutes has positive implications for pt convenience and healthcare resource savings, without compromising safety or efficacy.

P688

TWO DOSES OF POLATUZUMAB VEDOTIN (POV, ANTI-CD79B ANTIBODY-DRUG CONJUGATE) PLUS RITUXIMAB (R) IN PATIENTS WITH RELAPSED/REFRACTORY (R/R) FOLLICULAR LYMPHOMA (FL): DURABLE RESPONSES AT LOWER DOSE LEVEL

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Background: Based on early evidence of cumulative toxicity of PoV at a dose of 2.4 mg/kg (Morschhauser ASH 2014; NCT01691898), a dose of 1.8 mg/kg dose was explored.
Aims: To report updated results of the dose comparison. Safety and efficacy of PoV after 8 treatment cycles were also analyzed.
Methods: Patients (pts) with R/R FL received PoV at 2.4 mg/kg or 1.8 mg/kg with R 375mg/m^2, q21d until progression or unacceptable toxicity. Five pts with R/R FL from the Phase 1 study (Palanca-Wessels ASH 2013) treated with PoV 2.4 mg/kg were included in the analysis. Data at completion of PoV treatment were compared with data after 8 cycles.
Results: Forty-five pts received PoV+R (25, 2.4 mg/kg; 20, 1.8 mg/kg). Median follow-up was 14 mo. for 2.4 mg/kg *vs* 8 mo. for 1.8 mg/kg. When limited to the first 8 treatment cycles, median follow-up was similar at 6 mo. for both groups. Baseline characteristics were balanced between the two cohorts, except for age (median 68 yrs 2.4 mg/kg, 62 yrs 1.8 mg/kg) and tumor volume (SPD 1824 mm^2 at 2.4 mg/kg, 2655mm^2 at 1.8 mg/kg). Overall, 40% (10/25, 2.4 mg/kg) and 50% (10/20, 1.8 mg/kg) of pts were refractory to their last treatment. At data cut-off for this analysis, pts received a median of 10 and 9.5 treatment cycles in the 2.4 mg/kg and 1.8 mg/kg groups, respectively, with median dose intensities after cycle 8 of 88% and 99%, respectively. Safety is shown in the Table 1. Peripheral neuropathy (PN) was more frequent with PoV 2.4 mg/kg, and discontinuation (d/c) rates due to all causes were 56% *vs* 30% with the 1.8 mg/kg dose. After 8 treatment cycles, d/c rates were similar for both doses (28% *vs* 25%). An 84-year old pt in the 2.4 mg/kg cohort died 2 mo. after cycle 12 due to pulmonary congestion.

Table 1. Safety profiles of PoV+R for all treatment cycles and truncated after cycle 8

Adverse events, n (%) (MeDRA SOC)	PoV 1.8 mg/kg all cycles (N=20)	PoV 2.4 mg/kg all cycles (N=25)	PoV 1.8 mg/kg 8 cycles (N=20)	PoV 2.4 mg/kg 8 cycles (N=25)
Any AE Grade 3-4	10 (50)	13 (52)	10 (50)	13 (52)
Neutropenia	7 (35)	4 (16)	7 (35)	4 (16)
Febrile neutropenia	2 (10)	1 (4)	2 (10)	1 (4)
Serious AE	6 (30)	8 (32)	6 (30)	6 (24)
Deaths	-	1 (4)	-	-

AE leading to study discontinuation 6 (30) 14 (56) 5 (25) 7 (28) Grade 2-4 periph. neuropathy1 8 (40) 18 (72) 5 (25) 10 (40) Grade 3+ infection 1 (5) 3 (12) 1 (5) 2 (8) AE per CTCAE V4.03. AEs G3/4 ≥10% reported; 1 MedDRA SMQ peripheral neuropathy (wide).

ORR was similar in for both levels: 19/25 (76%) at 2.4 mg/kg, 15/20 (75%) at 1.8 mg/kg. CRs were achieved in 11/25 (44%) patients at 2.4 mg/kg and 2/20 (10%) patients at 1.8 mg/kg. Duration of response was 12 mo. for 2.4 mg/kg and not estimable for 1.8 mg/kg group. After 8 cycles, ORR remained similar; 64% for 2.4 mg/kg and 60% for 1.8 mg/kg. Median PFS was 15 mo. at 2.4 mg/kg and not reached in the 1.8 mg/kg group (Figure 1).

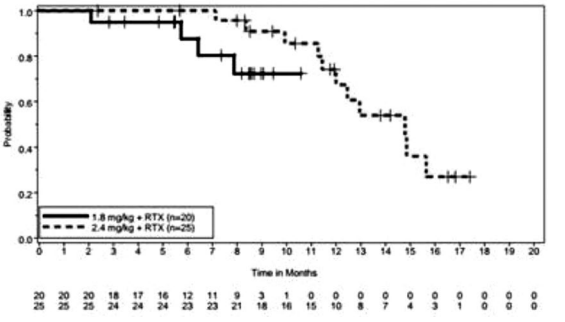

Figure 1. PFS for PoV+R (PoV at 2.4 mg/kg and 1.8 mg/kg).

Summary and Conclusions: PoV+R in R/R FL showed high ORR at both doses, with higher CR at 2.4 mg/kg. D/c rates, mostly due to cumulative PN, were high. AEs and d/c rates were reduced at both doses if only the first 8 cycles are considered *vs* those reported through study completion. The safety of PoV can be improved by shorter treatment and/or lower dose. Updated PFS will be presented.

P689

IDELALISIB EFFICACY AND SAFETY IN FOLLICULAR LYMPHOMA PATIENTS FROM A PHASE 2 STUDY

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Background: There is an unmet need for new treatment options in follicular lymphoma (FL), particularly for heavily pretreated, high-risk patients refractory to anti-CD20 and chemotherapy. Idelalisib, a PI3Kδ inhibitor, showed antitumor activity and acceptable tolerability as monotherapy in a pivotal phase 2, open-label study in indolent non-Hodgkin lymphoma (iNHL) refractory to rituximab (R) and an alkylating agent (NCT01282424).
Aims: This post hoc analysis evaluated efficacy and safety in the FL patient subset.
Methods: Double refractory patients with histologically confirmed iNHL received oral idelalisib 150 mg BID until disease progression (PD) or unacceptable tolerability; patients with FL (grade 1, 2, or 3a; n=72) were included in this analysis; all patients provided informed consent. Responses were evaluated by an independent review committee using standardized criteria. The primary endpoint was the overall response rate (ORR).
Results: At study entry, patients' median age was 62 y, 54% had a high-risk FLIPI score, 22% had bulky disease, and 17% had FL grade 3a. Median (range) number of prior treatments was 4 (2-12); 86% were refractory to their last therapy (32/50 to bendamustine). At data cutoff, median (range) treatment duration was 6.5 (0.6-31.0) mo, with 65 (90%) patients off treatment (PD, 38; adverse events [AEs], 15; investigator decision, 7; death, 5). Lymph node size decreased during treatment by ≥50% SPD in 57%. The ORR (95% CI) was 56% (43-67; P<0.001), including 10 complete responses (CR) and 30 partial responses. Kaplan-Meier (KM) - estimated median (range) time to response was 2.6 (1.6-11.0) mo, median response duration was 11 mo (27 mo in patients with CR), and progression-free survival was 11 mo, substantially longer *vs* the last regimen. Median overall survival (OS) was not reached; KM-estimated OS at 1, 1.5, and 2 y was 87%, 74%, and 68%. The most common AEs (any/grade ≥3,%) were diarrhea (51/14), cough (32/0), pyrexia (29/4), fatigue (28/0), and nausea (28/3). Rates of grade ≥3 transaminase elevation, pneumonitis, neutropenia, anemia, and thrombocytopenia were 14%, 4%, 22%, 3%, and 6%.
Summary and Conclusions: Idelalisib demonstrated rapid, durable responses that were substantially longer than those with the previous regimen with acceptable safety in highly refractory, relapsed FL patients with limited treatment options.
Supported by Gilead Sciences.

P690

IDELALISIB MONOTHERAPY RESULTS IN DURABLE RESPONSES IN PATIENTS WITH RELAPSED OR REFRACTORY WALDENSTROM'S MACROGLOBULINEMIA (WM)

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Background: Idelalisib (Zydelig), a selective oral inhibitor of PI3Kδ, demonstrated considerable anti-tumor activity in patients with relapsed/refractory iNHL in phase 1 (Flinn, 2014; Study 02) and refractory iNHL in phase 2 (Gopal, 2014; Study 09) trials. This analysis evaluates outcomes in the subset with WM.
Aims: To evaluate the combined safety and efficacy of idelalisib monotherapy in pts with WM in 2 clinical trials.
Methods: Eligible patients (pts) with WM included those with relapsed/refractory disease (phase 1), or those with disease refractory to both rituximab and an alkylating agent (phase 2). Idelalisib dosages were 150 mg QD, and 50 mg-200 mg BID (phase 1), and 150 mg PO BID (phase 2) and were administered continuously until disease progression. WM response was assessed by IgM levels and CT imaging (Owen, 2013).
Results: Enrolled pts, (phase 1 N=9; phase 2 N=10), had a median age of 63 and 60 years [range 42-83] and 78% and 80% were male, respectively. Patients had received a median of 4 prior regimens in both groups. Overall response rate (ORR) was 5/9 (56%), and 8/10 (80%; Table 1). Median time to minor or first response was 2 months, and most responses continued to improve over 6 months or longer. Median DOR was 32.8 months (phase 1) and not yet reached (phase 2). 67% have continued response at 2 years (phase 2). Median PFS is 33.3 months, and 22.1 months, respectively. Interestingly, >3 gram/dL improvements in hemoglobin were noted in 5/9 and 7/10 subjects, respectively, over 3-6 month timeframe. Grade ≥3 adverse events included increased ALT/AST 5/9, and 1/10, and diarrhea/colitis 1/9, and 3/10. One G3 ALT elevation and 1 G3 diarrhea resulted in study discontinuation.

Table 1.

	Phase 1 [Study 02] (n=9)	Phase 2 [Study 09] (n=10)
ORR, n (%), [95% CI]	5 (56%) [21-86]	8 (80%) [44-98]
CR	0	0
PR	1 (11%)	7 (70%)
MR	4 (44%)	1 (10%)
SD	2 (22%)	1 (10%)
PD	1 (11%)	1 (10%)
NE	1 (11%)	0

Summary and Conclusions: These combined data suggest single-agent idelalisib monotherapy is active in Waldenstrom's macroglobulinemia. Durable responses were seen in the majority of subjects. Marked improvements in hemoglobin level are also associated with response. The safety profile was acceptable and manageable, with no apparent disease-specific safety signals. Phase 3 clinical trials of idelalisib with combination therapy are in progress for pts with iNHL, including WM (NCT00710528, NCT01282424).

LB691

GADOLIN: PRIMARY RESULTS FROM A PHASE III STUDY OF OBINUTUZUMAB PLUS BENDAMUSTINE COMPARED WITH BENDAMUSTINE ALONE IN PATIENTS WITH RITUXIMAB-REFRACTORY INDOLENT NON-HODGKIN LYMPHOMA

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New Information: These data have been submitted to ASCO and ICML. The EHA submission contains additional data describing NHL subtypes and median duration of maintenance therapy, plus a PFS curve.
Background: Treatment options are limited and outcomes poor for patients with rituximab-refractory (Rit-ref) indolent non-Hodgkin lymphoma (iNHL). Bendamustine (B) has shown a median progression-free survival (PFS) outcome of 9 months and response duration of 10 months in Phase II trials in Rit-ref iNHL. Obinutuzumab (GA101/Gazyva [G]) is a glycoengineered type II anti-CD20 antibody with activity and an acceptable safety profile in Rit-ref iNHL shown in Phase I/II studies; obinutuzumab is not currently licensed in NHL. Preclinical studies have shown that combining G and B increases their activity; thus, this combination (GB) has potential for improved efficacy in comparison with B alone.
Aims: To evaluate efficacy and safety of GB *versus* B alone in patients with Rit-ref iNHL.
Methods: GADOLIN (NCT01059630) is a randomized, open-label Phase III study in patients with CD20-positive Rit-ref iNHL. In the control (B) arm, patients received single-agent therapy with B 120mg/m^2 (days 1 and 2, cycles 1-6); in the test (GB) arm patients received B 90mg/m^2 (days 1 and 2, cycles 1-6) in combination with G 1000mg (days 1, 8, and 15 of cycle 1 and day 1 of cycles 2-6) for up to six 28-day cycles. All patients gave informed consent. Non-progressing patients in the GB arm received further G monotherapy every 2 months for up to 2 years. The primary endpoint was PFS assessed by an independent radiology facility (IRF), with 80% power to detect a 43% improvement in median PFS.
Results: At a protocol-specified interim analysis, 396 patients were randomized to receive B (n=202 [198 were treated]) or GB (n=194). On February 4, 2015, the IDMC recommended to unblind the study and release the data to the scientific community as the primary endpoint (PFS) had been met. Baseline characteristics were balanced between the treatment arms and follicular lymphoma was the most common iNHL subtype (82.2% B *vs* 79.9% GB). The median age was 63 yrs and patients had received a median of two prior therapies. The median observation time was 20 months for B and 22 months for GB. IRF-assessed median PFS was 14.9 months for B and not reached (NR) for GB (hazard ratio [HR] 0.55, 95% confidence interval [CI]: 0.4-0.74; p=0.00011) (Figure 1).

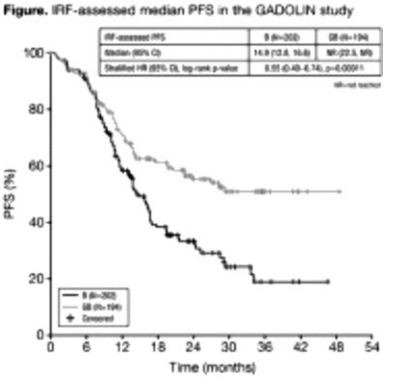

Figure 1. IRF-assessed median PFS in the GADOLIN study.

The median investigator-assessed PFS was 14 months for B and 29 months for GB (HR 0.52, 95% CI: 0.39-0.70; p<0.0001). There were no significant differences in IRF-assessed overall response rate (63.0% B *vs* 69.1% GB) or complete response (12.2% B *vs* 11.2% GB) at the end of induction, in IRF-assessed best overall response up to 12 months from the start of treatment (76.6% B *vs* 78.6% GB), or in preliminary overall survival (OS; median OS NR in either arm). The median duration of post-induction G monotherapy was 10.8 months, 74% of patients received at least one dose of G and 25% received all 12 doses. In the treatment period, there were fewer Grade ≥3 adverse events with B than with GB (62.1% B *vs* 68.0% GB), notably neutropenia (26.3% B *vs* 33.0% GB) and infusion-related reactions (3.5% B *vs* 8.8% GB), but more Grade ≥3 thrombocytopenia (16.2% B *vs* 10.8% GB), anemia (10.1% B *vs* 7.7% GB) and pneumonia (5.6% B *vs* 2.6% GB).
Summary and Conclusions: G combined with B (90 mg/m^2) followed by G maintenance significantly improved PFS *vs* B alone (120 mg/m^2) in Rit-ref iNHL. The clinically meaningful PFS improvement with GB is the first randomized evidence of benefit for a novel anti-CD20 antibody in Rit-ref iNHL.

Stem cell transplantation - Experimental

P691

RECURRENT TP53 ALTERATIONS IN PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA RELAPSING AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

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Background: Survival rates of many childhood malignancies at relapse -including patients relapsing with acute lymphoblastic leukemia (ALL) post allogeneic hematopoietic stem cell transplantation (allo-SCT)- remain very poor. These patient groups are the focus of the German-wide INdividualized therapy FOr Relapsed Malignancies in childhood (INFORM) initiative. Our group is focusing on post allo-SCT relapses since they exhibit a dismal prognosis despite various therapeutic efforts.
Aims: Since ALL relapses post allo-SCT escape both chemotherapeutic and immunologic therapies, they are assumed to display other genetic alterations compared to ALL relapses following conventional chemotherapy. The aim of our present study therefore is to identify the landscape of genetic alterations of ALL relapses post allo-SCT by whole-exome sequencing (WES). This will result in a better understanding of the pathobiology of these specific ALL cases and in more personalized treatment strategies through the identification of druggable targets. Achieving remission prior to secondary allo-SCT represents the primary clinical aim.
Methods: WES of germline and tumor DNA was performed on a HiSeq 2500 (Illumina). Obtained sequence reads were aligned to the human reference genome. Resulting variation calls were annotated by Variant Effect Predictor and imported into an in-house MySQL database. MuTect and VarScan2 were employed for identification of somatic nucleotide variations (SNVs) of each oncogenome.
Results: To address the genetic landscape of relapsed ALL following both chemotherapeutic and immunologic therapy, we sequenced five samples per patient: initial leukemia, first remission, first relapse, remission post allo-SCT, and relapse post allo-SCT. Since the latter generally occur in a situation of hematopoietic chimerism, it is important to have two genetic germline backgrounds (recipient=patient in first remission, stem cell donor=remission post allo-SCT) available subtracted to uncover those mutations exclusively defining the relapse post allo-SCT. To allow comparative analysis, we defined the following three "oncogenomes" (OGs): OG1 (initial leukemia), OG2 (first relapse), OG3 (relapse post allo-SCT). We report the results of the first 5 patients of our study. Patient age at initial diagnosis was 4-10 years (4x high, 1x medium risk; 3x B-, 2x T-ALL). Time from relapse diagnosis to allo-SCT (4x matched donor, 1x matched sibling donor) ranged from 3-5,5 months, relapse following allo-SCT occurred between 3-7 months, all patients died 1-4 months thereafter. Median numbers of leukemia-specific SN*Vs* in OG1-3 were 25, 42 and 69, respectively. More specifically, we identified 17 recurrently mutated genes present in OG3s of ≥2 patients (*e.g.* NOTCH1, TOP2A, WNT2). Of these, 9 were OG3 specific. Most notably, TP53 was mutated in 4/5 patients. One patient carried a TP53 germline mutation; 3 had novel TP53 mutations only present in their OG3s. Moreover, leukemic blasts showed plasticity concerning the mutational status of the nucleoside exporter NT5C2. Activating mutations in this gene were previously shown to drive chemotherapy resistance in relapsed ALL. We found a total of 3 SN*Vs* (1 known) in NT5C2 in the OG2s of 2/5 of our patients, but these disappeared again in the OG3s once selection pressure of maintenance chemotherapy employing nucleoside analogues had been withdrawn.
Summary and Conclusions: Comprehensive genetic analysis of the first 5 children with ALL relapsing post allo-SCT has shown leukemic cell plasticity (loss of acquired NT5C2 mutations) as well as identified several recurrent genetic alterations (*e.g.* NOTCH1, TOP2A, WNT2), most notably affecting TP53. Moreover, we have successfully established a bioinformatic analysis pipeline addressing the …two germlines" challenge in our post allo-SCT patient cohort.

P692

MESENCHYMAL STROMAL CELLS STIMULATE PROLIFERATION AND CYTOKINE PRODUCTION OF GROUP 3 INNATE LYMPHOID CELLS

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Background: Graft-*versus*-host disease (GvHD) remains a challenging complication after allogeneic hematopoietic stem cell transplantation (allo-HSCT). Transplantation of mesenchymal stromal cells (MSCs) is a frequently used secondary line of treatment for patients with steroid-refractory GvHD. The beneficial effects of MSCs in this context are mainly ascribed to their ability to modulate inflammation by inhibiting proliferation of (alloreactive) T cells. In addition MSCs might enhance tissue repair activities of other cells. We previously reported on the association between the activation status of a novel family of lymphocytes, called innate lymphoid cells (ILCs), and a reduced susceptibility to GvHD. Activated ($CD69^+$) ILC3s expressed the gut-homing marker $\alpha_4\beta_7$ and represent potent producers of the tissue-protective interleukin (IL)-22.
Aims: To test the hypothesis that MSCs may potentiate the tissue protective effects of ILC3s.
Methods: MSCs were harvested and expanded from bone marrow obtained from otherwise healthy human adults undergoing cardiac surgery with median sternotomy. ILC3s were freshly isolated from tonsils obtained from pediatric tonsillectomies. Co-cultures were performed in the presence of IL-2, for 5 days in a 1:1 ratio after which the cells were analyzed for proliferation (using a dye dilution assay) and cytokine production (by means of qPCR and intracellular cytokine stain).
Results: We observed an increased proliferation of ILC3s in the presence of MSCs (+/- pre-incubation with IFN-γ) as compared to a condition without MSCs (P=0.002). ILCs express CD127, the α-chain of the IL-7 receptor, which was downregulated upon co-culture with IL-7 producing MSCs. When IL-7 was blocked with neutralizing antibodies proliferation was significantly reduced. As compared to NK cells, ILC3s were found to have a low expression of genes encoding inhibitory cytokine receptors, such as the receptors for IL-10 and prostaglandin E2. Co-culture with MSCs resulted in an enhanced production of IL-22 (P=<0.0001) by $NKp44^+$ ILC3s. Also $NKp44^-$ ILC3s, which *ex vivo* express only low levels of IL-22, responded with a significantly enhanced IL-22 production. After co-culture with ILC3s, MSCs showed expression of the integrins vascular cell adhesion molecule 1 (VCAM1) and intracellular adhesion molecule 1 (ICAM1).
Summary and Conclusions: IL-22-producing $NKp44^+$ ILC3s represent the dominant ILC subset in the intestinal lamina propria and have important functions in mucosal homeostasis and immunity. IL-22 directly induces survival and proliferation of epithelial cells and stimulates secretion of antimicrobial peptides. Here we propose a mechanism for how MSCs may contribute to the control of GvHD, by promoting expansion and IL-22 production of tissue-restoring ILC3s. That MSCs and ILCs could serve as allies in combating GvHD is emphasized by the induced expression of the integrin VCAM1, a ligand for $\alpha_4\beta_7$, which we observed to be expressed on circulating ILCs before allo-HSCT. Further studies are needed to address the *in vivo* implications of these findings.

P693

ENDOTHELIAL CELL MICROPARTICLES-DELIVERY MIR-155 PROMOTES THE DEVELOPMENT OF AGVHD BY MODULATING T CELLS

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Background: Elevation of endothelial cell microparticles (EMPs) has been proved in acute graft-*versus*-host disease (aGVHD) patients after allogeneic hematopoietic stem cell transplantation (allo-HSCT) and recently found associated with the onset of aGVHD. However, how EMPs regulate T cells function and lead to the pathogenesis of aGVHD is rarely known.
Aims: We aimed to confirm that EMPs produced by injuried endothelial cells can regulate T cells function through carrying microRNA-155 (miR-155) in process of aGVHD by series of *in vivo* and *in vitro* experiments.
Methods: We first compared dynamic change of miR-155 of MPs in plasma of aGVHD patients, pre-aGVHD patients (+7d, +14d, +21d and +28d after allo-HSCT) and non-aGVHD patients using qRT-PCR. We then compared the expression of miR-155 in EMPs derived from TNF-α-stimulated endothelial cell line EA.hy926 cells and untreated EA.hy926 cells. Nextly, we transferred miR-155 agomir into EA.hy926 cells, collected EA.hy926 cells derived EMPs and co-cultured EMPs with T cells. Then we observed the fusion of EMPs and T cells by confocal laser-scanning microscope and measured the change of miR-155 level in T cells. Simultaneously, ELISA was used to detect secreted TNF-α, IL-4, IL-10 of T cells, Annexin V-FITC/PI double-labeled flow cytometry was used to detect apoptosis and Western blot was used to determine the caspase-3 and bcl-2 expressions of T cells. Survival rates of mice were calculated and scored clinical signs of aGVHD were determined in the C57BL/6 ($H\text{-}2^{b+}$) donor to BALB/c ($H\text{-}2^{d+}$) recipient MHC-mismatched aGVHDmodel, then miR-155 in MPs and in MPs-depleted plasma were examined in aGVHD mice.
Results: In this study, we found miR-155 level in MPs from peripheral blood was significantly higher in pre-aGVHD patients (+7d, +14d, +21d and +28d after allo-HSCT) compared with non-aGVHD patients and slightly decreased at aGVHD point. Similarly, in aGVHD mice, miR-155 in plasma MPs was sig-

nificantly higher on +3d, +7d, +14d after allo-BMT while slightly lower at aGVHD point. *In vitro,* TNF-α can induce EA.hy926 cells damage to release EMPs rich of miR-155, which was further demonstrated to be delivered into T cells and resulted in the up-regulation of miR-155 in T cells and the activation of T cells including decreased apoptosis and the transition from Th2 to Th1.This result was confirmed by the observation that EMPs derived from TNF-α and antagomir155 co-treated EA.hy926 cells increased caspase-3 expression and decreased bcl-2 expression in T cells.
Summary and Conclusions: Altogether, our data suggest that EMPs-delivery miR-155 is involved in the development of aGVHD through modulating T cell function. MiR-155 in EMPs might serve as an early predictive index as well as an early intervention target of aGVHD.

P694

PRESENCE OF KIR2DS1 RECEPTOR IN DONORS OF ALLOGENEIC HEMATOPOIETIC TRANSPLANTATION DUE TO MYELOID MALIGNANCIES IMPROVES OVERALL SURVIVAL IN RECIPIENTS WITH HLA-C2 ANTIGENS

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Background: Recognition of HLA-C2 antigen of recipient cells by the activating killer immunoglobulin like receptor (KIR), KIR2DS1 receptor on donor natural killer (NK) cell may lead to increased graft *versus* leukemia effect in patients with myeloid malignancies treated by allogeneic hematopoietic transplantation (HSCT) influencing disease free (DFS) and overall survival (OS).
Aims: The goal of the present study was to examine the effect of donor KIR status in conjunction with recipient HLA-C type on the outcome of HSCT.
Methods: In our cohort, 249 consecutive adult patients, who underwent first allogeneic HSCT (HLA-identical sibling n=117 and unrelated donor n=132) for a malignant myeloid condition, namely acute myeloid leukemia (AML, n=157), bi-phenotypic acute leukemia (n=10), chronic myeloid leukemia (n=24), myeloproliferative neoplasm (n=23) and myelodysplastic syndrome (n=35) at a single center between 2007 and 2013, were retrospectively analyzed. Median follow-up was 36 months (range 6-92 months). Genotyping for the presence of KIR genes was performed by an allele specific multiplex PCR using archived DNA samples. Low resolution HLA-C typing was performed by sequence specific oligonucleotides as part of the routine work-up prior to HSCT.
Results: The frequency of the donor KIR2DS1 gene in the entire cohort was 36.5% (91/249) while the distribution of HLA-C1/C2 groups was as follows: HLA-C1: 36.5% (91/249), HLA-C1/C2: 47.4% (118/249), HLA-C2: 16.1% (40/249). There was no difference in DFS or OS between patient subgroups stratified by the presence or absence of KIR2DS1 or HLA-C1/C2. As expected, patients with sibling donors showed significantly better DFS and OS. Further analyzes were performed exclusively focusing on the patient subgroup with KIR2DS1 positive donors (n=91). Within this subgroup, we found improved DFS and OS for patients carrying at least one copy of the HLA-C2 antigen (*i.e.* HLA-C2 homozygous and heterozygous patients combined, n=54) compared to those homozygous for the HLA-C1 antigen (n=47). DFS at 3 years for HLA-C2 carriers was 55.9% compared to HLA-C1 homozygotes with 39.4% (p=0.14). Similar comparison for OS showed 62.3% for the former and 42.3% for the latter subgroup (p=0.068). Upon performing a further stratification, we performed an identical comparison exclusively for the AML patient subgroup (HLA-C2 carriers: n=36 and HLA-C1 homozygous: n=22) indicating a 3 years DFS of 60.4% for HLA-C2 carriers compared to 45.1% for HLA-C1 homozygotes (p=0.19) and a 3 years OS of 71.7% for the former and 45.7% for the latter (p=0.047). In spite of the impressive differences of the above survival rates, due to the low case numbers, only OS among AML patients with KIR2DS1 and HLA-C2 combination was significantly better. Performing a multivariate analyzes by Cox regression among AML-patients considering age, sex, the type of conditioning (myeloablative or reduced intensity conditioning) and type of donor as covariates, the association of a favorable OS with the simultaneous presence of KIR2DS1 and HLA-C2 remained significant (hazard ratio=0.422, 95% confidence interval: 0.179-0.995, p=0.049).
Summary and Conclusions: Our results indicate that the combination of donor KIR2DS1 and recipient HLA-C2 may be a favorable genetic constellation in allogeneic HSCT for AML with respect to overall survival.

P695

ENDOTHELIAL MICROPARTICLE IS INVOLVED IN THE DEVELOPMENT OF ACUTE GVHD AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Endothelial microparticles (EMPs) are membrane-bound subcellular microparticles produced by endothelial cells in response to activation or apoptosis. Accumulating evidences have indicated that endothelial injury and EMPs release are initial steps in the development of acute graft-*versus*-host-disease (aGVHD) after allogeneic hematopoietic stem cell transplantation (allo-HSCT). However, the roles of EMPs in the feedback of endothelial injury and the development of aGVHD are poorly understood.
Aims: To investigate the role of EMPs in the development of aGVHD through evaluating their effects on endothelial cell survival and function.
Methods: Dynamic change of plasma EMPs was compared by flow cytometry in aGVHD patients and non-aGVHD patients (7, 14, 21 and 28 days after transplantation), aGVHD occurring point, and normal controls. Meanwhile, plasma TNF-αwas measured by ELISA among aforementioned groups. Primary human umbilical vein endothelial cells **(**HUVEC)and human umbilical vein endothelial cell line EA.hy926 were both treated with different concentrations of TNF-αto induce EMPs release. The level of EMPs in the supernatant was detected by confocal laser scanning microscopy and FCM with PE-labeled CD62E and FITC-labeled CD31. After treating EA.hy926 cells with 20μg/ml EMPs for 24h, we evaluated their apoptosis rates by Annexin V-FITC/PI double-labeled flow cytometry, intracellular nitric oxide (NO) production by Nitrate reduction enzymatic method, Fas and eNOS mRNA by Real-time PCR, and protein level of Fas, eNOS and phosphorylation-eNOS by Western-blot. Moreover, the angiogenic activity of EA.hy926 cells was measured by *in vitro* Matrigel tubulogenesis assay.
Results: The level of both serum EMPs and TNF-αin aGVHD group showed an increased tendency within 28d after allo-HSCT. Serum EMPs peaked at day 21, while TNF-αpeaked before EMPs. Moreover, the number of EMPs at day 21 was significantly higher in aGVHD group compared with non-aGVHD group (13819±1513 *vs* 2208±363/μL,P<0.05) at day 21. Significantly higher was found in the patients in aGVHD point group in comparison to the other groups including control groups, pre-aGVHD group and non-aGVHD group. *In vitro*, TNF-αwas found to stimulate endothelial cells to release EMPs in a dose-dependant manner. After treated with 100ng/ml TNF-α, the levels of $CD62E^{+}$EMPs increased significantly in both primary HUVEC and endothelial cell lines (HUVEC 27.7±0.54 *vs* 5.47±0.17; EA.hy926 36.5±0.63 *vs* 11.9±0.28%, P <0.05). In addition, the ratio of CD62E/CD31 of EMPs indicating that TNF-αinduced activated EMPs release instead of apoptosis EMPs. After treated with EMPs, EA.hy926 cells exhibited a significant increase of cell apoptosis rate (48.9±3.2% *vs* 7.8±1.2%, P <0.05), accompanied with an increase of Fas mRNA and protein expression. Meanwhile, they were found to produce less NO (77.54±4.52 μmol/gprot *vs* 30.85±3.20μmol/gprot, P <0.05) with decreased eNOS and phosphorylation-eNOS expression after treatment. And the tubule formation function was found weakened because the endothelial tubule length in EMPs-treated group were significantly shorter than that of control group (P <0.05).
Summary and Conclusions: At the onset of aGVHD, TNF-αinduced the damage of endothelial cells to release activated EMPs, which promote the development of aGVHD through inducing the apoptosis of endothelial cells and impair endothelial function.

P696

SIMPLE, EFFICIENT, AND SAFE PROCESSING OF FROZEN-THAWED UMBILICAL CORD-DERIVED MESENCHYMAL STROMAL CELLS APPLIED FOR THE TREATMENT OF GVHD

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Background: Human umbilical cord (UC) is a rich source for mesenchymal stromal cells (MSCs), and UC-MSCs have been reported to have multilineage potential including neural differentiation. In recent reports, MSCs have the ability to migrate to inflammatory tissues and suppress adverse immune reactions. BM-derived MSCs have been already applied for the patients with acute graft *versus* host disease (aGVHD) with promising efficacy. To avoid the potential risk of BM harvest from the donor, we intended to use umbilical cord (UC) as an alternative source for MSCs, especially for the treatment of GVHD in clinic. There were several problems in the processing of UC to be resolved. Here we introduced the new device in the explant method and found a suitable cryopreservation method of UC for clinical use.
Aims: The object of this study was to explorer an efficient and safe processing and quality evaluation methods for UC-MSCs GMP-grade banking for clinical use.
Methods: UC was collected after informed consent from the mother. MSCs (P1) were isolated from minimal size of fresh UC by an improved explants method using Cellamigo® to protect the fragments floating during the culture. The culture medium was refreshed every 3 days and the adherent cells and tissue fragments were harvested using trypsin. P1 MSCs were submitted for the quality control test including infection tests, chromosomal analysis, flowcytometry analysis and mixed lymphocyte reaction (MLR) assay to evaluate the immunosuppressive potency of the UC-MSCs. The remaining UC was cut in cross-section and incised longitudinally, immersed in the cryoprotectant StemCellbanker® and cryopreserved. After confirmation of the unit quality including 6-month baby's health, we thaw the UC and isolate MSCs by the above method

and continue to culture for further expansion in 5-stack Cellstack® chambers by P3. MLR was performed on UC-MSCs using CFSE-staining method, as *in vitro* functional assay. CFSE-labeled responder cells were analyzed by FACS. *In vivo* assay, we injected UC-MSCs into NOG mice with xenogeneic GVHD triggered by human peripheral mononuclear cells (MNC).
Results: The number of UC-MSCs isolated from 1 g of UC using the explant method with Cellamigo® was $2.9\pm1.4\times10^6$/g, which was significantly higher than that obtained without Cellamigo® ($0.66\pm0.53\times10^6$/g) (n=6, P <0.01) when cells reached 80-90% confluence. In addition, the processing time and incubation time required to reach 80-90% confluence were reduced in the improved explant method compared to the conventional method. Next, we compared the cryoprotectants to find a suitable cryopreservation method of UC tissue for clinical use. The highest yield of cells was obtained from frozen-thawed UC with serum- and xeno-free cryoprotectant, Stem-Cellbanker®, when compared with others. We obtained 1.5±0.96 MSCs from of frozen-thawed UC with Stem-Cellbanker® compared to those from fresh UC (n=4). UC-MSCs (P1) were further expanded in a large scale culture until P3. The expected final products showed more than 1×10^9 /g of original UC tissue, resulting in 30×10^9 per whole UC at least. The UC-MSCs showed spindle-shaped plastic adherent with positive CD105, CD73, CD90, CD44 and negative for CD45, CD34, CD14, CD19, and HLA -DR. They also have the ability of differentiation into adipocytes, chondroblasts *in vitro, although* osteoblasts differentiation was not enough. In MLR, 3rd party-derived UC-MSCs efficiently inhibited the responder T cells, triggered by allogeneic dendritic cells (DC). Furthermore, we found the xeno-GVHD mice treated with UC-MSCs showed a longer survival compared to the control.
Summary and Conclusions: We improved the explant method using stainless mesh resulting in the efficient and safe processing for isolation of MSCs from UC and developed the simple and safe cryopreservation method of UC tissue for clinical use. We demonstrated UC-MSCs have the immunosuppressive effect on the activated T cells in allogeneic 3rd party MLR setting. Conclusively, UC-MSCs may be a feasible alternative non-invasive source of BM-MSCs for GVHD treatment and can be considered for the prompt clinical applications.

P697

THE IMPACT OF NFAT INHIBITION ON NEUTROPHIL EFFECTOR FUNCTIONS IN PATIENTS AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Patients after allogeneic hematopoietic stem cell transplantation (HSCT) are threatened by opportunistic infection like invasive fungal diseases (IFD) partly due to immunosuppressive medication *e.g.* by calcineurin inhibitors like cyclosporine A (CsA). The nuclear factor of activated T cells (NFAT) is known as an important transcription factor downstream of calcineurin in the adaptive immune systems, but it also seems to play an important role in innate immune response by polymorphonuclear neutrophils (PMN), as indicated by recent data in rodent models.
Aims: The aim of our work is to elucidate the relevance of NFAT-dependent signals in PMN and the significance for antifungal immunity in human.
Methods: Firstly, isolated PMN from healthy donors were analyzed *in vitro* in absence or presence of CsA. In detail, we examined phagocytosis, L-selectin shedding, degranulation, generation of reactive oxygen species (ROS) and release of inflammatory mediators like IL-8 and TNF-α. After activation with lipopolysaccharide (LPS) and zymosan, phagocytosis, degranulation and L-selectin shedding were measured by flow cytometry using polychromatic microspheres and surface markers (CD11b, CD62L, CD66b). In addition, generation of ROS was analyzed by dichlorofluorescein assay (DCF), whereas activation-induced release of inflammatory mediators was measured by enzyme-linked immunosorbent assay (ELISA) and intracellular flow cytometry. Furthermore, migratory response of PMN was examined in a transwell migration assay. Secondly, blood samples of patients after HSCT under continuous CsA medication (n=17) and healthy volunteer donors (n=8) were analyzed *ex vivo* at two different time points after allogeneic HSCT (day 25-35 and 125-135) concerning their PMN effector functions as described above.
Results: CsA had no significant influence on expression of activation markers and shedding of CD62L *in vitro*. Moreover, no substantial influence of CsA on generation of ROS was detected compared to untreated controls. Along with that, activation-induced synthesis of IL-8 was not influenced in presence of CsA. However, CsA rather enhanced phagocytosis of PMN (83.5% +/- 1.7 (CsA) *vs* 71.0 +/- 1.5 (control), after stimulation with LPS), whereas their migratory capabilities were reduced (30.2% +/- 10.8 *vs* 40.1 +/- 0.9, spontaneous; 114.0% +/- 2.3 *vs* 79.8 +/- 0.6, yeast activated). Regarding the *ex vivo* analysis of HSCT patient and healthy donor blood samples, production of ROS was slightly decreased under CsA therapy (31.7% +/- 4.5 *vs* 41.4 +/- 7.0, zymosan). In contrast, CsA medication showed a stimulating effect on PMN phagocytosis which is in line with our *in vitro* data (54.2% +/- 4.1 *vs* 43.8 +/- 1.5, LPS), as well as on PMN activation (CD11b: 11897 MFI +/- 983 *vs* 9321 +/- 1279, LPS) and degranulation (CD66b: 22328 MFI +/- 2365 *vs* 9668 +/- 1359, LPS), whereas L-selectin shedding was unaffected. Furthermore, an increased production of IL-8 and TNF-α was detectable (IL-8: 18.7% +/- 4.3 *vs* 5.5 +/- 2.6; TNF-α: 20.9% +/- 3.4 *vs* 4.0 +/- 2.0, both zymosan).
Summary and Conclusions: We found an increased phagocytic activity *in vitro* and *ex vivo* in human PMN upon NFAT/calcineurin inhibition, whereas other effector mechanisms were unaffected or rather decreased *in vitro*. In addition, HSCT patients under CsA treatment displayed enhanced degranulation and inflammatory mediators production in PMN *ex vivo*. It is currently unclear whether these findings are clinically relevant for the innate immune response after HSCT and further studies are needed to distinguish this obviously multidimensional interaction of NFAT inhibition and innate immune response.

P698

INHIBITION OF TRANSFORMING GROWTH FACTOR BETA-ACTIVATED KINASE 1 SUPPRESSES ALLOREACTIVITY AND IMPROVES SURVIVAL IN A MURINE MARROW TRANSPLANTATION MODEL

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Background: Acute graft-*versus*-host disease (GVHD) is a major complication of allogeneic hematopoietic cell transplantation (HCT). Recently, much attention has been given to the role of innate immune responses in its pathogenesis. Host tissue damaged by HCT conditioning releases molecules that mediate danger signals, which bind to pattern recognition receptors on antigen-presenting cells, such as Toll-like receptors (TLRs), activate the innate immune response, and induce the upregulation of cytokines, leading to the amplification and maintenance of GVHD. The monocyte/macrophage is an important cell component in the innate immunity system; IL-32 augments the GVH reaction in contrast to the inhibitory effect of macrophage colony-stimulating factor (M-CSF). We analyzed the gene expression profiles of IL-32 and M-CSF-induced macrophages, and monocytes from HCT patients, and focused on transforming growth factor β-activated kinase 1 (TAK1), which plays a key regulatory role in various cytokine-mediated innate immunity signal transduction cascades, including the downstream signaling of TLRs.
Aims: The aim of this study was to clarify the roles of TAK1 on GVHD pathogenesis using TAK1 inhibitor *in vitro* and in an allogeneic mouse HCT model.
Methods: The present study was performed according to a protocol approved by the ethics committee of our institute, and informed consent was obtained from all the patients and healthy volunteers participating in the study. Transcriptional profiles of cytokine-induced macrophages from healthy volunteers and monocytes from post-HCT patients, as substitute for tissue macrophages (*N*=9; seven experienced GVHD, while two did not), were examined by microarray analysis and Gene set enrichment analysis (GSEA). Allogeneic mixed cell reactions (alloMCRs) were performed in the presence of 5Z-7-Oxozeaenol (OZ), a selective inhibitor of TAK1, or not. The impact of TAK1 inhibition by OZ on the production of the pro-inflammatory cytokines by LPS-stimulated monocytes was evaluated using a human proteome profiler array. In the MHC-mismatched mouse HCT model (donor, B6; recipient, BALB/c), recipient mice were given 5 μg of OZ from day 12 to day 25 to validate the effect of TAK1 inhibition on survival and GVHD severity.
Results: Gene expression signatures of M-CSF or IL-32 induced macrophages revealed difference in the TLR signaling pathway. Although the number of analyzed post-HCT patients was insufficient to make a definite clustering of monocyte gene expression, there were certain changes in the expression of TLR signaling molecules, depending on GVHD, and particularly the expression level of TAK1, a key molecule of the pathway, was elevated in monocytes from GVHD patients, accompanied by upregulation of TNFα, IL-1β, and IL-6. The TAK1 inhibitor, OZ, suppressed pro-inflammatory cytokine production in LPS-stimulated monocytes, and reduced lymphocyte proliferation in alloMCRs. OZ also reduced the donor T-cell response after dendritic cell (DC) stimulation in both donor DC and recipient DC. Finally, post-conditioning alteration of TAK1 activity significantly improved the survival of recipient mice compared with controls in the MHC-mismatched mouse transplant model (P <0.01) with a reduction in GVHD scores after day 16 (P <0.01).
Summary and Conclusions: Our findings suggest that TAK1 is a key molecule in post-transplant innate immune and inflammatory responses, and this could be a novel therapeutic target in GVHD.

P699

DISTINCT EXOSOMAL MIRNA EXPRESSION OF LATE ONSET GRAFT-*VERSUS*-HOST DISEASE IN ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Exosomes, small endosome-derived extracellular vesicles, are present in various biological fluids, such as plasma. They contain a wide range of functional proteins, mRNAs, and miRNA, thus have potential in facilitating molecular diagnosis. A recent study by Xiao *et al.* demonstrated that specific plasma miRNA signature could serve as an independent biomarker for the prediction, diagnosis, and prognosis of classic acute GVHD. Unlike classic acute GVHD, however, molecular mechanism of persistent, recurrent, late onset acute GVHD has not fully elucidated.
Aims: We therefore set out to determine clinically relevant exosomal miRNAs in patients developing late onset acute GVHD after HSCT.
Methods: We evaluated blood samples obtained from 10 patients who underwent HSCT for treatment of hematologic diseases between February 2012 and November 2013 at our institution. This study consisted of 5 patients with late onset acute GVHD (gut, n=2, liver, n=2, skin and gut, n=1), and 5 patients without GVHD (control). The diagnosis of acute GVHD was based on clinical symptoms or histologically proven by biopsy in the target organs. Patients with typical manifestations of acute GVHD occurring beyond 100 days after transplantation were considered as having late onset acute GVHD, according to the new classification made by NIH consensus criteria. Exosoms were isolated from plasma using a Total exosome isolation kit for plasma (Life Technologies). To identify exosomal miRNAs specific for late onset acute GVHD, we then compared exosomal miRNA expression between late onset acute GVHD and non-GVHD using TaqMan miRNA Array (Life Technologies). The validation of the microarray data was done by qRT-PCR. Expression levels of candidate miRNAs (miRNA-A, and B) was analyzed sequentially in a subset of late onset acute GVHD patients. Wilcoxon rank-sum test was used to determine statistical significance for comparisons between late onset acute GVHD groups and non-GVHD groups using R software. P values less than 0.05 were considered to indicate statistically significant differences.
Results: We found that exosomal miRNA are differently expressed in late onset acute GVHD compared to non-GVHD controls: a subset of miRNAs were significantly up-regulated in the exosomes of late onset acute GVHD (p□0.005) when compared with non-GVHD. Accordingly, the predicted target genes of the miRNA-A and B were obtained from MirTarBase: ERBB2, KLF13, CBFB for miRNA-A and DCX, RELN, BMI1, FBXW7 for miRNA-B were validated by luciferase reporter assay in the literature. The Database for Annotation, Visualization and Integrated Discovery (DAVID) Bioinformatics tools provided functions of the target genes for miRNA-A and B linked inflammation, T-cell activation, and immune system development and nucleotide-binding. Notably, the expression levels of the miRNA-A and B changed subsequently prior to the occurrence of clinical manifestations.
Summary and Conclusions: Although the number of patients in the current study is small, our study indicates that altered expression of certain exosomal miRNAs may play an important role in the occurrence of GVHD, therefore, may be helpful for as a potent predictive biomarker for managing patients with GVHD.

P700

B LYMPHOCYTES ARE IMPORTANT EFFECTOR CELLS IN ANTI-AML RESPONSES AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Unleashing the tumor-specific immune response by immunotherapies such as checkpoint inhibitors or allogeneic stem cell transplantation can result in long lasting tumor regression. It is thought that T cell responses are responsible for tumor rejection.
Aims: Here we tested the hypothesis that B cells contribute to tumor regression following immunotherapy by analysing the tumor-specific antibody repertoire in patients with acute myeloid leukemia (AML), a high-risk malignancy with a poor prognosis. These patients received an allogeneic hematopoietic stem cell transplantation (HSCT) that reset the immune system and led to potent graft *versus* leukemia (GvL) responses.
Methods: We selected three patients who despite the high-risk nature of their AML remained disease free after allogeneic HSCT. Of these patients we established antibody-producing clonal B cell lines following transduction of memory B cells from peripheral blood with BCL-6 and Bcl-xL and screened those for producing antibodies specifically binding to surface antigens on AML cell lines and AML blasts.
Results: A number of antibodies were identified that recognized various primary AML blasts isolated from newly diagnosed AML patients, but did not bind to healthy bone marrow, peripheral blood mononuclear cells or tissues such as liver, skin and colon. Some of the antibodies induced complement dependent cytotoxicity. Strikingly, other AML-specific antibodies (about 40% of the repertoire) induced direct cell death of cultured AML cell lines and of primary AML blasts, independent of complement or effector cells. Cytotoxicity of the antibodies was rapid, characterized by swelling and blebbing of the cells and occurred both at 37ºC and 4ºC, suggesting activation of a non-apoptotic pathway. Indeed, target cell death could not be prevented with pan-caspase inhibitors Z-VAD or Q-VD. These characteristics of cell death fit with killing of tumor cells through a necrotic pathway like oncosis. A possible target recognized by a number of these antibodies will be discussed.
Summary and Conclusions: Together, these data indicate that highly potent, tumor selective antibodies can be elicited following allogeneic HSCT in AML patients. The direct cytotoxic activities against tumor cells of a proportion of these antibodies suggest that they contribute to the GvL response.

P701

FEMUR TARGET CELL TRANSPLANTATION CAN BE ACHIEVED BY MAGNETISM-INDUCED CELL TARGET TRANSPLANTATION (MAGIC-TT) IN MURINE TRANSPLANTATION MODEL

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Background: Organ target cell transplantation will improve stem cell homing efficiency, as well as avoiding possible complications of cell diffused distribution. Here we set up a novel cell transplantation system named Magnetism-induced cell target transplantation (MagiC-TT). This work was supported by the National Natural Science Foundation of China (No. 30901367, 31070866), Outstanding Young Teachers' foundation of SMU (C1031694).
Aims: To explore the distribution and survival of donor cells transplanted by MagIC-TT *in vivo and ex vivo.*
Methods: 1) Magnetized cells: Two kinds of cells were used, C57BL/6 RFP+ mesenchymal stem cells (MSC-RFP) and C57BL/6 GFP+ CD45+ (CD45-GFP) cells. MSC-RFPs was bought from Cyagen Biosciences Inc, China, and were magnetized by self-made Au@Fe nano-particle, positive cells were sorted by MACS column. CD45-GFPs were sorted from C57BL/6 GFP-transgenic mice bone marrow by MACS anti-CD45 beads and column. 2) *Ex vivo* study: Both magnetized MSC-RFPs and CD45-GFPs and their wild type controls were study of cell morphology, cell proliferation, cell cycle and cell viability, as well as cells' migration to magnetism, transwell migration and matrigel migration assays. 3) *In vivo* study: To MSCs-RFP study, fifty C57BL/6 female GFP transgenic mice were divided into 3 magnetized cell groups and 2 non-magnetized cell groups, magnetized and wt MSCs were injected into the femur cavity of the mice. To the CD45-GFP cell study, forty C57BL/6 female mice (4 groups) were transplanted after 7.5Gy irradiation. At different time points after cells injection, FACS, bioluminescence, pathologic observation with fluorescence and confocal microscopy, together with real-time PCR were used to detect GFP and RFP cells in peripheral blood, bone marrow, liver, spleen, thymus and lung, etc. Femurs and humeri of recipients were decalcified with self-made semi-solid decalcification (SSD) to clarify the distribution of donor cells and their relationships with the microenvironment.
Results: 1) *Ex vivo* study: There were no differences between magnetized and non-magnetized MSCs or CD45 cells in cell morphology, cell proliferation, cell cycle and cell viability. The Fe particles exist within or on the surface of magnetized MSCs or CD45 cells observed by electric microscope. Within the magnetic field, magnetized cells can migrate through matrigel and transwell membrane much more efficiently, 174±22 *vs* 2±1 per 200X microscopic vision (P<0.0001); they also can migrate horizontally towards magnetism in matrigel. The most interesting is, magnetized MSCs even grow well on the roof of 24-plate (grows against gravity) in the culture medium. 2) In early hours after transplantation, lots of magnetized RFP+ or GFP+ cells exit within femur and knee joint in magnetized cells groups while few in the control groups, while many non-magnetized MSCs and CD45-GFP distributed in the lung while few in magnetized cell group. After few days without any magnetism, RFP+ or GFP+ were observed to migrate to the spleen, kidney, gut and other organs, showing the slowly release of target-transplanted-cells from femur.3) To the CD45-GFP study, the blood recovery in MagIC-TT group is much faster than the controls, especially for the platelet.
Summary and Conclusions: These results demonstrated that femur target cell transplantation can be achieved by MagIC-TT, which can be widely used for cell transplantation and cell therapy in the future.

Stem cell transplantation - Clinical 2

P702

COMPLEMENT-BINDING DONOR-SPECIFIC ANTI-HLA ANTIBODIES AND RISK OF PRIMARY GRAFT FAILURE IN HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Detection of donor-specific anti-HLA antibodies (DSA) has been associated with graft rejection in all forms of transplantation. The mechanism by which DSA increase the risk of graft failure remains unclear.
Aims: We hypothesized that complement-binding DSA are associated with engraftment failure in hematopoietic stem-cell transplantation.
Methods: We analyzed 122 haploidentical transplant recipients tested prospectively for DSA. Retrospective C1q testing was done on 22 allosensitized recipients.
Results: Twenty-two of 122 patients (18%) had DSA, 19 of which were females (86%). Seven patients with DSA (32%) rejected the graft. Median DSA level at transplant for patients who failed to engraft was 10,055 MFI *versus* 2,065 MFI for those who engrafted (p=0.007). Nine patients with DSA were C1q positive in the initial samples with median DSA level 15,279 MFI (range 1,554-28,615), compared with 7 C1q negative patients with median DSA level 2,471 MFI (665-12,254) (p=0.016). Of 9 patients, who tested positive for C1q in the initial samples, 5 patients remained C1q positive at time of transplant [all with high DSA levels (median 15,279, range 6,487-22,944)] and experienced engraftment failure, while 4 patients became C1q negative pre-transplant and all engrafted the donor cells (p=0.008) (Figure 1).

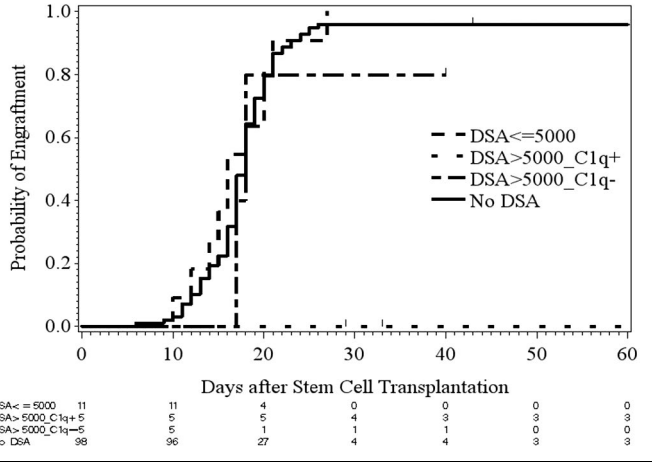

Figure 1.

Summary and Conclusions: Patients with high DSA levels (>5,000 MFI) and complement-binding antibodies (C1q positive) appear to be at much higher risk of primary graft failure. C1q should be assessed in patients with DSA prior to hematopoietic stem-cell transplantation. Reduction of DSA to non-complement binding levels might prevent engraftment failure in hematopoietic stem cell transplantation.

P703

DURABLE REMISSIONS AFTER SEQUENTIAL TRANSPLANTATION USING T-REPLETE NON-MYELOABLATIVE CONDITIONING FOR RELAPSED/ REFRACTORY ACUTE MYELOID LEUKAEMIA AND MYELODYSPLASIA

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Background: Allogeneic transplantation remains the only curative treatment for most patients with relapsed/refractory AML and high risk MDS. Conventionally, patients are treated with intensive chemotherapy to induce remission prior to transplantation. However, many patients cannot proceed to transplant using this approach because of toxicities, delayed engraftment and failure to achieve remission. The sequential allogeneic transplantation approach was introduced to address these concerns by giving intensive chemotherapy to reduce the leukaemic burden and then proceeding immediately to reduced intensity conditioned transplantation to provide a long-lasting graft-vs-leukaemia effect. To date, this approach has only been reported using T cell depleted conditioning in the context of myeloid disorders.
Aims: Following the low toxicity associated with our T-replete transplantation approach using non-myeloablative fludarabine cyclophosphamide conditioning in other clinical settings, we performed a prospective single-centre study using this transplant strategy as part of a sequential approach for patients with relapsed/refractory AML and high risk MDS.
Methods: Patients received daunorubicin (45mg/m^2 D-15-D-13) and cytarabine (1500mg/m^2 *bd* D-15-D-9) followed by fludarabine (25mg/m^2 D-6-D-2) and cyclophosphamide (1g/m^2 D-3-D-2). GvHD prophylaxis was with methotrexate and ciclosporin (CSA).
Results: 44 patients were enrolled on study between 2007 and 2014 after written informed consent. Median follow-up was 51 months. Median age was 53 years (range 23-68). Patients had refractory AML(50%), relapsed AML(30%), untreated AML(5%) and MDS(15%). Most patients (65%) had an intermediate or high co-morbidity index. No patients were in remission pre-therapy. All patients were transplanted from fully HLA-matched donors (50% sibling donors, 50% unrelated donors). All patients who survived beyond day 30 engrafted. Median time to neutrophil engraftment was 21 days. 3 year NRM was 38%, split equally between infection and GVHD. 35% developed acute GVHD (grade II-IV) and 33% of all assessable patients had chronic GVHD requiring immunosuppression, half of whom died from GVHD-related causes. Early deaths were more common in those over 60 years. Only one patient had CMV disease (colitis) and no other clinically significant viral complications occurred. No patients received donor lymphocyte infusions. With a median follow up of 5 years, the cumulative incidence of persistent or relapsed disease was 35%. 1 year and 3 year overall survival (OS) was 42% and 30% respectively. No factors impacted upon overall survival. Patients with adverse cytogenetics had a significantly higher risk of relapse (p=0.08). Patients with chronic GVHD demonstrated a trend to reduced relapse. Patients with relapsed AML and MDS had excellent outcomes with 5 yr OS of 42% and 67% respectively.
Summary and Conclusions: The rationale of using lower intensity chemotherapy and non-myeloablative conditioning followed by T-replete transplantation facilitated the treatment of an older, less fit patient group, whilst maintaining comparable outcomes to more aggressive strategies. This treatment approach is particularly effective in those with relapsed AML and MDS.

P704

SERUM FERRITIN IS INDEPENDENT OF DISEASE RISK INDEX FOR OVERALL SURVIVAL FOLLOWING ALLOGENEIC STEM CELL TRANSPLANTATION

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Background: Elevated serum ferritin has been associated with inferior survival and relapse outcomes following allogeneic stem cell transplantation (SCT). Serum ferritin is commonly used as a surrogate marker for iron overload. However, a recent meta-analysis showed no impact of liver iron concentration assessed by MRI on survival outcomes. The relationship between ferritin and survival outcome may be related to either the direct effect of iron on organ function or reflect the transfusion and/or comorbidities associated with pre-SCT disease status.
Aims: Correlation of iron studies pre-SCT with clinical outcomes.
Methods: We undertook a retrospective analysis of all allogeneic SCT recipients for haematological malignancy between 2000-2013. Iron studies were performed pre-SCT and Day 100, 12 months and 24 months post-SCT. We assessed overall survival (OS), relapse free survival (RFS) and non-relapse mortality (NRM) as a function of pre-transplant ferritin and transferrin saturation. Co-variates included in univariate analysis and then multivariate analysis if predictive of OS or RFS included age, gender, disease type, disease risk index (DRI), conditioning intensity, graft source, donor type, cytomegalovirus status, graft-*versus*-host disease (GVHD) prophylaxis and acute and/or chronic GVHD treated as time-dependent covariates.
Results: 602 patients were identified (Male n=339, Female n=263). The median age was 49 years. The main underlying diagnoses were acute myeloid leukaemia (AML) (n=223; 37%), Non-Hodgkin lymphoma (NHL) (n=96; 16%) and acute lymphoblastic leukaemia (ALL) (n=89; 15%). The pre-SCT conditioning regimen was myeloablative (n=372; 62%), reduced intensity (n=181; 30%) and non-myeloablative (n=49; 8%). Graft source was peripheral blood (PB) (n=476; 79%), bone marrow (BM) (n=96; 16%), cord blood (n=27; 4%) and both PB and BM (n=1; 0.2%). Donor type was sibling (n=362, 60%), unrelated (n=207; 34%), cord (n=26; 4%) and other family relation (n=7; 1%). Post-SCT GVHD prophylaxis was primarily with Cyclosporin and Methotrexate for the related and unrelated donor transplants and Mycophenolate mofetil for the cord blood transplants. DRI was low (n=53; 9%), intermediate (n=371; 62%), high (n=149; 25%), very high (n=5; 1%) and unable to be calculated in 24 (4%). The median ferritin levels were pre-SCT 1079ug/L, post-SCT D100 2074ug/L, 12 months 993ug/L and 24 months 740ug/L. A pre-SCT ferritin level above the median was associated with inferior OS (HR 1.84, 95%CI: 1.41-2.39) and increased relapse rates (HR 1.44, 95%CI: 1.07-1.93). High transferrin satura-

tions (>50%) were associated with inferior OS (HR 1.44, 95%CI: 1.09-1.90) but not with relapse outcomes (HR=1.10, 95%CI: 0.80-1.53). Univariate analysis determined DRI (High: HR 3.91, 95%CI: 2.08-7.35 and Very high: HR 6.4, 95%CI: 2.03-20.19 when compared to Low), disease type (NHL: HR 0.42, 95%CI: 0.27-0.66 when compared to AML) and chronic GVHD (HR 0.46, 95%CI: 0.34-0.63) as the other most significant variables impacting OS. On multivariate analysis, pre-transplant ferritin above median remained statistically significant for inferior OS (HR 1.89, 95%CI: 1.42-2.52) but lost its association with increased relapse risk (HR 1.28, 95%CI: 0.86-1.90) with DRI a strong confounding factor (High: HR=3.33, 95%CI: 1.44-7.71 and Very high: HR=10.35, 95%CI: 1.76-60.75). Pre-SCT ferritin level thresholds of 1000ug/L and 2500ug/L remained a significant predictor for OS. The main causes of death in the high/low ferritin groups were progressive disease (25%/14%), infection (7%/5%) and GVHD (6%/5%).

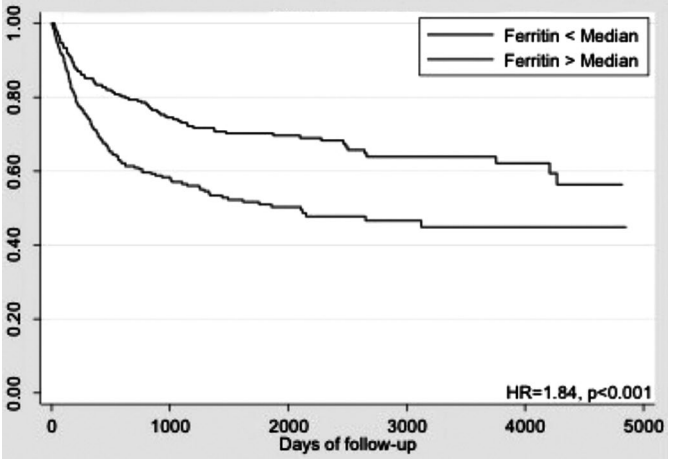

Figure 1. Kaplan-Meier survival estimates: by median ferritin.

Summary and Conclusions: A high pre-transplant serum ferritin remains an important prognostic variable in allogeneic SCT independent of DRI. Serum ferritin was a better predictor of survival outcome in comparison to transferrin saturation in our cohort of patients, although no significant prediction of relapse risk was found after adjustment for DRI.

P705

COMBINATION THERAPY WITH INOLIMOMAB AND ETANERCEPT FOR SEVERE STEROID-REFRACTORY ACUTE GRAFT-*VERSUS*-HOST DISEASE

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Background: After allogeneic stem cell transplantation (SCT) the occurrence of acute graft-*versus*-host disease (aGVHD) is an important cause of morbidity and mortality. Treatment of aGVHD consists of high-dose steroids in first-line with approximately a 50% response rate. However patients who prove refractory to this therapy, especially those who suffer from grade III-IV aGVHD with gastrointestinal involvement, have a very poor prognosis. Different therapeutic strategies have been used for second-line therapy, but responses have remained disappointing. In literature both inolimomab (monoclonal antibody targeting the interleukin-2 receptor subunit α that inhibits activated alloreactive T cells) and etanercept (TNFα-receptor fusion protein that scavenges TNFα) have shown promising results when used as monotherapy. Therefore combination therapy with these drugs might prove beneficial in patients with steroid-refractory aGVHD as two pivotal pathways in aGVHD pathogenesis are inhibited.
Aims: In this study we evaluated the efficacy and safety of second-line combination therapy, inolimomab and etanercept, for patients with steroid-refractory aGVHD. Primary endpoints were the overall response rate (ORR) on day 28 of second-line therapy and the 6-month estimated overall survival (OS).
Methods: Second-line treatment with inolimomab and etanercept started when steroid-refractory aGVHD was determined. Inolimomab was administered intravenously in a dose of 0.3 mg/kg daily from day 1 to 8 and a dose of 0.4 mg/kg every other day from day 9 to 28. Etanercept was given subcutaneously twice weekly at a dose of 25 mg during 4 weeks. Primary endpoints were overall respons rate (ORR) on day 28 of second-line therapy and the 6-month estimated overall survival (OS).
Results: From February 2010 to February 2014 21 patients with steroid-refractory grade III-IV aGVHD received treatment with the combination therapy inolimomab and etanercept. 19 had GI involvement and mean albumin levels at diagnosis were 27 g/l. Of the 21 patients 6 achieved a complete response (CR) and 4 patients a partial response (PR), bringing the overall response rate on day 28 to 48% (10/21). On day 56, the overall response rate was 33% with 5 patients retaining CR and 2 a PR. The estimated 6-month and 2-year overall survival were dismal; 24% (5/21) and 10% (2/21). Statistical significant risk factors for treatment response were determined namely aGVHD after donor lymphocyte infusion (p=0.01) and GVHD involving the liver stage 2-4 (p=0.03). There were no infusion-related adverse events. Infectious complications occurred frequently and consisted predominantly of bacteriemias with gut residing pathogens, invasive mould disease and CMV and EBV reactivation and disease.
Summary and Conclusions: Combination therapy with inolimomab and etanercept in patients with steroid-refractory grade III-IV aGVHD was moderately effective with regards to achieving a remission, but nevertheless overall survival was dismal, with only 10% of patients being alive at last follow up. New strategies are urgently needed with more effective drugs but also with approaches directed at prevention of refractory GVHD and incorporation of biomarkers that facilitate risk-adapted therapy.

P706

PURE RED CELL APLASIA IN MAJOR ABO MISMATCHED ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IS ASSOCIATED WITH SEVERE PANCYTOPENIA

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Background: The persistence of anti-donor isohemagglutinins in Major ABO mismatched allogeneic stem cell transplantation (HCT) leads to pure red cell aplasia (PRCA). In our previous report of 12 patients with PRCA, severe pancytopenia was observed in one patient who eventually had a second transplant (Aung *et al.* B J Haematol 2013 Mar; 160(6):798-805). Furthermore, ABO antigens are expressed on or adsorbed from plasma on granulocyte and platelets and production of these may be impaired by isohemagglutinins.
Aims: To further investigate this observation we analyzed a larger cohort of patients with PRCA to determine the frequency of pancytopenia and natural history of pancytopenia in patients with PRCA after major ABO incompatible HCT.
Methods: We reviewed 707 patients who received a major ABO-mismatched HCT between January 2003 and December 2012 at our institution. Pure red call aplasia was determined to be present when the bone marrow biopsy on post-transplant day 30 demonstrated absent or nearly absent erythroid precursors with absence of donor red cells on forward red cell typing of the recipient and the recipient being red cell transfusion dependent. Pancytopenia was defined as ANC $<1.5x10^9$/L or requiring G-CSF, Platelets $<50x10^9$/L or transfusion dependent, and PRCA with red cell transfusion dependence as above at 90 days after allogeneic SCT.
Results: 83 (11.7%) patients (29[35%] males: 54[65%] females) had PRCA and of these 13 (16%) (10 [77%] males]: 3[23%] females), median age 53 [range 27-66 years] had pancytopenia at day 90 after transplant. There was a female preponderance of PRCA patients, however a male predominance was noted in PRCA patients with severe pancytopenia. None of these patients had any other reason for persistent pancytopenia like CMV or other viral infection or use of drugs like ganciclovir or disease recurrence. On post-transplant day 90, median absolute neutrophil counts (ANC) was 0.93 K/UL with all patients having intermittent G-CSF. The median platelet count was 14 KL/UL (range 6-34) and all patients were platelet and red cell transfusion dependent. Of the 13 PRCA patients with pancytopenia, 2 (15%) received a second transplant due to persistent pancytopenia/graft failure, 4 (31%) patients did not recovered their platelet counts despite red cell and ANC recovery and died (3 from disease recurrence/1 from myocardial infarction). 1 (8%) patient was lost to follow up. In the remaining 5 (38%) patients, neutropenia and thrombocytopenia resolved after resolution of PRCA. Red cell recovery occurred at a median of 188 (97-549) days post-transplant, ANC recovered at median of 296 (145-743) days post-transplant, and Platelets recovered at median of 296 (190-1250) days post-transplant.
Summary and Conclusions: Severe pancytopenia is frequently (16%) associated with PRCA in major ABO incompatible HCT. Neutropenia and thrombocytopenia resolve after resolution of red cell aplasia in the majority of patients.

P707

DONOR CHIMERISM AS MARKER OF RELAPSES AFTER BONE MARROW TRANSPLANTATION.

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Background: Allogeneic hematopoietic stem cell transplantation is an effective treatment for different hematological malignances. Efficiency of donor cell engraftment is determined by donor chimerism evaluation, *i.e.* determining of genetically different cell populations coexistence in patient. We assume that the kinetics of chimerism in patients with hematologic malignancies, in turn, may be defined in

terms of relapse probability, and it could be used as a marker of minimal residual disease (MRD) after allogeneic transplantation of HSCs (allo-HSCT).
Aims: Evaluation of donor chimerism definition in assessing the likelihood of posttransplant relapses.
Methods: We have analyzed 440 allo-HSCT's in patients with various hematologic malignancies. For the donor chimerism monitoring we used standard panels of human DNA markers based on STR variability (short tandem repeats, microsatellites). The ratios of donor and recipient cells were calculated according to the data derived from fragment analysis, using the Genemarker program. The study was performed on days +15, +30, +45, +60 and +100 after transplantation.
Results: Achieving of full donor chimerism (95%) significantly increases the 5-year survival (p=0.001) and reduces the probability of disease recurrence (p=0.01). Among patients with mixed chimerism the stability of donor chimerism level is most important, being associated with lower risk of relapse (p=0.013). Thus, in patients with stable mixed chimerism, the markers molecular markers of MRD had no significant effect either on overall survival, or the likelihood of relapse (p=0.55 and p=0.34 respectively). Meanwhile, we had found an inverse correlation between the level of donor chimerism and expression of relapse markers, *i.e. WT1* (R=-0.4, p=0.0001) and *EVI1* (R=-0.16, p=0.03). Similar data were obtained when analyzing expression of the chimeric transcripts: *BCR-ABL* (R=-0.26, p=0.0001), *MLL-AF4* (R=-0.36, p=0.003), TEL-AML (R=-0.37, p=0.003). Hovewer, this dependence with other markers (*CBFB-MYH11*, *PML-RARα*, *RUNX1-RUNX1T1*) could be also obtained.
Summary and Conclusions: Monitoring of donor chimerism after allo-HSCT adequately reflects the dynamics of donor cells engraftment and may be used as an adequate method for predicting recurrence of the disease, but it can not be used as a single method in MRD analysis.

P708

PERIPHERAL BLOOD EOSINOPHIL COUNT AT DAY 28 AS A PROGNOSTIC INDICATOR IN ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Peripheral blood eosinophilia has been reported as a favorable prognostic indicator for patients with hematologic disease after allogeneic hematopoietic stem cell transplantation (HSCT). It has been reported to be associated with chronic Graft *versus* Host Disease (GVHD) and decreased relapse rate. Eosinophils play an important role in alloimmune reactions after HSCT. However, the relationship between eosinophilia after HSCT and acute GVHD is still controversial, and the clinical relevance of peripheral blood eosinophil count (PB-Eos) at the early phase after HSCT is not clear.
Aims: The purpose of this study was to clarify the clinical relevance of PB-Eos at the early phase after HSCT.
Methods: We enrolled 180 adult patients with hematological neoplasms who underwent their first HSCT between November 2004 and August 2013 at Niigata University Hospital and Nagaoka Red Cross Hospital in this study. Forty-four of the 180 patients were excluded from the analysis because of graft failure, using corticosteroids before day 28 after HSCT, or inadequate laboratory data. We analyzed retrospectively 136 patients, including 55 with acute myelogenous leukemia, 40 with acute lymphoblastic leukemia, 22 with myelodysplastic syndrome, 3 with myeloproliferative neoplasm, 2 with chronic myelogenous leukemia, 3 with adult T cell leukemia, 1 with mixed lineage leukemia, and 10 with malignant lymphoma. Median age of patients was 42 years (range: 16-66). Fifty-six patients received related stem cell transplantation, 50 received unrelated stem cell transplantation, and 30 received cord blood transplantation. Chi-squared analysis and Fisher's exact test were used to assess the relationship between PB-Eos and acute GVHD. Overall survival (OS) was based on Kaplan-Meier estimates. Cumulative relapse rate (RR) was based on Gray's estimates. Multivariate Cox regression for OS and Fine-Gray regression for RR were used to identify the independent risk factors.
Results: Median PB-Eos at day 28 was 48 /μl (range: 0-3078 /μl). We divided the patients into 2 groups, those with PB-Eos of more than 50 /μl and those with PB-Eos of less than 50 /μl at day 28. According to univariate analysis, PB-Eos of <50 /μl at day 28 tended to indicate a poor prognosis of OS (p=0.096). In multivariate analysis adjusting for age, grade 2-4 acute GVHD after day 28, disease status and conditioning regimen, and PB-Eos of less than 50 /μl at day 28 were independent poor prognostic factors for OS (HR: 1.86 (1.05-3.31), p=0.034). In patients with acute GVHD of any grade, PB-Eos of <50/μl at day 28 was an independent prognostic factor for OS (HR: 3.14 (1.34-7.34), p=0.0085) and RR (HR: 4.45 (1.65-12.06), p=0.003). In contrast, it was not a prognostic factor for OS or RR in patients without acute GVHD. Next, we analyzed the relationship between PB-Eos and acute GVHD. PB-Eos was not associated with development of grade 2-4 acute GVHD after day 28 by Chi-squared analysis (p=0.55) and grade of acute GVHD by Fisher's exact test (p=0.37). Furthermore, in patients with grade 2-4 acute GVHD after day 28, PB-Eos of <50/μl at day 28 was also an independent prognostic factor for OS (HR: 3.87 (1.12-13.37), p=0.033) and RR (HR: 4.88 (1.26-18.82), p=0.021).
Summary and Conclusions: PB-Eos at day 28 is an independent prognostic indicator for OS in HSCT. In particular, lower RR may contribute to better OS in patients with acute GVHD. The presence of eosinophils at the early phase of HSCT could predict a good prognosis in HSCT.

P709

AUTOLOGOUS STEM CELL TRANSPLANTATION WITH HIGH-DOSE MITOXANTRONE AND MELPHALAN IN PATIENTS WITH HODGKIN AND NON-HODGKIN LYMPHOMA: LONG-TERM SURVIVAL AND TOXICITY DATA

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Background: In patients with high risk or relapsed lymphoma, a satisfying response rate and long-term survival cannot be achieved by conventional chemotherapy regimens. Despite reports of high-dose chemotherapy regimens coupled with or without autologous stem cell transplantation (Auto-SCT), medical literature reporting long-term survival and toxicity data beyond 10 years is rare. In the autologous transplant setting, use of high-dose mitoxantrone coupled with high-dose melphalan as conditioning protocol (HDMTZMEL) is relatively less frequent, compared to the well known BEAM regimen.
Aims: In this study, we report disease-free (DFS), long-term overall survival (OS) and long-term toxicity in patients with lymphoma treated with HDMTZMEL conditioning regimen followed by Auto-SCT during the past 15 years (2000-2014).
Methods: 107 patients relapsed or resistant diffuse large B-cell NHL (DLBCL, n=49) or Hodgkin lymphoma (HL, n=58) underwent Auto-SCT after administration of the conditioning regimen consisting of high-dose mitoxantrone (60 mg/m^2) and melphalan (180 mg/m^2) following greater than 50% response to the salvage regimens; DHAP or ICE or IIVP and/or HD-VP16. Treatment did not include prophylactic cranial radiotherapy. Patients over 65 years of age and with CNS involvement prior to salvage regimen and refractory to both initial therapy and the salvage regimens were excluded. Patients had a median age of 39 (16-63) and proceeded with Auto-SCT only after CR or PR was achieved.
Results: Following salvage chemotherapy, 68 (63.5%) patients achieved CR, and 36 (36,4%) achieved PR. Transplant related mortality (TRM) rate at day +100 was%0,9 in all patients, with an OS rate of 72.4% and 67,2% at 5 and 10 years, respectively. In patients with DLBCL, 5 and 10 year OS were 70.4%, whereas patients with HL had 74.9% and 67.4% OS rates, respectively. Five-year DFS was calculated as 62.9% (66.8% for DLBCL, 61.8% for HL) and did not change at 10 years and beyond. No statistically significant difference in OS and DFS rates was found between patient groups. In the DLBCL group, relapse was not observed after 29 months, whereas late relapses were observed until 57 months in patients with HL. In both groups, OS at 15 years were identical to OS at 10 years. Patients transplanted in CR had an OS rate of 80.4% at 5 yr (78.8% for NHL, 82.7% for HL, 71.5% after 10 years) whereas patients transplanted in PR had an OS rate of 55.7% at 5 and 10 years (52.5% for NHL, 57.7% for HL) and beyond (CR *vs* PR, p=0.01 for NHL and p=0.3 for HL). Patients transplanted in CR had an DFS rate of 72.9% at 5 yr and beyond (76.2% for NHL, 71.9% for HL) whereas patients transplanted in PR had an DFS rate of 42.4% at 5 and beyond (43.8% for NHL, 44.9% for HL) and beyond (CR *vs* PR, p=0.004 for all patients, p=0.2 for NHL and p=0.04 for HL). Late complications and toxicities during the post-transplant period include; late relapses (>3 years post-transplant) among patients with HL (n=3), secondary malignancy between 1-3 years (n=2,1 AML, 1 MDS), delayed platelet engraftment (n=2), congestive heart failure (n=1) corresponding to a late toxicity rate of 2.8% (Figure 1).

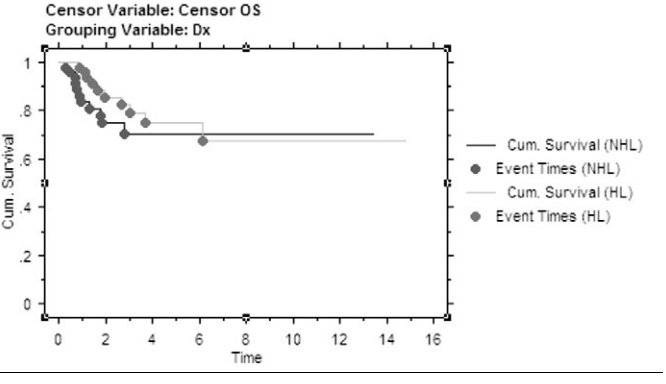

Figure 1. Kaplan-Meier cum. Survival plot for OS.

Summary and Conclusions: In patients with relapsed or resistant DLBCL and HL sensitive to pre-transplant salvage chemotherapy, HDMTZMEL conditioning regimen is capable of inducing high rates of OS and DFS rates with a low toxicity profile. In the DLBCL group, patients undergoing transplantation in CR have significantly higher overall survival and cure rate. In both DLBCL and HL groups, DFS is significantly better if the patients are transplanted in CR.

P710

RISK FACTORS FOR TRANSPLANTATION-ASSOCIATED THROMBOTIC MICROANGIOPATHY AND OUTCOME OF PATIENTS, FOLLOWING ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Transplantation-associated thrombotic microangiopathy (TA-TMA) is a feared complication of allogeneic hematopoietic stem cell transplantation (allo-HSCT) due to its high mortality. Endothelial damage and activation, attributed to multiple clinical factors, are thought to be the key elements in the induction of TA-TMA.
Aims: Our work focused on the assessment of the frequency, risk factors and prognostic impact of TA-TMA in allo-HSCT.
Methods: Data of 425 consecutive adult patients, who underwent first allo-HSCT for a malignant hematological condition at a single centre between 2007 and 2013, were retrospectively analysed.
The median age at transplantation was 43 years (range 19-73 years). The most common indications for the procedure were acute leukaemias. The donor stem cells originated from a sibling donor in 217/425 (51%) patients. Myeloablative conditioning (MAC) was administered to 267 (63%) patients, whereas 158 (37%) patients received reduced intensity conditioning. Graft-*versus*-host disease (GvHD) prophylaxis consisted of cyclosporin-, or tacrolimus-based regimens.
Results: The cumulative incidence of TA-TMA was 19% (80/425) in the entire cohort, and 65 (81%) patients developed TA-TMA within the first 100 post-transplant days. The majority of TA-TMA patients (83%) also suffered from acute GvHD (aGvHD), usually preceding TA-TMA. Several risk factors were identified in association with the manifestation of TA-TMA: aGvHD grade II-IV ($p<0.001$), unrelated donor type ($p<0.001$), MAC ($p=0.003$), tacrolimus-based GvHD prophylaxis ($p=0.003$) and cytomegalovirus (CMV) reactivation/disease ($p=0.004$). Overall survival rates of patients with TA-TMA were significantly lower compared to patients without the complication ($p<0.001$, 48-months overall survival 20+/-5 *vs* 52+/-3%). Among those affected by TA-TMA, the survival was poorer ($p=0.05$), if clinical signs of organ damage were present. Within the TA-TMA group, severe (grade III-IV) aGvHD influenced the survival the most adversely.
Summary and Conclusions: TA-TMA developed early following HSCT and was precipitated by several patient, and transplantation related risk factors, such as aGvHD, unrelated donor, MAC, tacrolimus administration and CMV infection. The survival of patients with TA-TMA was significantly poorer compared to patients without TA-TMA.
Our findings confirm that TA-TMA remains a severe complication of allo-HSCT, with the contribution of multiple factors and poor prognosis.

P711

REITERATED THERAPEUTIC DRUG MONITORING (TDM) TO BETTER CONTROL TARGETED EXPOSURE TO IV BUSULFAN IN INFANTS AND OLDER CHILDREN UNDERGOING HEMATOPOIETIC STEM-CELL TRANSPLANTATION (HSCT)

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Background: Busulfan (Bu) is the standard backbone for HSCT conditioning regimens (CR) worldwide. It has a narrow therapeutic window (TW), and graft rejection and toxicity may be related to Bu exposure. In infants, Bu exhibits large pharmacokinetic (PK) variability, and its metabolic clearance is non-linearly related to body weight (BW). In this setting, Bu dosage is stratified according to BW and commonly monitored once after the 1st of 16 total 2-hr infusions (96-hr exposure).
Aims: To optimize Bu duration/intensity exposure in children undergoing HSCT, we studied the possible contribution of double TDM of IV Bu in children receiving Bu-based CR for HSCT, by comparing the targeted and calculated exposure values after performing 1 PK (1st dose, PK1) and finally 2 PK (1st dose, PK1, and 9th dose, PK2).
Methods: In this single-centre observational study (05/2012-02/2015), 50 patients (Pts) receiving Bu based myeloablative CR for HSCT were prospectively included with median follow up of 12 months [0-33]. There were 33 males and 17 females. Median age was 16.5 months [1-193] and BW 11.0 kg [3-59]. Most Pts had non-malignant diseases, received allogenic HSCT and Bu-based CR in combination with fludarabine. The 1st dose and the tight and high TW were set up according to EBMT-ESID recommendations [ΣAUC=20,706-23,180 µmol/L*min]. Bu PK was assessed on 3 plasma samples/PK (LC-MS2 analysis) with area under the concentration-time curve (AUC) calculation from the 1st and 9th doses, using the NONMEM® software. AUC calculated after the 1st dose and extrapolated to the 16th one were compared by a Wilcoxon signed-rank test to the targeted AUC and to the sum of $AUC_{1\text{-}6}$ with TDM1 applied from the 7th dose with or without TDM2 applied from the 14th dose; $p\leq0.025$ was considered statistically significant.
Results: An initial median Bu posology of 1.16 mg/kgx4/day for 4 days was given. We demonstrate that: (a) calculated total AUC obtained from 2 PK differ significantly from those calculated after no PK ($p=2.7006$ E-6) or PK1 alone ($p=3.4750$ E-7), (b) double TDM allows achieving no difference between the targeted AUC desired by the medical staff in view of diseases *vs* calculated AUC ($p=0.8939$), (c) calculated total AUC obtained after no PK or PK1 alone significantly differed from targeted AUC. In 2/50 (4%) Pts, PK did not lead to change Bu dosage. In 9/50 (18%) and 12/50 (24%) Pts, dosage was modified after PK1 (TDM1 Group) or PK2 alone, respectively. In 27/50 (54%) Pts, changes were required twice, after PK1 and PK2 (Double-TDM Group). The median total doses of Bu were as follows: (a) a theoretical value of 220.8 mg (20.2 mg/kg) would have been given without any TDM (16 consecutive doses), (b) 194.7 mg (18.1 mg/kg) were administered in the TDM1 Group (16 doses), (c) 242.8 mg (19.5 mg/kg) were administered in the Double-TDM Group, preventing the unnecessary infusion of 20.0 mg of Bu *vs* PK1 alone in these Pts. In 15/50 (30%) Pts, a decision of discontinuation of Bu exposure was taken as the target desired by the medical staff was achieved after 13 (6 Pts) or 14 (9 Pts) doses; the mean total amounts of Bu were 273.2 mg (15.0 mg/kg) and 231.0 mg (15.6 mg/kg), respectively, *vs* 336.3 mg (18.5 mg/kg) and 278.2 mg (19.5 mg/kg) theoretically after TDM1 alone.
Summary and Conclusions: In this pediatric series, the double TDM of Bu is a relevant and feasible option to better achieve a narrow TW and to potentially minimize the risk of overexposure. Correlations with toxicities (VOD, aGVH), CR, OS are under analysis. Based on these data, the double-TDM procedure is routinely applied in our centre.

P712

FACTORS DETERMINING THE EFFECTIVENESS OF ACUTE GRAFT *VERSUS* HOST DISEASE PROPHYLAXIS AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION WITH MULTIPOTENT MESENCHYMAL STROMAL CELLS

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Background: Currently acute graft-*versus*-host disease (aGVHD) is one of the main problems after allogeneic bone marrow transplantation (allo-BMT) in patients with hematological malignancies. The new method involving the use of donors' multipotent mesenchymal stromal cells (MSC) was developed (ClinicalTrials.gov NCT01941394). In some cases prophylaxis of aGVHD with MSC failed. The reasons of failure could be either the result of particular qualities of donor-recipient interaction, patient status or characteristics of MSC samples.
Aims: The aim of the study was to analyze the main characteristics of MSC used for aGVHD prophylaxis based on the clinical outcome.
Methods: The study included 67 patients received allo-BMT from related donors after informed consent. The patients were randomized into 2 groups: first received standard prophylaxis of aGVHD (34) and the second (33) were additionally infused with MSC from the bone marrow of corresponding hematopoietic stem cells donor. MSC were cultivated in aMEM with 4% donors' platelet lysate. MSC were administered intravenously when the blood counts indicated recovery (peripheral blood leukocytes reached $1x10^9$/l). For analysis MSC were cultivated in standard conditions (aMEM, 10% fetal calf serum) for 3 passages. Relative expression level (REL) of 30 genes involved in proliferation, differentiation and immunomodulation was estimated by RT-qPCR in all MSC.
Results: Observation of the patients for 100 days (typical time of aGVHD manifestation) had revealed significant 3-time decrease in aGVHD development in the group of patients who received MSC (9.4% aGVHD grade II-IV) when compared to the standard prophylaxis group (29.4% aGVHD grade II-IV), $p=0.041$. MSC injection did not affect the engraftment as well as the relapse frequency. Overall survival of the patients was significantly higher in the MSC received group $p=0.048$. Three of 32 MSC samples had been ineffective for preventing aGVHD. Analysis of individual donor characteristics (gender, age, body mass index), found no significant differences between the MSC, effective and ineffective for preventing aGVHD. The cumulative MSC production of ineffective samples was 2 fold lower ($p=0.05$) than effective ones. REL of CSF1, FGFR1 and PDGFRB was 4 ($p=0.0005$), 2.5 ($p=0.02$) and 2.5 ($p=0.02$) fold decreased correspondingly in ineffective samples. REL of other 27 studied genes involved in proliferation, differentiation and immunomodulation did not differ significantly in MSC effective and ineffective for aGVHD prophylaxis.
The combination of predictors that characterize the most suitable for the prevention of aGVHD MSC samples was revealed by multiple logistic regression

analysis. A model calculating the probability of the success of MSC samples application was proposed:

$$logit(P)=2.5-0.26*CFH+0.02*IDO1-0.65*IGF1$$

where logit(P)=ln[P/(1-P)], P - probability of successful prophylaxis, CFH, IDO1 and IGF1 - REL of corresponding genes in tested MSC. Chi-square goodness of fit test p=0.0053. The calculated efficiency of this model was 95%. CFH and IDO1 are important for immunomodulation by MSC, while IGF1 is the gene associated with aging. So this statistical model seems reasonable for primary estimation of MSC efficiency for aGVHD prophylaxis.

Summary and Conclusions: The high variability of all analyzed characteristics among MSC from different donors was shown. By means of the proposed models ineffective MSC samples could be discharged. Such strategy hopefully will prevent the development of aGVHD in the maximum number of patients.

Stem cell transplantation - Clinical 4

P713

IMPACT OF HISTOLOGIC GRADE ON THE OUTCOME OF ALLOGENEIC STEM CELL TRANSPLANTATION IN FOLLICULAR LYMPHOMAS

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Background: Allogeneic stem cell transplantation (AlloSCT) represents an effective treatment for relapsed and refractory follicular lymphoma (FL) and it is the only curative treatment approach in this particular clinical setting. Indeed, graft *versus* lymphoma (GVL) effect is superior in patients affected by FL as compared to those affected by diffuse large B-cell lymphoma (DLBCL). Therefore, also patients with active disease at time of AlloSCT can achieve a long-term survival. Grade 3B FL, both for its histological features and clinical behavior, is generally considered as an aggressive lymphoma. However, it is completely unknown the outcome of patients with Grade 3B FL receiving AlloSCT.
Aims: We investigated the impact of histologic grade on the outcome of AlloSCT in relapsed/refractory FL patients
Methods: In this study, we retrospectively analysed a cohort of 62 FL pts transplanted in 5 Italian Institutions between December 2001 and November 2014. All patients fulfilled the following criteria: 1) confirmed histologic diagnosis of FL including revised histological grading of each case; 2) pre-transplant disease status and response evaluated by computed tomography (CT) and/or positron emission tomography (PET) and assessed according to criteria established by Cheson *et al.,* JCO 2007. Fifty (80.6%) patients had a diagnosis of grade 1-3A and 12 of Grade 3B FL. Twenty-eight (45%) patients were transplanted from related, 28 (45%) from unrelated and 6 (10%) from haploidentical donors. Thirty-three patients (53%) were characterized by the presence of active disease before AlloSCT [20 partial remission, 1 stable disease and 11 disease progression (PD)]. No significant pre-transplant clinical differences (age, disease status, previous chemotherapy lines, stem cell source and donor types) were observed in patients with grade 1-3A *versus* 3B.
Results: Considering the whole patient cohort, we confirmed the favorable outcome after AlloSCT with 5-years EFS, PFS and OS estimates of 83.6% (95% CI, 78.2% to 89%), 61.3% (95% CI, 54.7% to 67.9%) and 69.1% (95% CI, 63% to 75.2%), respectively. No differences in EFS, PFS and OS were observed among Grade 1, 2 and 3A. Conversely, Grade 3B FL patients were characterized by a worse outcome compared to the other FL in terms of 5-years EFS [24.2% (95% CI, 21.5% to 26.9%) *vs* 90.7% (95% CI, 86.2% to 95.2%); p=0.0004], PFS [11.1% (95% CI, 0.7% to 21.5%) *vs* 72% (95% CI, 65.3% to 78.7%); p<0.0001] and OS [42.8% (95% CI, 27.2% to 58.4%) *vs* 74.7% (95% CI, 68.3% to 81.1%); p=0.02] estimates. Among patients with active disease and grade 1-3A, AlloSCT confirmed its high efficacy with 5-years EFS, PFS and OS estimates of 75.2% (95% CI, 67% to 83.4%), 55.9% (95% CI, 46.6% to 65.2%) and 63% (95% CI, 56% to 71%) respectively. Conversely, all grade 3B patients with active disease before AlloSCT suffered an early relapse (p<0.01), suggesting a lower GVL efficacy compared to other FL grades. Grade 3B histology retained its independence for EFS, PFS and OS in multivariate analysis including: age, pre transplantation disease status, graft source (peripheral blood *versus* bone marrow), intensity of the conditioning regimen (RIC *versus* MAC), acute and chronic GVHD occurrence and previous autologous SCT.
Summary and Conclusions: Overall, these data confirm the favorable prognosis of relapse/refractory FL patients Grade 1-3A undergoing AlloSCT, including those with active disease. Conversely, our study suggests that 3B FL patients should be treated like DLBCL rather than FL. This different approach might be particularly important among patients with active disease before AlloSCT.

P714

ALPHA/BETA T-CELL DEPLETED ALLOGENEIC STEM CELL TRANSPLANTATION FROM MATCHED RELATED AND UNRELATED DONOR GRAFTS IN PATIENTS WITH HIGH RISK HEMATOLOGICAL MALIGNANCIES

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Background: The outcome of allo-SCT in patients with poor risk leukemia is still hampered by GVHD and relapse. The innate immune system has been reported to contribute to tumor control, with lower incidence of GVHD. Specific depletion of αβ T- cells - key players in the development of GVHD - will render NK cells and γδT cells within the allograft. Recently reported results have shown

the great promise of this approach in haploindentical transplantations. Within this study, we aim to extend αβT- cell depleted allo-SCT to patients with a mathed related donor (MRD) or matched unrelated donor (MUD).
Aims: An "Innate-Tx" (αβ T cell depleted allograft from MRD/MUD) is aimed to serve as a low toxicity allo-SCT platform for futee immune interventions. As such it should have reduced levels of GVHD as compared to T cell replete allo-SCT, withour an increase in clinical significant infections or relapse.
Methods: All patients with hematological malignancies and an indication for allo-SCT were eligible. Either HLA matched siblings or HLA matched (9 or 10/10) unrelated donors (MUD) were eligible. αβT-cell reduction was performed by negative selection with anti-αβTCR antibodies in combination with magnetic microbeads, using the automated CliniMACS device (Miltenyi Biotec, Bergisch Gladbach, Germany). The maximal contamination with αβT-cells was $5x10^5$/kg. The conditioning regimen consisted of: ATG (Genzyme®) 4 or 6 mg/m^2+fludarabine 120 mg/m^2+busilvex AUC=90 followed by αβT- cell depleted grafts from matched related or unrelated donors. No additional immune suppression was given post allo-SCT.
Results: A ~4 log depletion of αβT-cells has been observed in the product with a recovery of ~75% of $CD34^+$ cells. The combination of ATG/fludarabine/busilvex was well tolerated with a hematological recovery within 3 weeks. Most patients received a pre-emptive low dose DLI ($1x10^5$ T cells/kg). Primary engraftment (chimerism >95%) was observed in all patients (n=14). Immune reconstitution primarily consisted of NK cells, showing a contraction around 4 months post SCT. In addition, γδT cells were present at normal numbers up to 6 months post SCT, whereas the adaptive immune repertoire shows a delayed reconstitution. Under this "innate control", no increase in CMV or EBV reactivations has been observed so far. Up to date, none of the patients developed aGVHD >grade II, none of the patients developed a relapse and no treatment related deads have been observed.
Summary and Conclusions: Here we show that an conditioning regimen of ATG/busilvex/fludarabin in combination with an αβT- cell depleted allo-SCT from MRD/MUD results in a swift reconstitution of innate cells (NK cells and γδT cells) the first 6 months post transplantation, followed by a subsequent reconstitution of the adaptive immune repertoire. After a median follow-up of 5 months, we observed low levels of aGVHD, without a concomitant increase in graft failure and infections. To address the incidence of cGVHD, relapse rate and overall survival a longer follow-up is required. However, the low incidence of aGVHD in this protocol suggests that this transplantation strategy can serve as a platform for subsequent interventions such as administration of tumor specific drugs, vaccination or tranfer of tumor specific T cells.

P715

OUTCOMES OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANT IN PATIENTS WITH MDS/MPN OVERLAP SYNDROMES- A SINGLE INSTITUTIONAL EXPERIENCE

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Background: Clonal myeloid neoplasms with overlapping features of myelodysplastic syndrome (MDS) and myeloproliferative neoplasms (MPN), based on the 2008 WHO criteria, are categorized as MDS/MPN overlap syndromes. This subgroup includes well-defined entities such as, chronic myelomonocytic leukemia (CMML), juvenile myelomonocytic leukemia (JMML) and atypical chronic myeloid leukemia (aCML), along with lesser-defined entities such as, refractory anemia with ring sideroblast and thrombocytosis (RARS-T) and MDS/MPN- unclassifiable (MDS/MPN-U). Clinical outcomes are variable and allogeneic hematopoietic stem cell transplant (allo-HCT) remains the only potentially curative option.
Aims: To describe transplant and survival outcomes in patients with MDS/MPN overlap syndromes that underwent allo-HCT.
Methods: After IRB approval, consecutive patients with MDS/MPN overlap who underwent allo-HCT from 1990 to 2014 were identified and data was retrospectively abstracted.
Results: Of 51 patients (males: 62%) with MDS/MPN overlap, 36 had CMML (70%), 6 JMML (12%), 8 MDS/MPN-U (16%) and 1 aCML (2%). Median age was 54 years (range, 18- 68 years) in adult patients and 38 months (range, 3- 72 months) in JMML patients. (a) CMML: Of 36 patients (median age 51 years, range 66-18 years), 19 (52%) had CMML-1 (13 myeloablative conditioning, MAC; 6 reduced intensity conditioning, RIC) and 17 had CMML-2 (9 MAC; 8 RIC). The proportion that proceeded directly to transplant without prior chemotherapy was similar between MAC (12 patients/ 55%) and RIC (8 patients/ 57%). Post-transplant CR was achieved in 23 (64%) patients (MAC: 15, RIC 8). Median follow-up was 21 months (range 0.7- 191) with 2 years overall survival of 44% (n=16) and non-relapse survival of 36% (n=13). Common causes of non-relapse mortality (NRM) were related to chronic GVHD (27%) and sepsis (27%). 27 patients (75%) experienced acute-GVHD (grade 3/4: 9, 33%) and 22 (61%) had chronic-GVHD. More patients with chronic-GVHD died due to non-relapse causes (71% NRM *versus* 40% in those without chronic-GVHD). In multivariate analysis, engraftment failure (n=1, p <0.001) and higher HCT-comorbidity index (HCT-CI) were independent predictors of survival (HCT-CI >3, n=23; HCT-CI ≤3, n=13; p=0.03), and importantly, none of the conventional risk stratifying models predicted survival (Patnaik BJH 2014). (b) JMML: All 6 patients underwent MAC allo-HCT (5 peripheral blood stem cell transplant (PBSCT) and 1 umbilical cord blood transplant, (UCT)). Acute-GVHD occurred in 1 (grade 2) and chronic-GVHD in 2 patients. Median follow-up was 11 years and at last follow-up, 1 patient had died (1.8 years post-transplant) due to sepsis following bowel obstruction. 2 patients relapsed in the first year post-transplant and achieved remission with subsequent transplants. (c) MDS/MPN-U: Of the 8 patients (5 PBSCT, 1 BMT, 2 UCT), 6 (75%) underwent RIC allo-HCT. 7 patients (88%) experienced acute-GVHD (grade 3/4: 3, 43%) and 4 had chronic-GVHD. Median follow-up was 15 months. At last follow up, 4 patients (50%) were alive, 3 died due to relapse (3 months, 5 months, 19 months) and 1 died in remission due to immune mediated lung injury. (d) aCML: One female with aCML received PBSCT following MAC. She died 6.7 years post-transplant due to chronic-GVHD (Table 1).

Table 1. Clinical and laboratory features of CMML patients who have undergone allogeneic stem cell transplant.

Variable	Myeloablative conditioning n = 22 (61%)	Reduced intensity conditioning n = 14 (39%)	*P value*
Age in years; median (range)	49 (26- 62)	55 (18- 66)	**0.0038**
Males; n (%)	13 (59)	10 (71)	0.45
World Health Organization morphological subtype; n (%)			
Chronic myelomonocytic leukemia- 1	13 (59)	6 (43)	0.34
Chronic myelomonocytic leukemia- 2	9 (41)	8 (57)	
Pre-transplant hemoglobin g/dL; median (range)	9.7 (7.6- 14.4)	10.5 (9- 12.3)	0.11
Pre-transplant WBC x 10^9/L; median (range)	1.8 (0.5- 14.7)	3.1 (0.1- 47.4)	0.27
Pre-transplant ANC x 10^9/L; median (range)	1.4 (0.02- 11.33)	1.89 (0- 27.7)	0.13
Pre-transplant AMC x 10^9/L; median (range)	2 (0.15- 19.6)	3.1 (0.69- 23.5)	0.32
Pre-transplant platelets x 10^9/L; median (range)	36.5 (8- 295)	65 (18- 606)	0.23
Splenomegaly at diagnosis; n (%)	4 (18)	4 (29)	0.46
Spanish cytogenetic risk stratification; n (%)			
High	6 (27)	3 (21)	
Intermediate	5 (23)	2 (14)	0.68
Low	11 (50)	9 (65)	
MD Anderson prognostic risk categories; n (%)			
High	2 (9)	3 (21)	
Intermediate- 1	8 (37)	3 (21)	0.66
Intermediate- 2	3 (13)	2 (15)	
Low	9 (41)	6 (43)	
Mayo model prognostic risk categories; n (%)			
High	12 (54)	8 (57)	
Intermediate	7 (32)	2 (14)	0.36
Low	3 (14)	4 (29)	
French-American-British classification			
Myelodysplastic syndrome type	12 (55)	5 (35)	0.26
Myeloproliferative neoplasm type	10 (45)	9 (64)	
Hematopoietic stem cell transplant comorbidity index (range)	3 (0- 10)	2 (0-8)	0.35
Pre-transplant therapy; n (%)			
Best Supportive Care	8 (36)	2 (14)	
Hypomethylating Agent	2 (9)	4 (28)	0.17
Cytoreductive Therapy	12 (55)	8 (58)	
ABO compatibility types; n (%)			
Compatible	13 (59)	9 (64)	
Major	0	4 (29)	**0.012**
Minor	5 (23)	1 (7)	
Bidirectional	1 (4)	0	
Unknown	3 (14)	0	
Cytomegalovirus compatibility; n (%)			
Compatible	8 (36)	7 (50)	
Non-compatible	10 (46)	7 (50)	0.11
Unknown	4 (18)	0	
Graft source; n (%)			
Peripheral blood	14 (63)	14 (100)	
Bone marrow	7 (32)	0	**0.009**
Double cord blood	1 (5)	0	
Donor type; n (%) "N" evaluable= 35			
Matched sibling donor	15 (71)	4 (29)	
Matched unrelated donor	4 (19)	9 (64)	**0.021**
Mismatched unrelated donor (9/10)	2 (10)	1 (7)	
Graft versus host disease prophylaxis; n (%)			
Cyclosporine	4 (18)	2 (14)	
Cyclosporine + Methotrextate	14 (64)	3 (22)	**0.0026**
Tacrolimus + Methotrextate	2 (9)	9 (64)	
Unknown	2 (9)	0	
Date of diagnosis- date of transplant; median days (range)	202 (62- 706)	221 (80- 855)	0.22
Red blood cell engraftment in days; median (range)	28 (19- 152)	32 (19- 78)	0.96
Neutrophil engraftment in days; median (range)	21.5 (13- 51)	21 (13- 32)	0.41
Platelet engraftment in days; median (range)	18.5 (10- 42)	16 (3- 26)	0.53
Graft failure; n (%)	0	1 (7)	0.164
Acute graft versus host disease; n (%)	17 (77)	10 (71)	0.399
Acute graft versus host disease; overall grade (range)	1 (0-4)	2 (0-4)	0.70
Chronic graft versus host disease; n (%) "N" evaluable= 33			
No chronic graft versus host disease	6 (27)	5 (36)	
Mild	4 (18)	2 (14)	0.53
Moderate	3 (14)	3 (22)	
Severe	8 (36)	2 (14)	

Key: WBC, white blood cell count; ANC, absolute neutrophil count; AMC, absolute monocyte count; TNC, total nucleated cells.

Summary and Conclusions: Allo-HCT is a viable treatment option for patients with MDS/MPN overlap syndromes, with 3-year combined disease-free survival rates of 32% in adults with CMML and aCML, 43% in JMML and 1-year disease-free survival rate of 62% in adults with MDS/MPN-U. In patients with CMML, engraftment failure and co-morbidity index have prognostic implications, while conventional prognostic models were unable to predict transplant outcomes.

P716

FEASIBILITY OF LENALIDOMIDE MAINTENANCE AFTER NONMYELOABLATIVE ALLOGENEIC TRANSPLANTATION (NMAT) IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

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Background: NMAT is an effective therapy for relapsed/refractory CLL patients (pts). Results and safety using the FCR (fludarabine, cyclophosphamide, rituximab) regimen were previously described (Khouri *et al.* Cancer 2011; 117:4679-

88). About 50% of pts with refractory disease at study entry required immunomanipulation (IMM)[early immunosuppression withdrawal after NMAT and donor lymphocyte infusion (DLI)]. This is a significant cause of morbidity and graft-*versus*-host- disease (GVHD). Maintenance with lenalinomide post NMAT may improve outcomes by promoting the immune-mediated graft-*versus*-tumor effect and lessen the need for IMM.
Aims: To assess the feasibility (dose, toxicity and GVHD) of lenalidomide maintenance after NMAT in CLL.
Methods: This is a phase II randomized study that included relapsed/refractory CLL pts who were eligible to undertake NMAT after FCR conditioning [later replaced with the advent of the BFR regimen (bendamustine, fludarabine, rituximab) in 2011 (Khouri *et al. Blood* 2014; 124:2306)]. Pts were randomized at day 90-100 post NMAT to receive lenalinomide if they had persistent active CLL, engrafted donor cells, and no active GVHD, with ANC≥1,500/μL and platelets ≥70,000/μL. Lenalidomide was initiated at 5mg every other day (qod) with an increase to 10mg/day if tolerated.
Results: Of the 38 pts studied, 17 (48%) met the eligibility for randomization. Characteristics of non-randomized pts (n=21; Group A), pts randomized to receive lenalinomide maintenance (LM+) (n=8, Group B), or not (n=9, Group C) were similar and included median age (60, 55 and 56 years in the 3 groups, respectively), sex distribution, and percent of pts with ≥3 prior therapies (60% *vs* 38% *vs* 44%, respectively), percent of pts with β2-microglobulin ≥3 mg/L at study entry, refractory disease (75% *vs* 50% *vs* 67%, in the 3 groups respectively), unmutated status (100% *vs* 67% *vs* 100%, in the 3 groups respectively), donor/recipient CMV, sex-mismatched distributions and donor type (matched unrelated in 65%, 50% and 56%, in Groups A,B and C, respectively). Presence of 17p deletion was detected in 30% of Group A and 22% in Group C; none of the pts randomized to LM+ had 17p deletion. FCR was the NMAT conditioning in 55%, 75% and 78% of pts in the 3 groups, respectively, and the remaining pts received BFR. One pt refused LM+ after randomization. Maximum tolerated dose for LM+ was 5 mg qod in one pt due to dermatitis, 5 mg/day in 2 pts (cytopenia in one; liver toxicity and cytopenia in the other), 10 mg/day in 4 pts (1 opted to stop at 2.5 months and one died of pneumonia at 2 months; one achieved complete remission (CR) and stopped LM+ at 4 months due to rash and pt later relapsed; and one took 10 mg/day for 1 year without achieving CR. The need for IMM was 53%, 57% and 56%, in groups A, B, and C respectively. DLIs were needed in 10% *vs* 38% and 22%, respectively. The incidence of acute grade 2-4 GVHD was 47% for group A, and 50% *vs* 11% for groups B and C (P=0.11). The incidence of chronic GVHD was 40% *vs* 42% *vs* 59% in the 3 groups, respectively. Treatment-related-mortality at 1-year was 30% *vs* 12.5%, and 11%, in Groups A, B and C, respectively. With a median follow-up time of 31 months (range, 13-65), the 3-year overall survival rates for groups A,B and C were 42% *vs* 56% *vs* 63.5%, respectively (P=0.6) and the progression-free survival rates were 30% *vs* 25% *vs* 67%, respectively. The use of BFR resulted in a trend for a better OS (2-year 83% *vs* 54%) and PFS (64% *vs* 33%) than the FCR regimen.
Summary and Conclusions: This trial suggests that maintenance lenalidomide after NMAT for CLL is not feasible and that alternative strategies are needed to treat persistent disease . The preliminary results after BFR conditioning are encouraging and a larger trial is ongoing to confirm these findings.

P717

ENGRAFTMENT SYNDROME (ES) AFTER ALLOGENEIC STEM CELL TRANSPLANTATION: A HIGH INCIDENCE IN HAPLOIDENTICAL SETTING
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Background: Engraftment syndrome, a clinical entity presented with non-infectious fever, skin rash within peri-engrfatment period, has been described in patients receiving various types of allogeneic stem cell transplantation (SCT). However, ES in haplo setting remains poorly characterized, and the prognosis and appropriate management are unclear.
Aims: Here, we attempted to determine the incidence, manifestations, and outcomes of ES after haploidentical SCT.
Methods: We compared the incidence of ES in 516 patients received haploidentical SCT and in 393 patients received HLA-identical sibling SCT, between Octorber 2001 and Octorber 2012 were collected retrospectively. ES was described as unexplained fever and skin rash within 24 h of the start of neutrophil recovery by Maiolino's criteria.
Results: The incidence of ES was significantly higher in haploidentical type than the other one (21.9% *vs* 2.0%, $P < 0.001$). Major symptomes included fever (119/121, 98.3%), skin rash (98/121, 81.0%) and diarrhea (51/121, 42.1%), with the median time of +10 (6-20) days. Significantly higher median increases in CRP level were observed in 13 samples of ES group than in 38 samples of the non-ES group (99.0 *vs* 13.9 mg/L, $P<0.001$). Similarly, the results showed higher plasma concentration of C3 in the ES group compared with non-ES group (meadian: 1.30 *vs* 1.16 g/L, $P=0.003$). ES patients had a significantly higher incidence of grade II-IV aGVHD and than the non-ES group (42.1% *vs* 24.6%, $P<0.001$). No difference of non-relapse mortality or overall survival were noted between ES group and non-ES group in each transplant setting.
Summary and Conclusions: Higher incidence of ES was observed in haploidentical type, but it did't predict poor clinical outcomes.

P718

A PHASE 3, RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED TRIAL OF PLERIXAFOR+G-CSF *VS* PLACEBO+G-CSF TO MOBILIZE ≥5 X 106 CD34+ CELLS/KG IN CHINESE NHL PATIENTS FOR AUTOLOGOUS TRANSPLANTATION
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Background: Plerixafor, a small molecule chemokine receptor 4 (CXCR4) antagonist, has been shown to increase mobilization of CD34+ cells for Caucasian non-hodgkin's lymphoma (NHL) patients in a Phase 3 trial.
Aims: This Phase 3 trial was conducted to confirm efficacy and safety of plerixafor in Chinese NHL patients.
Methods: Patients in first or second complete remission or partial remission received morning granulocyto colony stimulating factor (G-CSF, 10μg/kg/day). Since Day 4 evening, patients started plerixafor 0.24 mg/kg/day or placebo (1:1 ratio) in addition to G-CSF treatment for a maximum of 4 apheresis days. Apheresis could be discontinued if optimal target ($5x10^6$ CD34+ cells/kg) was reached earlier.
Results: One hundred patients were randomized. A total of 12 patients (3 in plerixafor arm and 9 in placebo arm) discontinued mainly due to adverse events (1 in plerixafor arm and 7 in placebo arm). Patients' demographics and baseline characteristics were similar between groups. The primary endpoint was met as 62% (31/50) patients in the plerixafor group, and 20% (10/50) patients in the placebo group achieved optimal target ($p<0.0001$). 88% patients with plerixafor and 66% patients with placebo reached minimum target ($2 X 10^6$ CD34+ cells/kg). Median time needed to achieve optimal target was 2 days in plerixafor group and not reached in placebo group. Median time to engraftment of neutrophil (10 days for both groups) and platelet (19 days *vs* 17 days) were similar between groups. The incidence of adverse events was similar between groups (62.7% *vs* 63.3%). Neither unexpected adverse events nor serious adverse events were observed in treatment period.
Summary and Conclusions: This study demonstrated that plerixafor plus G-CSF was superior to G-CSF alone to mobilize CD34+ cells for autologous transplantation in Chinese NHL patients. Patients who received plerixafor plus G-CSF were statistically significantly more likely to achieve both optimal and minimum CD34+ cells collection targets and in less time than patients receiving G-CSF alone. Plerixafor plus G-CSF was a safe and well tolerated mobilization regimen in this Chinese NHL population.

P719

INFLUENCE OF CYP3A5 GENE POLYMORPHISMS ON PHARMACOKINETICS OF TACROLIMUS MODIFIED-RELEASE ONCE-DAILY AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION
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Background: Modified-release, once-daily dosage of tacrolimus (Tac-QD) has been developed to provide more convenient dosing and to improve patient adherence. Tac-QD is confirmed to have equivalent safety and efficacy as twice-daily tacrolimus (Tac-BID), and the renal dysfunction side effect of Taq-QD is the same as Taq-BID. Tac-QD is strongly affected by cytochrome P450 (CYP) 3A4/5, which is expressed in the small intestine and hepatocytes. The blood concentration of tacrolimus in a patient who has received renal transplantation with CYP3A5 **3/*3* (poor metabolizer ; PM) is higher than CYP3A5 **1/*1* or **1/*3* (extensive metabolizer ; EM) patients. Furthermore, the azole antifungal agents, fluconazole, itraconazole and voriconazole, are routinely used as prophylaxis against Aspergillus infection and administered to HSCT recipients receiving tacrolimus, and those azole antifungal agents potently inhibit the activity of CYP3A. 24 patients who underwent allogeneic HSCT were

enrolled. Before HSCT, CYP3A5 **3* allele was detected using a PCR-restriction fragment length polymorphism method. After HSCT, continuous infusion of 0.03 mg/kg/day tacrolimus was administered to prevent acute graft-*versus*-host disease (aGVHD). On days 20-30 after transplantation, patients were switched to Tac-QD administered about 4 times orally. We collected venous blood samples just before (C_{0h}) to determine the blood concentration of Tac-QD and check the organs dysfunction. From day 7 after oral administration of Tac-QD, 21 patients were administered orally azole antifungal agent. After the co-administration of azole antifungal agent, we were subjected to the same test. Before and after the co-administration of azole antifungal agent and Tac-QD, blood concentrations of tacrolimus were determined by the chemiluminescence magnetic microparticle immunoassay.

Aims: We identified influence of CYP3A5 gene polymorphisms on Tac-QD in patients after hematopoietic stem cell transplantation (HSCT).

Methods: 24 patients who underwent allogeneic HSCT were enrolled. Before HSCT, CYP3A5 **3* allele was detected using a PCR-restriction fragment length polymorphism method. After HSCT, continuous infusion of 0.03 mg/kg/day tacrolimus was administered to prevent acute graft-*versus*-host disease (aGVHD). On days 20-30 after transplantation, patients were switched to Tac-QD administered about 4 times orally. We collected venous blood samples just before (C_{0h}) to determine the blood concentration of Tac-QD and check the organs dysfunction. From day 7 after oral administration of Tac-QD, 21 patients were administered orally azole antifungal agent. After the co-administration of azole antifungal agent, we were subjected to the same test. Before and after the co-administration of azole antifungal agent and Tac-QD, blood concentrations of tacrolimus were determined by the chemiluminescence magnetic microparticle immunoassay.

Results: 14 patients have CYP3A5 **3/*3* (PM) and 10 patients have CYP3A5 **1/*1* or **1/*3* (EM). Before the co-administration of azole antifungal agent, we did not recognize significant differences between CYP3A5-PMs and CYP3A5-EMs about C_{0h} and dose of tacrolimus. However, after the co-administration of azole antifungal agent, although CYP3A5-PMs had a reduced dose of Tac-QD than CYP3A5-EMs (p=0.041), C_{0h} of tacrolimus was higher with a significant difference (p=0.034). Additionally, acute renal dysfunction occurring in patients with elevated C_{0h} of tacrolimus was remarkable. The risk factors of acute renal dysfunction were CYP3A5-PM (p=0.024) and C_{0h} with azole antifungal agent co-administration of tacrolimus (p=0.020). C_{0h} of tacrolimus which is a measure of the onset acute renal dysfunction was 10 ng/ml. The virus infection and fungal infection showed no significant differences between CYP3A5-PMs and CYP3A5-EMs.

Summary and Conclusions: In this study, we recognized that C_{0h} of tacrolimus with azole antifungal agent co-administration in CYP3A5-PMs was higher than that of CYP3A5-EMs, and acute renal dysfunction was occurred. Therefore, when we administer Tac-QD with azole antifungal agent co-administration in CYP3A5-PMs, Tac-QD dosage should be individualized based on therapeutic drug monitoring and CYP3A5 genotype information.

P720

Abstract withdrawn

P721

INTERFERONA: A POTENTIAL EFFECTIVE TREATMENT FOR MINIMAL RESIDUAL DISEASE IN ACUTE LEUKEMIA/MYELODYSPLASTIC SYNDROME AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Preemptive intervention with donor lymphocyte infusion (DLI) based on minimal residual disease (MRD) status is one of the most important methods for relapse prophylaxis in acute leukemia patients receiving allogeneic hematopoietic stem cell transplantation (HSCT). However, some patients could not receive further DLI because of provider refusal; in addition, some patients disagree to receive DLI after HSCT. Interferon (IFN)-α may be the potential alternative option for MRD positive patients after allogeneic HSCT.

Aims: The safety and efficacy of preemptive IFN-α were investigated and were compared with those of preemptive DLI in patients who were minimal residual disease (MRD) positive after allogeneic HSCT.

Methods: Patients receiving allogeneic HSCT were eligible if they had acute leukemia or myelodysplastic syndromes and MRD turning positive after HSCT. The patients who could receive DLI were assigned to a preemptive DLI group (n=49). The patients who could not receive DLI or be disagree to receive DLI after HSCT received preemptive IFN-α(n=25). MRD was monitored according to leukemia-associated immunophenotypes and genes. Informed consent was obtained from all patients, and the study was conducted in accordance with the Declaration of Helsinki. The study protocol was approved by the Ethics Committee of Peking University People's Hospital.

Results: A total of 25 patients received preemptive IFN-α, and the median duration of IFN-αtherapy was 35 days (4-180 days). Nine patients relapsed, and 1 patient died from severe pneumonia. The 2-year cumulative incidence of chronic graft-*versus*-host disease (cGVHD) after HSCT was 88.0% and 59.1% in IFN-α and DLI groups (*P*=0.006). The MRD status after preemptive intervention was comparable between IFN-α and DLI groups, and the 2-year cumulative incidence of relapse was 33.7% and 39.4%, respectively (*P*=0.812). The 2-year cumulative incidence of non-relapse mortality was 4.0% and 2.1%, respectively, in IFN-α and DLI groups (*P*=0.678). The 2-year probabilities of disease-free survival was 67.5% and 58.5%, respectively, in IFN-α and DLI groups (*P*=0.711). In multivariate analysis, early-onset MRD, persistent MRD after intervention, and non-cGVHD after intervention were significantly associated with poorer clinical outcomes.

Summary and Conclusions: Preemptive IFN-α may be the potential alternative option for MRD-positive patients who could not receive preemptive DLI after HSCT.

P722

INCIDENCE, RISK FACTORS, AND OUTCOME OF EARLY HEMOSTATIC COMPLICATIONS IN PATIENTS AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION: A RETROSPECTIVE MULTICENTER STUDY OF 551 PATIENTS

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Background: Hemostatic disorders are common and potentially fatal complications in patients undergoing allogeneic hematopoietic stem-cell transplantation. However, Limited data exists on early diagnosis and prevention of these complications.

Aims: To explore the incidence, risk factors, and outcome of early hemostatic complications in patients after allogeneic hematopoietic stem cell transplantation.

Methods: In this study, 551 allogeneic transplantation recipients were enrolled to investigate the incidence, risk factors, and outcome of thrombotic or bleeding complications in the first 100 days after transplantation.

Results: Of all the patients, 261 cases (47.4%) developed bleeding events, the cumulative incidence of minor, moderate, and severe bleeding was 28.9%, 14.9% and 3.8%, respectively. The incidence of thrombotic complications was 4.5% (25/551 cases), consisting of 15 cases of veno-occlusive disease, 7 thrombotic microangiopathy, 2 pulmonary embolism, and 1 deep vein thrombosis. Risk factor analysis demonstrated that veno-occlusive disease, II-IV acute graft-*versus*-host disease and cord blood transplantation were independent predictors for bleeding complications in multivariant analysis and platelet counts less than 10×10^9/L were significantly associated with severe bleeding. Meanwhile, mismatched donor, polyomavirus BK infection, cytomegalovirus infection and II-IV acute graft-*versus*-host disease were potential risk factors for late-onset hemorrhagic cystitis, while total body irradiation conditioning regimen and high-risk disease status prior to transplantation were significantly associated with the occurrence of thrombotic disorders. Severe hemorrhage and early-onset of thrombotic disorders independently increased the mortality of allogeneic transplantation recipients. However, late-onset hemorrhage cystitis did not appear to affect the survival rate significantly.

Summary and Conclusions: Our study demonstrated that hemostatic complications following transplantation have much high mortality. Therefore, early diagnosis and therapeutic intervention of hemostatic complications are crucial to improve the outcome of allogeneic hematopoietic stem cell transplantation recipients.

Gene therapy, cellular immunotherapy and vaccination

P723

A PROSPECTIVE CLINICAL TRIAL OF PROLONGED AUTOLOGOUS ERYTHROPOIETIN (EPO) SECRETION FROM TARGTEPO IN EPO-DEPENDENT END STAGE RENAL DISEASE PATIENTS SHOWED EPO INDEPENDENCE FOR OVER 8 MONTHS

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Background: Recombinant human Erythropoietin (rHuEPO) along with iron supplementation corrects anemia in most patients with End Stage Renal Disease (ESRD) but is associated with supra-physiological peak serum concentration (C_{max}) of EPO and may cause thromboembolic complications in these patients. The Transduced Autologous Restorative Gene Therapy system (TARGT™) is an *ex-vivo* gene therapy platform that provides autologous, continuous protein or peptide therapy in the physiological range. The system consists of several 2x30 mm pieces of dermal tissue (Micro-Organ, MO), extracted under local anesthetic in which its fibroblasts cells are transduced with a viral vector containing a gene of interest. $TARGT_{EPO}$ refers to a TARGT that provides EPO. After culture, and measurement of *in-vitro* EPO production, one or more transduced MOs secreting EPO ($TARGT_{EPO}$s) are re-implanted subcutaneously as required for delivering the target dose. Patients are treated with local steroid injection to stabilize secretion. The system allows dose flexibility and the TARGTs may be added or removed according to the *in-vivo* secretion levels.
Aims: We present here initial results from an-ongoing open label ascending dose exploratory clinical study of $TARGT_{EPO}$ in patients with anemia due to ESRD.
Methods: This study was approved by the Ethics Committees of the participating sites in Israel and the $TARGT_{EPO}$ product was cleared for clinical testing by the Israeli Ministry of Health. All patients have signed an informed consent prior to enrollment in the study. We have completed the enrollment of patients in the first out of 3 cohorts (the low dose) with 6 EPO-dependent patients treated with a total of up to 3 $TARGT_{EPO}$ units each, secreting a total of 25 IU/Kg/Day of autologous EPO. All patients continued their previous regimen of intravenous supplemental iron.
Results: Efficacy data in this ongoing study is being continuously assessed as per protocol. Patient follow-up post implantation is ongoing, while the first patient implanted has completed 8 months of follow up with stable EPO secretion and stable Hb. Results obtained from the 6 patients enrolled thus far suggest stabilization of serum EPO levels at the physiological range of ≤20 mIU/ml and the resulting Hb levels between 9-12 g/dL without rHuEPO or transfusion while $TARGT_{EPO}$s are still functioning. Comparative analysis of serum EPO levels revealed significantly lower C_{max} with $TARGT_{EPO}$ compared to rHuEPO. Also, comparison of extrapolated Area Under the Curve (AUC) of rhEPO *vs* actual $TARGT_{EPO}$ AUC, revealed that $TARGT_{EPO}$ maintained Hb within the desired range in these patients with an order of magnitude smaller exposure to EPO compared to rHuEPO. This observation may have significant clinical benefits. No treatment related serious adverse events have been reported.
Summary and Conclusions: $TARGT_{EPO}$ is a promising novel therapy for anemia and the TARGT platform may have promise for other diseases requiring protein or peptide based treatment.

P724

ADOPTIVE IMMUNOTHERAPY OF MULTIPLE MYELOMA (MM) WITH ALLOGENIC CAR T-CELLS TARGETING CS1: ENHANCEMENT OF CAR ACTIVITY THROUGH CS1 GENE INACTIVATION IN EFFECTOR CELLS

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Background: Chimeric antigen receptor (CAR)-redirected T-cells have been successfully used in patients with refractory leukemia by targeting the CD19 molecule, yielding long-term durable remissions and raising hopes that a wider application of CAR technology may lead to a new paradigm in cancer treatment. Nevertheless, the CAR approach is restricted to targeting antigens expressed at the tumoral cell surface but absent from T-cells, since CAR expression will deplete antigen-expressing T-cells and thus reduce the chances to efficiently produce CAR specific T-cells. Multiple Myeloma (MM) is a B-cell neoplasia characterized by clonal expansion of malignant plasma cells in the bone marrow, and although currently available therapies can improve overall survival it still remains incurable in most patients. Immunotherapy against MM is one area in which extensive research is being made, with novel antigenic targets being considered to drive eradication of plasma cells. Among these, the CS1 glycoprotein is highly expressed on tumor cells from most of patients with MM, making it an attractive antigen for CAR targeting.
Aims: Expression of CS1 on normal CD8+ T-cells is potentially an obstacle for the development of CAR T-cells targeting CS1. The objective of this work was therefore to evaluate if CS1 expression on T-cells could have an impact on CAR activity.
Methods: To address this limitation, Transcription Activator-Like Effector Nuclease (TALEN) gene editing technology was used to inactivate the CS1 gene in T-cells, prior to transduction with a lentiviral vector encoding an anti-CS1 CAR.
Results: We will present data showing that non-gene-edited T-cells expressing an anti-CS1 CAR display limited cytolytic activity against MM cells expressing the CS1 antigen as well as a progressive loss of CD8+ T-cells in the effector cell population. In contrast, CS1-gene-edited CAR cells display significantly increased cytotoxic activity with the percentage of CD8+ T-cells remaining unaffected. Experiments in an orthotopic MM mouse model are currently ongoing, in order to investigate the impact of CS1 gene disruption on *in vivo* antitumoral activity.
Summary and Conclusions: Gene editing technology can also be used to manufacture T-cells from third-party healthy donors to generate allogeneic "off-the-shelf" engineered CAR+ T-cell-based frozen products. We have previously demonstrated that TALEn mediated inactivation of the TCRa constant (TRAC) gene can be achieved at high frequencies and eliminate the potential for edited T-cells to mediate Graft *versus* Host Disease (GvHD). Furthermore, multiplex genome editing can lead to the production of double KO (TRAC and CS1) T-cells, allowing large scale manufacturing of allogeneic, non alloreactive CS1 specific T-cells that display enhanced antitumor activity. This technology therefore offers the possibility of developing an off-the-shelf cell therapy product that would be immediately available for administration to a large number of MM patients.

P725

EVALUATING HUMAN T-CELL THERAPY OF CYTOMEGALOVIRUS ORGAN DISEASE IN HLA-TRANSGENIC MICE

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Background: Reactivation of human cytomegalovirus (HCMV) can cause severe disease in recipients of hematopoietic stem cell transplantation. Although preclinical research in murine models as well as clinical trials have provided 'proof of concept' for infection control by pre-emptive CD8 T-cell immunotherapy, there exists no predictive model to experimentally evaluate parameters that determine antiviral efficacy of human T cells in terms of virus control in functional organs, prevention of organ disease, and host survival benefit.
Aims: Here we introduce a novel mouse model for testing HCMV epitope-specific human T cells. In this, we combined the well-described murine model of mCMV infection of the immunocompromised host with the strong T-cell immunogenicity of the HLA-A*0201 restricted HCMV epitope $pp65_{495-503}$ NLVPMVATV (NLV). We generated a chimeric recombinant mCMV expressing the NLV epitope (mCMV-NLV) during the infectious cycle to allow organ manifestation of the infection in the natural host similar to that seen in immunocompromised patients.
Methods: For construction of the chimeric virus mCMV-NLV we integrated the coding gene sequence of the HLA-A2.1 restricted $pp65_{495-503}$ peptide epitope NLVPMVATV (NLV) of HCMV into the immediate-early (IE)2/m128 gene of mCMV-Δm157. To determine *in vivo* antigenicity of mCMV-NLV and protective activity of NLV epitope-specific T cells, we infected sublethally irradiated HLA-A2.1 transgenic NOD/SCID/IL-2Rγc$^{-/-}$ mice transgenic (NSG/HHD) by intraplantar injection with $1x10^5$ PFU of mCMV-NLV, a dose that would be lethal after approximately 12 to 17 days. Shortly thereafter, we adoptively transferred murine or human NLV-specific T cells in order to mimic the clinical setting of pre-emptive therapy, and analyzed viral titers in spleen and lungs as well as numbers of infected cells in livers of individual mice on day 11.
Results: After infection of immunocompromised NSG/HHD mice, mCMV-NLV resulted in a rapid systemic infection that could be effectively combated by adoptively transferred murine and human NLV-specific CD8 T cells as well as by human CD8/CD4 T cells transduced with an NLV-specific T-cell receptor. Upon transfer, the T cells infiltrated host tissues in an epitope-specific manner, confining the infection to nodular inflammatory foci. This resulted in a significant reduction of viral load, prevention of organ pathology, and prolonged survival. Collectively, these data demonstrated that NLV-specific human T cells mediate HCMV NLV-specific immune control in mCMV-NLV infected NSG/HHD mice, even across the human-mouse species barrier.
Summary and Conclusions: The model has proven its potential for a preclinical testing of the protective antiviral efficacy of HCMV epitope-specific human T cells in the evaluation of new approaches to an immunotherapy of CMV disease.

P726

NOVEL HUMAN IL-15 SUPERAGONIST PROMOTES LONG-TERM PERSISTENCE AND REACTIVITY OF HUMAN EBV- AND LEUKEMIA-SPECIFIC CD8+ T CELLS WITH STEM CELL MEMORY AND CENTRAL MEMORY PROPERTIES IN HUMANIZED MICE

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Background: Adoptive cellular therapy (ACT) using leukemia-reactive T cells has evolved as a promising strategy to improve antileukemic immunity. However, durable clinical responses are often hampered by limited sustained reactivity of fully differentiated effector T cells or graft-vs-host disease when HLA-matched donor-derived T cells are applied. In addition to the transfer of leukemia-reactive stem cell memory (T_{SCM}) and central memory (T_{CM}) T cells, IL-15 has emerged as a promising cytokine to improve ACT as it facilitates the *in vitro* generation of leukemia-reactivity from naive $CD8^+CD45RA^+$ precursors, supports engraftment of adoptively transferred $CD8^+$ T cells in lymphopenic hosts and promotes homeostasis and formation of T cell memory.
Aims: In the current study we therefore investigated whether a novel IL-15 superagonist could promote engraftment, expansion, persistence and cytolitic acvtivity of human $CD8^+$ T cells in humanized NSG mice.
Methods: A novel high affinity human IL-15 superagonist (IL15N72D-IL15Rα/Fc referred to as ILR-Fc) was generated by exchanging asparagine (N) to aspartic acid (D) at position 72 reported to increase the biological activity of IL-15. The modified IL-15 sequence was then linked to the IL-15-binding Sushi-domain of IL-15Rα. To improve bioavailibility of the IL-15 complex the truncated IL-15Rα was fused to the Fc-domain of human IgG1. HLA-A2 reactive $CD8^+$ T cells were generated by repetitive stimulation of human $CD8^+$ T cells isolated from peripheral blood mononuclear cells (PBMC) by MACS® with HLA-A2 expressing K562 transfectants. To generate Epstein-Barr Virus (EBV) and acute myeloid leukemia (AML)-specific T cells *in vitro*, naive $CD8^+CD45RA^+$ T cells were isolated using the naive T cell isolation kit (Miltenyi Biotec) and repetitively stimulated with autologous, EBV-peptide loaded dendritic cells and peptide loaded PBMC or with HLA-matched primary AML blasts and feeder cells in the presence of a optimized cytokine cocktail and the Gsk3 inhibitor TWS119, which modulates the Wnt-signaling pathway resulting in strong enrichment of T cells with a T_{SCM} and T_{CM} phenotype. Phenotypic and functional analyses *in vitro* were performed by FACS and IFN-g ELISPOT- or ^{51}Cr-release assays, respectively.
Results: The Expi293 Expression System™ (Invitrogen) was applied to produce soluble ILR-Fc. Following transfection and expansion of modified 293T cells growing in suspension protein-A affinity based purification of ILR-Fc resulted in ≥1mg recombinant protein/25 ml culture. *In vitro* bioactivity analyses of ILR-Fc as compared to IL-15 (N72D) produced in E coli revealed that ILR-Fc was ≥10 fold more active when tested on M07e cells. To test for growth promoting effects on T cells in a lymphopenic environment, we first injected ILR-Fc into NSG mice previously humanized by engraftment of human $CD34^+$ hematopoietic stem cells and observed robust expansion of primarily human $CD8^+$ T cells. Strong effects on expansion of splenic $CD8^+$ T cells could be further confirmed following injection of ILR-Fc into C57BL/6 mice. Moreover, upon transfer of EBV- and AML-reactive $CD8^+$ $CTL_{SCM/CM}$ into NSG mice engrafted with autologous EBV-immortalized B cells or HLA-matched AML blasts, resp., we observed prolonged persistence and superior reactivity in mice receiving weekly i.p. injections of 60 ug ILR-Fc/mouse (equivalent to 10 ug IL-15) when compared to mice receiving T cells and 10 ug E. coli-derived IL-15. This extended bioactivity was partially attributed to an increased half-life of ILR-Fc in the serum as determined by IL-15 ELISA. In addition, ILR-Fc treatment resulted in strongly reduced AML burden and regression of EBV-lymphoma. Finally, adoptively HLA-A2-specific cytototxic $CD8^+$ T lymphocytes (CTL) caused xenogeneic GvHD in NSG mice in the presence of ILR-Fc when compared to mice injected with $CD8^+$ CTL alone.
Summary and Conclusions: These studies demonstrate that ILR-Fc has potent and prolonged bioactivity to facilitate $CD8^+$ T cell persistence and activity in lymphopenic hosts and might thus serve as a valuable tool to improve selective $CD8^+$ T cell-mediated immunotherapy.

P727

DENDRITIC CELL VACCINATION IN POSTREMISSION THERAPY OF AML: RESULTS OF A CLINICAL PHASE I TRIAL AND OF PRECLINICAL STUDIES TESTING COMBINATORIAL APPROACHES

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Background: Immunotherapy is a promising treatment strategy to eradicate residual chemorefractory leukemic cells in AML patients. We are currently conducting a proof-of-concept phase I/II (6+14 patients) clinical trial using *next-generation* dendritic cells for postremission therapy of AML (NCT01734304). Besides therapeutic vaccination, immune checkpoint modulation represents another strategy to enhance anti-tumor immune responses, which is now being tested in various hematological malignancies.
Aims: The primary endpoint of the clinical trial is feasibility and safety, and secondary endpoints are immunological responses and disease control, with a particular focus on MRD conversion. In preclinical studies we examine combinatorial approaches using DCs together with immune checkpoint modulation to augment T cell activation and thus the efficacy of DC vaccination.
Methods: *Next-generation* DCs are generated in a GMP-compliant 3-day protocol including a TLR7/8 agonist containing maturation cocktail. DCs are loaded with ivt-RNA encoding WT1 and PRAME as leukemia-associated antigens and CMVpp65 as an adjuvant and surrogate antigen. Patients are vaccinated intradermally with $5x10^6$ DCs of each antigen up to 10 times within 26 weeks in weekly to monthly intervals. To test the combinatorial approach, DCs and autologous T cells are cocultured *in vitro* with or without antibodies blocking inhibitory ligand-receptor interactions (PD-L1, PD-1 or LAG-3) or an immunomodulatory drug (lenalidomide). IFNγ secretion and proliferation are measured by FACS-based assays.
Results: Eight patients have been enrolled into the trial so far. In 6/6 cases, leukapheresis yielded sufficient monocytes to generate DCs for clinical application. DCs secreted IL-12p70, migrated, expressed all three antigens and activated antigen-specific T cells *in vitro*. Three patients have completed the vaccination schedule. Delayed-type hypersensitivity (DTH) responses at the vaccination site were observed in 4/4 patients, but no grade III/IV toxicities. Multimer analyses revealed the induction of antigen-specific T cell responses in 2/2 patients: a 10-fold increase of WT1-specific and a 6-fold increase of CMVpp65-specific T cells in a CMV-seronegative patient were detected. TCR repertoire analyses by next-generation sequencing revealed an enrichment of particular clonotypes at DTH sites, supporting the induction of antigen-specific immune responses. A patient with an impending relapse during the vaccination schedule was treated with a combination of DC vaccination and 5-azacytidine, resulting in MRD conversion. In preclinical studies, the blockade of PD-L1, PD-1 or LAG-3 in DC-T cell cocultures resulted in 1.4-, 2- or 5.9-fold augmented IFNγ secretion. Addition of lenalidomide induced a 5.4-fold increase in IFNγ secretion. All four agents additionally enhanced T cell proliferation.
Summary and Conclusions: We conclude that *next-generation* DCs are capable to induce tumor-specific immune responses *in vivo*. Moreover, our *in vitro* studies suggest that the efficacy of DC vaccination can be enhanced by concurrent immune checkpoint modulation.

P728

COMBINATION OF IBRUTINIB AND ANTI-CD19 CHIMERIC ANTIGEN RECEPTOR T CELLS FOR THE TREATMENT OF RELAPSING/REFRACTORY MANTLE CELL LYMPHOMA (MCL)

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Background: The Bruton Tyrosine Kinase (BTK) inhibitor Ibrutinib displays considerable activity in mantle cell lymphoma (MCL), yet approximately 30% of patients do not respond and among responders only one third experience complete remission (CR). Furthermore, the therapy invariably leads to drug resistance and a median response of 17.5 months (Wang ML, NEJM, 2013). Infusion of autologous T cells transduced with chimeric antigen receptors (CAR) against the B-cell specific CD19 antigen (CART19 or CTL019) leads to dramatic clinical responses in the majority of patients with acute lymphoblastic leukemia and chronic lymphocytic leukemia (Maude SA, NEJM, 2014; Porter D, NEJM, 2011). The role of CTL019 in MCL, where bulky disease may impair T cell infiltration, has not yet been established. Ibrutinib has shown particular efficacy in reducing tumor masses while mobilizing neoplastic B cells into the peripheral blood.
Aims: We sought to investigate the combination of two novel potent approaches, ibrutinib and CTL019, for treatment of MCL.
Methods: MCL cell lines were obtained from ATCC (Mino, Jeko-1, SP-49) while MCL-RL was generated in our laboratory from a pleural effusion of a MCL patient. CTL019 were produced as previously described by lentiviral transduction of healthy donor T cells using an anti-CD19-CD3zeta-41-BB construct (Milone M, Mol. Ther., 2009). For *in vivo* experiments, ibrutinib was dissolved in HP-beta-cyclodextrin/H_20 and administered in the drinking water to mice with established disease, usually from 7 days after tumor injection until the end of the experiment.
Results: *In vitro* studies with established MCL-derived cell lines and MCL-RL show a variable response to ibrutinib with an IC50 ranging from 10 nM to

10 μM as determined in the MTT conversion assay. MCL-RL was the most sensitive cell line with an IC50 of 10nM, similar to primary MCL. These different cell lines could therefore be used to model both ibrutinib-sensitive and ibrutinib-resistant MCL. Both ibrutinib-sensitive and ibrutinib-resistant cell lines, but not the CD19-negative control MOLM14 cell line, when exposed to allogeneic CTL019 strongly activated the T cells as detected by CD107a degranulation, cytokine production and CFSE proliferation assays. *In vitro* assays with MCL cell lines and different doses of CTL019 with increasing concentration of ibrutinib demonstrated an additive tumor killing with the combination CTL019 and ibrutinib. However, supra-physiologic doses of Ibrutinib (>/=1 uM) impaired cytokine production and T cell proliferation *in vitro*; these concentrations of ibrutinib are not typically reached in the clinical setting. In order to confirm these results *in vivo* we established a novel MCL model, using the MCL-RL cell line. Intravenous injection of MCL-RL cell line transfected with the GFP/luciferase gene into immunodeficient NOD/SCID-gamma chain knockout (NSG) mice resulted in 100% MCL engraftment in liver and spleen, with eventual dissemination into lymph nodes and bone marrow. Treatment with three different doses of allogeneic CTL019 (0.5, 1 and 2 million/mouse) led to a dose dependent anti-tumor effect. A similar dose response to CTL019 was also observed in the ibrutinib resistant Jeko-1 cell line. We treated MCL-RL xenografts *in vivo* with different doses (0, 25 and 125 mg/Kg/day) of ibrutinib, with a median overall survival respectively of 70, 81 and 100 days ($p<0.001$). Importantly, a direct *in vivo* comparison of the highest ibrutinib dose (125 mg/kg) and CTL019 showed a significantly improved tumor control for mice treated with CTL019. MCL-RL xenografts were treated with vehicle, ibrutinib, CTL019 or the combination of CTL019 and ibrutinib. The combination resulted in an improved tumor control with 80% of mice achieving long-term disease-free survival (Figure 1A). In addition, we found that mice treated with ibrutinib had higher numbers of circulating CTL019 cells (Figure 1B).

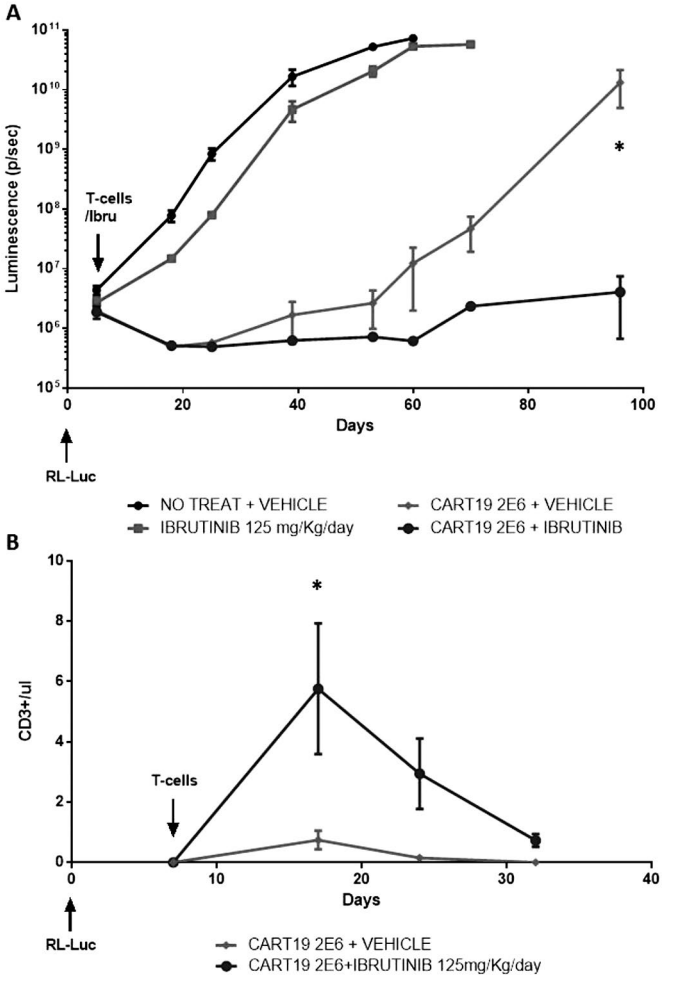

Figure 1.

Summary and Conclusions: This preclinical model demonstrates for the first time that a small molecule inhibitor of B cell receptor signaling (ibrutinib) can be combined with CTL019 in a rational manner that augments the anti-tumor effect and leads to enhanced survival.

P729

INTERIM ANALYSIS OF PHASE I/II TRIAL IN HIGH-RISK PEDIATRIC PATIENTS POST ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANT (HSCT) TREATED WITH *EX VIVO* EXPANDED DONOR-DERIVED ADENOVIRUS-SPECIFIC T CELLS

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Background: Adenovirus (ADV) is a cause of significant morbidity and mortality after pediatric HSCT. Antiviral drug therapy is routinely given for ADV viremia in HSCT patients, but can be associated with significant toxicity. While antiviral drug therapy may attenuate viremia, T cell reconstitution is required for sustained viral clearance. Adoptive virus-specific immunotherapy offers the possibility of durable viral control in the post transplant setting.
Aims: In order to assess the safety and tolerability of an ADV cell therapy (ACT) Cell Medica is evaluating an expanded anti-ADV T cell product in a clinical trial in three centers in children who reactivate ADV after T cell-depleted transplantation. Here we report on the clinical experience of the first five patients treated in the ASPIRE clinical trial (EUDRA CT 2011-001788-36).
Methods: Patients were consented to receive ACT on reactivation of ADV (defined as two consecutive positive PCRs>1000 copies/mL) post-transplant, in addition to routine antiviral drugs. Cell administration did not occur until 28 days post-transplant due to continuing *in vivo* persistence of conditioning antibodies. The primary dose was $1x10^4$/kg total CD3+ T cells. Patients were monitored post adoptive cellular therapy up to 180 days. If the patient exhibited significant levels of ADV viremia requiring treatment ≥4 weeks post-infusion they were eligible to receive a secondary maximum target dose of $1x10^5$/kg total CD3+ T cells and were monitored for a further 180 days. Lymphocytes for the expansion process were sourced from the transplant donor either from peripheral blood or a 5 mL aliquot of the original stem cell donation. The one-touch rapid expansion process for generation of this ACT was based on exposure of T cells to overlapping peptides covering the entire hexon V protein and 10 days expansion in the presence of cytokines; cells were then harvested and cryopreserved. The ADV-specific T cells were formulated in 4.5% human serum albumin and 10% dimethylsulfoxide with ≥$1x10^2$ ADV-specific T cells per kg body weight of recipient. Study endpoints included incidence and severity of GvHD, cytopenias and grade 3-4 adverse events.
Results: Patients were consented to receive ACT on reactivation of ADV post-transplant. All five patients analyzed cleared viremia post ACT. Two SAEs were reported; one pancreatitis and one GvHD (skin GvHD stage III; gut GvHD stage II). Additionally, one Suspected Unexpected Serious Adverse Reaction (SUSAR) was reported which was subsequently downgraded following attribution to astrovirus encephalitis.

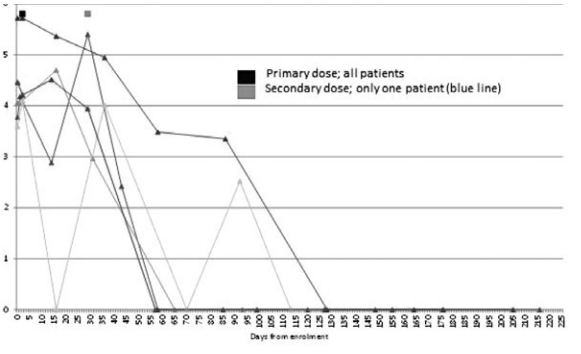

Figure 1. Log PCR *vs* days from enrolment.

Summary and Conclusions: In a small number of initial subjects ADV viremia in pediatric patients post HSCT was controlled following ACT. A number of AEs and SAEs were reported, in keeping with expectations for this patient population. The trial is ongoing.

P730

LIPOSOME-BASED SPECIFIC TARGETING OF A STAT3-INHIBITOR TO CANCER-PROMOTING MACROPHAGES: A NOVEL THERAPUTIC PARADIGM IN MULTIPLE MYELOMA

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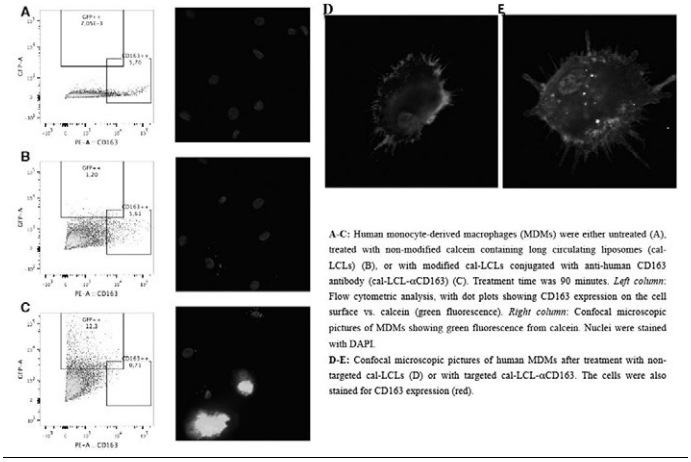
Background: Tumor-associated macrophages (TAMs) play an important role

in cancer by suppression of adaptive immunity and promotion of angiogenesis and metastasis. In multiple myeloma, as well as other haematological malignancies, there is an association between infiltration of CD163+ macrophages in the tumor microenvironment and poor outcome. This association has also been found for most other human cancers. Importantly, expression of the haemoglobin scavenger receptor CD163 is increased on TAMs and other alternatively activated macrophages (M2). Activity of the transcription factor STAT3 is increased in both stromal and malignant cells, and inhibition of STAT3 in tumor-associated myeloid cells has been proposed as a target for novel anti-cancer therapy. Inhibiting STAT3 could potentially repolarize TAMs towards a pro-inflammatory (M1) phenotype, and restore anti-tumor functions. *In vivo,* ablation of the *Stat3* gene, specifically in cells of the haematopoietic system, has been shown to elicit a strong anti-tumor immune response in a mouse model of melanoma. Therefore, CD163-targeted inhibition of STAT3 within TAMs may be a novel treatment paradigm in malignant diseases.

Aims: We aimed to produce a novel drug, long-circulating liposomes (LCLs) containing a STAT3 inhibitor, target this drug specifically to M2 macrophages with high CD163 expression, and evaluate the effect on STAT3 activation in the cells.

Methods: *In vitro,* monocyte-derived macrophages (MDMs) were targeted using a liposome-based system, in which the molecule/drug of interest was packaged within LCLs. LCLs containing either green fluorescent protein calcein (cal-LCLs) or a STAT3-inhibitor (STAT3i-LCLs) were produced using microfluidics technique. Subsequently, LCLs were modified by conjugation with anti-human-CD163 antibodies (LCL-aCD163). MDMs were cultured in RPMI-1640 medium containing 10% foetal calf serum, 10ng/mL M-CSF, and 1ng/mL GM-CSF, and were matured for 7-8 days before experiments were performed. For confocal microscopy, MDMs were cultured on cover slips. For STAT3-activation experiments, MDMs were first treated with the STAT3 inhibitor, and then stimulated with 50ng/mL IL-10 for 15min to induce STAT3 activation. Cells were then fixed, permeabilized, stained with anti-human-P-Y705-STAT3 PE conjugated monoclonal antibody, and analysed on a LSR Fortessa flow cytometer.

Results: Stable cal-LCLs and STAT3i-LCLs were produced, with a radius of 50-80 nm (measured by dynamic light scattering). Here, we show that cal-LCL uptake was markedly higher in MDMs with the highest CD163 expression (the 5-10% most CD163 expressing cells). Using a validated flow cytometric assay for detection of activated (phospho-Y-705) STAT3, we evaluated the ability to inhibit this activation in human MDMs. After stimulation with IL-10 the median fluorescence intensity (MFI) for P-Y705-STAT3 PE increased from ~150 to 290. This increase could only be partially reduced by free STAT3 inhibitor or non-targeted STAT3i-LCLs, but with the targeted STAT3i-LCL-aCD163, we were able to reduce the STAT3 activity to the unstimulated level, or below (MFI ~100 to 150).



A-C: Human monocyte-derived macrophages (MDMs) were either untreated (A), treated with non-modified calcein containing long circulating liposomes (cal-LCLs) (B), or with modified cal-LCLs conjugated with anti-human CD163 antibody (cal-LCL-αCD163) (C). Treatment time was 90 minutes. *Left column*: Flow cytometric analysis, with dot plots showing CD163 expression on the cell surface vs. calcein (green fluorescence). *Right column*: Confocal microscopic pictures of MDMs showing green fluorescence from calcein. Nuclei were stained with DAPI.

D-E: Confocal microscopic pictures of human MDMs after treatment with non-targeted cal-LCLs (D) or with targeted cal-LCL-αCD163. The cells were also stained for CD163 expression (red).

Figure 1.

Summary and Conclusions: We have shown that LCLs can be effectively targeted to the most CD163-expressing cells (which includes TAMs), and that this delivery system can be used to effectively inhibit STAT3 within human MDMs *in vitro*.

In perspective, this may lead to the first *in vivo* experiments of targeted inhibition of STAT3 specifically within TAMs.

P731

ANTIBODY-TARGETED CYCLODEXTRIN NANOPARTICLES DELIVER SIRNA AND ACHIEVE THERAPEUTIC GENE SILENCING IN BLOOD FROM ACUTE MYELOID LEUKAEMIA (AML) PATIENTS

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Background: Delivery of small interfering RNA (siRNA) using multifunctional nanoparticles has been investigated to treat solid cancers; however the application of this approach to blood cancers is at an early stage. Modified cyclodextrins have successfully delivered siRNA in a range of *in vivo* pre-clinical animal models of disease including prostate cancer [1], inflammatory bowel disease [2] and Huntington's disease [3].

Aims: The aims of this study are: to formulate an antibody (Fab) targeted cyclodextrin (CD) nanoparticle (NP) for siRNA delivery to treat AML, to assess the efficacy of this NP *in vitro* using KG1 (AML) cells and *ex vivo* using AML primary patient samples, and to evaluate the NP as an adjuvant therapy for traditional chemotherapy.

Methods: An amphiphilic cationic CD was synthesised as previously described [4]. This CD was complexed with siRNA and co-formulated with a PEGylated lipid (DSPE-PEG). The resulting NP was tagged with a Fab to give CD.siRNA.DSPE-PEG-Fab (NP-Fab) targeting the CD123 receptor overexpressed on AML stem cells. Incorporation of Fab was assessed by SDS-PAGE. The NP-Fab was characterised for particle size, surface charge and stability using a Malvern Zetasizer Nano ZS. Agarose gel retardation assay was performed to determine the ability of nanoparticles to bind siRNA and protect it from serum degradation. *In vitro* toxicity was examined in KG1 cells using the MTT assay. *In vivo* toxicity was assessed in C57BL/6 mice using ELISA and haematoxylin and eosin (H & E) staining. Competitive internalisation of nanoparticles was evaluated using FACS. Confocal microscopy was used to investigate intracellular trafficking of nanoparticles. Targeted gene knockdown was quantified using real-time qPCR and western blotting. Anti-leukaemia efficacy was investigated in KG1 cells and AML primary cells using a CCK-8 Kit and a colony-formation assay.

Results: The incorporation of DSPE-PEG into CD.siRNA NP significantly increased the size and reduced the charge, further incorporation of Fab significantly increased the size but did not affect the charge. The NP-Fab had a size of ~200 nm with a nearly neutral surface charge of 4 mV, and resisted aggregation in OptiMEM. The NPs displayed minimal *in vitro* and *in vivo* toxicities. Pre-treatment with free Fab significantly ($P<0.05$) reduced uptake of the antibody-targeted NP in KG1 cells, suggesting specific ligand-receptor mediated internalisation. Confocal microscopy data indicated that endosomal escape of antibody-targeted NP was time-dependent. The NP-Fab incorporating Bromodomain-containing protein 4 (BRD4) siRNA displayed significantly ($P<0.05$) higher gene knockdown efficiency in KG1 cells and AML primary cells compared to the siRNA delivered by the commercial cationic lipid vector, Lipofectamine 2000. Use of the Fab-targeted NP in combination with Cytarabine (Ara-C) resulted in a synergistic therapeutic response, achieving up to 90% growth inhibition of primary cells.

Summary and Conclusions: The antibody-targeted CD-based NP containing therapeutic siRNA is a novel therapeutic strategy with potential to treat AML, particularly when combined with chemotherapeutics. Future work is underway to investigate pharmacokinetics, tissue distribution, and anti-cancer efficacy in a pre-clinical AML mouse model.

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P732

CLINICAL GRADE ACTIVATED NATURAL KILLER PRODUCTS FOR ADOPTIVE IMMUNOTHERAPY AGAINST HIGH-RISK MALIGNANCIES

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Background: Natural Killer (NK) cells may exert a graft-*vs.*-leukemia/tumor effect without concomitant severe graft *vs* host disease. Different clinical-grade highly active human NK cell products are being used against malignancies.

Aims: Our study investigates the NK cell recovery, T cell depletion, phenotype and cytotoxicity of three different ways of good manufacturing practice (GMP)-grade NK cell products.

Methods: In four different clinical trials, we have infused three different activated NK cell products obtained after: i) enrichment from healthy haploidentical donors peripheral blood mononuclear cells (PBMNCs) collected by non-mobilized apheresis by two steps procedure, depletion of CD3+ cells and selection of CD56+ cells, using CliniMACS device and *ex-vivo* stimulation with IL-15 overnight (NKIL15), EudraCT 2009-010016-15; ii) expansion from autologous PBMNCs from multiple myeloma patients using as feeder K562 cells expressing interleukin (IL)-15 and 4-1 BB Ligand (K562-mb15-41BBL), (NKAE), EudraCT 2012-000514-11; iii) or expasion from healthy haploiden-

tical PBMNCs obtained by whole blood using the same feeder K562 cellular line, EudraCT 2012-000054-63 and EudraCT 2012-005146-38. The final cell products were analyzed for microbiological test and by flow cytometry for CD3+CD56- and CD3-CD56+ cells. In addition, haploidentical NKAE products were analyzed for the surface expression of CD25, CD69, NKG2D, DNAM and NCRs by tye appropiate combination of flurochrome-conjugated monoclonal antibodies. The NK cytolitic activity was tested in a 2h BATDA release assay against K562, A673, Jurkat and U266 in an 8:1 E/T ratio.

Results: Thirty-three activated NK cell products consisted on haploidentical NKIL15 (n=8), haploidentical NKAE (n=11) and autologus NKAE (n=14) were infused on 17 patients suffering from advanced malignancies (6 pediatric refractory sarcomas, 7 pediatric refractory acute leukemias, and 5 adults patients suffering from high risk multiple myeloma, respectively) on 4 different clinical trials. Safety. No microbial contamination was detected in any product. BCR-ABL1 quantification was negative after infusion in all patients treated with NKAE activated with K562 feeders. From NKIL15 the median NK cell recovery was 25% (range 10-41%) and the T cell depletion was 5.34x10-5 (5.1 log). From NKAE the expansion of CD56+CD3- cells after 14 days of culture was 268.47-fold (range 28- to 1547-fold) after 14 days of coculture from donors and 74.4-fold (range 13- to 551-fold) from multiple myeloma patients. Expansion of CD3+ T cells was minimal (median recovery 2.31-fold; range 0.13- to 5-fold from haploidentical donor and 0.36-fold; range 0.0001- to 1.7-fold from patients PBMCs). The CD56+CD3- cells, NK cells, obtained from NKIL15 and NKAE products were similar. However, CD56-CD3+ cells, T cells, were significantly lower in NKIL15 product, Figure 1. Haploidentical NKAE product expanded higher NK cells but also significantly higher T cells than autologous NKAE product (p=0.002). The surface expression of the activating receptors CD69, CD25, NKp44,NKp46, NKp30 and DNAM was up-regulated, Figure 2. NKAE and NKIL15 products had a significantly higher lytic activity than unstimulated NK cells (97 and 75% *versus* 42%; 34 and 57% *versus* 23%, 40-60% *versus* 19% and 60-52% *versus* 21%) in K562, A673, Jurkat and U266, respectively.

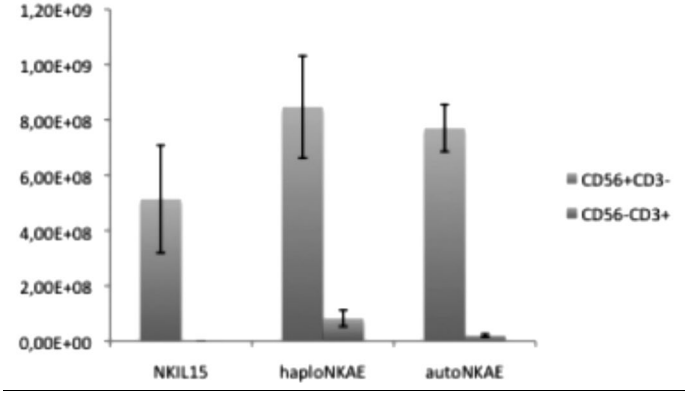

Figure 1.

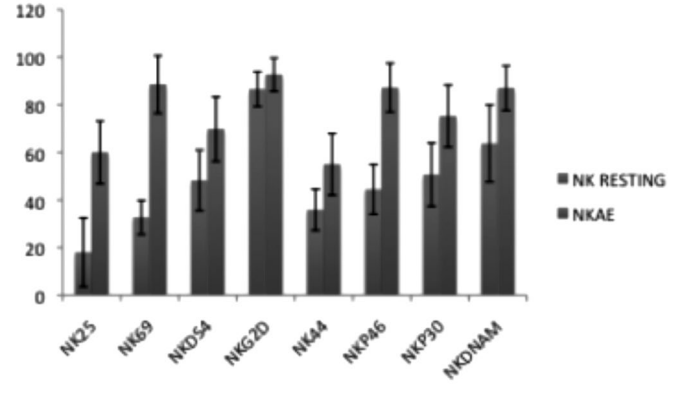

Figure 2.

Summary and Conclusions: In conclusion, our results indicate that different procedure of GMP-grade purification of highly activate NK cells with extremely low T cell content are feasible for clinical use. The T cells contained are lower in NKIL15 and autologous NKAE products than in haploidentical NKAE products.

P733

IMPROVED SAFETY AND EFFICACY OF A HIGH-AFFINITY A2.1-RESTRICTED SINGLE-CHAIN P53-SPECIFIC TCR FOR ADOPTIVE IMMUNOTHERAPY

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Background: Adoptive transfer of T cells retrovirally transduced with a tumor-associated antigen (TAA) -specific T cell receptor (TCR) has shown promise in the treatment of cancer patients. Mutation and overexpression of the p53 tumor suppressor protein are the most common genetic alterations in human cancers. We used HLA-A2.1 transgenic mice to generate a high-affinity CD8-independent TCR specific to the widely expressed human p53(264-272) tumor-associated antigen. However, a safety concern of TCR gene transfer is the pairing of natural and introduced TCR chains resulting in the potential generation of self-reactive T cells.

Aims: We aim at improving adoptive T cell-based immunotherapy by generating optimized specific TCR format with reduced potential for TCR mispairing-associated self-reactivity and enhanced tumor recognition.

Methods: To overcome TCR mispairing formation, we designed a single chain (sc) TCR format by connecting the variable TCRa domain to the TCRb chain via a short peptide linker co-expressed with a truncated TCRa constant domain. The TCR sequences were cysteine-modified, codon-optimized and cloned into one single 2A-based retroviral vector. The choice of target tumor antigens for cancer immunotherapy is of central importance as the transfer of high-avidity T cells may potentially induce severe on-target toxicity by damaging normal cells expressing the antigen. Here, we evaluated the safety issues raised by the risk of p53TCR gene transfer-associated on/off-target toxicities in A2.1 transgenic mouse models of adoptive transfer.The antitumor efficacy was assessed in syngeneic and NodScid IL-2R gamma chain-null mice (NSG) xenograft models.

Results: *In vitro* studies showed that, scTCR-modified $CD4^+$ and $CD8^+$ T cells displayed similar high-avidity compared to the full-length TCR, as determined by peptide titration in cytotoxicity assays and were able to mediate specific lysis of p53 mutant $A2.1^+$ tumor cells. However, in contrast to the full-length TCR, the optimized scp53TCR did not result in mispairing-mediated lethal off-target autoimmunity *in vivo*. We next assessed the potential of scTCR-modified T cells to cause on-target autoimmunity, using $A2.1K^b$ transgenic mice expressing human p53 (Hupki). We observed that transfer of scTCR-specific T cells did not result in a depletion of hematopoietic compartment, as mice recovered normal white blood cell counts and survived without any sign of toxicity. Importantly, high-avidity scTCR-modified T cells were able to eradicate established tumors in syngeneic and xenograft mouse models.

Summary and Conclusions: Together, our study provided evidence that an optimized high-affinity scTCR-specific for the broadly expressed tumor-associated antigen p53(264-272) can eradicate tumor cells without inducing off/on-target toxicities in murine models of adoptive T-cell transfer. These data demonstrate the improved safety and therapeutic efficacy of high-affinity scp53TCR for a broad-spectrum immunotherapy of malignant disease.

Red cells: Clinical

P734

HYDROXYUREA FOR B-THALASSEMIA: A META-ANALYSIS

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Background: β-thalassemia is one of the most common inherited diseases worldwide. Severe forms of β-thalassemia require life-long blood transfusions, resulting in iron overload with multi-organ morbidity and mortality. Hydroxyurea (HU), an oral chemotherapeutic drug, is anticipated to decrease the need for transfusions, either completely or partially by raising hemoglobin levels and thus decreasing the short and long term complications of chronic transfusions.
Aims: To evaluate the clinical efficacy and safety of HU in β-thalassemic patients of any age.
Methods: *Search strategy:* We searched MEDLINE, EMBASE, Cochrane Central Register of Controlled Trials (CENTRAL), ongoing trials registers, and major preceding conferences. Hand searches were also conducted using reference lists from primary studies. *Selection criteria:* Randomized controlled trials (RCTs) and observational studies (sample size ≥5) assessing the clinical efficacy of HU alone for three months or longer, for the treatment of patients with β-thalassemia were included. *Data collection and analysis:* Two authors acted as reviewers and independently assessed study quality and extracted data from the included studies. β-thalassemia classification and response evaluation outlined in the following Table 1. Effect size was estimated as a proportion (responders over sample size). All data was analyzed using Stata, Version 13.0.

Table 1.

Disease	Subgroup	Key features	Response assessment
β-thalassemia major (β-TM)	1) Classical β-TM; or 2) Severe E/ β-thalassemia	Life-long transfusion needs	1. Complete response (CR): complete cessation of transfusion 2. Overall response (OR): ≥50 reduction of transfusion
Severe non-transfusion dependent β-thalassemia (severe NTDβT)	1) Classical β-Thalassemia intermedia; or 2) Moderate E/ β Thalassemia	≥4 transfusions/year or every 3 months & not meeting β-TM definition	1. CR: as above 2. OR: as above
Mild NTDβT	1) Classical β-thalassemia intermedia; or 2) Mild E/ β Thalassemia	No or <4 transfusions/year	Response rate (RR): ≥1g/dL increase in Hemoglobin (Hb)

Results: For β-TM, 11 observational studies involving 620 patients were included. HU was associated with a significant decrease in transfusion need with CR of 41% (95% CI, 25-58%) and OR of 71% (95% CI, 56-84%). In severe NTDβT, 8 (1 RCT & 7 observational) studies involving 305 patients were analyzed. HU was associated with a significant decrease in transfusion need with CR of 55% (95% CI, 34-75%) and OR of 79% (95% CI, 69-88%). For mild NTDβT, 14 (1 RCT and 13 observational) studies involving 344 patients were included. HU therapy was effective in raising Hb by 1g/dL from baseline in 54% (95% CI, 43-65%). All of the studies had several limitations, such as small sample size, lack of comparison group, under-reporting of data and methods, and being mostly observational studies. Adverse events (AEs) were transient and improved with temporary cessation of the drug and/or adjustment of the dose.
Summary and Conclusions: HU appears to be effective in the management of β-thalassemia by decreasing the need for chronic blood transfusions completely or partially in a significant number of patients. It appears to be well tolerated and associated with mild and transient AEs. Patients with β-thalassemia may benefit from a trial of HU, though large RCT assessing efficacy should be done to confirm the findings of this meta-analysis.

P735

PHENOTYPIC VARIATIONS IN SICKLE CELL DISEASE: AN EXPLORATION OF THE ROLE OF THE ENVIRONMENT AND SKIN MICROBIOME ON LEG ULCERS

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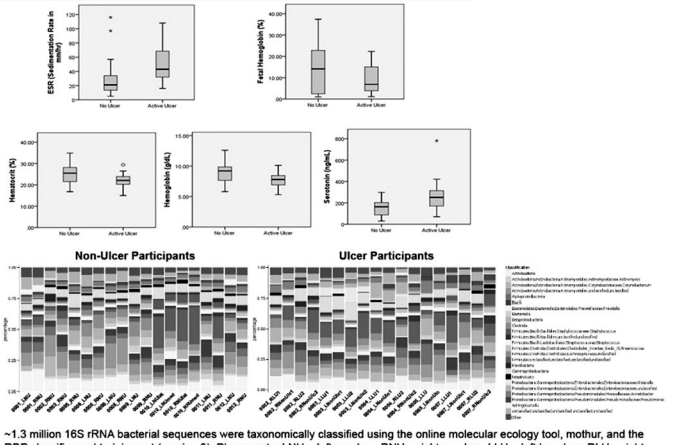
Background: There is extensive phenotypic variation in SCD, a monogenetic inherited disorder that affects millions worldwide. Fetal hemoglobin and alpha chain number have been identified as genetic disease modifier, but alone do not explain the marked interpatient and intrapatient variability of acute and chronic complications, which can affect every organ. Efforts have been hampered by the difficulty in precisely defining clinical phenotypes, such as acute pain crisis, and acute chest syndrome.Leg ulcers are a debilitating complication of SCD, associated with severe anemia, chronic pain, elevated TRV, priapism, thrombophilia, renal insufficiency, and a negative predictor of survival. They are easy to identify and recall both by the patient and the health care provider. Their prevalence in patients with SCD is 10 times higher than in the general population and varies in different geographic locations
Aims: We hypothesize that leg ulcerations in SCD are multifactorial and include contributions from the skin microbiome, genetic and environment factors, both physical and social.
Methods: The study will recruit 60 adults with SCD, 30 with and 30 without leg ulcers in a NHGRI sponsored trial. Clinical, laboratory, and social characteristics are prospectively obtained. Samples for microbiome analysis are collected at the center of the wound, the periwound, and the opposite leg. Microbial DNA was extracted, the 16S rRNA molecular marker sequenced (V1V3 region), and the PCR products purified and quantified for sequencing using the Illumina MiSeq Benchtop Sequencer platform.
Results: Laboratory measures from the first 35 participants in the study were significantly different between the leg ulcer and non-leg ulcer groups: lupus anticoagulant, glucose, hematocrit, hemoglobin, platelet count, ferritin, hemoglobin reticulocyte, ESR, and serotonin ($p<0.1$). The leg ulcer group had higher mean values for absolute reticulocyte count, serotonin, and ESR ($p=0.42$, $p<0.05$, and $p<0.1$, respectively), and significantly lower values for hemoglobin (Hb), hematocrit (hct) and fetal hemoglobin (HbF) at $p<0.05$, $p<0.05$, and $p<0.1$, respectively. For the first 12 participants who had their microbiome sequenced preliminary analysis of the relative abundance of bacteria on the malleoli of SCD participants with and without leg ulcers reveals that the bacterial community structure differs in participants with leg ulcer and non-ulcer. No significant differences were found for demographic characteristics between the active and no leg ulcer groups (Figure 1).



~1.3 million 16S rRNA bacterial sequences were taxonomically classified using the online molecular ecology tool, mothur, and the RDP classifier and training set (version 9). Please note: LNU – left no ulcer, RNU – right no ulcer, LLU – left leg ulcer, RLU – right leg ulcer, LNonUlc – left non ulcerated skin, and RNonUlc – Right non ulcerated skin.

Figure 1.

Summary and Conclusions: Clinical and laboratory differences exist in patient with SCD and leg ulcers, when compared to patients without ulcers. The microbiome of patients who have leg ulcers is different from patients that do not develop them and could contribute to their occurrence. Stress levels are higher, as demostrated by higher levels of serotonin and so are markers of inflammation (ESR). Family and education variables do not seem to responsible for this end organ complication.

P736

PERICARDIAL EFFUSION IS A MARKER OF INCREASED CARDIAC MORTALITY IN THALASSEMIA MAJOR PATIENTS

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Background: In different types of not-hematological diseases the presence of a small pericardial effusion (PE) was associated with worse survival even after adjustment for patient characteristics, suggesting that it is a marker of underlying disease. In thalassemia major (TM) pericardial effusion was shown to be

one of the manifestations of heart disease but its potential prognostic importance has never been investigated in the modern era. Cardiovascular Magnetic Resonance (CMR) by cine SSFP sequences was demonstrated to be extremely sensitive to even a small amount of PE.
Aims: This is the first prospective study evaluating if the presence of pericardial effusion is associated with increased cardiac mortality in TM.
Methods: 1259 patients (648 females, mean age 31.02±8.64 years) enrolled in the Myocardial Iron Overload in Thalassemia (MIOT) were prospectively followed from their first Magnetic Resonance Imaging (MRI) scan. CMR was used to quantify myocardial iron (MIO) overload by a multislice multiecho T2* approach and to assess biventricular function parameters and to detect PE by cine SSFP sequences.
Results: PE was present in 25 (2.0%) patients. Patients with and without PE were comparable for age and ratio of men/women. At the baseline, the percentage of patients with MIO (global heart T2* value <20 ms) was comparable between patients with and without PE (12.0% *vs* 28.7%; P=0.074) and left ventricular and right ventricular ejection fractions were not significantly different between the two groups. Mean follow-up (FU) time was 44.55±20.35 months and there were 15 deaths. Nine death were due to cardiac causes. Cardiac mortality was greater for patients with PE compared to those without an effusion (8.0% *vs* 0.6%, P=0.013). PE was a significant predictive factor for cardiac death (hazard ratio-HR=19.25, 95%CI=3.96-93.66, P<0.0001). PE remained a significant prognosticator for death also in a multivariate model including MIO (PE: HR=35.24, 95%CI=6.59-188.57, P<0.0001 and global heart T2* <20 ms: HR=6.89, 95%CI=1.60-29.69, P=0.010) (Figure 1).

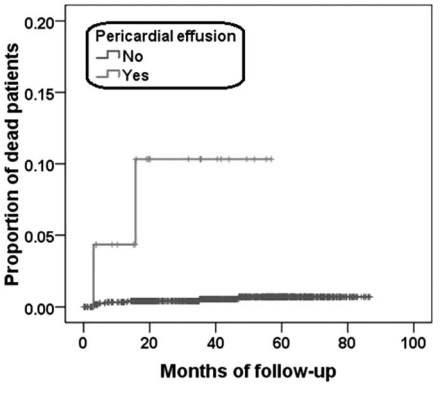

Figure 1.

Summary and Conclusions: PE is quite rare in TM patients and it is not related to myocardial iron overload. An important role in the development of PE could be played by the 'iron-induced' pericardial siderosis but, due to the limitations of the current non-invasive CMR techniques, we were not able to address this issue. PE was found to be a strong predictor for cardiac death, independently by the presence of myocardial iron overload. The non-invasive diagnosis of pericardial effusion is important for a more complete definition of the cardiac involvement of TM patients. The increased risk of cardiac death associated with PE may be used along with other clinical characteristics when estimating a patient's prognosis and monitoring.

P737

OUTCOMES OF A COHORT OF CHILDREN WITH HEMOGLOBINOPATHY RECEIVING ORAL IRON CHELATORS FOR TREATMENT OF TRANSFUSIONAL IRON OVERLOAD IN TURKEY: RESULTS OF 3-YEAR FOLLOW-UP STUDY

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Background: Tissue damage due to iron overload is an ineluctable complication in chronically transfused children with hemoglobinopathies. Lifelong iron chelation therapy is therefore necessary to reduce iron accumulation; however, compliance issues associated with parenteral treatments remain a challenge. Oral iron chelators (OIC) provide an attractive alternative due to their longer half-life, which permits once daily or three times a day dosing regimens.
Aims: We aimed to investigate the outcomes of two oral iron chelators, namely, deferiprone (DFP) and deferasirox (DFX) for transfusion dependent children with hemoglobinopathies in Turkey.
Methods: This multicenter, nation-wide, prospective, non-interventional, 3 year follow-up study evaluated OIC for the treatment IOL. Patients aged 2-18 years with beta-thalassemia major (bTM) or sickle cell anemia (SCA) who were suffering from TIO and on OIC treatment were included. Recommended assessments included demographics, laboratory and imaging results related to TIO, and serious adverse events.
Results: Hereby, we present 3 year follow-up results of this prospective study. Of 474 patients included from 30 centers, 450 (95%) (50.7% female, mean [standard deviation-SD-] age: 9.3 (4.1) years) were diagnosed as bTM whereas 24 (5%) (33.3% female, mean [SD] age: 9.9 (4.2) years) as SCA. Of the patients, 338 (71.3%) completed the study. Results of key laboratory parameters are summarized in Table 1. There were 165 (48.8%) patients with hemoglobin level of ≤9 g/dL at month 36. Serum ferritin (SF) levels significantly decreased with DFX therapy; however, no significant change was observed with DFP therapy. This discrepancy can be explained with the low patient numbers on DFP treatment (n=35). The rate of patients with SF levels of <1000 ng/mL was increased from baseline to month 36 (DFX: 19% to 37% *vs* DFP: 9% to 26%). Additionally, the mean DFX dose was increased from 26.3±6.1 mg/kg to 29.4±10.6 mg/kg on month 36. As compared to <20mg/kg dose, patients who were on ≥20mg/kg of DFX had a significant decrease in SF level. According to the Cox regression analysis, DFX alone was 2.23 times (95% CI: 1.281-3.867) more successful in reducing SF levels of patients below 1000 ng/mL as compared to DFP. There was a positive correlation between SF level and alanine aminotransferase (ALT) for both DFX and DFP.

Table 1. Results of selected laboratory parameters.

	n	Baseline Mean±SD (Median)	n	Month 6 Mean±SD (Median)	n	Month 12 Mean±SD (Median)	n	Month 18 Mean±SD (Median)	n	Month 24 Mean±SD (Median)	n	Month 30 Mean±SD (Median)	n	Month 36 Mean±SD (Median)	p
Hemoglobin (g/dL)															
DFX	437	9.0±1.2 (9.0)	426	8.9±1.1 (8.9)	399	8.9±1.2 (8.9)	379	9.0±1.1 (9.0)	358	9.0±1.1 (8.9)	339	8.84±1.08 (8.8)	314	9.05±1.02 (9.1)	0.068
DFP	34	9.0±1.3 (8.85)	33	9.3±1.0(9.3)	33	9.3±1.0 (9.2)	33	9.1±0.9 (9.2)	33	9.2±1.2 (9.3)	28	9.25±0.89 (9)	24	9.42±1.33 (9.35)	0.300
Serum Ferritin (ng/mL)															
DFX	418	2063.2±1316.3 (1775.5)	384	1926.5±1404.5 (1593.0)	359	1728.9±1206.8 (1426.0)	357	1753.3±1272.2 (1463.0)	329	1653.4±1302.4 (1300.0)	310	1601.85±1295.97 (1267.85)	294	1634.86±1272.6 (1250.5)	<0.001
DFP	34	2529.1±1464.5 (2128.5)	33	2270.1±1234.6 (1905.0)	32	2210.4±1178.1 (2157.5)	31	2320.5±1717.4 (2105.0)	32	2308.1±1472.1 (1989.0)	26	2004.05±1401.38 (1598.5)	23	1910.97±1243.19 (1572)	0.932
Alanine aminotransferase (U/L)															
DFX	417	34.7±31.9 (25.0)	391	36.0±36.1 (24.0)	365	32.3±33.9 (21.0)	347	30.2±31.8 (21.0)	347	30.4±42.6 (19.0)	320	28.71±40.68 (17)	298	23.99±21.79 (16.59)	<0.001
DFP	32	40.4±50.3 (22.5)	30	38.2±32.2 (25.5)	30	31.1±24.9 (25.5)	33	37.1±35.6 (28.0)	31	40.3±36.2 (24.8)	28	26.83±14.22 (23)	24	37.52±32.1 (26.5)	0.680
Serum creatinine (mg/dL)															
DFX	427	0.40±0.14 (0.40)	375	0.41±0.13 (0.40)	354	0.42±0.13 (0.41)	340	0.44±0.12 (0.43)	345	0.45±0.13 (0.44)	322	0.46±0.13 (0.45)	304	0.47±0.14 (0.46)	<0.001
DFP	31	0.39±0.08 (0.40)	30	0.42±0.12 (0.40)	29	0.44±0.08 (0.43)	31	0.47±0.09 (0.46)	31	0.47±0.10 (0.50)	28	0.49±0.12 (0.495)	24	0.48±0.11 (0.5)	<0.001

DFX: Deferasirox, DFP: Deferiprone, SD: Standard deviation.

Summary and Conclusions: This study comprises the overall prospective largest pediatric cohort including bTM and SCA patients suffering from TIO and under OIC treatment. In DFX treated patients receiving >20mg/kg, a decrease in SF was observed; however, it was lower than that reported in randomized trials most likely due to few patients receiving the recommended DFX dose of over 30 mg/kg to gain negative iron balance. There was no clinically significant change in liver or kidney function in these patients receiving DFX or DFP. About half of the patients had a hemoglobin level of ≤9 g/dL after 36-month follow-up, revealing that such patients still do not receive optimal transfusion regimens according to current guidelines. Results of three-year follow-up of the study demonstrate that in order to achieve optimal therapeutic goals, it is important to personalize the treatment through active dose adjustments and address potential compliance issues that can arise from multi-year oral treatments.

P738

RARE INHERITED ANAEMIAS: A CLINICAL-GRADE VALIDATED TARGETED RESEQUENCING PANEL YIELDS HIGH DIAGNOSTIC RATES AND IMPROVES TARGETED MANAGEMENT

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Background: There is a substantial need for improved molecular-diagnostic testing of patients with rare inherited anemias, whether isolated or in the context of pancytopenia. Due to the rarity of these disorders, no routine testing is available. In practice, clinicians must refer patient samples to multiple specialist centers for diagnostic investigations, which often leads to delays in diagnosis and high costs without the certainty of obtaining a molecular diagnosis. Next generation sequencing (NGS) methodology offers an avenue to overcome many of these issues. Targeted resequencing panels have been used for patients with rare anaemias in a research setting but technical and scientific barriers have hampered the transfer of this technology into a routine diagnostic setting. These include the extensive validation required for diagnostic tests and bioinformatic validation of coverage to highlight regions where pertinent mutations may have been missed. Finally, there is limited information regarding the clinical utility of this type of resequencing strategy in this patient group.
Aims: To meet this need we developed and validated a method for the routine diagnosis of congenital red-cell disorders by NGS with the aims of (1) demonstrating the feasibility of achieving clinical-grade validation for a targeted resequencing panel, (2) developing and providing an open-source tool for evaluation of DNA sequence coverage in a clinical setting, (3) assessing the diagnostic yield of the strategy in the diagnosis of congenital anemia/pancytopenia and (4) assessing the clinical utility and impact on management of results generated by this approach.
Methods: We designed a resequencing panel to target 34 genes known to cause anemia and/or pancytopenia [Diamond-Blackfan anemia (DBA), dyskeratosis congenital (DKC), Schwachman-Diamond syndrome (SDS), sideroblastic anemia, congenital dyserythropoietic anemia (CDA) and the enzyme deficiencies G6PD, PKLR and pyrimidine 5' nucleotidase] and used it to assess patients with unexplained anemia in whom acquired causes have been excluded. We also developed an open-source tool for evaluation of sequence coverage in a diagnostic clinical laboratory setting that determines the discoverability of all known mutations in each gene for every individual sample, a functionality currently unavailable in commercial software.
Results: We validated diagnostic findings from NGS with Sanger sequencing and microarray, and found 100% specificity and 99.7% sensitivity (95% CI 97.9-99.9%). We then analysed 57 clinical samples. A molecular diagnosis was made in 24 cases (42%), corresponding to a total of 34 mutations of which 21 were novel. The clinical utility of our diagnostic approach is apparent as accurate molecular diagnosis led to changes in patients' therapies such as splenectomy for patients with PK deficiency or trial of steroids for Diamond-Blackfan anemia.
Summary and Conclusions: We have developed a robust and useful platform for routine molecular diagnosis across the spectrum of congenital anemias and shown a high diagnostic yield, which translated into clinical utility by targeting therapies appropriately.

P739

A PROSPECTIVE CROSS-SECTIONAL STUDY ON OPTHALMOLOGIC COMPLICATIONS AMONG PATIENTS WITH SICKLE CELL DISEASE IN LEBANON

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Background: Sickle cell disease (SCD) is a hereditary disorder characterized by abnormal β-globin chain synthesis leading to vaso-occlusion, hemolytic anemia, recurrent infections and end organ failure. Vaso-occlusive effects on the vascular eye bed may be detectable before patients report visual symptoms.
Aims: This study aims at determining the prevalence and risk factors of ophthalmological complications in a Lebanese SCD cohort and delineating the importance of early detection of asymptomatic eye findings in this cohort.
Methods: This is a prospective observational ongoing cross-sectional study of a SCD cohort in North Lebanon. Ophthalmologic examination consisted of bilateral best-corrected visual acuity (BCVA) measurements, slit-lamp examinations, direct and indirect ophthalmoscopy and wide-field retinal photographs. Fundus fluorescein angiography (FFA) was performed in patients with reduced BCVA or retinal photograph abnormalities. Frequencies and descriptive data were generated using Statistica.
Results: 79 patients, 40 females, 39 males, mean age 15.6±10.5 years were tested. 70%, 28.5% and 1.5% had HbSS, Sβ and SD. Mean steady state hemoglobin, LDH, and ferritin were 8.6±0.87 g/dl, 520.1±203 U/L and 924±1028 ng/ml, respectively. 62% were on hydroxyurea and 86% had history of intermittent blood transfusions. BCVA was >20/50 in 97% and between 20/50 and 20/100 in 3%. Anterior and posterior segment findings were detected in 44% and 48% of patients respectively. The most common anterior segment findings were comma shaped conjunctivae and pallor in 70% and 36%. Posterior segment manifestations included salmon patches in 10.5%, black sunbursts in 31.5%, retinal vascular tortuosity in 26.3% and disc sign in 10.5%. Proliferative sickle retinopathy (PSR) was present in 39.5% of patients, mean age 17.5 years, age range 4 -35 years. Stages 1, 2, 3 and 4 were seen in 5%, 23.6%, 8% and 3% of patients. No patient had stage 5 PSR. Patients with anterior and posterior segment findings had a statistically significant older mean age than those who did not (19.6±10.4 and 18.6±11.5 years *versus* 12.3±9.6 and 13.2±8.7 years, p<0.05). Anterior manifestations were significantly more frequent among patients who had cholecystectomy compared to those who did not (63% *versus* 36.8%, p<0.05), those with a median hematocrit lower than 25.9% than those with higher hematocrit (59% *versus* 31.5%, p<0.05) and in those with a higher transfusion load (60.8 *versus* 21.8 transfusions, p=0.02). A significantly higher level of black sunbursts was found in patients who had cholecystectomy compared to those who did not (32% *versus* 11%, p<0.05).
Summary and Conclusions: Ophthalmologic complications are frequent among young Lebanese patients with SCD and tend to increase with age. Despite the small sample size, the high rate of PSR at a very young age is worrisome. The higher frequency of ophthalmic complications in patients with cholecystectomy, low hemoglobin and high transfusion rate suggest a possible underlying hemolytic etiology besides the well established vaso-occlusive etiology. Early identification of affected patients particularly those at high risk for PSR through effective screening can help introduce timely targeted treatment thereby improving visual outcomes and preventing vision loss.

P740

PREVALENCE OF G6PD DEFICIENCY IN PATIENTS WITH SICKLE CELL DISEASE AND IMPACT ON DISEASE SEVERITY IN LEBANON

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Background: Glucose-6-phosphate dehydrogenases (G6PD) and sickle cell disease (SCD) occur due to defects in hemoglobin stability or structure, respectively. Sickle cell trait and G6PD deficiency are found in 2.1% and 2.09% of the Lebanese population. To date, the rate of co-inheritance of both SCD and G6PD deficiencies in Lebanon has not been determined.
Aims: The primary objective is to determine the co-inheritance rate of G6PD deficiency and SCD in Lebanon. The secondary objective is to delineate the impact of G6PD deficiency co-inheritance on SCD related morbidity and mortality.
Methods: Patients with SCD, followed in 2 comprehensive SCD referral centers in mid and North Lebanon were tested for G6PD deficiency using spot test. All abnormal tests were confirmed by repeat testing. High pressure liquid chromatography (HPLC) was utilized for diagnosis of SCD. Pertinent demographic, clinical, and hematologic information was collected. Statistical analysis was done using Statistica (v. 12.5). Statistical significance was set for p <0.05.
Results: A total of 121 patients with SCD, 57% males, 43% females, mean age 12.5±11.3 years, median follow-up 12.6 years, were screened for G6PD deficiency. 72.5% had HbSS, 21.5% $S\beta^0T$, 4.2% $S\beta^+T$ and 1.6% SD, respectively. 6/121 (5%) patients were found to be deficient for G6PD. Hematologic and clinical features are listed in Table 1.

Table 1. General clinical and laboratory characteristics of SCD patients.

HbS at diagnosis	71±14.9 %
HbF at diagnosis	20.5±12.3 %
HbA1 at diagnosis	6.2±13 %
Mean steady state hemoglobin	8.7±1 g/dL
Mean steady state LDH	493.3±188 unit
Mean steady state indirect bilirubin	1.8±1 unit
Mean steady state total bilirubin	1.9±1.1
Mean steady state heptaglobin	0.5±1.7 unit
Mean steady state reticulocyte	9.5±4.8 %
Mean steady state serum ferritin	610±783 unit
N with acute splenic sequestration(ASS)	44.50%
Patients who have undergone cholecystectomy	53%
Age at cholecystectomy (years)	14.4±23
Mean number of transfusions	24.9±41
Hepatomegaly present	53%
Hydroxyurea treatment	61.60%

Steady state hemoglobin, LDH, haptoglobin, and ferritin were 8.7±1 g/dL, 493±188 unit, 0.5±1.7 and 610±783 ng/dcl, respectively. Acute splenic sequestration (ASS), cholecystectomy and persistent splenomegaly beyond 6 years of age were seen in 44.5%, 26% and 20% of patients, respectively. 40% of patients had splenectomy (surgical in 13% and auto-splenectomy in 26%). Mean HbA1 at diagnosis and steady state ferritin of SCD patients were significantly different among G6PD deficient and G6PD wildtype patients. Specifically, HbA1 was 21.3% in G6PD deficient patients *versus* 5% in wild type (p=0.01);

and steady state ferritin was 1410.9 unit in G6PD deficient patients, while it was 568.6 unit in G6PD wildtype SCD patients (p=0.0002). Number of transfusions, age at cholecystectomy, steady state hemoglobin, LDH, heptaglobin, reticulocyte count or bilirubin were not statistically different between the two groups. In Fisher's exact test analysis, we did not find a correlation between G6PD status and each of gender, splenomegaly, splenectomy, ASS, cholecystectomy or hepatomegaly. Three SCD patients (2.5%) who had wildtype G6PD died during the course of this study but was independent on G6PD status.
Summary and Conclusions: This is the first study on SCD and G6PD deficiency co-inheritance in Lebanon. G6PD deficient SCD patients had significantly higher steady state ferritin levels despite no increased rate of transfusions. Hence, it is possible that G6PD deficiency can alter the iron regulatory and storage pathways via mechanisms that are still to be elucidated. However, this warrants that SCD patients with G6PD deficiency should be monitored more closely for increased iron overload complications.

Infectious diseases, supportive care 2

P741

ANTIFUNGAL PROPHYLAXIS IN HAEMATOLOGY PATIENTS: FREQUENCY AND DRUG CLASS OF CHOICE ACCORDING TO TYPE OF HAEMATOLOGICAL MALIGNANCY (AFHEM STUDY)

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Background: The mortality rate associated with invasive fungal disease (IFD) remains very high (≥40%). Patients in haematology units are at high risk of IFD, and more acurate data is needed on medical practice and use of antifungal therapies.
Aims: An objective of this study was to describe the frequency and characteristics of systemic antifungal prophylaxis strategy in French patients suffering from haematological malignancy and hospitalized in a haematology unit in routine medical practice.
Methods: Multicenter, cross-sectional, prospective French observational study conducted in 24 hematology units (717 beds including 368 sterile beds) over 5 consecutive days on adult or paediatric patients with hematological malignancies and hospitalized during this period.
Results: Overall, 494 patients who signed a consent form were classified into 4 groups according to their risk of Invasive Fungal Infections (IFI). Group 1 includes patients who received allogeneic hematopoietic stem cell transplant (HSCT) (N=147), Group 2 patients suffering from AML or MDS who did not receive allogeneic HSCT (N=131), Group 3 patients suffering from ALL who did not receive allogeneic HSCT (N=71) and Group 4 all the patients not included in the groups 1, 2 or 3 (N=145). 246 patients received systemic antifungal therapeutic strategy. Among them, the proportion of patients who received prophylactic strategy was for Group 1: 95/119 (79.8%), Group 2: 56/78 (71.8%), Group 3: 12/19 (63.2%), Group 4: 24/30 (80.0%). Half of the patients from groups 2 and 3 presented neutropenia for at least 10 days when enrolled in the study compared to 17% for groups 1 and 4. 82% (78/95) and 73% (41/56) of the patients from groups 1 and 2 were placed in sterile room (either in laminar flow sterile room or Immunair™ bed or in highly purified room) compared to 50% (6/12) of the patients from Group 3 and 54% (13/24) from Group 4. In Group 1: 75% (71/95) of patients received an azole, mostly fluconazole (44/71); 13/95 received echinocandins, mostly caspofungin (11/13); 11/95 received polyenes. In Group 2: 89% (50/56) of patients received an azole, mostly posaconazole (35/56); 6/56 polyenes and 1/56 echinocandins. In Group 3: 33% (4/12) of patients received oral fluconazole; 3/12 posaconazole, 3/12 polyenes and 3/12 echinocandins. In Group 4: 92% (22/24) patients received an azole, mostly posaconazole (16/24); 1/24 polyenes and 1/ 24 echinocandins.
Summary and Conclusions: Prophylaxis is now the leading strategy used in hematology, whatever the disease conditions of the patients with overall 76% of treated patients receiving it. Overall, prophylaxis is mainly based on oral azoles : fluconazole and posaconazole. Fluconazole is still used in high risk patients (allogeneic HSCT) and low risk patients (Group 4). Posaconazole is mainly used in AML/MDS patients, but also in ALL patients not receiving HSCT where its use is off-label. Echinocandins are mainly used in high risk patients. Caspofungin is the most-used echinocandin although it is not licensed for prophylaxis.
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P742

INVESTIGATING CYTOPENIA MAY REVEAL BONE MARROW METASTASIS: A SINGLE CENTER EXPERIENCE FROM THE HEMATOPATHOLOGIST POINT OF VIEW

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Background: Bone marrow (BM) involvement by non-hematopoietic neoplasms usually occurs as a late event during the course of oncologic diseases. Most patients with BM metastases have hematological abnormalities, such as one or more cytopenias.
Aims: BM biopsy is a key diagnostic tool to investigate unexplained persistent cytopenias. Limited information is reported in the literature about the incidence of BM metastasis during the course of solid malignancies, with considerable variability depending on the different types of neoplasms.

Methods: A total of 10736 BM trephine biopsies were examined at the Hematopathology Laboratory of the Azienda Ospedaliero-Universitaria Policlinico of Modena, from January 2004 to December 2014. After processing, all samples were stained with Hematoxylin-Eosin (H&E), Giemsa and Silver impregnation (Gomori reaction). After morphologic evaluation, the appropriate immunohistochemical panel, including cytokeratin (CK) mix (AE1-AE3), was performed. Further characterizations of non hematopoietic neoplasms were carried out in collaboration with the Pathologic Anatomy laboratory.
Results: Solid cancer metastasis was documented in 107 of all 10736 BM biopsy samples (0.99%). Concomitant BM aspirates and touch imprints were evaluable in 92 (86%) cases. Median patient age was 68 years (range 7-88 years; excluding a pediatric case, the interval span was 37-88 years). Males and females were 49 (46%) and 58 (54%) of the 107 cases, respectively. The most frequent metastatic tumors were, in order of frequency, as follows: breast (39.25%), gastrointestinal (16.82%), lung (13.08%) and prostate (12.15%) carcinomas. Among them, a previous diagnosis of solid cancer was missing in 25 cases (23.36%). Primary tumor site was determined in 16 out of these latter 25 cases (64%), namely breast 5 (20%), lung 4 (16%), kidney 2 (8%), prostate 1 (4%), neuroendocrine 2 (8%), gastro-intestinal 1 (4%) and cervix 1 (4%). In the remaining 9 cases (36%), the rapid deterioration of clinical conditions contraindicated further diagnostic investigations. However, epithelial origin was documented according to CK mix positive staining, without other specific markers. Cytopenias were the most common hematological finding, namely, in order of frequency: anemia (75.7%), thrombocytopenia (64.5%), and more rarely, leukopenia (23.4%). Of note, pancytopenia was present in 13.1% of patients only. The finding of BM metastases predicted unfavorable outcome, with a median survival from observation of 7 weeks (range 0-361 weeks). This wide range was conditioned by the presence of few breast cancer patients still responsive to hormone therapy (15 women (14%) surviving more than one year).

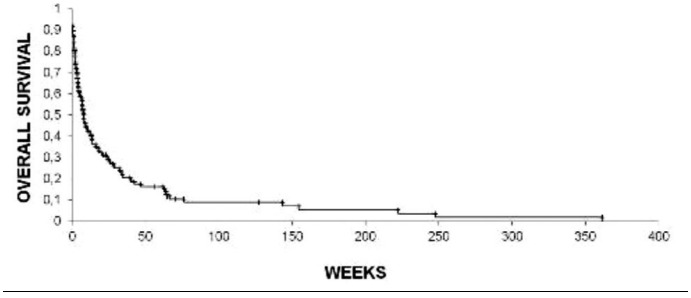

Figure 1. BM metastases patients (n=107).

Summary and Conclusions: BM biopsy is an invasive procedure mainly used for the diagnosis of hematopoietic disorders. Involvement of the BM by non-hematopoietic lesions can incidentally be observed by the hematopathologist. Our case series highlighted that persistent unexplained cytopenias in solid cancer patients can be the first indicator of BM metastases. Although rarely, the finding of BM metastasis can lead to the first diagnosis of solid organ malignancies. However, patients with BM metastasis have a poor outcome and, for most of them, only supportive care can be considered.

P743

DORIPENEM *VERSUS* MEROPENEM AS THE FIRST-LINE MONO-THERAPY IN HIGH-RISK FEBRILE NEUTROPENIC PATIENTS WITH ACUTE LEUKEMIA: A RANDOMIZED, CONTROLLED TRIAL

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Background: Febrile neutropenia (FN) is often observed in chemotherapy of hematological malignancy (HEM) such as acute leukemia (AL). Carbapenem antibiotics having a potent and broad antimicrobial activity against both gram-positive and gram-negative bacteria including Pseudomonas aeruginosa, are good candidates as the first-line therapy drug in high-risk FN patients. In the IDSA guideline 2010 on the use of antimicrobial agents in FN, mono-therapy with an anti-pseudomonal beta-lactam, such as piperacillin-tazobactam (TAZ/PIPC), cefepime (CFPM) or carbapenem [only meropenem (MEPM) and imipenem-cilastatin (IPM)], is recommended as an empirical therapy for high-risk FN patients. Doripenem (DRPM) is a newer carbapenem with few data available as for the efficacy and safety in the setting of FN patients.
Aims: We conducted a randomized, cooperative group and open-label trial comparing DRPM (1.0 g every 8 hours) with MEPM (1.0 g every 8 hours) as the first-line empirical antibacterial therapy for high-risk FN patients with AL and myelodysplastic syndrome (MDS).
Methods: One hundred and forty-six hospitalized high-risk FN patients with HEM (AML 96, ALL 23, APL 13, MDS-AML 9, MDS (RAEB-2) 5 cases) during or after chemotherapy were randomized to each drug group (DRPM, 72; MEPM, 74). The study drug was started to administer as a mono-therapy and continued at least for 5 days without severe drug toxicity, and the efficacy and safety were evaluated.
Results: The overall response rate at 7 days in DRPM and MEPM group were not significantly different (DRPM: 67.6%, MEPM: 52.9%, respectively, P=0.098). Both the resolution of fever by mono-therapy at day 3 to 5 (DRPM: 56.9%, MEPM: 47.0%, respectively, p=0.26), and survival at day 30 (DRPM: 98.4%, MEPM: 98.5%, respectively, p=0.312) were not significantly different in the two groups. The cases needed for anti-MRSA agent and antifungal agent tended to be observed less frequently in DRPM rather than in MEPM group [DRPM: 36.9%, MEPM: 50.0% (p=0.185), DRPM: 26.1%, MEPM: 32.3% (p=0.535), respectively]. Only grade 1-2 adverse events were observed in both groups (liver dysfunction, renal dysfunction, diarrhea and rash), and they were less often in DRPM group significantly (DRPM: 29.8%, MEPM: 40.8%, respectively, p=0.046). These adverse events were clinically acceptable in the two groups, and most of patients could continue the treatment by both study drugs.
Summary and Conclusions: Our clinical study suggested that DRPM had the non-inferiority of efficacy in comparison with MEPM as the first-line therapy in high-risk FN patients with AL and MDS, and both drugs could be well tolerated clinically.

P744

THE RATE OF HUMAN HERPES VIRUS 6 INFECTION IN ACUTE LEUKEMIA AND LYMPHOMA PATIENTS

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Background: Human herpes virus 6 (HHV-6) is increasingly detected in hematopoietic stem cell transplantation (HSCT) recipients and in patients who recover after chemotherapy courses. HHV-6 may cause serious complications such as encephalitis, myelosupression, pneumonitis, hepatitis, cystitis, unexplained fever, rash and is associated with graft-*versus*-host disease. The virus DNA is detected in blood of 40% to 60% patients after HSCT, HHV-6 infection - in 10-15% of such patients. DNA polymerase chain reaction (qPCR) is the common method of HHV-6 detection. HHV-6 infection lacks specific clinical syndromes and is often revealed with CMV infection/reactivation. On time diagnostics and early beginning and prolonged antiviral treatment (ganciclovir, foscarnet or cidofovir) may favorably affect the patient survival and neurologic symptoms.
Aims: To estimate the frequency of HHV-6 infection reactivation in patients (pts) with acute leukemias and aggressive lymphomas after chemotherapy and auto-HSCT.
Methods: For the period from January 2013 to December 2014 in the Department of Hematological Oncology and BMT 68 pts (23 ALL, 42 AML, 3 DLBCL) were analyzed. Auto-HSCT was carried out in 6 cases. Blood, bone marrow, cerebrospinal fluid and urine samples were collected in patients who had febrile neutropenia and other infection complications after courses of chemotherapy or auto-HSCT. HHV-6 DNA was detected in biological fluids by realtime PCR. The lower limit of detection for the assay was 500 copies/mL.

Table 1.

Patients	Ds	Male/Female	Age	Auto-HSCT	+CMV	Symptoms& infections	Period of treatment (Ganciclovir)	Reccurency HHV-6	Total Treatment iv
1 pt	AML	M	42	-	-	Febrile fever, cytopenia	3 weeks	+	6 weeks
2 pt	ALL	M	24	+	-	Encephalitis	2,5 weeks	+	9 weeks
3 pt	ALL	F	34	+	+	Febrile fever, cytopenia	4 weeks	-	4 weeks
4 pt	ALL	M	37	+	-	Hemorrhagic cystitis	4 weeks	-	4 weeks
5 pt	DLBCL	M	54	+	-	Hemorrhagic cystitis	4,5 weeks	-	8 weeks
6 pt	DLBCL	F	55	-	-	Febrile fever, cytopenia	4 weeks	-	4 weeks

Results: HHV-6 DNA was detected in 35% cases (n=24; 9/24 ALL, 13/24 AML, 2/24 DLBCL). Four out of 24 pts were after auto-HSCT. Only in 8,8% (6/68) of cases (including 4 pts after auto-HSCT) we associated the HHV-6 detection and clinical symptoms (3 ALL, 2 DLBCL, 1AML). Prolonged cytopenia (more than 40 days) and febrile fever had 3 pts, cystitis 2 pts, encephalitis 1 pt. (Table 1). All 6 pts were treated with ganciclovir (10mg/kg iv) during 4 weeks with conversion to valganciclovir (per os) during the next 2 weeks. The median ganciclovir treatment time was 4 weeks but in 2 cases of recurrent infection the treatment was prolonged until 6 (6-9) weeks. Early cessation of therapy (after 2 weeks) caused viremia reccurence and viral load increasing of HHV-6 in 2 pts. The clinical symptoms fully disappeared after 3-5 days of treatment. The median time to virus clearance was 4 (4-8) weeks.
Summary and Conclusions: Our study showed high frequency of HHV-6 DNA detection (35%) in immunocompromised pts, only 8,8% had HHV-6 associated infection, most of them were after auto-HSCT. Early conversion therapy to valganciclovir per os caused reccurent HHV-6 infection (33%). Thus, HHV-6 infection required long treatment.

P745

IMPACT OF DECTIN-1 POLYMORPHISMS ON SUSCEPTIBILITY OF INVASIVE FUNGAL DISEASE IN PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background: Patients with acute myeloid leukemia (AML) who undergo induction chemotherapy are at high risk for invasive fungal disease (IFD). Dectin-1 (Dendritic cell-associated C-type lectin-1), a C-type lectin family member represents one of the most important and intensively studied pattern recognition receptor (PRR) of the innate immune system and single nucleotide polymorphisms (SNP) in the Dectin-1 gene have been associated with an increased risk of infectious complications. In detail, the heterozygous Dectin-1 variant Y238X coding for an early stop codon has been associated with invasive aspergillosis (IA) in recipients of hematopoietic stem cell transplantation.
Aims: We sought to investigate the impact of two different Dectin-1 SNPs on developing IA in 164 patients with newly diagnosed AML during anthracycline-based induction chemotherapy (cytarabine combined with idarubicin or mitoxantrone). The occurrence of infectious complications (sepsis, general pneumonia, IFD) were analyzed.
Methods: Genotyping of Dectin-1 SNPs (rs16910526 and rs7309123) was performed by TaqMan method and pyrosequencing in 164 adult AML patients. IFD was defined according to the EORTC/MSG consensus guidelines. Multiple logistic regression analyses were applied to evaluate the association between Dectin-1 polymorphism and infectious events.
Results: We could demonstrate that patients carrying the homozygous Dectin-1 SNP rs7309123 (G/G genotype, n=43 patients) revealed a significantly higher risk for developing both pneumonia in general (p=0.018, odds ratio (OR): 2.46; 95% confidence interval (CI): 1.2 - 4.9) and pulmonary IFD (p=0.023, OR: 2.3; 95% CI: 1.2 - 4.5). In detail, 24 patients harbouring homozygous Dectin-1 SNP developed pneumonia in general whereas 18 of them fulfilled criteria for IFD. No significant correlation was observed between the occurrence of sepsis and either SNP. Importantly, no differences were found between patients with heterozygous Dectin-1 SNP rs16910526 (Y238X, T/G genotype, n=13 patients) and wild type carriers with regard to all analyzed infectious complications.
Summary and Conclusions: To our best knowledge, this study represents the first analysis demonstrating that a haplotype containing the Dectin-1 SNP rs7309123 influences the risk of developing invasive fungal disease in patients with acute myeloid leukemia undergoing induction chemotherapy.

P746

ANTIFUNGAL PROPHYLAXIS WITH POSACONAZOLE IN ACUTE MYELOID LEUKEMIA AND MYELODYSPLASTIC SYNDROME PATIENTS TREATED WITH INTENSIVE CHEMOTHERAPY. A MULTICENTER RETROSPECTIVE STUDY OF REAL-LIFE PRACTICE

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Background: Patients diagnosed with acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS) who receive intensive induction chemotherapy to achieve complete remission (CR) are considered to be at high risk for developing invasive fungal infection (IFI) during the neutropenic phase (NP). IFI occurs in up to 10% of patients diagnosed with AML/MDS on induction treatment, and is fatal in approximately 40% of cases. Moreover, IFI may compromise the subsequent chemotherapeutic strategies. Difficulty of making a rapid diagnosis of the fungal disease and the high mortality associated, have led to use primary antifungal prophylaxis (PAP) to prevent the occurrence of IFI
Aims: To collect the experience with posaconazole as PAP in patients diagnosed with AML or MDS in hospitals from our environment (Madrid-Spain). Specific goals of this study were to assess IFI rates in our patients (incidence of proven or probable IFI during the NP) the efficacy of this prophylaxis, the rate of adverse events, infection-associated mortality, and overall mortality
Methods: A multicenter, observational, retrospective study of incidence of IFI in patients diagnosed with AML and MDS, who received posaconazole as PAP, and treated with intensive induction chemotherapy, was carried out in 11 hospitals in Madrid (Spain) between 2009 and 2012. In order to achieve a minimum 100 evaluable patients each hospital collected around 10 consecutive AML/MDS patients showing deep neutropenia defined as <500 neutrophils/µL for more than 7 days and <100 neutrophils/µL for more than 4 days. All patients received posaconazole, oral suspension, 200 mg three times daily, at least for one day. Patients follow-up was stopped when a new chemotherapy treatment was started, after 30 days-off posaconazol, or if the patient died
Results: 102 patients fulfilled the inclusion criteria. 63% were male with a median age of 61 years. Diagnosis was AML 94% (8% in first relapse) and MDS 6%. Anthracycline-based chemotherapy was used in 92% of patients; CR was achieved in 72% and partial remission in 10%. All patients developed <500 neutrophils/µL with a median duration of 23 days (range 8-99) and <100 neutrophils/µL for a median of 16 days (range 4-84). Posaconazole was used a median of 19 days (range 1-90). In 48 patients (47%) PAP was successful, but 35 (34%) needed a change in antifungal therapy: empirical treatment due to maintained fever in 21 patients (21%) and to treat IFI in 14 patients (IFI was diagnosed as possible in 10, probable in 3 and proven in 1) In all cases, lung was the organ involved. Switch from posaconazole a different intravenous prophylaxis due to mucositis, or poor oral intake, was a further cause of PAP failure in 13% of patients. Drug discontinuation because of liver or gastrointestinal toxicity, was observed in 5% of patients. Overall mortality was 6% (bacterial infections in 3 patients; thrombotic and hemorrhagic events in 2 patients, and 1 patient due to progression leukemia) with no IFI attributable mortality in our patients.
Summary and Conclusions: Despite the limitations of this retrospective, multicenter, real-life study, PAP with posaconazole reduced the incidence of probable and proven IFI to 4% of patients diagnosed with AML and MDS and treated with intensive induction chemotherapy. Nevertheless empirical antifungal treatment was maintained in a fever-driven approach in 21% of the patients. Poor oral intake represents a further handicap with this PAP that might be solved in the near future with the new upcoming drug presentation

P747

COMMUNITY ACQUIERED RESPIRATORY VIRUS IN ADULTS PATIENTS WITH HEMATOLOGICAL DISEASE: CLINICAL CHARACTERISTICS AND OUTCOME IN RSV AND HPIV INFECTION

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Background: Community-acquired respiratory viruses (CARV) are a frequent cause of respiratory infections in immunocompromised patients with an increased risk for progression to pneumonia and fatal outcome. The diagnosis of these infections are moving from the classical techniques (virus isolation by cell culture, direct antigen detection) to nucleic acid amplification test usually directed to several viruses.
Aims: The aim of this study was to describe CARV infections in haematological patients based on multiplex PCR DNA microarray platform, focusing on Respiratory Syncytial Virus (RSV) and Human Parainfluenza Virus (HPIV) infections due to their higher frequency and clinical relevance.
Methods: We have analysed retrospectively 570 specimens from 211 consecutive haematological patients with clinical symptoms of respiratory infection from November 2012 to April 2014. The collected samples, nasopharyngeal exudates and/or bronchoalveolar lavages (BAL), were tested with the CLART® Pneumovir assay (Clinical Array Technology, Genomica, Spain) that detect 19 CARV*s.* Patients were classified as upper (URTID) or lower respiratory tract infection disease (LRTID) according to the published criteria of the Fourth European Conference Infections in Leukaemia (ECIL-4) (Hirsch HH, *et al.* Clin Infect Dis. 2013;56(2):258-66).
Results: There were 195 positive cases (34%) in 570 specimens from 211 haematological patients. Ninety nine patients had a positive result at least once. Viruses detected were: 64 RSV, 35 HPIV, 64 rhinovirus, 8 metapneumovirus, 2 enterovirus, 2 coronavirus, 2 bocavirus, 4 adenovirus and 14 patients with several viruses isolate in the same sample. Fifty-nine patients were diagnosed with RSV or HPIV URTDI/LRTID (Table 1).
All patients with RSV were diagnosed from November to April. Nevertheless, HPIV were diagnosed during all the year with a maximum incidence in June (6 cases). Twenty-nine (49%) patients were treated with oral ribavirin, mostly

when RSV was diagnosed. Patients with no treated RSV, had solved the symptoms when positive test was informed. Ribavirin dosage used was that recommended in ECIL-4. Median ribavirin treatment duration was 14 days (1-35). Treatment was well tolerated. There were two engraftment delays in two transplantation patients with RSV and LRTD (one autologous and one allogeneic). One of them died of Pseudomonas aeruginosa pneumonia without neutrophil engraftment. There were two deaths (6%) due to RSV pneumonia. One death occurred in an AML patient treated with chemotherapy without any other microbiological documentation. The other fatal case occurred in a T-NHL patient hospitalized for autologous stem cell transplantation with CMV coinfection. Both patients received ribavirin since RSV infection diagnosis. No deaths related to HPVI were described. In the whole series time to solve symptoms was 14 (3-59) days and time to achieve a negative test was 20 days (7-119).

Table 1. Patients with RVS or HPIV disease.

		RSV	HPIV
Number of cases	59	32	27
Age, median years (range)	57 (21-90)	59 (21-90)	55 (23-82)
Gender, male / female	26 (44%) / 33 (56%)	15 (47%) / 17 (53%)	11(41%) / 16 (59%)
Underlying haematological disease Acute leukemia Lymphoma, CLL Multiple Mydoma Myelodisplastic syndrome Other	 20 (34%) 20 (34%) 9 (15%) 7 (12%) 3 (5%)	 10 (31%) 14 (44%) 3 (9%) 4 (12%) 1 (3%)	 10 (37%) 6 (22%) 6 (22%) 3 (11%) 2 (8%)
Hematopoietic Stem Cell Transplantation (HSCT) Allogeneic PB-HSCT Autologous PB-HSCT	27 (46%) 23 (85%) 4 (15%)	15 (47%) 12 (80%) 3 (20%)	12 (44%) 11 (92%) 1 (8%)
Presentation of respiratory disease Cough Fever Rhinorrhoea Dyspnoea Odynophagia	 37 (63%) 28 (47%) 20 (34%) 7 (12%) 4 (7%)	 20 (62%) 11 (34%) 11 (34%) 4 (12%) 3 (9%)	 17 (63%) 17 (63%) 9 (33%) 3 (11%) 1 (4%)
Coinfection Bacterial Virus Fungal Bacterial + other	21 (36%) 9 (43%) 7 (33%) 2 (9%) 3 (14%)	13 (41%) 8 (62%) 3 (23%) 0 2 (15%)	9 (35%) 1 (12.5%) 4 (15%) 2 (25%) 1 (12.5%)
CARV reinfection	7 (12%)	3 (9%)	4 (15%)
Type of infection URTID LRTID	 42 (71%) 17 (29%)	 21 (66%) 11 (34%)	 21 (78%) 6 (22%)
Lymphopenia <300/µl	11 (20%)	8 (25%)	3 (12%)
Ribavirin treatment	29 (49%)	24 (75%)*	5 (20%)*
Interval, symptoms-diagnosis	5 (1-20)	5 (1-20)	5 (2-14)
Interval, diagnosis-resolution	14 (3-59)	16 (6-59)	11 (3-21)
Interval, diagnosis-testnegative	20 (7-119)	15 (7-119)	25 (16-38)

URTID: Upper respiratory tract infection
LRTID: Lower respiratory tract infection
* Statically significant

Summary and Conclusions: CARV infection is a frequent cause of respiratory tract infectious disease. The most important CAR*Vs* by clinical relevance and frequency are RSV and HPIV. In our series LRTID was diagnosed in 34% patients with RSV and 22% patients with HPIV but fatal complications occurred only in RSV infection (6%) in spite of treatment. Oral ribavirin is a treatment option, but can be associated with engraftment delay. More studies are necessary to established treatment criteria, dosage, duration and efficacy of ribavirin treatment in this population. The value of follow-up PCR tests in this patient population neither has been established.

P748

LEVEL OF PENTRAXIN-3 IN PATIENTS WITH ACUTE LEUKEMIA IN SEPTICEMIA AND ITS PROGNOSTIC VALUE

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Background: In acute leukemia, sepsis is a potentially fatal complication. Pentraxin 3 is a protein rapidly produced in response to primary inflammatory signals. It shows high levels in sepsis, especially associated with vascular and end organ failure.
Aims: The study aimed to measure the level of Pentraxin 3 in septicaemia in patients with acute leukemia and correlate its level to higher risk of complications compared to C-reactive protein (CRP).
Methods: The study took place from January, 2012 to December, 2013 including 60 acute leukemia patients with sepsis, during severe neutropenia (ANC<500) and after receiving chemotherapy. Samples were analyzed for serum CRP and plasma PTX3 on days 1, 2 & 3 by ELISA. Blood cultures were done to all patients.
Results: The studied group had a male to female ratio of 1:1, age ranged from 18-62 years (median of 40 years). 41 patients had Acute Myeloid Leukemia while 19 had Acute Lymphoblastic Leukemia. High Pentraxin 3 levels on first day of sepsis is a strong indicator for poor prognosis (septic shock, mortality and coagulopathy outcomes) compared to CRP ($p<0.01$). PXT3 showed sensitivity of 100% and specificity of 70% for prediction of poor prognosis where CRP showed 88.5% and 60.5% respectively (Table 1 and Figure 1).

Table 1. Area under the curve represents significance, sensitivity and specificity of Pentraxin 3 and CRP in day 1 among patients in sepsis.

Test Result Variable(s)	Area	Std. Error(a)	Asymptotic Sig.(p)	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
Pentraxin day 1	0.920	0.057	0.001*	0.809	1.032
CRP day 1	0.778	0.109	0.022	0.565	0.991
			Sensitivity %	Specificity %	
Pentraxin day 1	3.3500		100	70	
CRP day 1	89.00		88.5	60.5	

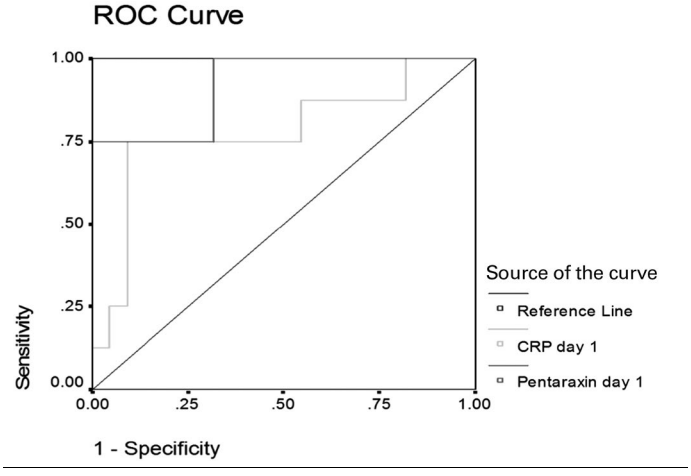

Figure 1. ROC curve to determine the sensitivity and specificity of CRP and Pentraxin 3 at day 1.

Summary and Conclusions: PXT3 is highly recommended in diagnosis of sepsis in patients with acute leukemia during neutropenia and shows high sensitivity and specificity in prediction of poor prognosis (septic shock, mortality and coagulopathy outcomes) in comparison to CRP.

Non-malignant hematopoietic disorders

P749

IDENTIFICATION OF CLONALITY IN IDIOPATHIC HYPEREOSINOPHILIA USING TARGETED EXOME SEQUENCING

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Background: Idiopathic hypereosinophilia (IHE) has been a disorder of uncertain etiology, characterized by persistent elevation of blood eosinophil count exceeding $1.5x10^3$/uL. So far, the diagnosis has been made through the exclusion of either reactive or clonal causes of hypereosinophilia. When end-organ damage due to eosinophilic infiltration is present, idiopathic hypereosinophilic syndrome (IHES) is diagnosed. IHE and IHES are heterogeneous diseases and have not been studied well so far. The nature of eosinophilic proliferation is still unclear, whether benign or clonal, but WHO classification of hematologic neoplasm described IHE and IHES with defined diagnostic criteria in the section of myeloproliferative neoplasm.
Aims: Purpose of this study was to investigate the frequency of somatic mutations in patients with IHE/IHES through targeted exome sequencing.
Methods: Bone marrow was collected from a total of 20 patients fulfilling WHO 2008 criteria for IHE (n=8) and IHES (n=12) along with 15 patients diagnosed with reactive eosinophilia as a control group. In all patients, fluorescence *in situ* hybridization (FISH) for *BCR/ABL1*, *PDGFRa, PDGFRb*, and *FGFR1* was performed for excluding myeloproliferative neoplasms associated with eosinophilia with known cytogenetic aberrations. We designed capture sequencing for 88 genes known for recurring mutation in hematologic malignancies using target capture by Sureselect (Agilent, Santa Clara, CA, USA) followed by sequencing using IlluminaHiSeq 2500. The sequencing reads were analyzed based on the pipeline of bioinformatics tool. Candidate mutations were confirmed by Sanger sequencing and also sequenced using matched saliva samples (germline) from patients.
Results: The average coverage of target regions was >800-fold. The targeted exome sequencing analysis revealed 193 substitutions and insertion/deletions (Indels), median 3 per patient (range 0-86). Of these 193mutations, we identified somatic mutations in 4 genes according to interpretation algorithm; *MPL* (2/20, 10%)*, SCRIB* (2/20, 10%), *ASXL1* (3/20, 15%), and *STAG2* (3/20,15%). The findings above highly suggest that these genes are likely related to pathogenesis of hypereosinophilia.
Summary and Conclusions: Four recurrent mutations were identified in 6 patients (30%) by targeted exome sequencing analysis. Due to a limitation of small number patients enrolled in the present study, a future larger validation cohort is needed to test the frequency of the identified variants. We suggest that mutation analysis of the 4 genes (*MPL, SCRIB*, *ASXL1* and *STAG2*) could be help to unveil clonal proliferation of eosinophil and here we propose them as a potential candidate for the diagnostic work-up for hypereosinophilia. Furthermore, this discovery of somatic mutations in IHE/IHES will provide a baseline data for the tailored treatment of hypereosinophilia.

P750

DEVELOPMENT OF A NOVEL ANTI-NEUTROPENIC FACTOR CLINICAL CANDIDATE FOR HEMATOPOIESIS DEFICIENCIES

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Background: A novel anti-Neutropenic factor (ANF) consisting of a pegylated granulocyte colony-stimulating factor with improved pharmacokinetic/pharmacodynamics (PK/PD) properties was developed through a combinatorial PEGylation approach to create a clinical candidate ideally suited for treating conditions of severe neutropenia.
Aims: Analyze ANF and marketed therapeutic(s) in parallel to identify unique properties of ANF thereby expediting selection of a superior clinical candidate for specific human clinical trials.
Methods: A series of *in vivo* and *in vitro* pre-clinical studies were conducted in comparison to marketed forms of filgrastim (F) or PEG-filgrastim (PF). Following pre-requisite and unremarkable GLP-toxicology studies, a phase 1 clinical study was conducted in healthy volunteers (signed, consenting) to assess safety and tolerability of ANF as well as the PK and PD. Subcutaneous, single dose treatment of ANF (or PF) with ascending dose were evaluated in a double-blind study that included PK, neutrophil and CD34+ analytical parameters to determine if pre-clinical ANF findings could also be observed within healthy human subjects. ANF was evaluated in a dose-escalation (5 - 50µg/Kg) study with each cohort including randomized treatment and controls. PF was administered at the single 6mg dosage (80-100µg/Kg), single-use syringe as a standard of care supplied by manufacturer.
Results: Rat neutropenia model dosage study results indicated the blood PD parameters of ANF were significantly superior to both F and marketed PF. Area under the curve (AUC) kinetic analysis showed the absolute neutrophil count (ANC) of ANF was equivalent at 4X lower dosage (25 *vs* 100µg/Kg) and yielded significantly higher ANC than marketed PF when administered at equivalent 100µg/Kg dosage. A follow-on *in vitro* human neutrophil maturation study was conducted to evaluate CD34+ stem cell maturation effects of ANF compared to PF. Similar to rat study findings, ANF yielded a 4-6 fold increase in *de novo* neutrophil (CD66+) counts. Phase I clinical interim safety results were unremarkable, with no severe adverse events in any cohorts (ANF or PF). The ANF PK/PD parameters within the phase 1 study were similar to pre-clinical findings. PD results (ANC and CD34+) were markedly prolonged in the ANF treatment groups even at the lowest cohort. Mean ANC counts for all ANF cohorts showed an ANC C_{max} between 162 - 177hrs in contrast to PF that reached max concentrations at 52hrs. AUC analysis showed that ANF at 10µg/Kg was equivalent to PF at 100 ug/Kg (standard of care) demonstrating a >8-fold potency effect over PF in healthy volunteers. Peripheral blood CD34+ counts also yielded similar results; average ANF C_{max} was prolonged by 2 days over PF (7 *vs* 5 days). PK results were prolonged with ANF in both the pre-clinical rat and phase-1 human study that correlated with the PD results.
Summary and Conclusions: Collectively these data showed that ANF, a novel anti-neutropenic factor, has unique, prolonged PK/PD attributes as compared to PF, and these qualities may provide an improved clinical benefit in further clinical studies. Phase 2 efficacy studies in severe neutropenia are planned to further understand the potential clinical applications and benefits of ANF.

P751

PRECLINICAL PHARMACOKINETIC/PHARMACODYNAMIC RELATIONSHIPS FOR AG-348, AN INVESTIGATIONAL SMALL-MOLECULE ACTIVATOR OF PYRUVATE KINASE

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Background: Pyruvate kinase (PK) deficiency is a rare, autosomal recessive, inborn error of metabolism caused by a functional deficiency of the R isoform of PK (PK-R). It is characterized by life-long hemolytic disease and serious long-term comorbidities such as poor growth and development in children, iron overload, and multi-organ dysfunction. Current treatments are palliative only, and there are no approved drugs that directly target the underlying enzyme defect. PK-deficient red blood cells display metabolic changes related to defective glycolysis, including a build-up of upstream intermediate, 2,3-diphosphoglycerate (2,3-DPG) and a deficiency in the product, adenosine triphosphate (ATP). AG-348 is a first-in-class, oral, small molecule allosteric activator of PK-R. AG-348 treatment of PK-deficient red blood cells *ex vivo* increased PK-R enzyme activity and induced metabolic changes consistent with increased glycolytic activity, including reductions in 2,3-DPG levels and increases in ATP levels. First-in-human, phase 1 trials of AG-348 in healthy volunteers have recently been completed.
Aims: Pharmacokinetic/pharmacodynamic (PK/PD) studies were conducted to evaluate the effect of AG-348 on red blood cell metabolism *in vivo* in PK-R wild-type (WT) mice, rats, and monkeys.
Methods: PK-R WT C57/BL6 mice, Sprague Dawley rats, and cynomolgus monkeys were administered single or multiple (twice daily) doses of AG-348 by oral gavage for 3 and 7 days (mice and rats) or 5 days (monkeys). Dose levels tested were 1 mg/kg, 10 mg/kg, 50 mg/kg, and 150 mg/kg in mice and rats, and 3 mg/kg, 10 mg/kg, 25 mg/kg, and 70 mg/kg in monkeys. After the first and last dose, blood samples were collected to evaluate plasma drug exposure and pharmacodynamic (PD) markers in whole blood, including 2,3-DPG and ATP levels, and PK-R activity.
Results: In WT mice, rats, and monkeys, a single dose of AG-348 resulted in AG-348 exposure-related increases in PK-R activity concomitant with a reduction in 2,3-DPG levels, but no significant change in ATP levels. After multiple doses of AG-348, the increase in PK-R activity and decrease in 2,3-DPG was comparable to that observed after a single dose. In contrast to the single-dose study, ATP levels increased in an exposure-related manner after multiple doses (Figure 1). *In vitro* IC_{50} values for purified red blood cell PK-R activity correlated well with estimated *in vivo* potencies in mice, rats, and monkeys.
Summary and Conclusions: *In vivo* PK/PD studies in WT mice, rats, and monkeys found no significant species-related differences in pharmacokinetics following single and multiple oral doses of AG-348 and demonstrated an indirect PK/PD relationship across all three species. In whole blood samples, robust changes in all three PD markers (2,3-DPG, ATP, and PK-R activity) were observed following multiple doses, which correlated with AG-348 plasma exposure. Changes in PD markers showed good cross-species consistency, and there was good correlation of *in vivo* and *in vitro* data. These studies in mice, rats, and monkeys effectively predicted the changes in 2,3-DPG and ATP levels observed in single and multiple ascending dose clinical studies in healthy volunteers.

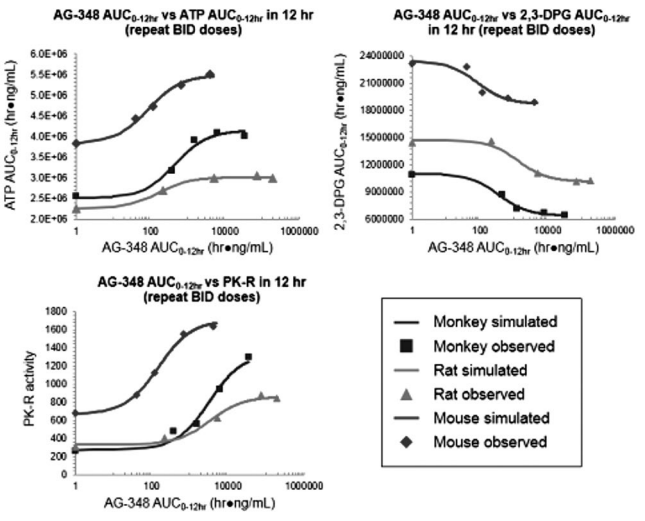

Figure 1. Correlation of AG-348 plasma exposure and ATP, 2,3-DPG and PK-R activity in WT mice, rats, and monkeys following multiple, twice daily (BID) dosing.

P752

EFFICACY AND SAFETY OF ELTROMBOPAG IN PATIENTS WITH MODERATE, SEVERE AND VERY SEVERE APLASTIC ANEMIA

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Background: Aplastic anemia is a rare, life-threatening, bone marrow failure disorder in adults with few treatment options, particularly for those who are not bone marrow transplant candidates. Eltrombopag is an orally bioavailable, synthetic, small molecule, non-peptide thrombopoietin-receptor agonist that interacts with the transmembrane domain of the thrombopoietin receptor (TPO-R). As with thrombopoietin, the binding of eltrombopag to the TPO-R initiates a number of signal transduction events which leads to an increase in the production of mature megakaryocytes and platelets. Eltrombopag has been shown to be effective in a limited number of patients to increase platelet counts in patients with moderate, severe, and very severe aplastic anemia, and to reduce the need for frequent platelet transfusions.
Aims: The primary aim of this study is to evaluate the efficacy of eltrombopag in patients with moderate to very severe aplastic anemia. Efficacy is defined as a stable platelet count of 50,000/μL or more during any 4 week period within the possible 12 weeks while on study. The secondary aims include recording adverse events, identifying maximal platelet counts, hemoglobin and absolute neutrophil counts, odds of responding during treatment, and proportion of patients achieving at least twice their baseline platelet value at any point during the study.
Methods: This is a prospective, phase II, investigator-initiated clinical research trial. After IRB approval, patients meeting inclusion criteria and having platelet counts that dropped below 30,000/μL or with clinically significant bleeding were enrolled with informed consent. Data collected included: serial complete blood counts, peripheral smears, complete metabolic panels, pharmacokinetic testing of eltrombopag, serum thrombopoietin levels and urinalysis.
Results: Nine patients have been enrolled in the trial, and 8 have completed at least 12 weeks of therapy and 4 weeks of follow up. One patient withdrew from the study due to a concomitant liver injury, not study related. Thrombopoietin level at the time of study entry was available for 8 of the 9 patients; average 2,863pg/mL (range 1452-4000), reference range 7-99 pg/mL. Efficacy: 3 patients (38%) had a complete response (platelet count of >50 K/μL) and 1 patient (12%) had a partial response (decreased need for platelet transfusion) during treatment and in the first 4 weeks of follow up. The minimum effective eltrombopag dose was 150mg po daily. The average time to increase in platelet count was 7 weeks, range 2-12 weeks. All patients who had a response maintained the response at time of censure (average 4-43 weeks. In the 3 patients who responded, a mean increase in hemoglobin (9.5 to 10.6 g/dL) occurred and liberation from red blood cell transfusions was observed and 2 had improvement of neutrophil count to >1.5 K/μL. 2 additional patients showed improvement in absolute neutrophil count; therefore, 4 of 8 patients had increases from baseline to liberation from granulocyte colony stimulating factor therapy (0.9 to 3.0 K/μL). No serious adverse events were observed. No patient stopped the drug due to side effects of the medication.
Summary and Conclusions: Eltrombopag at a dose of 150-300mg daily is safe in individuals with aplastic anemia with 50% achieving reduction or elimination of need for supportive platelet transfusions. An additional subset of 25% of patients who did not meet the primary study endpoint experienced improvement in neutrophil count, and/or hemoglobin level, with an overall response rate of 75%.

P753

HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS: SINGLE CENTER EXPERIENCE

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Background: Hemophagocytic lymphohistiocytosis (HLH) is a rare disorder which presents as prolonged fever, severe cytopenia and hepatosplenomegaly. It is classified as primary (genetic) and secondary (acquired) HLH according to the underlying etiology. If left untreated, patients with HLH survive for only a few months.
Aims: There were few studies related with HLH series. As the reported series continue knowledge about this rare disease will increase and make a contribution to advence its prognosis. In this study we present our experience with patients with HLH.
Methods: We retrospectively analyzed data of 46 HLH patients who were admitted to the Pediatric Hematology Department of Dr. Behçet Uz Children's Hospital, Izmir, Turkey between July 2008 and October 2014. Patients were diagnosed based on the revised diagnostic criteria criteria (HLH-2004) of the International Histiocyte Society.
Results: Of 46 patients, six (M/F=3/3) were defined as primary and 40(M/F=23/17) were secondary HLH. Consanguinity rate was 35%. Perforin, sytaxin, and RAB27A mutations were detected in three, two, and one of primary HLH patients, respectively. Malignancy, infection and rheumatologic diseases were related in 5 (11%), 29 (63%) and 12 (26%) of secondary HLH patients. The mean age of primary and secondary HLH patients was 2.3(0.2-11.0) years and 5.5(0.2-17.0) years respectively (p=0.074). Fever, organomegaly, hyperferritinemia, hemophagocytosis, hypertrigylseridemia, hypofibrinogenemia were seen 100.0%, 85.0%, 85.0%, 52.2% and 54.3% respectively. The *central nervous system* was involved in a total of 11(23.9%) patients; 10 had *seizure and one had visual loss.* The median follow-up duration was 10(1-84) months. Five year survival rate fort he patients was 79%. Hematopoetic stem cell transplantation *were performed* in five primary HLH patients.
Summary and Conclusions: This study present clinical findings of patients with primary and secondary HLH. We think that this study will contribute the data related with HLH which is rare and fatal disorder.

P754

MANAGEMENT OF CYCLIC NEUROPENIA: DIAGNOSIS AND THERAPY

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Background: Cyclic neutropenia (CyN) is a hematologic disorder characterized by regular oscillations of the bone marrow maturation leading to peripheral blood neutrophil counts with consistent, usually 21-day, periodicity. Other blood cells, *e.g.* platelets, may also show a cyclic pattern. CyN is inherited as an autosomal dominant disorder and mainly attributed to mutations in *ELANE* (formerly *ELA2*). Other gene mutations may also cause blood cell periodicity related with regular fevers and infections, *e.g. tumor necrosis factor receptor gene* (TRAPS) or *MEFV* (*MEditerranean FeVer*). *ELANE* mutations are also responsible for a substantial proportion of congenital neutropenia (CN) with persistent low neutrophil counts.
Aims: Investigation of the natural course and management of cyclic neutropenia.
Methods: The Severe Chronic Neutropenia International Registry (SCNIR) collected data on 80 patients with CyN (37 with *ELANE* mutation, 9 *ELANE* negative, 34 not tested) since 1994. Eleven families with a total of 24 patients were identified.
Results: *Diagnosis:* It is most important to discriminate between CN and CyN, since patients with *ELANE* associated CN have a high risk of leukemic transformation whereas patients with *ELANE* associated CyN do not, although some mutations are associated with both phenotypes, even in the same kindred. To document the cyclic pattern of blood counts 3 CBCs per week over a period of six weeks are required to proof CyN. Depending on the cycle phase, bone marrow morphology may change between normal and maturation arrest of granulopoiesis. Intriguingly, one patient harbored both *ELANE*- and *TNFR1A*-mutations suggesting there is an overlap of patients suffering from Cyclic Neutropenia and Periodic Fever Syndrome. *Treatment:* The efficacy of daily subcutaneous Granulocyte-Colony Stimulating Factor (G-CSF) has been proven in randomized controlled trials with congenital, cyclic and idiopathic neutropenia. Sixty-seven patients (83,8%) received G-CSF at a median dose of 1,94 μg/kg/d. Long-term follow-up indicates that these patients experience fewer mouth ulcers, febrile events and (bacterial) infections when treated with recombinant human granulocyte-colony stimulating factor (rHuG-CSF), despite a persistence of the cycling of blood counts. However, the cycle length and duration of nadir is shortened with treatment. G-CSF can be administered every day or every other day for patients with cyclic neutropenia. Ordinarily it is easier to start with

a very low dose, 0.5 to 3 mcg per kilogram per day given on a daily basis, going up gradually to find the lowest effective dose. Both children and adults respond well to G-CSF and adverse events are relatively infrequent. The most common acute adverse events associated with G-CSF therapy are bone pain and headache. Less frequent administration of larger doses can intensify bone pain, so weekly or twice weekly injections are seldom tolerated, and increasing the frequency of administration may ameliorate the bone pain. Treatment responses tend to be quite stable once an effective dose for the individual patient is found. The dose should be gradually adjusted for body weight in growing children, with monitoring of the response. *Supportive care:* Chronic periodontal disease may occur in untreated CyN. In addition to sufficient neutrophil counts, good dental hygiene and regular professional treatment are necessary to prevent tooth loss. Psychosocial support can help alleviate the psychological and financial burdens of a chronic illness, and can be provided both by health professionals and by patient/parent groups which are established in many countries already.
Summary and Conclusions: CyN patients benefit from G-CSF treatment. Correct disgnosis is important for the long-term management and counseling.

P755

RELATION BETWEEN GLUTATHIONE S TRANSFERASE GENES (GSTM1, GSTT1 AND GSTP1) POLYMORPHISMS AND CLINICAL MANIFESTATIONS OF SICKLE CELL DISEASE IN EGYPTIAN PATIENTS

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Background: Sickle cell disease (SCD) is characterized by clinical manifestations that resulted from sickling of Hb S, which is augmented by accumulation of oxygen free radicals that cause oxidation of Hb S. Defective normal antioxidant protective mechanism predispose to clinical manifestations of SCD like vaso-occlusive crisis (VOC) and acute chest syndrome (ACS). The glutathione system plays an important role in the removal of endogenous products of peroxidation of lipids and so protects cells from the deleterious effects of oxidative stress. Impairment of glutathione system due to genetic polymorphisms of glutathione S transferases (GSTs) genes is expected to increase risk of SCD clinical manifestations. GSTs genes polymorphisms may have ethnic-dependent polymorphism frequencies. The association of *GSTM1*, *GSTT1* and *GSTP1* polymorphisms and various diseases such as cancer and inflammatory diseases has been proved in various studies.
Aims: Understanding the impact of GSTs gene polymorphisms on severity of SCD manifestations, may help to improve the quality of life of SCD patients, by using prophylactic measures that decrease oxidative stresses, such as antioxidants, in patients at risk of developing sever manifestations due to impairment of their defense reductive mechanisms, namely patients who carrying the predisposing GSTs genotypes. So in this study, we aimed at outline the relation between GSTs gene polymorphisms and severity of SCD manifestations.
Methods: In this study, we measured the frequency distribution of the 3 GSTs genes polymorphisms (GSTM1, GSTT1 and GSTP1) in 100 Egyptian adult SCD patients and 80 corresponding controls.GSTM1 and GSTT1 genotypes was determined by multiplex polymerase chain reaction (PCR) with using a housekeeping B globin gene as internal control. GSTP1 polymorphism were determined with a polymerase chain reaction-restriction fragment length polymorphism assay [PCR-RFLP]. Patients were followed up prospectively between June 2012 and June 2014 to evaluate the relation between GSTs genes polymorphisms and the frequency of complications of SCD, in the form of severity of anemia, number of VOC attacks, acute chest syndrome and pulmonary hypertension. The patients were assessed for the severity of anemia by the average number of units of packed red cells (PRCs) transfused per year. The average number of VOC per year was documented.
Results: In our study, the frequency distribution of the GSTs null genotypes was found in 58% of SCD patients, with the highest prevalence for GSTM1 (42%), followed by GSTT1 (32%). Absence of both GSTM1 and GSTT1 genes was found in 16% of SCD patients. Regarding GSTP1 polymorphisms distribution, highest prevalence was for wild genotype (59%), followed by heterozygous genotype (35%) then homozygous genotype (6%). There was no statistically significant difference in the distribution of the GSTs gene polymorphisms between SCD patients and the controls. GSTM1 null genotype was significantly associated to ACS and VOC (P value=0.03 and 0.01 respectively), it also associated to pulmonary hypertension and high requirement of blood transfusion, but not of statistical significance. GSTT1 null genotype was associated with significantly increased requirement of blood transfusion (P value=0.01), however risk of pulmonary hypertension, ACS, and VOC were not increased significantly (P value=0.1, 0.8 and 0.2 respectively). Absence of both GSTM1 and GSTT1 genes significantly predisposed to pulmonary hypertension (P value=0.04). Non wild GSTP1 polymorphism not predisposed to clinical manifestations of SCD in the form of pulmonary hypertension, ACS, frequent VOC or increased requirement of blood transfusion (P value=0.7, 0.6, 0.9 and 1 respectively).
Summary and Conclusions: In this study, the null status of GSTM1 and GSTT1 genes were identified as important risk factors for developing sever clinical manifestations of SCD. Further studies are needed to confirm our findings. Identifying GSTM1 and GSTT1 null genotype as risk factors for developing sever manifestations of SCD may help to categorize SCD patients whom are genetically at risk of developing sever manifestations due to defective antioxidative defense mechanism, in order to minimize severity of their symptoms by using prophylactic antioxidants and other measures that improve their reductive defense mechanisms.

P756

CASES OF SECONDARY HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS: A SINGLE CENTER EXPERIENCE

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Background: Hemophagocytic lymhohistiocytosis (HLH) is a life-threatening hematologic disorder characterized by fever, hepatosplenomegaly or splenomegaly, and cytopenias that accompany with elevated liver enzymes, hyperferritinemia, hypertriglyceridemia, and hypofibrinogenemia. There are two types as primary (congenital) and secondary. HLH may ocur secondary to infections, malignancies, immun deficiency syndromes, and rheumatologic and metabolic disease.
Aims: To describe the profile and the treatment of children with secondary hemophagocytic lymphohistiocytosis (HLH).
Methods: Secondary HLH, who were admitted to the Department of Pediatrics, Istanbul Faculty of Medicine Hospital, from January 2003 through December 2014, were retrospectively evaluated.
Results: There were 38 secondary HLH cases, of that 49% were females and the mean age was 6.7±4.2 (range: 1-17.0) years. Primary causes were distributed among patients as follows: sixteen cases with rheumatological diseases, eleven with malignancies, seven with infectious diseases, one with metabolic disorder, one with epilepsy, one with hemolytic anemia, and one with chronic granulomatous disease. Mean ferritin value was 22693.8±26312.3 (range: 1114-100 000) ng/mL. Six cases (15.7%) were treated with high dose methylprednisolone (HDMP), three (7.9%) with intravenous immunoglobulin (IVIG) only; four (10.5%) with HDMP+plasma exchange (PE); 10 (%26.3) with HDMP+PE+IVIG; 12 (31.5%) with HDMP+IVIG; 2 (5.2%) PE+IVIG, and one (2.6%) with standard steroid treatment.
Summary and Conclusions: HDMP is a very effective treatment especially for macrophage activation syndrome. In case of progressive disease adding PE +/- IVIG is an important issue.

P757

MYCHOPHENOLATE MOFETIL AND SIROLIMUS AS TREATMENT OF AUTOIMMUNE LYMPHOPROLIFERATIVE DISEASE IN CHILDREN: A SINGLE CENTER EXPERIENCE

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Background: Autoimmune Lymphoproliferative Syndrome (ALPS) is an erratic disorder with wide phenotypic expressions that vary from asymptomatic pictures to very severe clinical manifestations. Steroids and intravenous immunoglobulin are commonly used to switch off the disease, although they may not be effective or may require long-term administration to maintain remission, which may lead to severe side effects. Mycophenolate mofetil (MMF) and sirolimus have been used as alternative option in this setting but experiences are still limited.
Aims: To evaluate the efficacy and toxicity of the use of MMF and sirolimus in children for treatment of ALPS in a single center
Methods: ALPS was defined according to the revised criteria of the consensus conference held in 2009. Clinical records of all ALPS patients followed at our Center were revised. Complete and partial response (CR, PR) was defined as follows. Lymphoproliferation: lymphonodes/spleen volume reduction with (CR) or without (PR) return to normal size. Platelets >100.000 (CR), or 20-100x10^3/mmc (PR) and doubled number compared to the baseline. Haemoglobin: grade 2 but no need of transfusion (PR). Neutrophil: >1,5 (CR), or 0,5-1,5x10^3/mmc (PR) and double number compared to the baseline.
Results: From January 2002 to December 2014, 26 subjects (50% females, 50% males) aged 0.1-40 yrs (median 10.5) with probable (35%) or definitive (65%) diagnosis of ALPS were studied. 18/26 (70%) of patients showed cytopenia, and the remaining 8 patients (25%) suffered from lymphoproliferation with or without other symptoms (fever, asthenia, headache). 6/26 (23%) did not need any treatment. Sixteen out of the remaining 20 (80%) patients were initially treated with steroids: 3/16 (19%) of them responded, 13 (81%) did not. 4/20 (20%) patients received MMF (3) and Azatioprine (1) as first

line therapy and achieved a CR and PR in 3 and 1 cases, respectively.12/13 non-responders to steroids received MMF as 2nd (8), or further line (4) therapy and achieved a complete (7) or partial (5) response. The remaining child received and responded to sirolimus. Three patients who partially responded MMF where shifted to sirolimus with the aim to further improve the response. Two of them achieved a CR and the remaining one maintained the PR. Overall, 16/16 patients who received MMF and 4/4 who received Sirolimus responded to the treatment. Both drugs were well tolerated. Median age at last follow-up (FU) was 17 years (range 6.3-49.6) and median duration of fup was 5.2 years (range 0.4-12).
Summary and Conclusions: This is the largest report on children receiving MMF and Sirolimus shows that they are safe and effective in ALPS. Results must be validated by clinical trials.

P758

ADULT DISSEMINATED LANGERHANS CELL HISTIOCYTOSIS IN THE US: A POPULATION-BASED STUDY USING THE SURVEILLANCE, EPIDEMIOLOGY, AND END RESULTS (SEER) PROGRAM
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Background: Adult disseminated Langerhans Histiocytosis (AD-LCH) is an extremely rare multisystem histiocytic disorder of the dendritic cells. Accurate estimates of its incidence and survival are important for assessing optimal patient care and prognosis. However, most of the current data are in the pediatric population. Comparative epidemiologic study of AD- LCH in a large population is lacking and clinical studies remain limited to small series.
Aims: To investigate the epidemiology and outcomes of patients with AD-LCH. using the Surveillance, Epidemiology, and End Results (SEER) Program database.
Methods: SEER, a program of the U.S. National Cancer Institute, collects cancer incidence and survival data from population-based cancer registries covering approximately 28% of the US population. We identified AD-LCH cases using ICD-O-3 histology code 9754/3 who were >18 years of age at the time of diagnosis. We calculated the incidence rates (case/1,000,000) using the 2000-2009 SEER 18 registries and age-adjusted those to the US 2000 standard population. Patient level data were analyzed to determine demographic findings and clinical outcome. To measure the relative risk of subsequent malignancies compared to the general population, we calculated a standardized incidence ratio (SIR) for each type of second and higher primary cancer (observed/expected). We used SEER*Stat (v 8.1.5) for incidence and SIR statistical calculations.
Results: In the years 2000-2009, a total of 40 cases of AD-LCH were reported in the SEER database with the overall incidence of 0.07 per 1,000,000 individuals (95% CI: 0.05 - 0.10). The incidence according to racial groups (adult and pediatric cases combined) was: Whites 0.22 (95% CI: 0.18 -0.25), Black 0.08 (95% CI: 0.04-0.14), American Indian/Alaska Native 0.16 (95% CI: 0.03-0.6), and Asian/Pacific Islander 0.21 (95% CI: 0.12-0.32). The incidence was significantly lower among Black compared to White population (rate ratio: 0.35; 95% CI: 0.17-0.67; p<0.001). The incidence was similar among both sexes (male/female ratio=1.03) and the median age at diagnosis was 46 years (range, 20-86 years). Males were more likely to be diagnosed at a younger age compared to females (36 *vs* 51 years; p=0.01). After a median follow-up of 83 months (range, 16-323), 19 patients have died. The causes of death were malignancies (7 patients), vascular diseases (6 patients), accident (1 patient) and 'other causes' (5 patients). The median overall survival was 255 months (Figure 1A). The relative survival was similar between patients diagnosed in the 1978-1993 and 1994-2009 eras (Figure 1B). Nine patients developed more than one malignancy including acute myeloid leukemia (AML; 2 patients), lung cancer (1), and non-Hodgkin lymphoma (1). Compared to the general population, AD-LCH patients had an increased risk of developing AML (SIR 58.6; p<0.05).

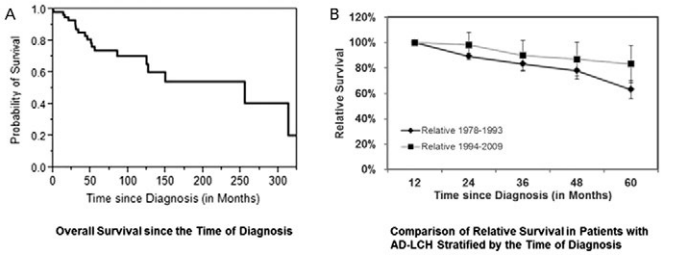

Figure 1.

Summary and Conclusions: AD-LCH is a very rare malignancy that is less common among the Black population. The prognosis is relatively good with overall median survival of more than two decades after diagnosis. There is an increased risk of subsequent development of AML.

P759

MULTICENTRE OBSERVATIONAL PROSPECTIVE COHORT STUDY LOOKING AT CARDIOVASCULAR RISK AND PURE RED CELL APLASIA ASSOCIATED WITH EPOETIN THETA IN 1039 PATIENTS WITH CHRONIC KIDNEY DISEASE
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Background: In 2010 the EU Authorities recommended to limit haemoglobin (Hb) levels within the range of 10 to 12 g/dL for adults when giving Erythropoiesis-Stimulating Agents (ESAs) to patients with chronic kidney disease (CKD). In addition, rare cases of pure red cell aplasia (PRCA) have been reported through post-marketing surveillance.
Aims: The primary objective was to assess the incidence and severity of cardiovascular events including ischemic stroke in patients treated with epoetin theta (EPO θ) for anaemia associated with CKD. The secondary objective was to monitor the safety of EPO θ, including PRCA.
Methods: In this observational, non-interventional, cohort study 55 study centres from 4 European countries observed 1039 consecutive patients with advanced or end-stage renal disease who received EPO θ for at least 6 months. Up to 4 visits could be documented at study start, month 2, 4 and 6 respectively. Spontaneous as well as reportable adverse events (RAEs) were collected, the latter being grouped as "cardiac disorders", "cardiac failure", "myocardial infarction", and "ischaemic stroke" with respective defined subterms. Furthermore, EPO θ doses and available Hb values were recorded. For a post-hoc analysis, classification by 33.3% and 66.6% percentiles resulted in 3 tertiles for individual mean Hb levels: low Hb level (≤10.7 g/dL), medium Hb level (>10.7 to ≤11.47 g/dL) and high Hb level (>11.47 g/dL) and for mean weekly EPO θ doses: low dose (≤62 IU/kg per week), medium dose (>62 to ≤125 IU/kg per week) and high dose (>125 IU/kg per week), respectively.
Results: 101 RAEs were documented in 89 patients (8.6%) with an event rate of 0.0992 events per ½ person year. 64 patients died (6.1%), but none of the deaths were related to EPO θ. Among the Hb classes, the lowest incidence of RAE was found at medium Hb level (6.2%) compared to low (11.3%) and high Hb level (7.8%). The incidence for ischemic stroke is more than double in the high Hb level class (1.5% *vs* 0.6% for low and medium Hb level). The incidence of any RAEs was higher in the high dose group (10.1%) compared to medium (8.0%) and low dose group (7.6%). Constellations associated with higher risk for any cardiovascular RAE or ischaemic stroke are high dose/high Hb (13.3%), high dose/low Hb (12.6%), and low dose/low Hb (12.1%). Lower risk for RAEs is associated with medium Hb (high dose/medium Hb: 3.8%, low dose/medium Hb: 5.3%). The event rate for Adverse Drug Reactions (ADRs) that affected terms other than the predefined reportable ADRs was 0.0081 events per ½ person year. No cases of PRCA were reported in this study.
Summary and Conclusions: This observational study confirmed that the lowest approved effective EPO θ dosage and dose frequency should be used to reach an individual target Hb level in the medium Hb range of 10 to 12 g/dL. These results confirm the prescribing recommendations and support a favourable risk-benefit ratio for treatment with EPO θ.

Immune thrombocytopenia

P760

ADJUSTED CORTICOSTEROID RISK FUNCTION OF SEVERE INFECTION IN PRIMARY IMMUNE THROMBOCYTOPENIA ADULTS. A NATIONWIDE NESTED CASE-CONTROL STUDY.

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Background: Corticosteroid (CS)-related infection risk in immune thrombocytopenia (ITP) is unknown.
Aims: The objective of this study was to assess the CS risk function of severe infection in persistent or chronic primary ITP adults, adjusted on other immunosuppressive treatments (rituximab, splenectomy, immunosuppressants) and intravenous immunoglobulin exposures.
Results: We identified 161 cases (including 9 opportunistic infections). The model with the best goodness of fit was CS exposure during the month before the index date (OR: 2.48, 95% CI: 1.61-3.83). The risk existed from doses ≥5 mg PEQ daily (*vs* <5 mg: 2.09, 95% CI: 1.17-3.71). No impact of previous cumulative exposure was measured.
Summary and Conclusions: Maintaining supraphysiological CS dose is associated with the risk of severe infection in persistent or chronic primary ITP patients.

P761

ELTROMBOPAG SAFETY AND EFFICACY FOR PRIMARY CHRONIC IMMUNE THROMBOCYTOPENIA IN CLINICAL PRACTICE

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Background: Eltrombopag is a thrombopoietin receptor agonist (TPO-RA) drug. It is considered effective and safe in chronic immune thrombocytopenia (ITP) patients. However, the results from clinical trials could not accurately reflect what happens in the clinical practice.
Aims: To evaluate the efficacy and safety of eltrombopag in primary chronic ITP in a real world setting.
Methods: 164 primary chronic ITP patients from 33 Spanish centers who had been treated with eltrombopag were retrospectively evaluated. Nevertheless 12 patients were excluded from the final analysis because they were not treated for primary chronic ITP.
Results: The median age of our cohort (72% women) was 63 (IQR, 45-75) years. The median months with ITP diagnosis was 81 (IQR 30 - 192). The median number of therapies prior to administration of eltrombopag was 3 (IQR, 2-4), including splenectomy (68%) and rituximab (23%). At the time of eltrombopag start, 45 of 152 (30%) patients were receiving concomitant treatment for ITP, mainly corticosteroids and/or immunoglobulins (29%). Forty-six (30%) patients had bleeding signs/symptoms the month before treatment start. The median platelet count at eltrombopag initiiation was $22x10^9$/L (IQR, 8-$39x10^9$/L). Platelet responses were observed in 135 of 152 (88.4%) patients. The proportion of patients achieving platelet response was quite similar regardless of age (86.7% and 91.4% for patients <65 years-old and ≥65 years-old, respectively), gender (93.4% and 76.7% for women and men respectively), splenectomy status (87.5% and 89.4% for splenectomised and nonsplenectomized patients, respectively), use of concomitant ITP medication at baseline (90.9% and 87.9% for patients with and without concomitant baseline treatment), bleeding at eltrombopag initiation (82.6% and 91.5% for patients with and without bleeding, respectively) and baseline platelet count (85.9% and 90.9% for patients with less than and more than $20x10^9$/L platelets, respectively). Demographic (age, gender), baseline disease characteristics (platelet count, splenectomy status, concomitant ITP treatment or presence of bleeding) were examined as possible predictors of platelet response. However, no predictive factor was statistically significant. The median of days to platelet response was 12 (95%CI, 9-13). The proportion of cumulative platelet response time observed during the 15-months period under examination was 75.2%.
28 patients (18.4%) experienced adverse events, mainly grade 1-2. The commonest adverse effects reported were diarrhea and headache. Seven patients had hepatobiliary laboratory abnormalities. One patient had superficial venous thrombosis and one patient had myelofibrosis grade II.
Summary and Conclusions: This abstract describes the results of the Spanish Eltrombopag ITP registry. To the best of our knowledge, this is the largest "real-world" primary chronic ITP eltrombopag treatment reported study.
Here we describe eltrombopag efficacy and safety after a 15 months follow-up for a clinical practice group of 152 adult primary chronic ITP patients. Our "real-world" results are in line with the excellent results of eltrombopag in ITP clinical trials[11]. Eltrombopag was well tolerated in our study. We only observed 18.4% of adverse events. 98% of them were grade 1-2.

P762

IDENTIFICATION OF B CELL SUBSETS IN PATIENTS WITH IMMUNE THROMBOCYTOPENIA

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Background: Immune thrombocytopenia (ITP) is an autoimmune disorder characterised by antibody-mediated platelet destruction. The pathogenesis of ITP remains poorly defined, resulting in untargeted treatment and additional side effects. It has been evident that B cells have multiple functions in the regulation of immune responses, besides the production of allo-antibodies.
Aims: We investigated the phenotype of B cells in ITP patients.
Methods: Using a twelve-colour flow cytometry, we identified the frequency of $CD19^+$ B lymphocytes, $CD24^+CD38^+$ transitional cells, $CD24^-CD38^+$ plasmablasts, $CD27^-IgD^+$ naïve B cells, $CD27^+IgD^+$ non-class switched, $CD27^-IgD^-$ class switched, $CD21^+CD27^+$ resting, $CD21^-CD27^+$ activated and $CD21^-CD27^+$ exhausted memory B cells. We included 21 ITP patients and 10 healthy controls after obtaining informed consent. Patients were separated into different groups based on current and past treatment. 11 patients were not on any treatment at the time of blood sampling, 8 patients had received rituximab treatment and 4 patients used trombopoietin receptor agonists.
Results: When taken as a whole group, patients with ITP had a significantly decreased ($p<0.05$) frequency of plasmablasts, CD27+ class switched, non-class switched and resting memory B. Naïve B cells and exhausted memory B cells were significantly increased in ITP patients compared to controls. ITP patients who were on no treatment showed the same distribution of B cell subsets. Patients who had received Rituximab between 1 and 7 years previously had significantly reduced plasmablasts, CD27+ class switched, non-class switched and resting memory B cells when compared to ITP patients who had never received Rituximab. Frequency of naïve B cells and exhausted memory B cells was significantly increased in this group. In contrast, patients who used thrombopoietin receptor agonists had almost normal B cell subsets. There remained a significant increase in the frequency of exhausted memory B cells in this cohort. All other subtypes were not significantly different to controls.
Summary and Conclusions: Overall, there seems to be a shift from mature B cell to immature B cells in ITP patients compared to controls. This might suggest exhaustion of memory B cells, caused by constant stimulation of memory B cells by auto-antigens. Our findings indicate a major role for medication, in the change of B cell distribution, with an unexpected long term effect on memory B cells many years after rituximab therapy despite no clear evidence of increased infection, whilst trombopoietin receptor agonists may have an improvement in B cell phenotype. Further understanding might give a better insight in the effects of different therapies and this could cause the need for revision of current therapeutic options.

P763

BETA2 GLYCOPROTEIN I EXPRESSION IN MONOCYTES IS DECREASED IN IMMUNE THROMBOCYTOPAENIA AND REGULATED BY IL-6

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Background: Numerous reports have indicated dys-regulated expansion of cytokine profiles from specific T-cell subsets in immune thrombocytopaenia (ITP). In particular, elevated interleukin-6 (IL-6) levels inversely correlate with platelet count. β2-glycoprotein I (β2-GPI) is a plasma glycoprotein that acts as a complement regulator (Gropp *et al.* Blood. 2011) and might be involved in the pathogenesis of ITP. Recent studies have proven the presence of β2-GPI mRNA in human peripheral blood monocytes. Whether monocytes can be 'educated' by IL-6 to regulate β2-GPI expression remains unknown. The role of anti-IL-6R antibodies in controlling β2-GPI/IL-6 imbalance in ITP needs to be exploited.
Aims: To elucidate the reciprocal relationship between β2-GPI and IL-6, and evaluate the potential role of anti-IL-6R antibodies in regulating β2-GPI in patients with ITP.
Methods: Twenty-four consecutive patients with primary ITP with 24 healthy donors were enrolled in this prospective study. The levels of serum IL-6 and plasma β2-GPIwere measured using ELISA. The purity of human peripheral blood monocytes obtained from ITP patients and healthy donors was assessed by cytofluorimetric analysis using FITC-conjugated anti-CD14 antibodies. Intracellular β2-GPI and IL-6 expression was determined by Western blot and flow cytometry. DNA fragmentation was assessed by propidium iodide staining followed by flow cytometric analysis. β2-GPI and IL-6 mRNA expression in monocytes was assessed by RT-PCR. To further determine the role of IL-6, monocytes were pre-treated with IL-6 or anti-IL-6R antibodies *in vitro*. β2-GPI mRNA expression and β2-GPI levels in monocytes and cell supernatants were determined.
Results: β2-GPI was down-regulated and IL-6 was up-regulated in both peripheral blood samples and CD14+ monocyte lysates in patients with ITP compared with healthy controls. Negative correlations were observed between β2-GPI and IL-6 levels in peripheral blood samples and monocytes. To further confirm the negative correlation between the expression of IL-6 and β2-GPI, flow cytometry with staining intracellular antigens revealed an increased frequency and number of monocytes with lower β2-GPI expression and positive higher IL-6 expression. To eliminate the possibility that increased β2-GPI expression may be due to cell apoptosis, we analysed DNA fragmentation by cytofluorimetric analysis in the two groups, and no significant hypodiploid peaks were detected. Decreased β2-GPI expression in cell lysates from healthy donors pre-treated with IL-6 *in vitro* was observed, and this level did not significantly differ with the levels observed in ITP patients. Down-regulated secretion of this protein in cell supernatant was also observed. More importantly, β2-GPI mRNA expression was inhibited in monocytes. Decreased expression of β2-GPI at the molecular and genetic levels was reversed by the addition of anti-IL-6R antibodies.
Summary and Conclusions: Our results are the first to suggest that patients with ITP may have decreased β2-GPI expression in monocytes, which was inhibited by persistently high IL-6 expression. The effect of anti-IL-6R antibodies in correcting imbalanced β2-GPI/IL-6 levels may suggest the promoting therapeutic potential of anti-IL-6R antibodies in ITP patients.

P764

THE UNITED KINGDOM IMMUNE THROMBOCYTOPENIA (UK ITP) REGISTRY: PRELIMINARY FINDINGS ON BLEEDING EVENTS EXPERIENCED BY ITS PARTICIPANTS

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Background: Primary immune thrombocytopenia (pITP) is a rare bleeding disorder in which platelets are destroyed through immune-mediated mechanisms. This leads to a reduction in platelet count and at particular low level there is an increased susceptibility to bleed. There is much heterogeneity in outcome for individuals with low platelet counts, which can range from no bleeding to severe intracranial haemorrhages. What influences its occurrence and its severity is not well understood.
Aims: The Registry was set up to provide much required evidence on the natural history of ITP, occurrence of comorbidities and treatment effectiveness. The main purpose of this study is to investigate the occurrence of bleeding in this cohort and identify likely contributory and preventative factors.
Methods: The Registry relies on the contribution of haematology teams across the country to provide adequate data to conduct its studies. To date, it has 62 centres throughout the UK but aims to expand internationally to increase recruitment and improve collaboration. The findings reported here are from univariate analyses which were preliminary to a more in-depth study.
Results: These initial findings were obtained on a cohort of 1454 (58.2% females; 76% European ancestry) participants with a median age at diagnosis of 49.2 [interquartile range (IR) 30.8, 63.9; mean 47.9] years old. Their mean platelet count at diagnosis was $38.8x10^9$/L. Among participants who reported bleeding episodes before and around diagnosis of pITP, 53% were cutaneous bleed (*e.g.* petechiae, purpura, and haematoma), 24% were haematomas affecting 'not specified' sites, 20% affected the eye, 20% occurred in the oral cavity, 19% were epistaxis, 11% were 'not specified' mucocutaneous bleed and 6% occurred in the gastrointestinal tract (exc. oral). Cutaneous bleed continued to be one of the commonest complaints (36%) after ITP diagnosis (at which point treatment may have been started) whereas the distribution of the other sites' bleed stayed almost the same as pre-diagnosis. Intracranial bleed was experienced by 0.7% before and around ITP diagnosis and 1.3% after. Advancing age and being female were found to be significantly associated with bleeding after ITP diagnosis.
Summary and Conclusions: Skin-related bleed appeared to be the most complaint at and after diagnosis of ITP. Whether any types/sites of bleed were related to different platelet counts levels and other characteristics remained to be established. The associations depicted in this analysis were noteworthy for further stratified analysis by certain characteristics (*e.g.* gender) and treatments as well as for identifying likely confounders/proxy/explanations (*e.g.* anticoagulation therapy used in the treatment of DVT) before engaging in more elaborate multivariate analysis.

P765

THYROID DYSFUNCTION IN IMMUNE THROMBOCYTOPENIA: MORE THAN AN ASSOCIATION

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Background: Immune thrombocytopenia is characterized by immune mediated platelet destruction in addition to suppression of platelet production by the bone marrow (BM) megakaryocytes leading to variable degree of thrombocytopenia and bleeding manifestations. It could be newly diagnosed, persistent or chronic and primary or secondary. Primary ITP is an autoimmune disorder characterized by isolated thrombocytopenia in the absence of other causes or disorders that may be associated with thrombocytopenia. Secondary ITP includes those associated with antiphospholipid syndrome, infection with Helicobacter pylori, hepatitis C, HIV, lymphoproliferative disorders, systemic lupus erythematosis, thyroid dysfunction etc. In these cases, treatment of the underlying condition helps in recovery of platelet counts and thus prevents bleeding. Mild-moderate thrombocytopenia is found commonly in patients with hyperthyroidism due to reduced non immune platelet survival. Similarly, mild thrombocytopenia occurs in some patients with hypothyroidism due to impaired production.
Aims: To study the prevalence of thyroid dysfunction in Immune thrombocytopenia.
Methods: This was a prospective observational study, which included patients with age >12 years, diagnosed as ITP on the basis of peripheral smear and/or bone marrow examination. They were evaluated for secondary ITP. Serum T4 and TSH levels and Anti thyroid peroxidise antibodies were done by electro-chemi-illuminence test. All patients were treated as per standard guidelines, with regular follow up for assessement of bleeding and platelet counts. Those with treatable secondary causes like H. Pylori or thyroid dysfunction were treated accordingly in addition to immunosuppressive therapy for ITP. Females with severe thrombocytopenia and menorrhagia were also given hormonal treatment.
Results: A total of 168 patients of immune thrombocytopenia were enrolled, with thyroid function tests available in 146 patients. Mean age was 30.6 years, with 67.8% females (n=114). Most of our patients were having either persistent ITP (n=42, 25%) or chronic ITP (n=101, 60%), with a median duration of symptoms being 2 years. Abnormal T4 levels were present in 29 patients (27 hypothyroid and 2 hyperthyroid). Total 26 patients had abnormal TSH. Of these, antithyroid peroxidase antibodies were demonstrated in 9 patients (31%) with abnormal T4. Raised antithyroid antibodies were present in 14 patients (54%) with raised TSH. These were significantly associated with both abnormal T4 (p value 0.038) and TSH (p value 0.021). Subclinical hypothyroidism was present in 14 patients. Hypothyroidism (high TSH, or low T4) were significantly associated with presence of anti-nuclear antibodies (p value 0.011). Presence was abnormal thyroxine levels (p value 0.04), abnormal thyroid stimulating hormone levels (p value 0.039) and presence of anti thyroid peroxidase antibodies (p value 0.02) were associated with chronic or persistent ITP. None of these were associated with response to treatment.
Summary and Conclusions: Thyroid dysfunction was present in 43 patients (29%). Both hypothyroidism (clinical and overt) and hyperthyroidism were seen, latter being less common. Mildly raised T4 can be due to effect of estrogen containing pills prescribed frequently to females with menorrhagia. Estrogens bind to thyroid hormone binding protein and cause elevated total T4 levels, free T4 levels are likely to be normal in these cases. Disproportionately low TSH can be seen with patients on glucocorticoids. Most of our patients had been on steroids before they presented to us, thus explaining the abnormal relation in T4 and TSH. Association of hypothyroidism and antinuclear antibodies highlights that two autoimmune diseases tend to occur together. Presence of antithyroid peroxidase antibodies in these cases suggests autoimmune nature of thyroid dysfunction and perhaps common occurence of multiple autoimmune disorders in these patients. Due to increased prevalence of thyroid dysfunction in ITP patients, thyroid function tests should be a part of secondary ITP work up at diagnosis and periodically thereafter.

Screening with TSH alone is likely to be insensitive in ITP patients and we suggest combined T4 and TSH for this screening. Results of treatment of thyroid disease and impact on platelet count have been variable.

P766

ELEVATED SERUM THROMBOPOIETIN LEVEL IN IMMUNE THROMBOCYTOPENIA IN PREGNANCY

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Background: Thrombopoietin (TPO), the ligand of c-mpl protein, is the major regulator of megakaryocyte maturation and platelet production. Up to 10% of all pregnancies would present thrombocytopenia, most of which are caused by gestational thrombocytopenia (GT). Immune thrombocytopenia (ITP) appears in 1 to 2 per thousand pregnancies, which may lead to bleeding complications in both the mothers and their infants. Previous studies showed that TPO levels were mildly increased or within the normal range in ITP patients. However, little data is available about the TPO levels of ITP in pregnancy or gestational thrombocytopenia.
Aims: To determine the serum levels of TPO in ITP in pregnancy, gestational thrombocytopenia, healthy pregnancy, age-matched nonpregnant ITP and controls. And to assess the value of serum TPO levels for differential diagnosis of thrombocytopenia in pregnancy.
Methods: Blood samples were obtained from ITP in pregnancy, gestational thrombocytopenia, healthy pregnancy, age-matched nonpregnant ITP and nonpregnant healthy control at Qilu Hospital, Shandong University. Platelet count was determined by complete blood count (CBC). Serum TPO concentration was analyzed by a commercially available ELISA kit, according to the manufacturer's instructions. This study was approved by the Medical Ethical Committee of Qilu Hospital, Shandong University. Informed consent was obtained from each patient.
Results: The TPO result and the platelet count of different groups are summarized in the Table 1. In all these five groups, ITP in pregnancy displayed the highest TPO level (P<0.01). The serum TPO level in ITP in pregnancy was significantly elevated compared with that in GT (P<0.01). It might be helpful to differentiate the two disorders by TPO level since TPO level in 26/31 ITP in pregnancy was 500 pg/ml or higher, but none of the GT patients' TPO level exceeded 500 pg/ml. Furthermore, the level in GT was mildly higher than healthy pregnancy (P<0.05). ITP in pregnancy presented a markedly higher TPO level than nonpregnant ITP (P<0.01). Numerous studies over the past two decades had documented that TPO levels were high when thrombocytopenia was due to megakaryocyte deficiency, and low when due to increased platelet destruction. Taken together the previously published studies and our data, it could be inferred that the pathogenesis of ITP in pregnancy might be different from nonpregnant ITP. The TPO level in nonpregnant ITP was slightly higher than nonpregnant healthy control, which was generally in consistence with most of the previous studies. The platelet count in healthy pregnancy was mildly lower than nonpregnant healthy control. Meanwhile, the healthy pregnancy had slightly higher TPO level than nonpregnant heathy control. We did not find a correlation between platelet count and thrombopoietin level in any group.

Table 1.

	ITP in pregnancy n=31	gestational thrombocytopenia n=27	healthy pregnancy n=29	age-matched nonpregnant ITP n=25	nonpregnant healthy control n=27
Plt count ($\times 10^9$/L), median (range)	20, (9-43)	81, (53-97)	238, (203-277)	27, (1-49)	278, (221-297)
TPO (pg/ml), mean ± SD	1350 ± 646	197 ± 62	65 ± 23	92 ± 44	38 ± 16

Summary and Conclusions: TPO levels might be useful for differential diagnosing ITP in pregnancy from other causes of thrombocytopenia in pregnancy. These findings need to be further explored.

P767

CHRONIC IMMUNE THROMBOCYTOPENIA IN CHILDHOOD: EVALUATION OF THE DATA OVER 30 YEARS BASED ON THE REVISED CRITERIA OF INTERNATIONAL WORKING GROUP

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Background: Childhood immune thrombocytopenia (ITP) is usually a self-limited acquired bleeding disorder lasting from a few weeks to several months. About 20% of children with newly diagnosed ITP develop a chronic course. The International Working Group (IWG) recently standardized the terminology and definitions of ITP.
Aims: We evaluated the data of children with chronic ITP based on the revised criteria to investigate the natural course of this disease.
Methods: The medical records of 564 children diagnosed with ITP from 1978 to 2014 were reviewed according to the IWG criteria and data of the patients with chronic ITP were included.
Results: Eighty-six patients (M/F: 1.09) with chronic ITP were included. Median age at admission was 6 years (range: 1-16 years). Median platelet count at admission was $20x10^9$/L (range: 1-$4x10^9$/L). The most frequent bleeding symptoms at admission were petechia and/or echimosis (86%), followed by mucosal bleedings (39.5%) such as epistaxis (24%), gingival/oral mucosal bleeding (15%), severe menstrual hemorrhage (1%), gastrointestinal bleeding (2.3%), hematuria (3.5%) and subconjunctival hemorrhage (1%). Intracranial hemorrhage was not observed in none of patients at admission or on follow-up. Bone marrow examination was performed in 65% of the patients; megakaryocytes were increased in 80% and decreased in 1.7% and normal in the remainder. Chronic infections were detected in two patients (Hepatitis B carrier (n:1) and *Helicobacter pylori* infection (n: 1)), 1 patient was diagnosed with SLE and 1 patient was diagnosed with Evans syndrome. Laboratory results showed direct coombs test and anti-nuclear antibody positivity in 8% and anticardiolipin antibodies in 1% of the patients. Spontaneous remission (>$100x10^9$/L platelet count) was detected in 28% of the patients and the mean duration time to spontaneous recovery was 3.5 years. Twenty patients (23%) underwent splenectomy. The platelet counts increased to >100000/mm3 in 90% of the patients after splenectomy. None of the patients who had splenectomy had complication related to surgery. During follow-up period treatment was given to patients with bleeding or platelet count <$20x10^9$/L. Treatment options were steroids (n:68), IVIG (n:38), anti-D immunoglobulin (n:12), rituximab (n:5), dapsone (n:3) and vincristine (n:1).
Summary and Conclusions: The etiology remains unknown in children with chronic ITP. Our data of 30 years showed that platelet counts recovered spontaneously in one-third of the patients about 3.5 years after the diagnosis. Splenectomy is a safe and effective treatment option in children as in adults and may be considered in selected cases.

P768

RESPONSE LOSS AND DEVELOPMENT OF NEUTRALIZING ANTIBODIES DURING LONG-TERM TREATMENT WITH ROMIPLOSTIM IN PATIENTS WITH IMMUNE THROMBOCYTOPENIA (ITP)

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Background: In ITP patients (pts) failing immunosuppressants, an alternative approach is to enhance platelet (plt) production stimulating thrombopoiesis. Clinical development of first-generation thrombopoietic agents (TPO) was halted due to induction of neutralizing antibodies (Abs) against both recombinant and endogenous TPO. Romiplostim (R) is a second-generation TPO receptor agonist (TPO-RA) with no sequence homology to TPO, thus avoiding the risk of eliciting cross-reacting Abs . However, Kuter *et al.* reported development of neutralizing Abs to R in 2/291 treated pts. Both pts were tested after their plt counts fell and in both cases these neutralizing Abs were no longer present at re-testing after withdrawal of R. No pt had Abs that cross-reacted with eTPO (1).
Aims: To describe prevalence and time course of response loss to R and the results of neutralizing Abs testing in a group of ITP pts treated at 2 Centers.
Methods: 44 pts received R between Sept 2009 and Dec 2014 for ITP not responsive to immunosuppressants. All pts are enrolled in a local Italian data base (REL-ITP data base), and informed consent to clinical data use is given at enrollment. Whenever feasible, blood samples of pts losing response to R were collected and sent for R neutralizing Ab testing (performed as immunoassay), according to R manufacturer instruction.
Results: Among our pts, 5/44 (11%) were non responders to R. An additional 32% of pts (14/44) although responders, are not evaluable since: 2 lost to follow up; 3 discontinuation due to SAE (1 bone marrow fibrosis; 2 thrombotic events); 5 bridge to splenectomy; 3 late responders to either splenectomy or rituximab. Therefore a total of 25 pts are evaluable for loss of response. Of these pts: 11/25 discontinued R after achieving a stable response and are in follow up, off any therapy; 8/25 are currently receiving R with stable response; 6/25 have lost response while on therapy. Outcome of these 6 pts is summarized in Table 1. Neutralizing R Abs testing could be carried out in 4 of 6 pts (#1 to 4) and 3/4 (# 1,2,3) tested positive. These Abs did not cross react with eTPO. 4/6 pts went into long-term remission when switched to a different therapy: #1 rituximab, #2 and #3 splenectomy, # 5 eltrombopag; 2/6 pts (#4 and 6) were heavily pre-treated, splenectomized pts and never regained a response. Pts #2 and #3 were re-tested for Abs, at 9 and 7 mos from first detection respectively and both tested negative.
Summary and Conclusions: The second generation TPO-RA romiplostim is an effective and well tolerated therapy for ITP pts not responding to immunosuppressants. Pts seldom discontinue treatment due to adverse events but over time a fraction of responding pts need dosage increase in order to maintain their plt count (1) or are unable to maintain a sustained response (2). Response loss doesn't seem to be so rare an event accounting for 24% of pts in our series. Similarly to Kuter's data (1), in 3 of our pts abrupt

loss of response was associated with development of neutralizing Abs to R, not cross-reacting with endogenous TPO and resolving after R withdrawal.

References
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2. Kuter DJ, Macahilig C, Grotzinger KM *et al.* Treatment patterns and clinical outcomes in patients with chronic immune thrombocutopenia (ITP) switched to eltrombopag or romiplostim. Int J Hematol DOI 10.1007/s12185-014-1731-7

Table 1.

n	Sex, yr	Previous ITP treatment	Duration of Rom before LR	Type of response before LR	Ongoing dose of Rom before LR	Clinic at LR	anti-Rom Ab	Ab re-test	Action taken at LR	Outcome
1	F, 38	IVIG, DEX, P	11 months	CR	2 mcg/kg	Sudden drop plt count, no response with higher Rom doses	Positive	n.a	RTX	CR, off therapy
2	F, 38	IVIG, DEX, P, Elt	12 months	CR	8 mcg/kg	Sudden drop plt count, no response with higher Rom doses	Positive	Negative (after 9 months)	IVIG, spl	CR
3	M, 19	IVIG, DEX, P	11 months	CR	9 mcg/kg	Sudden drop plt count, no response with higher Rom doses; sudden diffuse skin reaction after Rom injection	Positive	Negative (after 7 months)	Elt as "bridge" to spl	CR
4	M, 42	Spl, P, DEX,	4 months	R	10 mcg/kg	sudden drop plt count	negative	n.a	Elt	NR
5	F, 56	IVIG, DEX	5 months	unstable R	6 mcg/kg	Sudden drop plt count, no response with higher Rom doses	n.a	n.a	RTX, Elt	CR in Elt
6	M, 61	IVIG, P, VCR, RTX, spl	12 months	unstable R	10 mcg/kg	Drop, no more resp	n.a	n.a	Elt	NR

IVIG: intravenous immunoglobulin; DEX: dexamethason; RTX: rituximab; Spl: splenectomy; P: prednisone; VCR: vincristine; R: response; NR: no response; CR: complete response; LR: loss of response; Ab: antibodies; Rom: Romiplostim; Elt: Eltrombopag; n.a: not available

P769

LONG-TERM FOLLOW-UP SPLENECTOMY FOR IMMUN THROMBOPENIA PURPURA

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Background: Splenectomy was the first effective treatment for ITP, reported nearly 100 years ago. At many centers splenectomy is still the second line treatment for patients with ITP who have failed corticosteroids. The surgical approach has been improved by laparoscopic splenectomy, offered to most patients.
Aims: We report here the long-term outcome of a large cohort of ITP splenectomized patients. Criteria for response were defined as follows: complete response (CR), all post-splenectomy platelet counts>150×109/L without ever receiving treatment after splenectomy; partial response (PR), all other patients with platelet counts >50×109/L without therapy after splenectomy; failure, platelet counts <50×109/L or patients who received therapy after splenectomy.
Methods: We retrospectively analyzed the data on 159 patients (64 males, 95 females) who underwent splenectomy for ITP between 1999 and 2014. 53 underwent laparoscopic splenectomy, introduced in our center from 2006. Mean age was 31years (7→63 years), 43 were less than 16 years. Platelets before splenectomy was de 27.000 ($10.10^3 \rightarrow 37.10^3$/µl). The median interval from diagnosis to splenectomy was 19 months with a range of 5-39 months. Diagnosis has been reconsidered in 4 patients: splenic lymphoma 2, familial thrombopenia 2.
Results: One hundred forty three patients (90%) achieved a complete remission the first month after splenectomy. There was 1 death in operative period. The immediate postoperative complications: infection 3, thromboembolic events in 4 patients. Accessory spleens were identified 11 cases, 9 from laproscopic splenectomy. After the first year: CR 132(85%), partial remission 32 (20%), failure 23(16%). After the second year, among 113 patients followed, we observed 24 cases of failure. After 5-year: 51 patients (32%) were lost follow-up, Among 108 patients, 17 Failure, 15 PR. Seventy-six (70%) remained in a prolonged complete remission with a persistently normal platelet count, never having received any postsplenectomy treatment. Seven patients of the complete responders to surgery relapsed after an initial complete response. All 17 patients in the failure group received prednisone after splenectomy, 2 received rituximab, 3 received cyclophosphamide, 1 received high-dose dexamethasone. Any patients died of hemorrhage during neither follow-up nor cases of overwhelming post-splenectomy infection.
Summary and Conclusions: This study shows that splenectomy is a safe procedure and effective in approximately two thirds of patients with chronic ITP. However, the durability of responses, the ability to predict who respond will remain uncertain. Further studies are required to establish whether surgery-sparing treatments of chronic ITP, such as high-dose dexamethasone, anti-D and anti-CD20 immunoglobulins, thrombopoietin mimetics, have similar or even superior efficacy, risk and cost ratios compared to splenectomy.

Bleeding disorders

P770

WHOLE EXOME-SEQUENCING TO IDENTIFY GENETIC RISK FACTORS OF INHIBITOR IN KOREAN SEVERE HEMOPHILIA A PATIENTS

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Background: The development of inhibitor against factor VIII is a serious complication in the treatment of hemophilia A. *F8* mutation type plays an important role in inhibitor development, however, patients who share the same *F8* mutation with discordant inhibitor status suggest the presence of other genetic risk factors. Studies on genetic determinants other than *F8* mutation yielded inconsistent results, therefore the genetic background of the risk remain largely unknown.
Aims: This study was designed to investigate the genetic risk factors underling the development of inhibitor in Korean severe hemophilia A patients by whole exome sequencing with next-generation sequencing.
Methods: A total of 89 severe hemophilia A patients (55 with inhibitors and 34 without inhibitors) underwent sequencing of the exome on NextSeq500 (Illumina) following capture by SureSelect Human All Exon V5 UTR kit (Agilent). Mapping of reads was performed on reference genome (hg19) by BWA and variant calling by GATK. Initially, we screened *F8* mutations and the candidate mutations were confirmed by Sanger sequencing, MLPA and intron22 inversion test. Then we performed case(inhibitor)-control(non-inhibitor) association studies using SNP-set Kernel Association Test (SKAT-O test, gene-based test) and mixed-effect logistic regression analysis (EMMAX).
Results: A mean depth of 81X was achieved and the mean 63,103 variants were identified per patient. Large deletion, nonsense, small insertion/deletion, missense mutation and inversion 22 of *F8* were found in 10, 11, 20, 18 and 22 patients, respectively. All the patients with large deletions and all except one case with nonsense mutations developed inhibitors. We found the 8 immune genes (*IL28RA, SMARCAL1, HLA-B, TNFRSF10C, RAG1, PLXNC1, ITGAD, LAIR1*) at P<0.05 by SKAT-O test and 95 immune related variants at P<0.05 by EMMAX. When we narrowed the patients with inversion 22, we identified ten immune related variants at P<0.05.
Summary and Conclusions: This study reveals new candidate genes associated with the risk of development inhibitor in Korean hemophilia A patients.

P771

SCREENING FOR AN ACQUIRED VON WILLEBRAND SYNDROME IN PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA

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Background: Acquired von Willebrand syndrome (AvWS) is a rare bleeding disorders but it is particularly frequent in myeloproliferation. ET(essential thrombocythemia), one form of myeloproliferation, is characterised not only by thrombotic but also by bleeding complications. It is very important to know if AvWS may cause bleeding tendency in ET patients.
Aims: The aim of the study was to look for AvWS in ET patients using a screening evaluation of vWF antigen and activity and factor VIII activity.
Methods: We have examined 106 patients with ET(80 females and 26 males, mean age 54(23-82). In 22 patients(20,75%) bleeding episodes occurred . We have evaluated factor VIII level (FVIII) and von Willebrand factor antigen (VWF:Ag) and activity. In order to evaluate von Willebrand factor activity we have used two reagents: von Willebrand Factor Activity kit Hemosil™, and von Willebrand Factor Collagen binding kit Technoclone. In the first method a specific anti -vWF monoclonal antibody directed against glycoprotein Ib receptor reacts with the vWF in patient plasma. In the second method we evaluated the ability of vWF in binding to collagen. If all values : VWF:Ag, VWF:Activity and FVIII are within the normal range, AvWS may be excluded. If at least one parameter is abnormally low diagnosis of AvWS is probable. To differentiate the type of AVWS(1, or 2A, 2B or 2M) we calculate the ratios: 1:VWF Activity/VWF:Ag, 2:FVIII/VWF:Ag.
Results: We have detected: low level of VWF:Ag in 8 (8,42%) patients, low VWF: activity measured by Hemosil reagent in 19 (20,43%) patients, low VWF collagen binding activity in 27(27,42%) of patients. In a group of 12 patients low levels of both forms of vWF activities have been observed. We

have detected low factor VIII level only in 3 (3,13%) patients. Summing up, we have discovered abnormalities in 34 patients. According to AvWS definition, the diagnosis is based not only on laboratory abnormalities but also on clinical symptoms. Based on this we have diagnosed AvWS in 9 (41%) patients from the group of ET patients with bleeding episodes.
Summary and Conclusions: In 41% of ET patients presenting bleeding episodes this tendency may be caused by AvWS. We propose to use a screening evaluation mentioned above, to avoid bleeding complication connected with AvWS.

P772

INTEREST OF A NEW RAPID COMMERCIAL ASSAY FOR THE ANALYSIS OF VON WILLEBRAND MULTIMERS IN AGAROSE GEL

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Background: The analysis of von Willebrand factor (vWF) multimers is necessary for the classification of hereditary and acquired forms of von Willebrand disease (vWD). Only a few specialized laboratories are skilled enough to perform this analysis due to the complexity of the method itself and to its very slow turnaround time (2- 3 days). In fact, vWF multimers are usually separated in "Home- made" discontinuous SDS agarose gel (difficult to produce) followed by a western blotting step. Multimers are then identified by immunofluorescence or other staining techniques.
Aims: We present here the use of a new commercial method for multimer analysis, which is rapid (6 hours), easy to perform (no western blot), reproducible (ready to use SDS agarose gel). This method assesses the overall size distribution of vWF multimers (low, intermediate, high and very high molecular weight).
Methods: vWD were diagnosed on quantification of vWF by both immunological (vWF:Ag) and functional assays based on ristocetin cofactor activity (vWR:Co), bleeding time (BT), platelet count, ristocetin induced platelet aggregation (RIPA). Plasma from patients diagnosed with different von Willebrand disease (vWD) types were loaded and separated by the new assay, in continuous SDS agarose gel system (no stacking and running gel) within 110 mn Multimers were probed in gel by immunofixation using horse-radish peroxide (HRP) conjugated to a rabbit anti-vWF (90 min). Visualisation of multimers was achieved by colorimetry using commercially available TTF1/TTF2 reagents. Curves were produced using the manufacturer's gel scanner and interpretation software.
Results: The results obtained with this new method are shown in the figure. In normal plasma at least 9 to 12 main multimer bands can be detected (line C). Line 1 corresponding to a type 1 vWD in basal conditions (vWF:Ag=49%; vWR:Co=47%) shows a weaker coloration than controls, but all the multimers were present. Coloration of all the multimer bands increased after desmopressin infusion (lane 2; vWF:Ag=178%; vWR:Co=188%). Lanes 3 and 4 correspond respectively to the plasma of a patient with a suspected acquired vWD (avWD) in basal conditions (vWF:Ag=49%; vWR:Co=28%) and after purified vWF infusion (vWF:Ag=101%; vWR:Co=58%). There was an absence of high molecular weight multimers (HMW) in both samples, strengthening the hypothesis of an avWD with an accelerated turn-over of HMW multimers of vWF concentrates. Line 5 shows the plasma of a pregnant woman (38th week/1st gestation) who reported unusual bruises from childhood. BT was very prolonged, and a discrepancy was noted between vWF:Ag (225%) and vWR:Co (86%). There was an absence of HMW multimers and a large excess of vWF dimers, suggesting a type 2:C. Lane 6 corresponds to the plasma of a pregnant woman who reported a familial history of vWD (24th week/1st pregnancy). BT was very prolonged, vWF:Ag was 132% and vWR:Co slightly slower 77%):Co. The absence of HMW multimers as well as RIPA results suggested a type 2:A.

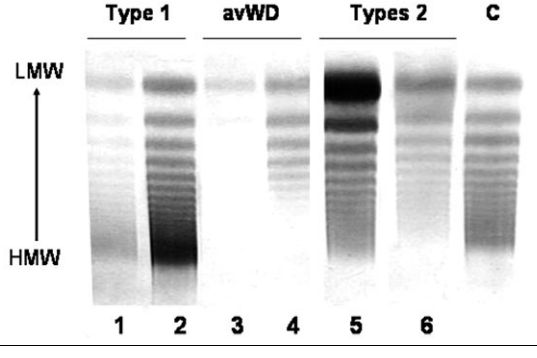

Figure 1.

Summary and Conclusions: The method for vWF multimer analysis described here is simple to carry out, produces results within 6 hours, and is performed on a commercially available instrument. This is particularly valuable in emergency situations such as preterm labor or urgent surgery, when the patient has no document specifying its vWD type. This technique which is the first line multimer analysis would also help for extensive screening and studies.

P773

DENGUE VIRUS NONSTRUCTURAL PROTEIN 1-INDUCED ANTIBODIES CROSS-REACT WITH HUMAN PLASMINOGEN AND ENHANCE ITS ACTIVATION

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Background: Dengue virus (DENV) infection is the most common mosquito-borne viral disease, which can cause life-threating dengue hemorrhagic fever and dengue shock syndrome (DHF/DSS). Abnormal activation of the coagulation and fibrinolysis system is one of the hallmarks associated with DHF/DSS patients. However, the mechanism to cause hemorrhage in DHF/DSS is still elusive. In previous studies, plasminogen (Plg) cross-reactive antibodies which can recognize DENV nonstructural protein 1 (NS1) were found in dengue patients. However, it is unclear whether these antibodies are indeed induced by DENV NS1.
Aims: To test whether dengue NS1 indeed can induce antibodies crossreact with human Plg and enhance its activation.
Methods: We immunized mice with recombinant NS1 from both bacteria and drosophila to test whether NS1 can induce Plg-cross-reactive antibodies.
Results: Results from NS1-immunized mice sera indicated NS1 immunization induced antibodies which could cross-react with Plg. To study the effects of these NS1-induced Plg-cross-reactive antibodies on fibrinolysis, we isolated several Plg cross-reactive anti-NS1 monoclonal antibodies (mAbs) from these mice and found some of these mAbs could enhance Plg activation. In addition, epitope mapping by phage-displayed random peptide library revealed that one of these mAbs (2A5) could recognize NS1 C-terminal residues 305-311 which share sequence homology with Plg region 609-616. Synthetic peptide of NS1 305-311 could inhibit both 2A5 and its antigen-binding fragment (Fab) binding to and enhancing Plg activation.
Summary and Conclusions: Our results suggest that DENV NS1 can induce Plg cross-reactive antibodies through molecular mimicry, which can enhance Plg activation and contribute to the pathogenesis of DHF/DSS.

P774

GENERAL DECREASE OF CIRCULATING MICROPARTICLES IS RELATED TO THE PRESENCE OF FVIII INHIBITORS IN HEMOPHILIA A PATIENTS

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Background: The cell activation in the blood is associated with plasma membrane remodeling, resulting in the shedding of membrane-derived microparticles (MPs) rich in accessible phosphatidylserine and in specific cell-surface antigens. There are limited data about the role of MPs in hemophilia A [HA] and especially in FVIII inhibitors development.
Aims: This study aimed to assess the MPs dosage in patients with HA and positive [HAα-FVIII(+)] (n=34) or negative [HAα-FVIII(−)] (n=68) for inhibitors.
Methods: The study was conducted in Fundação Hemominas, Belo Horizonte, MG, Brazil. The recruitment of participants and the methodology of the study were approved by the Brazilian ethics committees. MPs were analyzed by flow cytometry using the classical MPs marker, annexin, and fluorochrome-labeled monoclonal antibodies against specific cell surface markers: T cells (CD3); platelets (CD41a); granulocytes (CD66); leukocytes (CD45); erythrocytes (CD235a); and endothelial cells (CD51/61).
Results: The meaning of circulating MPs were markedly increased in HAα-FVIII(-) when compared to HAα-FVIII(+) in T cells (33.58±15.08 *vs* 13.20±14.70; p<0.01); platelets (203.10±450.00 *vs* 50.86±54.64; p=0.03); granulocytes (12.05±7.95 *vs* 6.78±6.08; p<0.01); leukocytes (241.10±419.30 *vs* 71.29±45.87; p=0.02); endothelial cells (31.57±11.42 *vs* 22.22±11.41; p<0.01) (MPs/μL±standard deviation). In contrast, the meaning MPs/μL of erythrocytes were higher in HAα-FVIII(+) (12.64±10.01 *vs* 8.58±9.53; p<0.01).
Summary and Conclusions: We found recently that HAα-FVIII(−) patients has increased activation of all leukocytes and synthesis of pro-inflammatory cytokines. These data could explain the increased presence of MPs in these patients. Increases in circulating MPs counts may be a good prognostic for

inhibitors clearance and could be used as an important biomarker for the inhibitors development. More research is needed to fully understand the role of MPs in hemophilia A. Financial support: Fundação Hemominas, Fapemig, CAPES and CNPq.

P775

MICROVESICLES ARE A POTENTIAL BIOMARKER FOR THROMBOSIS AND DIC IN ACUTE LEUKEMIC PATIENTS: A CROSS-SECTIONAL STUDY

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Background: Thrombosis is a common complication of patients with malignancies. Patients with hematological malignancies have a 28 fold increased risk to develop venous thromboembolism (VTE). Among patients with acute myelogenous leukemia (AML), the incidence of VTE is 5.2%, and among patients with acute lymphoblastic leukemia (ALL), the incidence of VTE is 4.5% in the first two years of disease. Despite their influence on mortality and morbidity, the mechanisms inducing a hypercoagulable state are not fully understood to date. Multifactorial causes include physic immobility, chemotherapy adverse effects and the overexpression of several procoagulant substances by cancer cells (cytokines, cysteine protease and tissue factor). Recent studies strongly demonstrate that cancer cells shed microvesicles (MVs) harboring tissue factor (TF-MVs). These TF-M*Vs* have a primary role in thromboembolism by expression of procoagulant phospholipids (PL) such as phosphatidylserine, and TF. Tissue factor, the most potent initiator of the coagulation cascade, plays a critical role in hemostasis. Moreover several studies strongly suggest that TF-M*Vs* and their activity may have prognostic value in identifying cancer patients with increased risk of developing venous thromboembolism (VTE) or disseminated intravascular coagulation (DIC)
Aims: The aim of this study is to find a link between procoagulant state in patient with acute leukemia and M*Vs* procoagulant activity (PCA). The other aim is to assess if MVs-PCA could be a good biomarker to predict thrombotic events.
Methods: Blood samples from 38 patients with acute leukemia newly diagnosed were obtained at Day-0 (before treatment), D-3 and D-7 (3 and 7 days after treatment). Extracellular vesicles were isolated and concentrated by ultracentrifugation. EVs-PCA and E*Vs* associated tissue factor activity was measured a commercial bio-immunoassay (Zymuphen MP-TF®), respectively.
Results: Among acute leukemic patients, 3 patients have an increased E*Vs* PCA at D-0 and 2 of them developed a thrombotic event. The remaining patients without thrombotic events (n=35) do not show an increased EVs-PCA. One patient has an increased extracellular vesicles procoagulant activity at D-3 and develop a DIC at D-5. Three patients have an increased EVs-tissue factor activity (>2pg/ml), among these, two developed a thrombotic event and one had haemorrhage. A plasma from patient with induced DIC has no increased extracellular vesicles tissue factor activity from D-0 to D-7 (<2pg/ml).
Summary and Conclusions: This study shows the link between thrombotic events and MVs-PCA, and suggests the role of M*Vs* derived from leukemic blast and others cells in procoagulant state in AL. Moreover, M*Vs* PCA could have a predicting value for venous thromboembolism and DIC in patients with acute leukemia and could inform haematologists for the thrombosis prophylaxis.

P776

THERAPEUTIC APPROACH TOWARDS SYMPTOMATIC THROMBOCYTOPENIA IN DENGUE HEMORRHAGIC FEVER

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Background: Immune-mediated destruction of platelets is thought to be the mechanism of thrombocytopenia seen after the viremic phase of dengue haemorrhagic fever (DHF). Immuno-suppressants such as steroids, immune globulin and Anti D immunoglobulin are effective in the treatment of this type of immune thrombocytopenic purpura.
Aims: To evaluate the efficacy of oral Prednisolone in the rate of resolution of thrombocytopenia and monitoring of complications in patients recovering from Dengue haemorrhagic fever.
Methods: A controlled study was carried out on diagnosed cases Dengue haemorrhagic patients presenting with sever thrombocytopenia and symptoms like confluent ecchymosis, epistaxis and purpuric rashes. This study was conducted in Ittefaq hospital (trust) Lahore, during the period of October to December 2013. Treatment group received steroids in two forms *i.e.* 1st line therapy prednisolone (1mg/kg) orally or as 2nd line therapy of initial I/V high dose (prednisolone) in pulse doses *i.e.* 40 mg bid for four days and later oral prednisolone as in 1st line therapy with omeprazole 20 mg bid in addition to standard treatment. Control group received standard supportive care only.
Results: A total of 341 suspected patients were admitted in hospital. Serological diagnosis was confirmed in 166 patients. CBC revealed platelet count $\leq 100x10^9$/l in 106 patients. A group of symptomatic febrile patients have platelet count $<20x10^9$/l was selected for therapeutic intervention. 1st line therapy (oral prednisolone was stated in 43 patients. In Fourteen patients 2nd line therapy (high dose dexamethasone pulse) therapy was instituted. Seven of them attained complete response whereas two patients achieved partial response. Four patients were shifted to Anti D therapy. Three deaths occurred during our study. Rest of all the patients improved and were discharged in due course of time.
Summary and Conclusions: This small scale preliminary study shows promising results in reducing the morbidity of patients in a relatively serious stage but large scale double blinded randomized controlled studies are needed before making recommendations on use of steroids in symptomatic thrombocytopenic patients with dengue haemorrhagic fever.

Venous and arterial thrombosis

P777

PREVALENCE AND PREDICTIVE MODEL OF MYELOPROLIFERATIVE NEOPLASM-RELEVANT MOLECULAR MARKERS IN ISCHEMIC STROKE PATIENTS

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Background: Myeloproliferative Neoplasm (MPN) has three major clonal molecular markers, including *JAK2*V617F, *CALR* and *MPL* mutation. MPN is also highly correlated with thromboembolic disease including ischemic stroke. However, the prevalence of these markers in patients with cerebral infarction is little known and the clinical characteristics of these patients remain unclear.
Aims: In this study, we aimed on the prevalence of *JAK2*V617F, *CALR* exon 9 and *MPL* mutation in patients with ischemia stroke. We also wanted to know if the clinical characters were different from unmutated patients with ischemic stroke.
Methods: In this study, more than 150 patients were enrolled. We used Allele-specific PCR (AS-PCR), real-time AS-PCR and Illlumina paired-end sequencing to detect the mutation including *JAK2*V617F, *CALR* exon 9 and *MPL* in the DNA of patients' granulocytes. Clinical characteristics were also recorded for comparison including age, gender, complete hemogram, thromboembolic events and risk factors of ischemic stroke including hypertension, diabetes mellitus, family history of stroke, atrial fibrillation, smoking history and hypercholesterolemia.
Results: In this study, more than 8% of stroke patients carried *JAK2*V617F mutation. two of these patients were homozygous for *JAK2*V617F mutation and had phenotype of essential thrombocytosis. If we excluded these 2 patients, the prevalence of *JAK2*V617F mutation was still more than 7%, which is higher than general population. Stroke patients harboring *JAK2*V617F mutation were mostly low allele burden without clinical phenotype of MPN. Only 2 patients carried heterozygous *CALR* mutation but the mutation didn't occur inside exon 9 nor affect KDEL amino acid domain of the calreticulin protein. No *MPL* mutation was detected in this study. We correlated patients' clinical parameters with their mutational status. Several characteristics were found closely related to the status of *JAK2*V617F mutation, including age, hemogram and stroke related risk factors. Using these parameters, we developed a scoring system to predict stroke patients at risk of having *JAK2*V617F clonal mutation. The scoring system composed of age older than 60 years, circulating white blood cell count greater than $10x10^9$/L, hemoglobin level greater than 14g/dL, platelet count greater than $400x10^9$/L and presence only 1 or no stroke factor. Each item give a score of 1, minimal score 0 and maximal score 5. Higher score, higher incidence of *JAK2*V617F mutation (Score 0-1: 2.8%; Score 2: 11.3%; Score 3: 20%; Score 4-5: 50%).
Summary and Conclusions: The prevalence of mutant *JAK2*V617F in stroke patients was higher than general population. Using our scoring system, identify stroke patients at risk of carrying *JAK2*V617F mutation is warranted.

P778

DENGUE VIRUS NON-STRUCTURAL PROTEIN 1 INDUCES VASCULAR LEAKAGE THROUGH MACROPHAGE MIGRATION INHIBITORY FACTOR SECRETION AND GLYCOCALYX DEGRADATION

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Background: Dengue virus (DENV) is a member of flaviviridae and it causes the most common mosquito-borne disease, including the life-threatening dengue hemorrhagic fever (DHF) and shock syndrome (DSS). In the pathogenesis of DHF/DSS, vascular leakage is the most characteristic feature, of which the mechanisms remain unclear. DENV non-structural protein 1 (NS1) is a highly conserved protein and its serum level is positively-correlated with the severity of the disease. Cytokine secretion, autophagy and disruption of endothelial barrier are factors cause vascular leakage, but how they are regulated by DENV NS1 has not yet been described.
Aims: The purpose of this study is to test whether DENV NS1 disrupted endothelial barrier function through macrophage migration inhibitory factor (MIF)-induced autophagy and glycocalyx degradation.
Methods: The *E. Coli* generated recombinant NS1 (rNS1) and *Drosophila* generated recombinant NS1 (CTK-NS1) were used. The expression and secretion of MIF in human microvascular endothelial cell line (HMEC-1) were determined by RT-PCR and ELISA. Endothelial permeability was determined by real-time cellular analysis and transwell permeability assay. *In vivo* permeability assay was demonstrated by Evans blue permeability assay and abdominal lavage in mice. The distribution of junction proteins and autophagy were observed by immunofluorescence assay and transfection of tf-LC3 construct. The degradation of glycocalyx was determined by MMP-9 expression in primary cultured human peripheral blood mononuclear cells (PBMC), heparanase-1 (HPA-1) protein level in HMEC-1 cells and CD138 concentration in mice peritoneal lavage and human patient serum samples.
Results: We first demonstrated that rNS1 induced vascular leakage *in vitro* and *in vivo*, and the permeability was rescued by treating heat-denatured rNS1 or co-treating rNS1 neutralizing antibodies. CTK-NS1 could also induce endothelial hyperpermeability. We then demonstrated that rNS1 induced the secretion of MIF in various cell types including HMEC-1 and PBMC. MIF concentration was also increased in the peritoneal lavage of rNS1-injected mice, and this phenomenon was rescued by co-treating NS1 neutralizing antibodies. In addition, rNS1 induced the LC3-I-to-LC3-II conversion and phosphorylated mTOR reduction, which indicated autophagy formation. The conversion of EGFP to mRFP in tf-LC3 transfected HMEC-1 cells further revealed that rNS1 induced the formation of autophagolysosome and autophagic bodies. The disarray of VE-cadherin and ZO-1 in HMEC-1 was induced by rNS1 and could be reversed by either MIF or autophagy inhibitors, which indicated the involvement of MIF and autophagy. Atg5 knockdown HMEC-1 stable clone could resist rNS1-induced hyperpermeability, indicating the importance of autophagy in endothelial permeability. Both MIF inhibitors and autophagy inhibitors rescued rNS1-induced endothelial hyperpermeability *in vitro* and *in vivo*, which further confirmed the mediation of MIF and autophagy in rNS1-induced vascular leakage. In patient serum samples, the increase in MIF and CD138 concentration were observed, indicating the correlation of glycocalyx degradation and vascular leakage. The glycocalyx degradation enzymes MMP-9 and HPA-1 were increased by rNS1 in PBMC and HMEC-1, indicating rNS1-induced glycocalyx degradation.
Summary and Conclusions: Taken together, these results suggest that NS1 may play pathogenic roles in DENV-induced vascular hyperpermeability through MIF secretion and glycocalyx degradation.

P779

PROVOKED VENOUS THROMBOEMBOLISM IN NON-CANCER PATIENTS: ARE THEY THE SAME?

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Background: Venous thromboembolism (VTE) can be classified as provoked if there is a clear identifiable provoking factor. Collectively they have been shown to be associated with a lower risk of recurrence, and are often treated in a similar fashion.
Aims: To evaluate the association between different categories of provoked VTE in our study population and their risk of recurrence
Methods: Retrospective evaluation of all non-cancer VTE from July 2011 to December 2012 at Austin and Northern Health, Melbourne, Australia. The follow up was continued through to December 2014.
Results: 779 VTE cases were identified during our study period of which 50.6% were female with a median age of 63 years. Cases with no provoking factors (n=303, 39%) had a higher risk of recurrence at 11.9% (RR 2.4, p<0.001) and were more likely to be subsequently diagnosed with malignancy (3.3%, RR 3.2, p=0.03). Cases with provoking factors (n=476, 61%) included surgery (35.1%), non-surgical causes of immobility or injury including hospitalizations (38.4%), travel greater than 4 hours (14%), estrogen-related (11%), catheter-associated (1%) and local inflammatory processes such as cellulitis (0.4%). Of the provoked cases, travel had the highest recurrence rate (10.6%), comparable to unprovoked VTE (RR 0.89, p=0.771). Non-surgical causes of immobility or injury had the second highest recurrence rate of 5.5% (RR 0.46, p=0.024), followed by surgery at 3.6% (RR 0.30, p=0.005). Estrogen-related VTE had a lower trend of recurrence at 1.9% (RR 0.16, p=0.067). None of the VTE associated with catheter or local inflammatory processes recurred during our follow up period, however numbers were small (n=7). Provoked VTE had a significantly shorter time to recurrence than unprovoked VTE, with 76% occurring within the first 12 months of the initial event (median 8 months *versus* 18 months, p=0.02). Whether an episode of VTE was provoked did not appear to influence the rate of VTE-related deaths (p=0.89).

Table 1. Provoking factors and risk of recurrence in VTE.

	Total	Recurrence	Percentage	Relative Risk	95% CI	P-value
Unprovoked	303	36	11.9%	1.00		
Provoked (overall)	476	24	5.0%	0.42	0.26 to 0.70	<0.001
Travel	66	7	10.6%	0.89	0.42 to 1.92	0.771
Immobility or injury (non-surgical)	183	10	5.5%	0.46	0.23 to 0.90	0.024
Surgery	167	6	3.6%	0.30	0.13 to 0.70	0.005
Estrogen related	53	1	1.9%	0.16	0.02 to 1.13	0.067
Catheter associated	5	0	0.0%	0.69	0.05 to 10.03	0.789
Local inflammatory process	2	0	0.0%	1.39	0.11 to 17.75	0.801

Summary and Conclusions: Provoked VTE is not a homogenous group, and recurrence rates depend on the identifiable provoking factor. Reversible factors such as surgery, estrogen exposure, catheter or local inflammatory process demonstrated lower risk of recurrence. The high risk of recurrence associated with travel raises the question of the validity of travel as a "true" provoking factor. Further studies are warranted to better define travel as a provoking factor, as this will influence the optimal treatment and future outcomes for travel related VTE.

P780

COAGULATION PROCESS AND FIBRINOGEN GENETIC VARIABILITY ARE STRONG RISK FACTORS FOR ATHEROGENESIS, MYOCARDIAL INFARCTION AND THE EXTEND OF THE DISEASE IN SUBJECTS SUSPECTED FOR STABLE ANGINA

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Background: It is well known that altered coagulation as well as fibrinogen genetic variability contribute to atherogenesis. In the present study we examined the combined effects of the rs1800790, rs2070011 fibrinogen polymorphisms on coagulation and endothelial function as well as the effect of coagulation on atherosclerotic manifestations.
Methods: We enrolled 422 patients with stable angina (SA) and 277 controls. The rs1800790 (G455A) and the rs2070011 (G58A) polymorphisms were estimated by appropriate genotyping. Fibrinogen and D-Dimer levels, as well as factors (f) V, X activity were measured by standard coagulometry techniques. Flow-mediated dilation (FMD) was assessed by brachial ultrasound.
Results: We have found the 455AA homozygosity was associated with raised fibrinogen levels in both groups (p=0.035 controls and p<0.001 SA). In addition, homozygotes for the 58A allele had lower fibrinogen levels in controls (p=0.038). Both the 58AA (p=0.027) and 455AA homozygotes (p=0.022) had higher levels of D-Dimer in the SA group. The 455AA homozygotes had also increased fV activity in the SA group (p=0.048). No effects were observed on fX activity and FMD. Further analysis revealed that fibrinogen levels were strongly associated with SA (1.005 [1.003-1.007], p<0.001) as well as the presence of MI (1.003 [1.001-1.005], p=0.001). Similarly, D-Dimer levels were also strong predictors of CAD (1.001 [1.001-1.002], p<0.001), but not of MI (1.000 [1.001-1.001], p=0.083). Surprisingly, when fV and fX activities were examined for possible associations with clinical presentations, we found that fV activity was associated with increased number of diseased vessels (1.016 [1.001-1.030], p=0.037), while fX activity was strongly related to MI (0.985 [0.973-0.997], p=0.013).
Summary and Conclusions: The rs1800790 variant increases significantly fibrinogen, D-Dimers levels and fV activity, contributing to atherogenesis. In addition, fibrinogen and D-Dimers levels are strong predictors of SA and MI. Interestingly, in our study we have shown for the first time to our knowledge that fV and fX are associated with the number of diseased vessels and the risk of MI respectively.

P781

LONGTERM CANCER RISK AFTER VENOUS THROMBOEMBOLISM

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Background: A venous thromboembolic event (VTE) may indicate an underlying cancer. In population based studies the likelihood of cancer is highest during the first year but a persistent increase has been reported also in the years thereafter. Data on the risk in patients who completed anticoagulant treatment and recommendations regarding screening are lacking.
Aims: To evaluate the long-term risk of cancer in a well-defined cohort of patients with an unprovoked VTE.
Methods: We prospectively followed patients with a first unprovoked VTE after discontinuation of anticoagulation and excluded those with long-term antithrombotic therapy, major thrombophilia or pregnancy. Study endpoint was diagnosis of cancer. The ratio of the observed cases and the number of cases expected based on national cancer incidence rates was calculated and expressed as standardized incidence ratio (SIR). The study was approved by the local ethics committee and all patients consented.
Results: 43 (3.6%) of 1188 patients (mean age 48 years, 638 women, median duration of anticoagulation 7.2 months) were diagnosed with cancer (colon 8, breast 7, lung 6, hematologic 6, prostate 4, skin 1, brain 1, others 10) during a median follow up of 70 months (range 3-216). Cancer patients were significantly older (57 *vs* 48 years, p<0,001) but did not differ from non-cancer patients regarding proportion of women (49 *vs* 54%), blood cell counts (hemoglobin 14.2 *vs* 14.2 g/dl; platelets 232 *vs* 238 G/l; leukocytes 6.5 *vs* 6.8 G/l), body mass index (both 27 kg/m^2) or D-Dimer (469 *vs* 463 µg/ml). The probability of cancer was 0.7% (95% CI 0.1-1.3%), 1.7% (95% CI 0.9-2.5%) and 3.0% (95% CI 1.8-4.2%) after 1, 2 and 5 years. The corresponding SIR did not significantly differ from expected rates in an age- and sex-matched general population (1.2, 1.3 and 1.0) (Figure1).

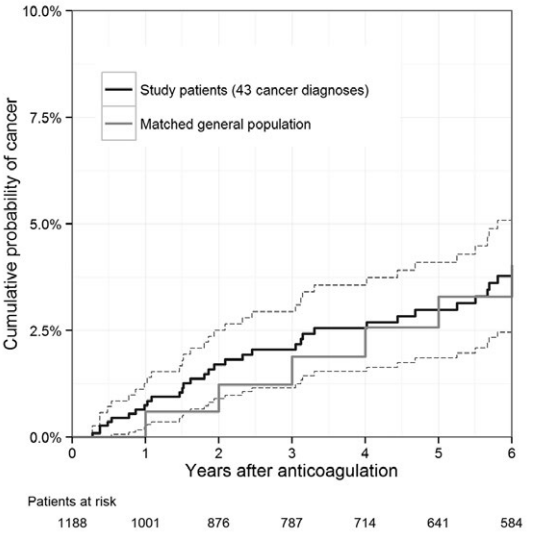

Figure 1.

Summary and Conclusions: In patients with unprovoked VTE the long-term incidence of cancer after anticoagulation is low. Cancer types are heterogeneous and specific screening strategies do not seem to be warranted.

P782

POLYMORPHISM OF GENES INVOLVED IN LIPID METABOLISM AS A RISK FACTOR FOR VENOUS THROMBOEMBOLISM

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Background: Venous thromboembolism (VTE) is one of the leading causes of morbidity and mortality in industrialized countries, and is known as a multifactorial disease. Along with the classic risk factors, significant role in the development of VTE has been assigned to genetic predisposition. Although conventional genetic risk factors have been involved in the disease process, VTE often occurs in the absence of known risk factors. Doubtless, some forms of dyslipidemia are risk factors for arterial thrombosis. However, based on the recent research, lipid metabolism disorders have a high probability to be implicated in pathogenesis of VTE.
Aims: The aim of this study was to investigate the role of allelic polymorphism of some genes involved in lipid metabolism: apolipoprotein E (ApoE), apolipoprotein CIII (ApoCIII), lipoprotein lipase (LPL) and hepatic lipase (LIPC), in patients with VTE.
Methods: We retrospectively analyzed DNA samples of 300 patients with VTE (147 men and 153 women, average age 39,9±12,4 years), including 200 individuals with the debut of VTE under the age of 45 years. Isolated deep vein thrombosis (DVT) has been diagnosed in 170 patients, whereas in 67 persons DVT complicated by pulmonary embolism (PE) ("DVT+PE"), and 63 individuals have had PE without signs of DVT (isolated PE). The control group consisted of 100 sex- and age-matched healthy persons without history of thrombosis. Polymorphism of the ApoE (E2/E3/E4), ApoCIII (C3175G and −455 T/C), LPL (Asn291Ser) and LIPC (-514 T/C) genes was determined by polymerase chain reaction with subsequent restriction digest. Statistical analysis was performed using the program GraphPad Prism 5.0. Differences in the distribution of alleles and genotypes were assessed by Fisher's exact test.
Results: The distributions of alleles and genotypes of analyzed genes in patients with VTE had no significant differences from those in healthy controls. Nonetheless, ApoE E3/E4 genotype was more frequently seen in men with thrombosis when compared to group of women (24,5% *vs* 15,0%; OR=1,8; 95%CI: 1,0-3,3; p=0,043). Significant increase in proportion of homozygotes for the LIPC −514T allele was observed in the "DVT+PE" group (69,2% *vs* 52,9% in patients with isolated DVT; OR=2,0; 95%CI=1,1-3,7; p=0,027). The ApoCIII −455CC genotype was more prevalent in patients who had their first episode of VTE after 45 years than in those with early-onset disease (26,5% *vs* 14,9%, respectively; OR=2,0; 95%CI=1,0-4,1; p=0,043). On the other hand, the proportion of homozygotes for the ApoCIII −455T allele was significantly higher in young patients (37,4% *vs* 22,0% in the group with late-onset VTE; OR=2,1; 95%CI=1,1-4,0; p=0,023).
Summary and Conclusions: Our data indicate that polymorphism of some genes affecting lipid metabolism could influence the risk and course of VTE. ApoE genotype E3/E4 is a potent risk factor for VTE development in men. Polymorphism −455 T/C of the ApoCIII gene could influence the age of VTE debut. Furthermore, the risk of PE development in patients with DVT may depend on their LIPC genotype.

P783

HIGH LEVELS OF FIBRINOGEN, BUT NOT FIBRINOGEN GENETIC VARIANTS PREDICT CORONARY ARTERY DISEASE IN SUBJECTS WITH HYPERTENSION AND DIABETES MELLITUS TYPE 2

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Background: It is well established that hypertension (HTN) and diabetes mellitus (DM) are strongly associated with coronary artery disease (CAD). In addition, controversial data exist related to the role of fibrinogen genetic variants in atherosclerosis-CAD. Therefore, we examined the effects of the rs180070 and rs2070011 fibrinogen polymorphisms on coagulation, inflammation and on the risk for CAD in patients with DM and HTN admitted with stable angina pectoris symptoms.
Methods: A total of 744 subjects were enrolled in 3-year period. Fibrinogen polymorphisms were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique. Fibrinogen, interleukin-6 (IL-6), high sensitivity C-reactive protein (hsCRP) CD40L, D-dimers and factors V, X activity were measured with appropriate laboratory methods.
Results: The AA homozygosity of rs180070 was associated with significantly higher levels of fibrinogen in both HTN and DM (p=0.05, p=0.04 respectively). The presence of AA genotype (rs180070) was also significantly associated with increased risk of CAD in the general population [OR: 3.2, 95% CI, (1.01-10.1, p=0.049)]. While it was associated with higher IL-6 an D-dimers, but not hsCRP levels in the general population. Interestingly, multivariate logistic regression analysis showed that fibrinogen levels >443 mg/dl were associated with higher risk for CAD [OR: 3.9, 95% CI, (1.7-9.4, p=0.002)] compared to levels <347 mg/dl in the general population. Similar associations were observed in HTN and DM patients. In hypertensive patients, higher fibrinogen levels >443 mg/dl [OR: 3.5, 95% CI (1.14-10.9, p=0.029), but not the AA genotype of rs180070 [OR: 3, 95% CI (0.78-11.9, p=0.11)] were independent predictors of CAD. Similarly, in DM only fibrinogen remained an independent predictor of CAD [OR 5.86, 95% CI (1.1-31.2, p=0.038)].
Summary and Conclusions: We have shown that the presence of AA genotype (rs180070) is associated with increased levels of D-dimers and IL-6 promoting atherosclerosis, as well as risk for CAD. However, elevated fibrinogen levels >443 mg/dl remain the most significant predictor of CAD both in HTN and in DM subjects.

P784

VENOUS THROMBOEMBOLISM IN NORTHEAST MELBOURNE, AUSTRALIA: EVALUATION OF EPIDEMIOLOGY, RISK FACTORS AND TREATMENT STRATEGIES IN THE WARFARIN ERA

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Background: Venous thromboembolism (VTE) is associated with significant morbidity, mortality and economic cost (approximately AUD$117 000 per person, *Access Economics 2008*). Despite advances in thrombosis management, management strategies remain heterogenous with a paucity of real-world VTE data.
Aims: To provide a holistic evaluation of VTE management in the warfarin era including identifying potential causal effects and complications.
Methods: Retrospective evaluation of VTE from July 2011 to December 2012, at two major hospitals in Northeast Melbourne, Australia.
Results: 1003 patients, with a median age of 63 (range 19-97) years and an overall male predominance (52%), presented with a total of 1025 episodes - 574 (56%) pulmonary embolism (PE), 422 (41%) deep venous thrombosis (DVT). 23% (n=234) had active malignancy at time of diagnosis with 14 patients (1.4%) subsequently being diagnosed. Median follow up was 33.5 (range 23-53) months. *Non-cancer patients:* 63% had provoked VTE and thrombophilia screen was performed in 41%. 20% had previous VTE and 7.5% reported positive family history. Median duration of anticoagulation was 6 and 7 months for DVT and PE respectively. Majority (90%) was on warfarin for long-term anticoagulation. 5% required other interventions - IVC filter (n=29) and thrombolysis (n=13). 19% had end-of-treatment repeat imaging and residual clot was observed in 56%. On extended follow-up, clot persistence was not associated with increased recurrence (OR 1.9 [0.6-6.2]). 7% had recurrent thrombosis with increased risk in unprovoked VTE (OR 2.2 [1.3-3.80], p=0.004). 60% of recurrence occurred within 12 months of initial VTE. 5.6% reported grade III/IV bleeding with 56% occurring within the first month of anticoagulation. Bleeding risk was independent of duration of anticoagulation. All-cause mortality rate was 14%. In the below knee DVT (BKDVT) sub-analysis, 1.5% was subsequently diagnosed with cancer, similar prevalence to those with major VTE (1.7%), defined as proximal DVT and/or PE. BKDVT were more likely to be provoked (71% *vs* 60%, p<0.001) and recurrence was similar to major VTE (9% *vs* 7%, p=0.36). Mortality rate was 5.5% with no thrombosis-related deaths. *Cancer patients:* These patients were older (67 *vs* 61 years, p<0.001) and had higher clot burden with more PE (64% *vs* 53%, p=0.004), proximal DVT (63% *vs* 46%, p=0.0008) and bilateral DVT (16% *vs* 5%, p<0.001). Metastatic cancer was associated with increased unprovoked events (p=0.015). Cancer patients were more likely to require IVC filters and lifelong anticoagulation (p<0.001). Recurrence/clot extension (17%) and Grade III/IV bleeding (10%) were more common (p=0.02). Mortality rate in this cohort was 68% with higher incidence of complications-related deaths (p<0.001).
Summary and Conclusions: Recurrence rate was 4% in the first 12 months with unprovoked VTE being a major risk factor. Cancer patients experience more complications with higher clot burden and all-cause mortality rate. Interestingly, BKDVT, often considered a minor VTE, had comparable rates of recurrence and subsequent cancer detection to major VTE suggesting that BKDVT may not be as benign as it seemed. Further evaluation of new treatment strategies as well as clinical and laboratory risk assessments are required to improve the management for VTE. This data will serve as an important baseline for future comparison in the new era of novel oral anticoagulants.

SIMULTANEOUS SESSIONS

Multiple myeloma: Clinical studies 3

S785

IBRUTINIB IN PREVIOUSLY TREATED PATIENTS WITH WALDENSTRÖM'S MACROGLOBULINEMIA IS HIGHLY ACTIVE, PRODUCES DURABLE RESPONSES, AND IS IMPACTED BY MYD88 AND CXCR4 MUTATION STATUS

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Background: MYD88^{L265P} and CXCR4^{WHIM} are highly prevalent somatic mutations in Waldenström's Macroglobulinemia (WM). MYD88^{L265P} triggers WM growth via Bruton's Tyrosine Kinase, a target of ibrutinib. CXCR4^{WHIM} mutations confer *in vitro* resistance to ibrutinib.
Aims: The primary aim of this study was to determine in a prospective, multicenter study the overall and major response rates of ibrutinib monotherapy in previously treated and symptomatic WM patients requiring therapy. Secondary aims of this study included determination of progression-free survival, drug safety, and impact of MYD88 and CXCR4 mutations on treatment outcome.
Methods: Symptomatic WM patients with >1 prior therapies were eligible. Ibrutinib (420 mg) was administered daily until progression, or unacceptable toxicity. Dose modifications for toxicity were permitted. Central pathology review was performed in all patients. Genotyping for MYD88 L265P and CXCR4 WHIM mutation status was performed in 63 and 62 patients, respectively.
Results: 63 patients with a median of 2 prior therapies (40% with refractory disease) were enrolled. Post-therapy, median serum IgM levels declined from 3,520 to 880 mg/dL; hemoglobin rose from 10.5 to 13.8 g/dL, and bone marrow involvement declined from 60% to 25% (p<0.01 for all comparisons). Overall and major response rates were 90.5% and 73.0%, and the median times to at least minor and major responses were 4 and 8 weeks, respectively. Overall responses were similar regardless of baseline age (<65 *vs.* ≥65 years), ECOG status (0 *vs.* ≥1), pre-therapy WM IPSS score, serum β_2-microglobulin levels (<3.0 *vs.* >3.0 mg/L), hemoglobin (<11 *vs.* >11 g/dL), serum IgM (<4,000 *vs.* ≥4,000 mg/dL), BM disease involvement (<50% *vs.* ≥50%), prior relapsed or refractory status, and prior lines of therapy (1-3 *vs.* >3). Fifty-six (88.9%) patients expressed MYD88^{L265P}, and 21 (33.9%) had CXCR4^{WHIM} mutations. Overall and major response rates were highest in patients with MYD88^{L265P}CXCR4$^{wild\text{-}type(WT)}$ (100% and 91.2%), followed by MYD88^{L265P}CXCR4^{WHIM} (85.7% and 61.9%), and MYD88^{WT}CXCR4^{WT}(71.4% and 28.6%) mutation status. The 2-year PFS (Figure A) and overall survival rates (Figure B) were 69.1% and 95.2%, respectively. Subset analysis showed that >3 prior lines of therapy, high pre-therapy IPSS score, and MYD88^{WT}CXCR4^{WT} mutation status associated with inferior progression-free survival. Grade >2 treatment-related toxicities included neutropenia (22.2%) and thrombocytopenia (14.3%) that were more common in heavily pre-treated patients; atrial fibrillation in patients with a prior arrhythmia history (3.2%); procedure-related bleeding (3.2%); and epistaxis related to marine oil supplements (3.2%). Serum IgA and IgG levels were unchanged, and treatment-related infections were infrequent.

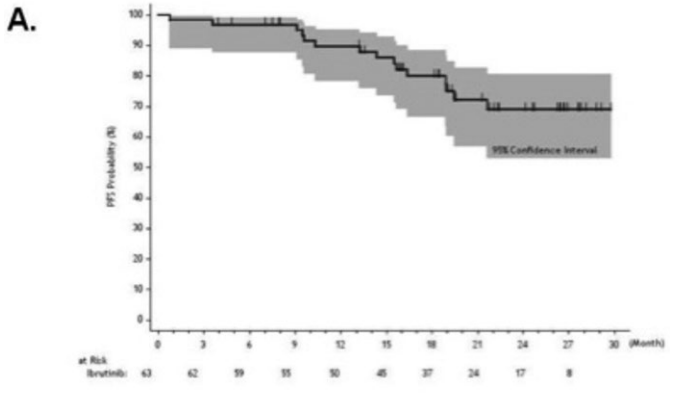

Figure 1. A

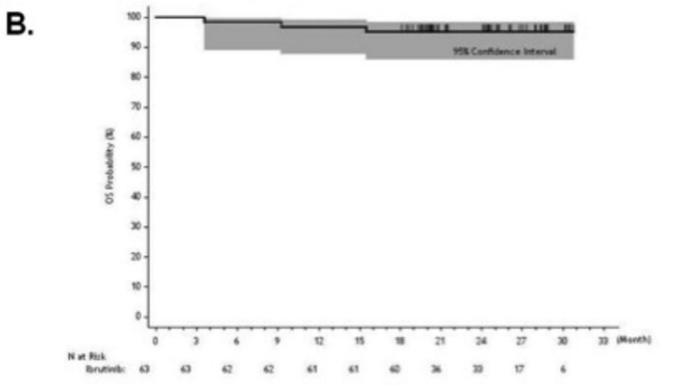

Figure 1. B

Summary and Conclusions: Ibrutinib is highly active in previously treated WM patients. An overall response rate of 90.5%, and 2-year progression-free and overall survival rates of 69.1% and 95.2%, respectively, was achieved with ibrutinib monotherapy. Ibrutinib responses were influenced by MYD88 and CXCR4 mutation status. Overall, treatment toxicity was moderate and no unexpected toxicities were observed.

S786

LOW IKZF1 EXPRESSION IS ASSOCIATED WITH FAVORABLE OUTCOME IN LENALIDOMIDE TREATED NEWLY DIAGNOSED MULTIPLE MYELOMA

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Background: Lenalidomide is an immunomodulatory drug (IMiD) with high activity in multiple myeloma (MM). IMiD binding to the CRBN E3 ubiquitin ligase results in targeted ubiquitination and degradation of the lymphoid transcription factors IKZF1 and IKZF3 and constitutes a unique mechanism of action for a class of drugs.
Aims: To determine whether expression levels of *CRBN*, *IKZF1*, and *IKZF3* are associated with outcome in lenalidomide treated patients (pts) with newly diagnosed MM.
Methods: We analyzed mRNA expression levels of *IKZF1*, *IKZF3*, and *CRBN* by real-time quantitative PCR (RQ-PCR) in CD138-selected pre-treatment samples from 60 pts with newly diagnosed MM uniformly treated within a phase II clinical trial of the German Myeloma Study Group (DSMM). Additionally, all pts were characterized for the presence of cytogenetic abnormalities using a comprehensive set of FISH probes. All patients received induction consisting of four cycles of lenalidomide, adriamycin and dexamethasone followed by high-dose melphalan (Mel-200) with autologous stem cell transplantation (aSCT). Genetically defined low- and intermediate risk patients received a second aSCT, while those pts with high-risk cytogenetics (presence of a del17p13, t(4;14) or t(14;16)) underwent allogeneic stem cell transplantation if a donor was available. All patients received lenalidomide maintenance for 12 months.

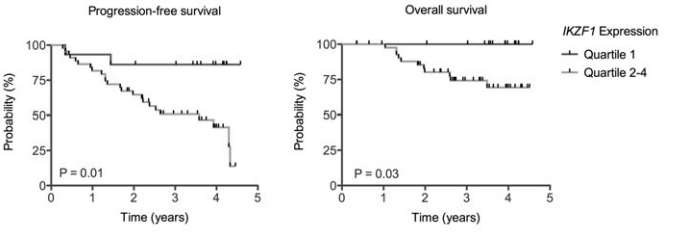

Figure 1.

Results: Median expression levels normalized to plasma cells from healthy volunteers of *CRBN*, *IKZF1*, and *IKZF3* were 0.66 (range, 0.12 - 2.86), 0.66 (0.16-2.92), and 0.52 (0.19-5.13), respectively. Expression levels of *CRBN*, *IKZF1*, and *IKZF3* were not affected by the presence of chromosomal aberrations (del13q14, del17p13, del9q34, +1q21, t(4;14), t(11;14), t(14;16)). Patients achieving a complete response (CR) or very good partial response (VGPR) had a trend towards a lower *IKZF1* expression than patients achieving a partial response (PR) (median *IKZF1* expression: 0.62 *vs* 0.84, p=0.07). Consistently,

lower *IKZF1* expression levels were associated with a better outcome: when segregating *IKZF1* expression levels into quartiles, pts with the lowest (Q1) quartile of *IKZF1* expression had a 3 year progression-free survival (PFS) of 86% compared to 51% in patients of the remaining quartiles (Q2-Q4)(Log rank test, p=0.01). This translated into a better overall survival (OS) (*IKZF1* Q1:100% *vs* *IKZF1* Q2-4:74% after 3 years, log rank p=0.03). In contrast, *CRBN* and *IKZF3* expression levels had no impact on response, PFS, or OS. Factors also associated with outcome included the presence of a del13q14 (PFS: p=n.s.; OS: p=0.04), del17p13 (PFS: p=0.001; OS=0.03), t(4;14) (PFS: p=0.009; OS: p=0.004) or +1q21 (PFS: p=n.s.; OS p=0.037).
Summary and Conclusions: In this cohort of uniformly treated newly diagnosed MM patients, *IKZF1* but not *IKZF3* or *CRBN* expression levels were associated with outcome. Low *IKZF1 m*RNA expression levels seem to identify a sub-group with a particularly good outcome.

S787

POST-TRANSPLANT CARFILZOMIB (KYPROLIS®), LENALIDOMIDE (REVLIMID®), AND DEXAMETHASONE (KRD) CONSOLIDATION IN NEWLY DIAGNOSED MULTIPLE MYELOMA (NDMM): EFFICACY AND TOLERABILITY OF EXTENDED TREATMENT

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Background: In a previous phase 1/2 study, extended treatment for 24 months with KRd provided a high rate of stringent complete response (sCR) of 55% and durable disease control with a 3-year progression-free survival (PFS) of 79% and overall survival (OS) of 96% after extended treatment (Jakubowiak *et al.* Blood 2012; Jasielec *et al.* ASH 2013). In this Phase 2 study we evaluate post-transplant extended treatment with KRd in NDMM (NCT01816971).
Aims: To assess the efficacy and tolerability of extended post-transplant treatment with KRd in NDMM pts who have received KRd induction and ASCT.
Methods: Pts with symptomatic NDMM per IMWG criteria and transplant candidates were eligible for enrollment. After 4 cycles of KRd induction and ASCT, pts received KRd consolidation treatment (70—90 days post-transplant) as 28-day cycles for 4 cycles: CFZ 36mg/m^2 IV on Days 1, 2, 8, 9, 15, 16; LEN PO Days 1-21 starting at 15mg with option to escalate after 1 cycle of consolidation; DEX PO 20mg Days 1, 8, 15, 22. After consolidation, KRd was given for additional 10 maintenance cycles, with modified CFZ schedule on Days 1, 2, 15, 16. LEN single-agent was recommended off protocol at best tolerated dose after KRd maintenance. Dose modifications were permitted to manage toxicities. The primary endpoint was sCR rate at the end of Cycle 8; an improvement of sCR from 30%, the rate observed at the end of 8 cycles without transplant, to 50% in this study will be considered promising for strategy of combining KRd with transplant.
Results: As of Feb 1, 2015, 28 of a total 55 enrolled pts have completed at least 1 cycle of KRd consolidation: 25 pts have completed KRd consolidation and initiated maintenance; 7 pts have completed KRd maintenance and initiated LEN single-agent off protocol. After transplant prior to the initiation of KRd consolidation, ≥VGPR was 96%, ≥near CR (nCR) was 46%, and sCR was 29%. After 4 cycles of consolidation, ≥VGPR was 100%, ≥nCR was 88% and sCR was 72%. The rate of negative minimal residual disease by 9-color flow cytometry at the end of 8 cycles was 88%. Responses deepened in 25 pts treated beyond at least 4 cycles of KRd consolidation with an additional 1 pt converting to nCR and 3 to sCR, resulting in ≥nCR 96% and sCR 84%. After a median follow-up of 14 months from the start of the KRd treatment (range 8-24), all 28 pts were alive and progression free. There were no new or unexpected toxicities post-transplant compared to the referenced above phase 1/2 trial of KRd without transplant. Enrollment continues with a target of 70 pts; 55 have been enrolled and updated results of pts receiving at least one cycle post-ASCT will be reported during the meeting.
Summary and Conclusions: Based on this analysis, extended post-transplant treatment with KRd can be considered for future evaluation in pts who have received induction regimens other than KRd. The preliminary data suggest that KRd with transplant has high efficacy compared to standard single-agent maintenance therapy post-transplant.

S788

IMPACT OF STABLE DISEASE AS A RESPONSE TO POMALIDOMIDE PLUS LOW-DOSE DEXAMETHASONE ON SURVIVAL OUTCOMES IN PATIENTS WITH REFRACTORY OR RELAPSED AND REFRACTORY MULTIPLE MYELOMA IN THE MM-003 TRIAL

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Background: Patients (pts) with refractory or relapsed and refractory multiple myeloma (RRMM) have poor overall survival (OS) outcomes after failure of bortezomib and lenalidomide treatment (Tx) regimens. Pomalidomide plus low-dose dexamethasone (POM+LoDEX) has been demonstrated to significantly extend OS in this pt population compared with high-dose DEX (HiDEX; hazard ratio [HR]=0.74 [95% CI, 0.56-0.97]; *P*=0.0285; San Miguel et al, *Lancet Oncol,* 2013) in the phase 3 MM-003 trial (clinicaltrials.gov identifier NCT01311687). Secondary analysis of MM-003 showed that the survival benefit of Tx with POM+LoDEX extended to pts who achieved minor response or better (San Miguel et al, ASH, 2013).
Aims: This post hoc analysis of MM-003 investigated OS based on response status with a focus on pts who achieved stable disease (SD) but not a response by certain landmark points in time.
Methods: Kaplan-Meier methods and unadjusted Cox regression models were used for landmark analyses at the start of cycles (C) 3, 5, and 7. Response status was captured over time by time-dependent survival analyses. For both approaches, survival of pts with SD was compared with that of pts with progressive disease (PD) or overall response (OR; ≥partial response) at the same point in their treatment.
Results: 302 pts randomized to POM+LoDEX were included in the analysis. Response rates at C3 were 38.4% SD, 14.6% PD, and 19.2% OR, with no response data for 27.8% of pts (mostly due to early discontinuation). The median OS from randomization by response status at C3 was 15.3 mos for SD (n=116), 6.3 mos for PD (n=44; hazard ratio [HR]=3.83; *P* <0.0001 *vs* SD), and 17.5 mos for OR (n=58; HR=0.75; *P*=0.320 *vs* SD). Median OS at C5 was 16.6 mos for SD (n=57), 11.0 mos for PD (n=31; HR=2.81; *P*=0.004 *vs* SD), and 18.0 mos for OR (n=56; HR=0.74; *P*=0.462 *vs* SD). Median OS at C7 was not reached for SD (n=40), 16.4 mos for PD (n=18; HR=2.66; *P*=0.080 *vs* SD), and 18.0 mos for OR (n=47; HR=0.90; *P*=0.843 *vs* SD). The time-dependent survival model showed a lower risk of death for pts with SD or OR *vs* PD (HR=0.27 [95% CI, 0.17-0.44] and HR=0.06 [95% CI, 0.02-0.16], respectively). The HiDEX Tx arm exhibited a lower response rate and shorter OS, which in combination with a smaller number of pts overall resulted in response groups too small for meaningful analysis.
Summary and Conclusions: POM+LoDEX–treated pts with SD at C3, C5, and C7 had OS comparable to pts who achieved OR. The OS of pts with either SD or OR at these time points was significantly longer than for those with PD.

S789

A PHASE I/IIA STUDY OF THE HUMAN ANTI-CD38 ANTIBODY MOR202 (MOR03087) IN RELAPSED OR REFRACTORY MULTIPLE MYELOMA

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Background: MOR202 is a HuCAL-derived fully human immunoglobulin G1 (IgG1) anti-CD38 antibody, with high efficacy in preclinical models of multiple myeloma.
Aims: To evaluate the safety and preliminary efficacy of MOR202 in adult patients with relapsed or refractory multiple myeloma.
Methods: This is an open-label dose-escalation study (3+3 design). The data of patients previously treated with ≥2 prior therapies, including an immunomodulatory drug and a proteasome inhibitor, are presented. Patients received 2-hour intravenous MOR202 every 2 weeks (q2w; 8 dose levels from 0.01–16 mg/kg) without dexamethasone, or 4 or 8 mg/kg (dose levels 6 and 7) weekly (q1w)+/-dexamethasone. MOR202 16 mg/kg (dose level 8) q1w+/-dexamethasone, and combination cohorts with lenalidomide+dexamethasone and pomalidomide+dexamethasone, are planned, as well as confirmation cohorts.
Results: As of December 31 2014, 38 patients had been treated; 29 and 9 patients in the q2w and q1w dose levels, respectively. Median age was 70 (44–80) years. The median number of prior treatment lines was 4 (2–10) for all patients. A total of 36 patients (94.7%) developed adverse events. The most frequently reported adverse events (>10%) of any grade were anemia (31.6%), fatigue (28.9%), white blood cell count decreased (21.1%), lymphocyte count decreased (21.1%), diarrhea (21.1%), nasopharyngitis (18.4%), and leukopenia (13.2%). Grade ≥3 hematologic adverse events were lymphocyte count decreased (15.8%), white blood cell count decreased (7.9%), leukopenia (5.3%), thrombocytopenia (2.6%), and lymphopenia (2.6%). Infusion-related reactions occurred in 13 patients (34%) receiving MOR202 without dexamethasone, mainly during the first infusion. All were grade 1–2 except for 1

patient (grade 3). There have been no treatment-related deaths. Pharmacokinetic data demonstrate a significant target-mediated drug disposition effect for most patients treated q2w. In 4 out of 6 patients in the q1w 4 mg/kg cohort, MOR202 trough levels show the start of target saturation. Only 1 patient (MOR202 0.15 mg/kg q2w) generated a transient anti-drug antibody response to MOR202.

Summary and Conclusions: The maximum tolerated dose has not been reached. MOR202 is safe and well tolerated. Pharmacokinetic data show the potential for full target occupancy in the majority of patients receiving 8 and 16 mg/kg q1w. These latter dose levels of MOR202 will be tested as monotherapy or in combination with dexamethasone, lenalidomide+dexamethasone, and pomalidomide+dexamethasone, in the upcoming cohorts. Efficacy analyses are ongoing.

CLL: Refining outcomes

S790

SAFETY AND EFFICACY OF THE BRUTON TYROSINE KINASE INHIBITOR IBRUTINIB IN PATIENTS WITH HAIRY CELL LEUKEMIA: INTERIM RESULTS OF A PHASE 2 STUDY

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Background: Effective therapy for classical hairy cell leukemia (c-HCL) refractory to purine nucleoside analog (PA) treatment is limited, and there is no accepted treatment standard for either treatment-naïve or relapsed variant hairy cell leukemia (v-HCL). Ibrutinib, an oral small molecule inhibitor of Bruton tyrosine kinase (BTK), has shown single-agent efficacy and acceptable tolerability in patients with various B-cell malignancies, including CLL, mantle cell lymphoma, and Waldenstrom macroglobulinemia. However, while BTK is expressed in HCL cells, the clinical activity of ibrutinib in this disease has not been previously assessed.
Aims: To characterize the overall response rate (complete+partial response) and safety of single-agent ibrutinib treatment for hairy cell leukemia.
Methods: This NCI/CTEP-sponsored, single-arm phase 2 study enrolled patients with c-HCL (unfit for or relapsed after PA) and v-HCL (relapsed or treatment-naïve). Eligible patients required treatment, had ECOG ≤2, no active infection, and preserved end-organ function. Patients received continuous oral ibrutinib (420 mg daily) in 28-day cycles. A second cohort at higher dose (840 mg daily) has been accrued after a pre-determined response endpoint was not initially met at the 420 mg dose. Response, including bone marrow biopsy with immunohistochemistry for minimal residual disease (MRD), is assessed after 8 and 12 cycles. Soluble interleukin-2 receptor levels (sIL2R) were measured serially and correlated with clinical outcome. Patients experiencing clinical benefit may continue ibrutinib indefinitely absent unacceptable toxicity or progressive disease.
Results: Data are presented for the initial 420 mg/day cohort (n=13). Enrolled subjects (1 treatment-naïve v-HCL, 1 relapsed v-HCL, 11 relapsed c-HCL) included 12 male and 1 female patients with median age 64 years (range: 43-76) and median 4 (range: 1-11) prior therapies. All relapsed patients had received prior PA, 5 (45%) were splenectomized, and 9 (82%) had prior rituximab. The most frequent (>20%) grade 3/4 adverse events (AEs) included lymphopenia (37%), hypophosphatemia (30%), neutropenia (23%), and infection (23%). Common grade 1/2 AEs included myalgias (61%), headache (38%), dizziness (38%), diarrhea (38%), arthralgias (30%), rash (30%), and fatigue (30%). Other hematologic AEs included grade 1/2 anemia (38%) and grade 1/2 thrombocytopenia (38%). In general, response has improved with continued treatment (Table). One MRD-negative complete response (c-HCL) and 5 partial responses have been observed (ORR 46%). Four additional patients (30%) with stable disease have experienced clinical benefit not meeting criteria for PR and continue on treatment. At median follow-up of 14.5 months, 9 patients (69%) remain progression-free on treatment, 3 patients (1 v-HCL, 2 c-HCL) have progressed and 1 patient (c-HCL) discontinued in cycle 8 after failing to resolve baseline neutropenia. Redistribution lymphocytosis (peaking at day 8) occurred in both v-HCL patients and 1 c-HCL patient with circulating disease at baseline. sIL2R levels were increased at baseline in all c-HCL patients for whom data were available and normalized in responders.

Tabella 1.

Response Category	Best Response (n=13)	Post-Cycle 8 (n= 11)	Post-Cycle 12 (n=11)
CR	1	0	1
PR	5	5	5
SD	6	6	4
PD	1	0	1

Summary and Conclusions: Ibrutinib is well-tolerated and can induce remissions, including MRD-negative complete remission, in hairy cell leukemia patients. The correlation between sIL2R levels and response will be investigated further. Accrual continues to the second cohort.

S791

THE INTERNATIONAL PROGNOSTIC INDEX FOR PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA (CLL-IPI)-AN INTERNATIONAL META-ANALYSIS

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Background: Chronic lymphocytic leukemia (CLL) is a disease with a highly heterogeneous course, ranging from mild to aggressive. Therefore, prediction of outcome is important in clinical practice to avoid too aggressive treatment in patients with a rather mild course of disease and to identify patients in whom standard treatment is likely to fail. In the era of more effective treatments for CLL, the established clinical staging systems [Binet/Rai] do not accurately discriminate between prognostic subgroups. Despite the introduction of several new prognostic markers, there is no system integrating the major clinical, biological and genetic variables into one widely accepted prognostic system.
Aims: To develop an internationally applicable prognostic index for CLL patients [CLL-IPI], we performed a comprehensive analysis of 26 prognostic factors including clinical, biological and genetic markers.
Methods: The data from 8 prospective randomized phase III trials from France, Germany, UK, USA and Poland comprised our full analysis set including 3472 previously untreated patients at early and advanced clinical stage with a median age of 61 years (yr) (range, 27 - 86) and a median observation time of 80 months. First line therapies included Fludarabine (F), F+Cyclophosphamide (FC), FC+Rituximab, FC+Campath, C+Cladribine, Chlorambucil and watch & wait. The full analysis set was randomly divided into training [2308 (67%)] and internal validation dataset [1164 (33%)]. Methods of multivariable survival statistics were applied including proportional-hazards Cox regression analyses. The main end point of all statistical analyses was overall survival (OS). Handling of missing data was performed with complete case analysis using a 4 step-down procedure accounting for the degree of completeness of data. *C*-statistics were calculated to evaluate the discriminatory value of the model (c=1 indicates perfect discrimination; c=0.5 is equivalent to chance). Subsequently, the model was externally validated in a third dataset comprised of 845 newly diagnosed CLL patients from Mayo Clinic [median age 62 yr (range, 25 - 89); 63 months median observation time].
Results: Based on 1192 (52%) patients from the training dataset, 5 independent predictors for OS were identified: age [cut off, 65 yr], clinical stage [Binet A/Rai 0 *vs.* Binet B-C/Rai I-IV], del(17p) and/or *TP53* mutation, *IGHV* mutation status (MS) and serum β2-microglobulin (B2M) [cut off, 3.5 mg/L]. Applying weighted grading of the independent factors based on the regression parameters, a prognostic index was derived separating 4 different risk groups [low (score 0-1), intermediate (score 2-3), high (score 4-6) and very high risk (score 7-10)] with significantly different OS [93%, 79%, 64% and 23% OS at 5 yr for the low, intermediate, high and very high risk group respectively, p<0.001; *C*-statistic c=0.724 (95% confidence interval (CI), 0.69-0.76)] (figure 1A). This multivariable model was confirmed on the internal validation dataset [575 (49%) patients; 91%, 80%, 52% and 19% 5-yr OS, p<0.001; c=0.777 (95% CI, 0.73-0.82)] (figure 1B). In the external Mayo dataset the 5-yr OS for the CLL-IPI risk groups were 97%, 91%, 68% and 21% [p<0.001; c=0.790 (95% CI, 0.74-0.85)] (figure 1C). Further, the CLL-IPI provided accurate estimation regarding time to first treatment within this cohort [81%, 47%, 30% and 19% patients free from treatment at 5 yr, p<0.001].

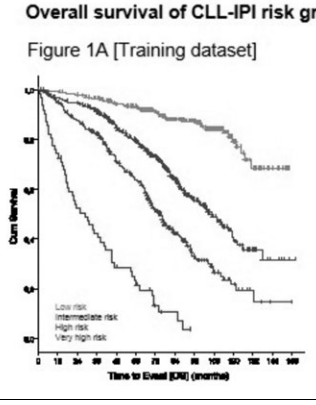

Figure 1A.

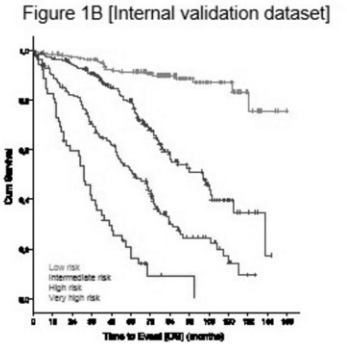

Figure 1B.

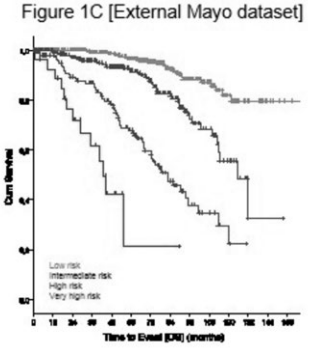

Figure 1C.

Summary and Conclusions: We report the development and validation of a weighted, integrated prognostic score and index derived from a broad number of established prognostic markers. The resulting CLL-IPI combines the most important genetic risk factors (*IGHV* MS, del(17p)/*TP53*) with traditional clinical stage, age, and B2M into an easily applicable prognostic index for CLL patients. Moreover, it both discriminates between prognostic subgroups and may help to inform current treatment recommendations in the future.

S792

THE LLR TAP ICICLLE TRIAL ASSESSING BIOLOGICAL RESPONSE TO IBRUTINIB IN CLL: IMMEDIATE DISEASE REDISTRIBUTION PRECEDES CELL CYCLE ARREST BY 2 WEEKS WITH REDUCED BONE MARROW INFILTRATION BY 6 MONTHS

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Background: Ibrutinib is a Bruton's tyrosine kinase inhibitor with efficacy in CLL. The IcICLLe trial (ISRCTN 12695354) is a feasibility study to investigate the biological response to ibrutinib and inform the design of subsequent randomised phase II/III trial(s). Primary outcome measures include assessment of the CLL cell levels and protein expression profile, particularly in relation to cell cycle and other CLL treatment targets, in the peripheral blood (PB) and bone marrow (BM). Toxicity, categorical response and survival are secondary outcomes.
Aims: To understand the biological impact of ibrutinib treatment and identify changes that may impact the design of therapeutic strategies or affect disease monitoring.
Methods: 40 participants with CLL requiring treatment (20 treatment-naïve and 20 relapsed/refractory) received ibrutinib (420 mg once daily) from registration until either achievement of <0.01% residual disease in bone marrow or disease progression. PB & BM samples are taken -0.5 (screening), 1 & 6 months with additional PB samples at 0 (baseline), 4 hours, 1, 2, 7, & 14 days and 2, 9, & 12 months (M). Markers assessed at each time point include CD19, CD20, CD45, CD5, CD10, CD305, kappa, lambda, CD23, CD43, CD81, CD79b, ROR1, CXCR4, CD49d, Ki67, CD62L, and CD38 with an additional 30 markers (including BCL2, ZAP-70 and IRF4) samples with paired PB & BM.
Results: Absolute peripheral B-cell counts increased immediately after the first dose, peaking at two weeks, and returning to baseline by month 2 (median 1.25, 1.44, 2.1, 1.19 fold absolute B-cell count relative to baseline at 4hrs,

24hrs, 2 weeks and 2 months after first dose respectively). There was no difference in bone marrow infiltration after 1 month of treatment but after 6 months, 7/8 evaluable patients had more than 50% reduction in PB CLL counts relative to baseline and bone marrow involvement was reduced with 3/8 achieving <30% BM CLL and 1 treatment-naïve patient achieving a CR. The proportion of CLL cells expressing Ki67 at baseline was slightly lower in the peripheral blood (median 3.5%, range 0.3-22.3%) than the bone marrow (median 3.9%, range 0.8-42%) but the difference was not significant and blood was a suitable site for assessing proliferative capacity. Ki67 expression initially increased, peaking at 24 hours after first dose (PB CLL Ki67+median 5.1%, range 0.4-16.4%), after which the Ki67 expression declined below quantifiable levels (<0.5%) in 15% of patients by week 1 and in more than 90% of patients at subsequent time points. Similarly in the BM, CLL Ki67 expression was not detectable in most patients after 1 month of treatment. Other changes in protein expression during treatment, including decreases in markers associated with proliferation in CLL (CD5, CD23, CD38, CD49d and IRF4), and changes in molecules involved in CLL cell trafficking and adhesion (increased CXCR4 and CD24 expression; decreased CCR7, CD31 and CD11a) followed the same kinetics as cell cycle arrest, *i.e.* differences emerged after 1-2 weeks of treatment and then stabilised during subsequent treatment. Concerning molecules involved in disease monitoring, CD19, CD5 & CD43 expression were mildly reduced but overall expression remained strong; CD81/CD79b expression did not change significantly; CD20/CD22 expression decreased, potentially enhancing discrimination from normal B-cells but this may affect optimal timing of anti-CD20 therapy; CD200 expression was also consistently decreased and these changes need to be considered when assessing residual disease levels.
Summary and Conclusions: The redistribution of CLL cells during ibrutinib therapy happens much more rapidly than any changes in proteins associated with proliferation, cell trafficking or adhesion. The majority of changes in CLL cells correlate with the loss of the proliferative fraction, mostly stabilising after one month of treatment.

S793

OVERALL SURVIVAL ANALYSIS ADJUSTING FOR TREATMENT EFFECT AFTER CROSSOVER IN A PHASE 3 STUDY EVALUATING IDELALISIB IN COMBINATION WITH RITUXIMAB IN RELAPSED CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

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Background: A Phase 3 trial evaluated idelalisib (IDELA)+rituximab (R) *vs* placebo (PBO)+R in patients (pts) with relapsed CLL (Furman, NEJM, 2014). Using an extension study design, pts on the control arm were allowed to receive IDELA after progression (PD). Estimating the true overall survival (OS) benefit of the active arm of randomized studies with such a crossover design represents a challenging statistical problem. Intent-to-treat (ITT) analyses considering data from pts after crossover as well as the approach of censoring subjects at the time point of crossover may significantly underestimate the OS benefit. Alternative analysis approaches like the rank-preserving structural failure time (RPSFT) model (Robins, 1991; Ishak, 2014) have successfully been used to estimate the true treatment effect in similar trials designs with crossover.
Aims: Estimate the treatment effect in OS adjusted for the impact of crossover
Methods: Three methods of OS analysis were compared (data cut-off date 01July2014): 1) ITT analysis calculating the OS according to initial randomization including data after crossover (as pre-specified), 2) Censoring subjects at the time point of crossover, and 3) RPSFT. Methods 1 and 2 commonly underestimate the true OS benefit. The RPSFT model estimates the counterfactual event time of the control arm that would have been observed without crossover and provides the estimate of treatment effect adjusted for the impact of crossover.
Results: 110 pts were randomized to each arm. 38% (n=42) of pts on PBO+R crossed over to receive single-agent IDELA following PD according to the initial study design. An additional 40% (n=44) of subjects on PBO+R crossed over to receive idelalisib in the absence of prior PD according to an amended protocol after the study was stopped due to overwhelming PFS efficacy. The median follow-up for the presented analyses was 12.5 months (mos) for pts initially on IDELA+R and 11.1 mos for PBO+R. There were 17 deaths in the IDELA+R group, and 40 deaths in the PBO+R group. Analysis results from each method are presented in the table below:

Tabella 1.

Method	HR (95% CI*)	Median OS (95% CI) in mos		p-value
		IDELA + R	PBO + R	
ITT	0.34 (0.19, 0.60)	NR (NR, NR)	20.8 (14.8, NR)	0.0001
Censoring at crossover	0.43 (0.20, 0.95)	NR (NR, NR)	NR (NR, NR)	0.0332
RPSFT	0.20 (0.10, 0.36)*	NR (NR, NR)	11.6 (8.2, NR)	<0.0001

NR=Not Reached; *95% CIs for hazard ratios were estimated by bootstrap method

Summary and Conclusions: IDELA+R demonstrated significant and consistent improvement in OS regardless of the analysis method. The median OS and HR as estimated by the RPSFT model reduced the bias associated with the idelalisib treatment effect compared to the ITT analysis or the censoring approach.

S794

BONE MARROW IS MORE SENSITIVE THAN PERIPHERAL BLOOD FOR DETECTION OF MRD IN CLL AND PROVIDES A MORE RELIABLE PREDICTION OF OUTCOME ACROSS DIFFERENT TREATMENTS

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Background: Minimal residual disease (MRD) in CLL is an independent predictor of progression-free (PFS) and overall survival (OS) with a high, intermediate and low risk of disease progression seen in patients with >1%, 0.01-1%, or <0.01% MRD respectively. Evaluating novel treatments currently requires assessment of PFS/OS in randomised controlled trials but the prolonged duration of remission means that this approach is slow. Assessing MRD as a trial endpoint could greatly speed the process; using peripheral blood (PB) would be preferable but antibody therapies preferentially deplete circulating disease to a variable extent and bone marrow (BM) is the more sensitive site for MRD detection.
Aims: To compare PFS/OS according to MRD level in PB and BM for different treatment and patient groups and determine the optimum site for MRD assessment.
Methods: MRD was quantified using multi-parameter flow cytometry according to ERIC consensus protocols with a limit of quantification of 10-4 / 0.01% or better.
Results: The MRD level in 609 paired PB & BM samples taken during or within 6 months of treatment (446 in clinical trials, see table) was concordant in 443 (72.7%) at MRD thresholds <0.01% / 0.01-1% / >1%. In 391 cases with <0.01% PB MRD, 85/391 (21.7%) had 0.01-1% CLL and 14/292 (3.6%) had >1% CLL in the paired BM sample. In 118 cases with 0.01-1% CLL in the PB, the paired BM sample showed >1% CLL in 63/118 (53.4%). The PB MRD level would have assigned a higher risk category than BM in only 4 cases, all of which had detectable BM disease. Discrepancies between PB & BM MRD levels were not consistent across treatment and patient groups. Refractory patients treated with alemtuzumab-based therapies typically showed a high proportion of discrepant results with >1 log differences while patients in CR/PR receiving alemtuzumab consolidation had few discrepancies; during Ibrutinib monotherapy (IcICLLE trial) there have been no discordant cases to date. To determine whether discrepant results impact on outcome, PFS/OS was evaluated according to PB & BM MRD in different trials. In the ADMIRE/ARCTIC trials, PB & BM MRD levels were discordant in 66/312 evaluable cases, all with ≥0.01% BM but <0.01% PB MRD. After a median follow-up of 3 years, MRD level was confirmed as a strong predictor of PFS and OS, independent of categorical response, with the presence of ≥0.01% MRD conferring a significantly poorer outcome (PFS: HR 4.6, 95%CI 2.7 to 7.7, p=<0.001). For patients in CR, and with ≥0.01% MRD (n=70), 3 year PFS was 68.7% compared with 86.6% for patients with <0.01% MRD (n=176). There was no observable difference in the PFS or OS for patients with <0.01% PB but ≥0.01% BM MRD compared to those with <0.01% PB & BM MRD. However, for those patients with MRD detected in the BM only, there was a median of 0.08% CLL and this would be predicted to yield observable differences in PFS only after >3 years follow-up. The results contrast with the outcome for 47 evaluable patients in the CLL202 CAMFlud trial where patients with <0.01% MRD in PB but ≥0.01% disease in BM had a poor outcome that was similar to those with ≥0.01% in both PB & BM, and both groups had significantly poorer PFS/OS than patients with <0.01% MRD in both PB & BM (with median 55 months follow-up for patients with ≥0.01% in PB & BM / <0.01% PB but ≥0.01% BM / <0.01% MRD in both PB & BM patients respectively, median PFS was 1.6 / 6.5 / 44.7 months and OS was 7.5 / 14 / not reached, P<0.001).

Tabella 1.

						Discrepant results between PB and BM (% of total paired samples)			
Trial	Situation	Treatment	Patients	Final MRD assesment	Paired PB & BM samples	All discrepant cases	PB <0.01% BM >1%	PB<0.01% BM 0.01-1%	PB 0.01-1% BM >1%
CLL 201	Relapsed refractory	FCM ±R	52	3 months post-treatment	14	1 (7.1%)	0 (0%)	0 (0%)	1 (7.1%)
CLL 202 CAMFlud	Relapsed refractory	Alemtuzumab and Fludarabine	50	End of treatment	46	18 (39.1%)	5 (10.9%)	4 (8.7%)	9 (19.6%)
CLL 207	Consolidation in CR/PR	Alemtuzumab	47	End of treatment	49	7 (14.3%)	0 (0%)	7 (14.3%)	0 (0%)
IcICLLe	All patients	Ibrutinib	40	End of treatment	18	0 (0%)	0 (0%)	0 (0%)	0 (0%)
MO20927	Untreated, less fit	Chlorambucil Rituximab	100	3 months post-treatment	7	4 (57.1%)	1 (14.3%)	1 (14/3%)	2 (28.6%)
ADMIRE & ARCTIC	Untreated, fit	FCR, FCMR or FCM-miniR	415	3 months post-treatment	312	85 (27.2%)	2 (0.6%)	64 (20.5%)	19 (6.1%)

Summary and Conclusions: Patients achieving <0.01% CLL have a better outcome than those with detectable MRD but the power of peripheral blood MRD eradication to predict outcome varies widely according to the type of therapy received and is poor with more potent antibodies. The most robust tissue and time-point for MRD assessment to predict long-term outcomes in CLL is the bone marrow at 3 months post-treatment. Our work justifies the use of MRD eradication from the marrow post-treatment as a surrogate end-point in clinical trials.

AML: Molecular profile and targeting

S795

COMPREHENSIVE GENOMIC PROFILING OF A LARGE COHORT OF ACUTE MYELOID LEUKAEMIA TO DISSECT ITS GENOMIC AND PHENOTYPIC COMPLEXITY

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Background: The characterization of recurrent chromosomal abnormalities in AML has paved the way for the incorporation of genetic lesions into clinical diagnostics and prognostication tools. However, clinical outcomes vary greatly and in the absence of informative lesions, a large proportion of patients are given an intermediate risk score. Recent profiling studies have characterized >50 recurrently mutated genes in AML. Considering the multitude of gene candidates, evaluations of the AML genomic landscape and its relationship to clinical trajectory must extend beyond single/few biomarkers studies and, importantly, involve large well-annotated datasets.
Aims: We sought to comprehensively evaluate the known driver mutations in AML; study molecular phylogenies that underpin AML transformation, and investigate how the composite genetic architecture determines phenotype and correlates to clinical response.
Methods: We study 1540 adult AML patients from three clinical trial cohorts of the AML-SG (HD98A, HD98B and 07-04) using a targeted re-sequencing approach. Taken together with cytogenetic analysis we identify 5,241 driver variants across 55 genes and 23 recurrent cytogenetic alterations. We incorporate these to diagnostic and clinical outcome data (median follow-up=2169 days), perform classification analysis to determine molecular subgroups, and apply variable selection models to characterize prognostic biomarkers.
Results: We find at least 1 driver mutation in 97% patients in the study, and importantly at least 2 in 85%. There was a long tail of infrequently mutated driver genes, with only 30% of the events seen in >10% of patients. The number of drivers increases with age and patients with normal karyotype have more mutations than patients with cytogenetic abnormalities. Using the fraction of reads reporting a gene mutation to distinguish subclonal from clonal mutations, we identify 690 samples with at least 2 driver mutations and subclonal heterogeneity and map recurrent preferences in order of mutation acquisition. We survey the composite genomic architecture and define 10 classes, which are broadly defined by inv(16); t(8;21), t(var;11q23), inv(3), t(6;9), bi-allelic CEBPA, NPM1/DNA hydroxymethylation, TP53/aneuploidies, chromatin/spliceosome and DNMT3A/IDH2 mutations, and collectively account for 85% of patients. The definition of each class extends beyond a single lesion and is rather determined by class-specific initiating events and co-operative lesions that are ordered in time. Many of these interactions extend to mutation clusters or hotspots within genes as evidenced by pairings between FLT3 TKD/ITD, NRAS c.12-13/c.61, IDH2 c.140/c.172 and CEBPA mono/bi-allelic mutations. As expected, evaluation of clinical phenotype and outcome associations identifies broad correlations between class, phenotype, response to induction chemotherapy and overall survival. Strikingly, we find that specific molecular contexts within each class (as defined by secondary and tertiary interactions) correlate with distinct and often opposing outcome trajectories and clinical phenotype ranging from excellent to highly adverse clinical outcomes. These relationships are underpinned by models of additive risk or suggestive of genetic epistasis. Using overall survival as a primary endpoint we evaluate the known landscape of driver mutations, and genetic interactions. We derive a comprehensive set of significant factors and identify at least three genetic interactions that retain significance ($q<0.01$) in the presence of other clinical and demographic variables. Importantly we identify at least one prognostic predictor in 70% of the patients and observe a three-fold increase in patients with 2 or 3 prognostic predictors relative to current molecular prognostication guidelines.
Summary and Conclusions: Taken together, we study an extended and well-annotated clinical cohort we demonstrate the potential of recent genetic findings to deliver improved classification and risk stratification algorithms that extend beyond single biomarker correlations, capture most AML patients and that are tailored to the individual patients genomic profiles.

S796

AKT PHOSPHORYLATION AT SERINE 473 IS A POWERFUL NOVEL BIOMARKER IN ACUTE MYELOID LEUKAEMIA, AND IS RELATED TO PROTEIN PHOSPHATASE 2A (PP2A) INHIBITION BY CIP2A AND SETBP1

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Background: Many malignancies are characterised by inappropriate substrate phosphorylation which causes aberrant proliferation and decreased apoptosis; this is often due to an overactive kinase *e.g.* BCR-ABL or FLT-3 in myeloid malignancy, or ERK or ErbB2 in solid tumours. What is less clear is why this effect is not simply countered by normal cellular phosphatases, of which the most important is protein phosphatase 2A (PP2A). PP2A is normally inhibited by SET, (which is stabilised by a binding protein SETBP1) and cancerous inhibitor of PP2A (CIP2A). We have shown that high CIP2A levels confer a poor outcome in chronic myeloid leukaemia (similar to findings in many solid tumours), and also that high CIP2A levels are associated with stabilisation of c-Myc and E2F1, as well as PP2A inactivation in CML. There are also data to suggest that high SETBP1 levels may confer a poor outcome in AML, presumably by PP2A inhibition via increased action of SET, though little is known of CIP2A in AML.
Aims: To investigate if CIP2A or network related proteins could predict clinical outcome in AML patients.
Methods: CIP2A and PP2A related proteins were investigated in 120 AML patient samples collected in the UK MRC/NCRI trials AML15, 16 and 17. These were stratified into intermediate or adverse risk, based on cytogenetics as previously defined (Grimwade *et al*, Blood. 1998: 92; 2322-33). Protein levels were studied in diagnostic mono-nuclear cells samples by flow cytometry. CIP2A levels in individual samples were then defined as either high or low based on receiver-operator characteristics analysis as previously described.
Results: Diagnostic CIP2A levels were significantly higher in younger intermediate than in adverse risk patients (p=0.004). In the intermediate risk group, overall survival was dominated by the survival from relapse. High diagnostic CIP2A was associated with high levels of c-Myc (p=0.003), inactive PP2A ($PP2A^{Y307}$, p=0.04) and activation of AKT as identified by its phosphorylation on serine 473 (AKT^{S473}) (p=0.0009). Interestingly, high diagnostic CIP2A was strongly associated with the presence of a FLT3-ITD mutation (p=0.009). For younger intermediate risk patients with high and low CIP2A levels, median survival from relapse censored at transplant was 56 days and 303 days respectively, and multivariate analysis adjusted for FLT3-ITD showed that a high CIP2A level predicted inferior survival from relapse (p=0.04, hazard ratio 4.02). Rates of high levels of CIP2A were lower in adverse risk patients but instead we found elevated levels of SETBP1, which acts via SET as an alternative inhibitor of PP2A. In younger patients with adverse cytogenetics, high SETBP1 levels predict for an inferior overall survival (p=0.02). High SETBP1 was also associated with secondary AML (p=0.06). In univariate analysis, high levels of AKT^{S473}were associated with poor survival in all patient groups. Multivariate analysis of known predictors of outcome (age, cytogenetics, white count, secondary leukaemia, FLT3-ITD) together with CIP2A and related proteins ($PP2A^{Y307}$, SET, SETBP1, c-Myc E2F1, AKT^{S473}, STAT5, and E2F1) was used to derive a prognostic model for survival. This ranked high levels of AKT^{S473} as the third most important predictor of outcome after age and cytogenetics.
Summary and Conclusions: AKT phosphorylation at S473 is a surrogate 'readout' of PP2A inhibition by either CIP2A (intermediate risk) or SETBP1 (adverse risk patients). The diagnostic AKT^{S473} level appears to be a novel biomarker for treatment outcome in AML. This should now be tested prospectively as part of a clinical trial.

S797

ASSOCIATION OF MIR29 RNA WITH RESPONSE AND SURVIVAL IN OLDER PATIENTS WITH ACUTE MYELOID LEUKEMIA (AML) TREATED WITH AZACITIDINE (AZA) OR CONVENTIONAL CARE REGIMENS (CCR)

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Background: DNA methyltransferases (DNMTs) mediate DNA methylation and contribute to epigenetic aberrancy in myeloid malignancies. The miR29 family of microRNAs directly inhibit the expression of DNMT-3A and -3B (Fabbri, *PNAS*, 2007). In a small study (N=23) of older patients (pts) with AML, higher levels of miR29b were significantly associated with response to decitabine (Blum, *PNAS*, 2010). The large (N=488) phase 3 randomized AZA-AML-001 study compared AZA and CCR treatment (Tx) in older pts with AML. AZA prolonged median overall survival (OS) by ~4 months (mos) (10.4 *vs* 6.5 mos; p<0.101) and improved 1-year survival (46.5% *vs* 34.2%) *vs* CCR (Dombret, EHA, 2014).
Aims: To investigate the relationship between miR29 family RNA levels and clinical endpoints (response and OS) in AZA-AML-001 pts.
Methods: Eligible pts were age ≥65 years with newly diagnosed AML (>30% bone marrow [BM] blasts) and had ECOG PS of 0-2, WBC count ≤15x10^9/L, and intermediate- or poor-risk cytogenetics. Pts received AZA (75mg/m^2/day [d] x7d/28d cycle) or a preselected CCR: intensive chemotherapy (standard 7+3 regimen), low-dose cytarabine (20mg SC BID x10d/28d cycle), or best supportive care only. RNA was isolated from BM mononuclear cells collected at screening from a subset of pts who gave informed consent for this exploratory biomarker analysis and had adequate BM mononuclear cells for analysis. Experimental miRNAs (miR29a, b, c) and control RNAs (RNU44, miR-16) were quantified by qRT-PCR. Associations between miR levels and response (IWG 2003) were assessed, defining responders as pts who achieved complete remission (CR) or morphologic CR with incomplete blood count recovery (CRi), and nonresponders as pts with partial response (PR), stable disease (SD), or progressive disease (PD) as their best response. Differences in baseline miRNA expression were compared by T-test within the AZA and CCR Tx arms. A 2-way ANOVA model was used to test the interaction between miR expression and Tx. miR expression as a continuous variable was also fit in a Cox model with and without strata in separate arms.
Results: The biomarker cohort comprised 156 of all 488 pts (32%; AZA n=83, CCR n=73). Baseline characteristics were well-matched in the biomarker *vs* non-biomarker groups, respectively, with median age 75 years in both groups, median BM blasts 50.5% *vs* 52.0%, and cytogenetic risks of intermediate 63.8% *vs* 66.5%, and poor 36.3% *vs* 33.5%. Baseline miR29a and miR29b expression levels were significantly higher in responders (CR+CRi) *vs* nonresponders (PR+SD+PD) with AZA Tx (p<0.004 & p<0.029, respectively), but not with CCR Tx (**Figure**). This difference for miR29a was confirmed in the 2-way ANOVA analysis of response and Tx (p<0.054). miR29a levels were also associated with OS (p<0.044) in the AZA arm, but not in the CCR arm, in stratified survival analysis. No significant associations between miR29b or miR29c levels and OS were seen in either Tx arm.

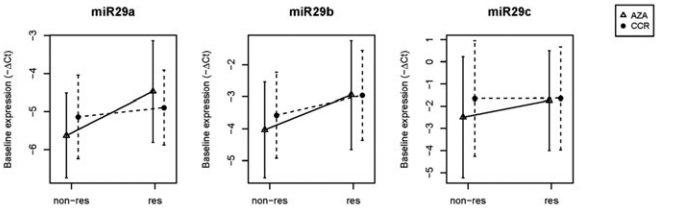

Figure 1. miR29a, b, c RNA expression levels (mean ±SD) in responders *vs* nonresponders.

Summary and Conclusions: In this exploratory analysis, higher expression levels of miR29a and miR29b were significantly associated with response to AZA Tx, and the association of miR29a levels with response was specific to AZA. Higher miR29a levels were also associated with improved OS in pts treated with AZA, but not in pts treated with CCR. As miR29a is implicated in regulating DNMT levels, an AZA target, these data are consistent with the concept that reduced pretreatment DNMT levels due to higher miR29a expression can improve response and survival with hypomethylating agents.

S798

RESULTS OF A FIRST-IN-HUMAN, PHASE 1/2 TRIAL OF ASP2215, A SELECTIVE, POTENT ORAL INHIBITOR OF FLT3/AXL, IN PATIENTS WITH RELAPSED OR REFRACTORY ACUTE MYELOID LEUKEMIA

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Background: FMS-like tyrosine kinase-3 (FLT3) mutations, including internal tandem duplication (ITD) and tyrosine kinase domain (TKD) mutations, are present in acute myeloid leukemia (AML) cells in up to 30% of patients. FLT3 inhibitors have shown promise as a therapeutic option for AML patients with

FLT3 activating mutations. *in vitro* analyses suggest that ASP2215 is a potent inhibitor of FLT3 and AXL tyrosine kinases and demonstrates activity against both FLT3-ITD and FLT3-TKD.
Aims: A Phase 1/2 trial investigated the safety, pharmacokinetics (PK), and clinical efficacy of ASP2215 in patients with relapsed or refractory (R/R) AML.
Methods: This open-label, first-in-human study, conducted in patients ≥18 years old with R/R AML, included 2 cohorts: dose escalation (Cohort 1) and dose expansion (Cohort 2). Cohort 1 was a modified 3+3 with accelerated titration design that evaluated ascending oral doses of ASP2215 (20–450 mg). Cohort 2 was a parallel multi-dose expansion cohort that was initiated based on the efficacy observed during dose escalation and patients were stratified by FLT3 mutation status. The primary endpoints included determination of the maximum tolerated dose (MTD) and the PK profile; clinical efficacy and FLT3 phosphorylation were evaluated as secondary endpoints. All patients provided informed consent. Data from an interim analysis are presented.
Results:: A total of 166 patients (Cohort 1, n=25; Cohort 2, n=141) with a median age of 64 years (range: 21–90) were enrolled between October 2013 and December 2014. ASP2215 MTD was determined to be 300 mg after 2 out of 3 patients dosed with ASP2215 treated at 450 mg experienced dose-limiting toxicities (grade 3 diarrhea and ALT/AST elevation). Plasma concentrations of ASP2215 increased with increasing dose, with t_{max} between 2 and 6 hours. Accumulation was observed following repeat dosing, with estimated $t_{1/2}$ values of 45 to 159 hours based on accumulation index. Approximately dose-linear PK parameters were observed over the dose range for both single and repeat dosing. Fatigue (13%), constipation (10%), anemia (8%), nausea (8%), diarrhea (7%), thrombocytopenia (6%), decreased platelet count (6%), vomiting (6%), dizziness (6%), peripheral edema (5%), increased transaminases (5%), and hypomagnesemia (5%) were the most commonly reported treatment-emergent adverse events considered "possibly" or "probably" related to drug. Further, no incidents of clinically significant QTc prolongation have been reported. Overall response rate (ORR, defined by composite complete and partial remissions) in patients with FLT3 mutations was 57% at doses between 20 and 300 mg, and 65% at doses of ≥80 mg; a lower ORR (11%) was observed in patients with wild type FLT3 (Table). In patients receiving doses ≥80 mg, a plasma inhibitory activity assay confirmed effective, consistent, and sustained FLT3 inhibition.

Tabella 1.

Response	FLT3 mutated		
	20–300 mg	≥80 mg	20–300 mg
	n=82	n=65	n=38
CR	4 (5%)	3 (5%)	0
CRp, incomplete platelet recovery	4 (5%)	4 (6%)	0
CRi, incomplete hematologic recovery	27 (33%)	25 (38%)	3 (8%)
PR	12 (15%)	10 (15%)	1 (3%)
Composite CR (CR+CRp+CRi)	35 (43%)	32 (49%)	3 (8%)
ORR (CRc+PR)	47 (57%)	42 (65%)	4 (11%)

All data presented as n (%).
Of the 166 enrolled patients, 120 patients (n=82 FLT3 mutations; n=38 FLT3 wild type) had evaluable efficacy data.
Abbreviations: CR, complete remission; CRc, composite complete remission; CRi, complete remission with incomplete hematologic recovery; CRp, complete remission with incomplete platelet recovery; ORR, overall response rate.

Summary and Conclusions: ASP2215, a potent oral inhibitor of FLT3/AXL, was well tolerated at doses of 20–300 mg/d in patients with R/R AML and showed a high degree of clinical activity in patients with FLT3-ITD and/or FLT3-TKD mutations. ASP2215 demonstrated a predictable PK profile consistent with once-daily dosing. Randomized phase 3 trials of ASP2215 dosed at 200 mg in newly diagnosed and R/R AML are planned.

S799

UPDATED RESULTS FROM THE SORAML TRIAL COMPARING SORAFENIB VERSUS PLACEBO IN ADDITION TO STANDARD THERAPY IN YOUNGER PATIENTS WITH NEWLY DIAGNOSED ACUTE MYELOID LEUKEMIA

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Background: Sorafenib is a multi-kinase inhibitor with activity against several oncogenic kinases that may play a role in the pathogenesis of acute myeloid leukemia (AML). We present updated results from the randomized placebo-controlled SORAML trial (NCT00893373) testing sorafenib versus placebo as add-on to standard treatment in AML patients ≤60 years.
Aims: The data focus on treatment adherence, efficacy in cytogenetic subgroups and relapse pattern.
Methods: The trial enrolled 276 patients with newly diagnosed AML from 18 to 60 years and suitable for intensive therapy. The treatment plan for all patients included two cycles of induction with DA (daunorubicin 60 mg/m^2 days 3-5 plus cytarabine 100 mg/m^2 cont. inf. days 1-7) or HAM (cytarabine 3 g/m^2 b.i.d. days 1-3 plus mitoxantrone 10 mg/m^2 days 3-5), followed by three cycles of high-dose cytarabine consolidation (3 g/m^2 b.i.d. days 1, 3, 5). Allogeneic stem cell transplantation (SCT) was scheduled for all intermediate-risk patients in first complete remission (CR) with a sibling donor and for all high-risk patients with a matched related or unrelated donor. Patients were randomized to receive either sorafenib (800 mg/day) or placebo as add-on to standard treatment in a double blinded fashion. Study medication was given on days 10-19 of DA I+II or HAM, from day 8 of each consolidation until 3 days before the start of the next consolidation and as maintenance for 12 months after the end of consolidation.
Results: Out of 276 enrolled patients, 267 received study treatment, 134 in the sorafenib arm and 133 in the placebo arm. Demographic and disease characteristics were equally distributed between the two arms; the incidence of FLT3-ITD was 17%. Toxicity-related drop-outs were slightly more frequent in the sorafenib arm with 32% *vs* 25% of patients commencing maintenance. After a median observation time of 36 months, the median EFS was 9.2 months in the placebo arm and 20.5 months in the sorafenib arm (p=0.013). Induction mortality was 4% in both arms, but cumulative incidence of relapse was significantly higher in the placebo arm. Subgroup analyses on EFS suggest a beneficial effect of sorafenib already after induction treatment. In multivariate analysis, sorafenib exposure, favorable cytogenetics and NPM1 mutation were associated with superior EFS whereas FLT3-ITD remained a negative prognostic factor. Median RFS after standard treatment plus placebo was 23 months and not yet reached after sorafenib treatment (p=0.017). The median OS had not been reached in either arm; the 3-year OS was 56% with placebo versus 63% with sorafenib (p=0.382). More placebo patients (N=66; 50%) than sorafenib patients (N=39; 29%) received a SCT in relapse due to more and earlier relapses with placebo, whereas relative SCT rates among relapses were similar (sorafenib: 75%; placebo: 82%). Detailed subgroup analyses will be presented. The risk for fever, diarrhea, bleeding events, liver toxicity, hand-foot syndrome and rash was significantly higher in the sorafenib arm.
Summary and Conclusions: In younger AML patients, the addition of sorafenib to standard therapy is associated with significant EFS and RFS prolongation independent of FLT3-ITD status. After the current follow up, the RFS benefit does not translate into an OS benefit, most likely due to potent salvage strategies mainly based on SCT. Ongoing data collection on salvage treatments, second CR and molecular profiling plus additional follow up will contribute to further explain the mechanism of action of sorafenib in younger AML patients.

Stem cell transplantation: Clinical 3

S800

MONOTHERAPY TREATMENT WITH A SINGLE DOSE OF THE CXCR4 ANTAGONIST BL-8040 AS A NOVEL METHOD FOR MOBILIZATION OF HUMAN HSPC AND MSC; RESULTS OF A PHASE I HEALTHY VOLUNTEERS STUDY

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Background: Hematologic malignancies often require hematopoietic cell transplantation (HCT). Randomized trials demonstrated the advantages of hematopoietic stem/progenitor cells (HSPC) mobilized from the peripheral blood (PB) compared to bone marrow (BM) for HCT. The most commonly used mobilization regimen utilize G□CSF (-/+Plerixafor), which requires 4-5 treatment days and often involves several apheresis sessions. In addition, the graft composition; CD34+, mesenchymal stem cells (MSCs), NK and T cell subsets may influence recipient outcomes in terms of engraftment, anti-tumor activity, GVHD, morbidity and mortality. BL-8040 (BKT140) is a novel CXCR4 antagonist that binds the receptor with high affinity and long receptor occupancy. Studies in mice demonstrated that single BL-8040 injection mobilized long term repopulating cells sufficient for transplantation. Results from a study in multiple myeloma patients showed that BL-8040 when combined with G-CSF enabled the collection of high number of CD34+cells in a single aphaeresis procedure. The potential of BL-8040 monotherapy as a mobilizing agent and its derived graft composition and quality is currently evaluated in a phase I clinical study in healthy volunteers (NCT02073019).
Aims: To test the potential of BL-8040 monotherapy as a mobilizing agent and its derived graft composition and quality.
Methods: The first part of the study was randomized, double-blind, placebo-controlled dose escalation. Each cohort included 8 subjects in a 6+2 design (0.5, 0.75 and 1 mg/kg). After obtaining informed consent, BL-8040 was administered SC QD on 2 consecutive days. Frequent PB samples were tested for the number of WBC, CD34+, B, T, and NK cells, MSC, MSC colony forming cells and HPCs colony forming cells. In the second part, 8 subjects are receiving single injection of BL-8040 (1mg/kg) and 4 hours later undergo a standard leukapheresis (18 L) procedure. The composition of the collected graft is being tested for the number of CD34+cells per kg, number and type of MSCs per kg, number and type of T, B, NK and dendritic cells.
Results: BL-8040 was found safe and well tolerated at all doses tested (0.5-1 mg/kg). The primary treatment related AEs were mild to moderate transient injection site and systemic reactions. BL-8040 triggered substantial mobilization of WBC to the circulation. The mean WBC count, of all patients receiving BL-8040, rose from a baseline of 6.3 to 29.7 X 10^9/L at 4 hr post dose. Dramatic mobilization of HSPC (CD34+cells) was observed across all doses tested. Mean CD34+count at baseline was 5.8/μL; four hours post dose, the count rose to a mean of 8, 37, 31 and 35 cells/μL and four hours post the second dose of BL-8040, it further increased to 9, 38, 46 and 58 cells/μL (placebo, 0.5, 0.75 and 1 mg/kg, respectively). BL-8040 administration resulted in rapid mobilization of MSC and HPC colony forming, T, B and NK cells. Preliminary results from the second part of the study indicate the potential to collect sufficient amount of CD34+cells following single apheresis session. Long receptor occupancy and long pharmacodynamic effect (≥24 hours post dosing) were also observed.
Summary and Conclusions: The current data demonstrate that BL-8040 is safe and well tolerated and induces rapid mobilization of HSPC and MSC. These results support BL-8040 monotherapy as an effective strategy to shorten the procedure length required to collect sufficient cells for HCT. In addition, treatment with BL-8040 is expected to yield a potent hematopoietic graft with unique cells composition and may also serve as a novel approach to collect MSCs for diverse indications. The final study results will be presented.

S801

IMMUNOMODULATORY EFFECT OF VITAMIN D AFTER ALLOGENEIC STEM CELL TRANSPLANTATION (ALLOSCT): RESULTS OF A PROSPECTIVE MULTICENTER CLINICALTRIAL. ALOVITA; EUDRACT: 2010-023279-25

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Background: Vitamin D receptor (VDR) is expressed on activated immune cells. Numerous preclinical studies, including models for solid organ transplantation, have shown that vitamin D (VitD) has a potent immunomodulatory effect although, by contrast, few reports have described an increased immune response upon exposure to vitD. In the alloSCT setting scanty information is available although an association between common polymorphisms of VDR and graft-versus-host disease (GVHD) has been described. In addition, different studies have also described the capability of vitD to induce differentiation of blasts in acute leukemia. In spite of these properties no clinical trial has been carried out to evaluate the role of VitD as an immunomodulatory agent after alloSCT.
Aims: Based on the potential benefit of vitD on the risk of GVHD but also considering previous studies describing an exacerbated immune response after exposure to the drug, the main aim was to determine the safety and to assess the effect of VitD supplementation after alloSCT on the incidence of GVHD.
Methods: We designed a multicenter prospective Phase I/II clinical trial with three consecutive cohorts of patients (n= 50 each group), receiving no vitD (control group, CG), 1000 IU/day po. VitD (low dose group, LDG) or 5000 IU/day (high-dose group, GDA) from day -5 to +100 post alloSCT. In addition, we measured plasma levels of VitD (on days -5, +1, +7, +14 and +21 after transplant), of serum th1/th2 cytokines (on days +1, +7, +14, +21, +56 and +100) and of WBC subpopulations including T cells (naive/memory/effector), activation markers for T cells, Treg, NK cells, B cells and dendritic cells by flow cytometry (on days +21, +56 and +100).
Results: Regarding characteristics of patients, we only observed significant differences between the 3 subgroups in terms of age, patients in LDG being slightly older. Calcineurin inhibitors plus methotrexate or MMF and FK506 plus rapamycin were used as GVHD prophylaxis. No T-cell depletion was allowed as GVHD prophylaxis. No significant differences were observed in terms of cumulative incidence of global and grade II-IV of acute GVHD (CG= 55.9% and 38.2%; LDG= 56.8% and 36.4% and HDG= 56.4% and 53.8%, respectively). By contrast, a significantly lower cumulative incidence of chronic GVHD (cGVHD) at 1 year was observed in LDG and HDG as compared to patients who did not receive VitD (33.7%, 25.9% and 66.7% for LDG, HDG and CG, respectively; p=0.04) (Figure 1). Multivariate analysis identified treatment with VitD as the only variable which significantly decreased the risk of cGVHD (p=0.03) (for LDG [HR=0.33, (95% CI=0.13-0.81), p=0.01] and for HDG [HR=0.42, (95% CI=0.16-1.1), p=0.07]. No significant differences were observed in terms of relapse or non-relapse mortality. With a median follow up of 1 year, overall survival was 70,6%, 74.2% and 70.4% for CG, LDG and HDG, respectively, p=0.76). Concerning the biological parameters monitored, plasma levels of vitD were higher among patients receiving the drug as compared to the control group, beyond day +21 (significantly for HDG). VitD modified the immune response after alloSCT for parameters evaluated, the most significant differences being a decreased percentage of B-cells for both LDG and HDG (p≤0.05), a markedly modified ratio of naïve/central memory/effector T cells, with a lower number of circulating naïve CD8 T cells for patients receiving vitD (10.1%; 9.61%; 16%, p=0.02 for LGC, HGD and CG respectively, p≤0.025) and a lower expression of CD40L as marker of activation upon exposure to vitD (24.4%, 39.5% and 49%, for LGD, HGD and CG, respectively, p≤0.02).

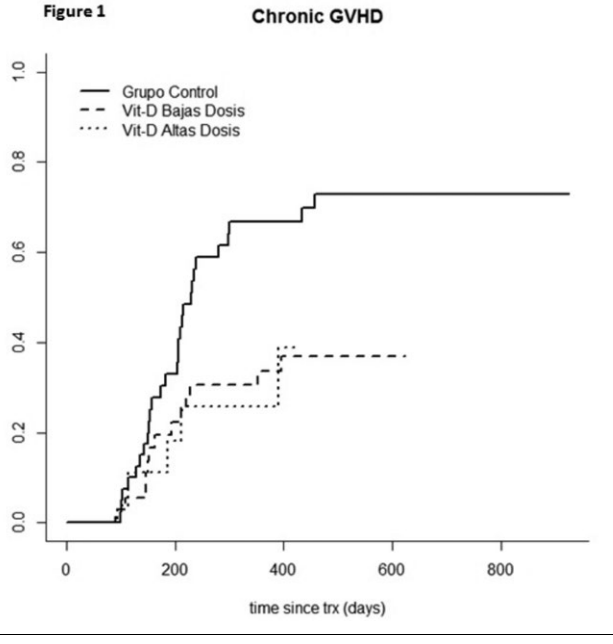

Figure 1.

Summary and Conclusions: This is the first prospective multicenter trial which analyzes the effect of vitamin D administration after alloSCT. A significantly lower incidence of cGVHD was observed among patients receiving vitD. Interestingly, vitD markedly modified the immune response after alloSCT.

S802

DONOR-DERIVED, CD19-DIRECTED, CAR-MODIFIED T CELLS INFUSED AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION AS PRE-EMPTIVE DONOR LYMPHOCYTE INFUSION IN PATIENTS WITH CD19+MALIGNANCIES

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Background: Allogeneic hematopoietic stem cell transplantation (HSCT) can be curative in a subset of patients with advanced B-lineage acute lymphoblastic leukemia (ALL), but relapse remains the main reason for treatment failure. Donor-derived, non-specific lymphocyte infusions (DLI) have been ineffectively infused and are associated with significant graft-versus-host-disease (GVHD). Chimeric antigen receptor (CAR)-modified T cells directed toward CD19 have demonstrated dramatic efficacy in patients with refractory ALL. However, responses are often associated with life-threatening cytokine release.
Aims: We hypothesized that infusing CAR-modified, CD19-specific T cells after HSCT as a directed DLI would be associated with less GVHD while providing cellular-based disease control, and may be associated with less cytokine released when administered in a minimal disease state.
Methods: We employed a non-viral gene transfer using the *Sleeping Beauty* (SB) transposon/transposase system to stably express a 2nd generation CD19-specific CAR (designated CD19RCD28 that activates via CD3z/CD28) in donor-derived T cells for patients with advanced $CD19^+$lymphoid malignancies. T cells were electroporated using a Nucleofector device to synchronously introduce two DNA plasmids coding for SB transposon (CD19RCD28) and hyperactive SB transposase (SB11). T cells stably expressing the CAR were retrieved over 28 days of co-culture by recursive additions of g-irradiated activating and propagating cells (AaPC) in presence of soluble recombinant interleukin (IL)-2 and IL-21. The AaPC were derived from K562 cells and genetically modified to co-express CD19 as well as the co-stimulatory molecules CD86, CD137L, and a membrane-bound version of IL-15.
Results: To date, we have successfully treated 16 patients with advanced $CD19^+$ALL (n=13) or NHL (n=3); 7 patients had active disease at time of HSCT. Donor-derived CAR^+T cells (HLA-matched sibling n=9; haplo-family n=5; double cord blood n=2) were infused at a median 64 days (range 42-91 days) following HSCT to prevent disease progression. Transplant preparative regimens were myeloablative, busulfan-based (n=8) or reduced intensity, fludarabine-based (n=8). All patients were maintained on GVHD prophylaxis at time of CAR infusion with tacrolimus, plus mycophenolate mofeteil for cord, plus post-HSCT cyclophosphamide for haplo donors. The starting CAR^+T-cell dose was 10^6 (n=7), escalated to 10^7 (n=6), and currently at $5x10^7$ (n=3) modified T cells/m^2 (based on recipient body surface area). Patients have not demonstrated any acute or late toxicity to CAR^+T cell infusions. Three patients developed acute grades 2-4 GVHD (liver n=1, upper GI n=1, skin=1) which was within the expected range after allogeneic HSCT. Eight patients have relapsed at a median of 90 days following HCT (range 68-185 days). Fifty percent of patients (n=8) remain alive and in complete remission (CR) at median 7.2 months (range 2.1-21.3 months) following HSCT.
Summary and Conclusions: We report the first human application of the SB and AaPC platforms to genetically modify clinical-grade cells. We demonstrate that infusing donor-derived CD19-specific CAR^+T cells in the adjuvant HSCT setting as pre-emptive DLI may provide an effective and safe approach for maintaining remission in patients at high risk for relapse. Modification of the CAR construct is underway in efforts to improve CAR T-cell *in vivo* proliferation and persistence.

S803

POLYMORPHISMS IN IMMUNOMODULATING GENES AND RISK OF INVASIVE ASPERGILLOSIS: RESULTS FROM THE ASPBIOMICS CONSORTIUM

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Background: Invasive Aspergillosis (IA) is a life-threatening infection caused by *Aspergillus* that mainly affects acute myelogenous leukemia and allogeneic stem cell transplantation (allo-SCT) patients. Recent studies suggest that immunomodulating single nucleotide polymorphisms (SNPs) may influence on the risk of developing the infection.
Aims: The purpose of this study was to assess whether 36 SNPs within 14 immune-related genes (*IL4, IL4R, IL8, IL8RA, IL8RB, IL10, IL12A, IL12B, IL13, IFNg, IFNgR2, CCR5, MIF and VEGF*) are associated with the risk of IA and whether a predictive model built with these variants might help to predict the disease risk.
Methods: We conducted a three-stage case-control association study of 742 high-risk patients, 146 of whom were diagnosed with proven or probable IA. This is one of the largest populations recruited so far to conduct genetic association studies.

Tabella 1. Association of immunoregulatory polymorphisms and invasive aspergillosis

Variant_dbSNP	Gene	OR (95% CI)[a]	P_{value}	OR (95% CI)[b]	P_{value}	OR (95% CI)[c]	P_{value}
rs2243248	*IL4*	1.15 (0.53-2.48)[d]	0.72	1.19 (0.63-2.26)	0.59		
rs2070874	*IL4*	0.72 (0.37-1.40)	0.33	0.91 (0.53-1.55)	0.72		
rs2243268	*IL4*	0.64 (0.35-1.18)	0.15	0.85 (0.52-1.38)	0.50		
rs2243290	*IL4*	0.67 (0.34-1.32)	0.24	0.67 (0.39-1.16)	0.14		
rs2057768	*IL4R*	0.91 (0.51-1.60)	0.74	1.20 (0.75-1.92)	0.44		
rs2107356	*IL4R*	**2.27 (1.20-4.27)[f]**	**0.012**	**2.05 (1.24-3.40)[f]**	**0.0063**	**1.99 (1.22-3.23)[f]**	**0.007**
rs1801275	*IL4R*	0.82 (0.46-1.45)	0.49	1.00 (0.63-1.59)	0.99		
rs4073	*IL8*	1.07 (0.61-1.89)	0.80	1.02 (0.64-1.61)	0.95		
rs2227307	*IL8*	**2.28 (1.15-4.52)[f][g]**	**0.019**	1.72 (1.00-2.94)[f]	**0.049**	**1.95 (1.19-3.21)[f][e]**	**0.010**
rs2234671	*IL8RA*	1.01 (0.43-2.39)	0.98	1.57 (0.80-3.08)	0.20		
rs1126580	*IL8RB*	1.18 (0.63-2.21)	0.60	1.50 (0.88-2.54)	0.13		
rs3024491	*IL10*	1.18 (0.64-2.16)	0.59	1.09 (0.67-1.78)	0.72		
rs3024496	*IL10*	1.43 (0.76-2.71)	0.26	1.16 (0.71-1.90)	0.55		
rs582054	*IL12A*	1.13 (0.58-2.21)	0.72	1.09 (0.64-1.84)	0.76		
rs3212227	*IL12B*	**0.46 (0.25-0.84)[g]**	**0.009**	**0.57 (0.35-0.93)[g]**	**0.021**	**0.63 (0.39-0.99)**	**0.046**
rs20541	*IL13*	**0.45 (0.24-0.85)**	**0.011**	0.76 (0.46-1.24)	0.26		
rs1800925	*IL13*	0.67 (0.38-1.20)	0.17	0.85 (0.54-1.36)	0.51		
rs1295686	*IL13*	**0.52 (0.29-0.96)**	**0.032**	0.73 (0.45-1.16)	0.18		
rs2069705	*INFG*	**0.53 (0.31-0.93)**	**0.026**	**0.56 (0.36-0.88)[g]**	**0.012**	**0.63 (0.41-0.96)**	**0.032**
rs1861494	*INFG*	0.70 (0.40-1.23)	0.22	0.74 (0.47-1.17)	0.20		
rs1059293	*INFGR2*	0.96 (0.49-1.86)	0.90	0.98 (0.57-1.67)	0.93		
rs9808753	*INFGR2*	1.18 (0.63-2.21)	0.63	1.10 (0.65-1.85)	0.72		
rs1799987	*CCR5*	1.35 (0.72-2.52)	0.34	1.40 (0.83-2.36)	0.20		
rs2734648	*CCR5*	1.24 (0.70-2.19)	0.47	1.07 (0.67-1.71)	0.76		
rs755622	*MIF*	0.92 (0.50-1.68)	0.78	1.38 (0.84-2.25)	0.20		
rs25648	*VEGFA*	0.99 (0.49-1.98)	0.97	1.11 (0.63-1.97)	0.72		
rs699947	*VEGFA*	1.37 (0.71-2.67)	0.34	1.28 (0.75-2.18)	0.35		
rs3024994	*VEGFA*	1.02 (0.45-2.34)	0.96	1.61 (0.86-3.03)	0.15		
rs3025035	*VEGFA*	1.44 (0.76-2.73)	0.27	1.31 (0.78-2.22)	0.31		
rs2146323	*VEGFA*	1.79 (1.00-3.20)[g]	**0.045**	**1.63 (1.02-2.61)**	**0.040**	1.51 (0.97-2.36)	0.064
rs3024997	*VEGFA*	1.26 (0.72-2.20)	0.41	1.04 (0.67-1.61)	0.87		
rs3025030	*VEGFA*	0.79 (0.42-1.52)	0.48	1.00 (0.58-1.70)	0.99		
rs998584	*VEGFA*	0.66 (0.36-1.20)	0.18	0.66 (0.41-1.06)	0.088		
rs6899540	*VEGFA*	0.70 (0.38-1.28)	0.24	0.84 (0.51-1.40)	0.50		
rs6900017	*VEGFA*	1.42 (0.72-2.79)	0.32	**1.76 (1.02-3.03)[g]**	**0.046**	1.48 (0.88-2.52)	0.15
rs6905288	*VEGFA*	0.83 (0.47-1.47)	0.52	0.83 (0.52-1.31)	0.42		

Abbreviations: OR, odds ratio; CI, confidence interval. Abbreviations: n/s, not specified; SNP, single nucleotide polymorphism; UTR, untranslated region. Estimates were adjusted for age, sex, country of origin, allo-SCT, and prophylaxis status (ever use of prophylaxis). P<0.05 in bold.
[a]Stage 1 (aspBIOmics population; N=330).
[b]Stage 1+2 (aspBIOmics + PCRAGA+Valencia+Salamanca populations; Phase 1+2; N=593 hematological patients).
[c]Overall (after extension with 149 non-HSCT patients; 48 of them with prophylaxis data).
[d]Estimates according a recessive model of inheritance.
[e]SNP also signifcantly associated with IA infection according a log-additive model of inheritance.
[f]Estimates calculated according a co-dominant model (homozygotes for the rare allele were not found).

Results: Overall, we found that the $IL4R_{rs2107356}$ and $IL8_{rs2227307}$ SNPs were associated with an increased risk of IA (P=0.007 and P=0.010, respectively) whereas the $IL12B_{rs3212227}$ and $IFNg_{rs2069705}$ variants were significantly associated with a decreased risk of developing the disease (P=0.046 and P=0.032; Table 1). Importantly, an allogeneic stem cell transplantation (allo-SCT)-stratified analysis revealed that the effect observed for the $IL4R_{rs2107356}$ and $IFNg_{rs2069705}$ SNPs was stronger in allo-SCT patients (P=0.0007 and$P$=0.0010, respectively) compared with those patients without transplantation. Although none of these associations remained significant after correction for multiple testing (P=0.0004), the association of$IL4R_{rs2107356}$ and $IFNg_{rs2069705}$ SNPs in allo-SCT patients remained marginally associated with the risk of IA infection. *In vitro* stimulation assays confirmed a relevant role of the $IL4R_{2107356}$ and $IL12B_{rs3212227}$SNPs in regulating *IL4R* and *IL12* levels. We found that $CD_{19}{}^+$B lymphocytes from carriers of the$IL4R_{2107356A/A}$ mutant genotype (n=7) tended to have an increased expression of *IL4R* protein when compared

with those B-cells from subjects harbouring the wild-type allele (n=7; *P*=0.08). Although carriers of the $IL4R_{2107356A/A}$ genotype also tended to have higher levels of *IL4R* in T-cells and monocytes, a substantial correlation could only be detected in B-lymphocytes, a cell subset where *IL4R* is highly expressed. We also confirmed that carriers of the $IL12B_{rs3212227C}$ allele showed an increased production of *IL12p70* after 24 and 48h of incubation with zymosan alone or in combination with LPS when compared with carriers of the $IL12B_{rs3212227A/A}$ genotype ($IL12B_{MUT\text{-}ZYM\text{-}24h}$=44.0±8.5 *vs.* $IL12B_{WT\text{-}ZYM\text{-}24h}$=30.1±2.2, *P*=0.087 and $IL12B_{MUT\text{-}ZYM\text{-}48h}$=81.9±9.4 *vs.* $IL12B_{WT\text{-}ZYM\text{-}48h}$=26.8±5.8, *P*=0.006 and $IL12B_{WT\text{-}ZYM+LPS\text{-}24h}$=34.6±3.0 *vs.* $IL12B_{MUT\text{-}ZYM+LPS\text{-}24h}$=73.3±4.0,*P*=0.0017). In addition, we found that patients harbouring the $IL12B_{rs3212227C}$ allele showed a substantially increased level of *IFNg* after 48h of incubation with Zymosan when compared with those subjects harbouring the wild type genotype ($IFNg_{MUT\text{-}ZYM\text{-}48h}$=501.4±38.1 *vs.* $IFNg_{WT\text{-}ZYM\text{-}48h}$=183.5±83.0, *P*=0.06). Finally, a predictive analysis also confirmed that a prediction model including SNPs significantly associated with IA showed a substantial improvement in the discriminatory ability to predict the disease when compared with a reference model including only demographic and clinical variables (AUC=0.659 *vs.* AUC=0.564; Figure 1).

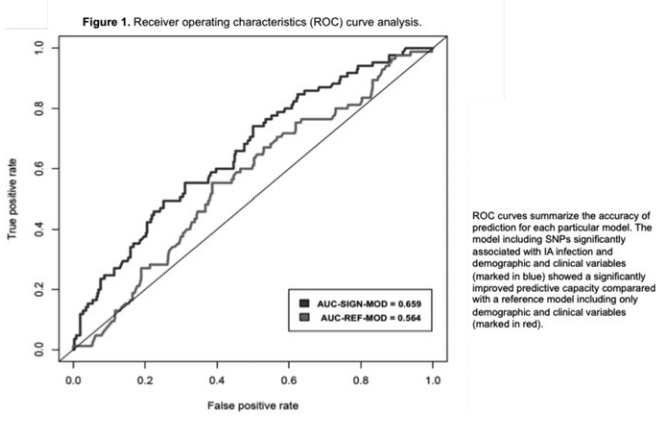

Figure 1. Receiver operating characteristics (ROC) curve analysis.

Summary and Conclusions: These findings suggest that SNPs within immuno-modulating genes influence on the risk of developing IA infection and might be used to predict the disease risk and to implement risk-adapted prophylaxis strategies.

S804

IMPACT OF PREVIOUS RITUXIMAB EXPOSURE IN FOLLICULAR LYMPHOMA PATIENTS INTENSIFIED WITH HIGH DOSE THERAPY AND AUTOLOGOUS STEM CELL TRANSPLANTATION: A RETROSPECTIVE ANALYSIS OF THE GELTAMO REGISTRY

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Background: High-risk Follicular Lymphoma (FL) patients intensified with high dose therapy and autologous stem cell transplantation HDT/ASCT may achieve prolonged remissions. The best timing for the procedure remains controversial. Addition of rituximab to conventional chemotherapy has brought a significant development, with several phase III randomized trials proving a benefit in favor of a rituximab-containing chemotherapy up front. The vast majority of studies of HDT/ASCT in FL have been performed in the pre-rituximab era, and its value now remains to be elucidated.

Aims: To analyze the impact of previos Rituximab exposure in patients intensified with HDT/ASCT in a retrospective study from the Spanish Geltamo Registry.

Methods: All FL patients undergoing HDT/ASCT from 1989 to 2007 and reported to the Spanish GELTAMO registry (n=655, mean age 47 years, male sex 49,6%) were eligible for this study. 203 patients (31%) received HDT/ASCT after achievement of 1st CR, 43% of them requiring more than one therapy line to achieve CR; 26% in 2ndCR, 5% in 3rdCR, 21,5% in 1stPR, 12,5% in chemo sensitive recurrence, and 5% with active disease. Of the 312 cases who were evaluable for FLIPI, 125 (38%) were in the high-risk group. Of the 332 who were evaluable for FLIPI II, 125 (40%) were in in the high-risk group. Of the 640 assessable for Rituximab exposure previously to ASCT, 184 (30%) had been treated with the anti-CD20 monoclonal antibody (R+Group). Overall survival (OS) and progression free survival (PFS) probabilities were calculated using Kaplan-Meier estimates. The log rank test was used for univariate comparisons.

Results: Median follow-up from HDT/ASCT was 14, 5 years for the Rituximab-naïve (R- Group) patients and 9 years for the R+Group. In the R+Group, patients were older (*P*=0.01) and there were more with ECOG >1 (*P*=0.05), with high LDH (*P*=0.02), with poor risk FLIPI II *(P*=0.05) and had a shorter length of follow-up (*P*<0.0001). In patients transplanted in 1st CR, there were more patients who received more than one line of therapy to reach CR in the R+Group (P=0.02). Globally, R+Group showed a better outcome than R-Group (median PFS not reached *vs* 74 months (*P*=0.0006); median OS not reached *vs* 221 months, respectively; (*P*=0, 02)). For patients transplanted in 2nd/3rd CR the benefit of Rituximab was remarkable: median PFS not reached *vs* 92 months (P=0.002); OS at 5 years 91%, OS at 10 years 86%, in the R+Group *vs* OS at 5 years 76%, OS at 10 years 63% for the R- Group, respectively (*P*=0.01). Surprisingly this benefit did not occur in patients transplanted in 1st CR, where R- patients showed even a better outcome (no statistically differences on PFS and OS at 5 years 91% *vs* 83%, OS at 10 years 84% *vs* 72% for the R- Group and R+Group, respectively;(*P*=0.02)).

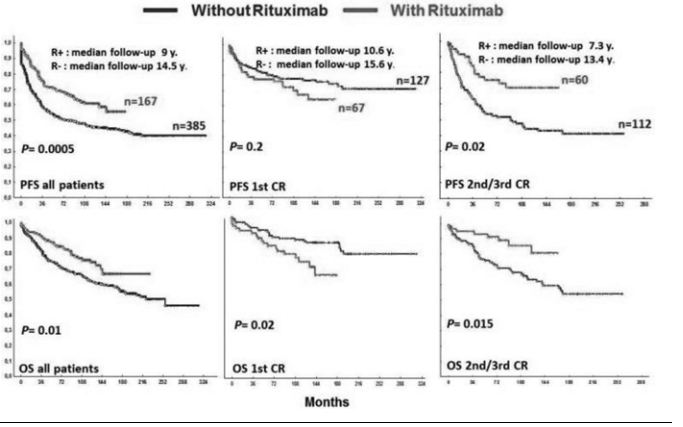

Figure 1.

Summary and Conclusions: Follicular lymphoma patients undergoing HDT/ASCT in 2nd / 3rd CR have a formidable outcome; treatment with rituximab seems to have a synergistic effect with HDT/ASCT in this population. R+Group transplanted in 1st CR, showed an excellent outcome, however it was no better than the obtained in R- Group. Worse initial prognostic factors in the R+Group, with more patients needing more than one line of therapy to reach the CR, could explain these data. In the Rituximab era, HDT/ ASCT should be considered in the therapeutic approach at first relapse for FL patients. Very high risk patients could, as well, benefit from a transplant in 1st CR. As well-balanced randomized studies are not easily feasible in this setting, we think that updating the results of the available studies could elucidate this question.

Progess in Hodgkin lymphoma therapy: Incorporation of novel agents and reduction of side effects

S805

GERMAN HODGKIN STUDY GROUP PHASE I TRIAL OF DOXORUBICIN, VINBLASTINE, DACARBAZINE, AND LENALIDOMIDE (AVD-REV) FOR ELDERLY HODGKIN LYMPHOMA PATIENTS-FINAL ANALYSIS

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Background: About 30% of all Hodgkin Lymphoma (HL) patients are ≥60 years old. ABVD is standard of care for these patients, although outcome and feasibility are poor, one limitation being bleomycin-induced pulmonary toxicity.
Aims: We thus replaced bleomycin with lenalidomide (Revlimid®), and initiated the AVD-Rev phase-I trial (NCT01569204) for 60-75 year-old patients with 1st diagnosis of early unfavorable- or advanced-stage HL, good performance status (ECOG/WHO ≤2), and no severe organ dysfunction.
Methods: Depending on stage and response at interim staging, patients received 4-8 cycles of AVD-Rev followed by radiotherapy with prophylactic anticoagulation (ASA or heparin). The daily lenalidomide dose for the first patient was 5mg; possible dose levels ranged from 5 to 40mg. Thromboembolism ≥CTC II°, hematological toxicity as severe cytopenia (ANC<500/µl >7days with G-CSF and thrombocytopenia <25.000/µl), and complications as neutropenic fever and prolonged therapy delay were considered as dose limiting toxicities (DLT) if they occurred during the first 4 cycles.
Results: 25 patients (median age: 67, range 61-76) and a CIRS-G comorbidity score of up to 7 points (range 0-7) were assigned to dose levels 5mg (n=1), 10mg (n=1), 15mg (n=1), 20mg (n=6), and 25mg (n=16). 15 patients were male, 68% had advanced stage-HL, and 80% had B-symptoms. After DLT evaluation of 20 patients, a pre-specified stopping criterion was reached with a recommended dose for a phase II trial of 25mg. Dose delivery was high with a median relative dose intensity of 97% (range 49%>104%; mean 91%). However, CTC III°-IV° toxicities occurred in all 22 patients treated at the 20mg and 25mg dose levels. DLTs were observed in 1 of 6 and 5 of 16 patients at 20mg and 25mg, respectively, and were mainly hematologic but included 3 thromboembolic events despite ASA prophylaxis. No DLT occurred in patients receiving <20mg lenalidomide. Of note in these vulnerable patients, no treatment related deaths occurred. Overall response rates were 80% for all patients (20/25) and 86% (19/22) for patients receiving ≥20mg lenalidomide. At 19 months median observation time, 5 patients had disease progression and 4 patients died. The one-year estimates for progression-free (PFS) and overall survival (OS) are 75% [95%>CI: 53-88%] and 92% [95%>CI: 71-98%], respectively. Final results on PFS and OS with two years follow-up will be presented at the EHA congress 2015.
Summary and Conclusions: AVD-Rev is toxic but feasible and highly effective in this vulnerable population of elderly HL patients.

S806

BENDAMUSTINE-CONTAINING REGIMEN (BEGEV) AS INDUCTION CHEMOTHERAPY PRIOR TO ASCT FOR RELAPSED/REFRACTORY HODGKIN LYMPHOMA

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Background: Salvage chemotherapy followed by high dose chemotherapy with autologous stem cell transplant (ASCT) is the standard of care for refractory/relapsed Hodgkin's lymphoma (HL r/r), leading to durable responses in approximately 50% of relapsed patients. The pre-transplantation achievement of CR is the strongest prognostic factor in this setting. The combination of gemcitabine and vinorelbine and ifosfamide (IGEV) demonstrated to be an highly active salvage regimen, with a high CR rate (48%) and low toxic profile. Bendamustine, as single agent, has been reported to induce objective responses in an high percentage of pts with HL recurring after autologous stem cell transplant (ASCT).
Aims: This study has been conducted with the aim of improving the CR rate of induction salvage chemotherapy in comparison to historical IGEV data by combining Bendamustine with Gemcitabine and Vinorelbine (BeGEV).
Methods: This was a Fleming's single-stage phase II multi-centric trial in HL r/r patients before ASCT. Aim of the study was to assess the activity of BeGEV in term of CR rate. Assuming an improvement of 15% (50 to 65%), with a 10% rejection error and a power of 85%, at least 35 CR out of 59 patients had to be observed. Secondary end points were overall response, cell mobilization, toxicity, progression free survival (PFS) and overall survival (OS). Patients were eligible if they had relapsed or refractory classic HL after first line chemotherapy. The treatment schedule was: Bendamustine (90 mg/sqm, days 2-3), Gemcitabine (800 mg/sqm, day 1 and 4) and Vinorelbine (25 mg/sqm, day) every 3 weeks for a total of 4 courses.
Results: Between August 2011 and March 2014, 59 consecutive patients with relapsed (45.8%) or refractory (54.2%) HL were enrolled. The median age was 33 years (range 18-68). Out of 59 enrolled patients, 43 (72.9%) achieved a CR, 6 (10.2%) a partial response (PR) for an overall response rate of 83.1%. The remaining 10/59 patients (16.9 %) were evaluated as non responders for: 1 (1.7%) stable disease, 8 (13.6 %), PD, 1 (1.7 %) toxicity. Adequate CD34+cell collection was achieved in 55 out of 57 (96.5%) mobilized patients. Considering the 49 responsive patients, 42 proceeded to ASCT (37/43 in CR, 5/6 in PR). The remaining 7 patients did not proceed to ASCT due failed mobilization (2 cases), allogenic transplant (1 case), physician's decision (2 cases), early relapse (1 case), and patient refusal (1 case). With a median follow up of 16 months, the PFS and OS at 2 years were respectively 50.9% and 68.8% for all series, without significant difference among relapsed and refractory patients. The 2-year PFS for the 49 responsive pts was 61% for CR and 63% for CR+PR, respectively. The 2-year OS reached 85.5% for CR+PR *vs* 14.3% induction failures (p value <0.001). Hematological and non-hematological side effects were acceptable. Out of 204 evaluated cycles, 23 (11%) had to be delayed and 4 (2%) reduced, respectively; only 1 patient stopped therapy for toxicity. Three non-hematologic G4 toxicity (2 infection and 1 hepatic failure unlikely related to treatment), were observed. No toxic death occurred.
Summary and Conclusions: The BeGEV regiment achieved a CR (72.9%) and overall response rate (83.1 %) overcoming the expected results. The study highlights the activity of this combination as well as the good toxicity profile and the high mobilizing potential. These data provide strong support for further development of BeGEV.

S807

MULTIVARIATE ANALYSIS OF PFS FROM THE AETHERA TRIAL: A PHASE 3 STUDY OF BRENTUXIMAB VEDOTIN CONSOLIDATION AFTER AUTOLOGOUS STEM CELL TRANSPLANT FOR HL

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Background: In the phase 3, randomized, placebo-controlled AETHERA trial, progression-free survival (PFS) was significantly improved with brentuximab vedotin (BV) *vs* placebo (HR=0·57, P=0·001) in Hodgkin lymphoma (HL) patients at risk of progression post-autologous stem cell transplant (ASCT).
Aims: A multivariate analysis was performed to determine which factors significantly influence PFS by investigator assessment.
Methods: After ASCT, 329 patients were randomized to receive BV 1.8 mg/kg q3wk (n=165) or placebo (n=164) for up to 16 cycles. The primary endpoint was PFS per independent review. Multivariate analysis using a Cox-proportional hazards model was developed on the following factors: treatment, age, sex, weight, geographical region, initial disease stage, time from diagnosis, no. of treatments pre-ASCT, chemosensitivity, response to frontline (FL) and salvage, type of FL therapy, prior radiotherapy, extranodal disease pre-ASCT, ASCT conditioning regimen, B symptoms at pre-ASCT relapse, number of risk factors, baseline ECOG, baseline lesions, and pre-existing peripheral neuropathy. Significant factors (p<0.05) were determined after a stepwise addition and elimination of nonsignificant factors from the model.
Results: Multivariate modeling indicated that factors significantly associated

with PFS by investigator assessment included: treatment (BV *vs.* placebo), salvage response, gender, number of treatments pre-ASCT, type of FL therapy, B symptoms pre-ASCT, and weight (see Table).

Tabella 1. Multivariate analyses of PFS by investigator assessment.

Effect	Hazard Ratio (95% CI)	P value
Treatment (BV vs Placebo)	0.44 (0.31, 0.62)	<0.001
Salvage response (CR vs PR/SD)	0.44 (0.30, 0.64)	<0.001
Gender (F vs M)	0.60 (0.43, 0.85)	0.004
No. treatments pre-ASCT (2 vs >2)	0.65 (0.47, 0.90)	0.010
FL therapy (ABVD vs BEACOPP/Other)	0.63 (0.44, 0.91)	0.013
B symptoms pre-ASCT (No vs Yes)	0.64 (0.45, 0.92)	0.015
Baseline weight (≤100 vs >100 kg)	0.58 (0.36, 0.94)	0.026

Summary and Conclusions: After adjustment for significant clinical factors in a multivariate regression analysis, consolidation treatment with BV significantly reduced the risk of treatment failure compared to placebo with a HR of 0.44. These results support the primary analysis.

S808

NIVOLUMAB IN PATIENTS WITH RELAPSED OR REFRACTORY LYMPHOID MALIGNANCIES AND CLASSICAL HODGKIN LYMPHOMA: UPDATED SAFETY AND EFFICACY RESULTS OF A PHASE 1 STUDY (CA209-039)

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Background: The PD-1 pathway functions as a checkpoint which limits T-cell mediated tumor immune responses. Nivolumab (NIVO), a fully human IgG4 monoclonal PD-1 blocking antibody, potentiates T-cell activity. Prior results from this study (median follow-up 40 weeks) showed that NIVO was tolerated and achieved an overall response rate of 87% in classical Hodgkin lymphoma (cHL), 40% in follicular B-cell lymphoma (FBL), 36% in diffuse large B-cell lymphoma (DLBCL) and 17% in T-cell non-Hodgkin lymphoma (T-NHL). The stable disease rate in multiple myeloma (MM) was 67%.
Aims: Herein, we report the updated follow-up and safety profile of this study.
Methods: Patients (Pts) were treated using a dose-escalation design (1 and 3 mg/kg) of NIVO administered every 2 weeks (wks) for 2 years. Responses were assessed using standard criteria. Primary endpoint was safety. The secondary endpoint was efficacy.
Results: 105 pts were enrolled (23 cHL, 31 B-NHL, 23 T-NHL, 27 MM and 1 chronic myelogenous leukemia). Pts were heavily pretreated with 88%, 78%, 68% and 66% of pts with cHL, T-NHL, B-NHL and MM, respectively, having received ≥3 prior regimens. Previous ASCT was reported for 75% of pts with cHL, 56% of MM, 13% of B-NHL and 9% of T-NHL. As of 1/8/2015, median duration of follow-up was 62 wks (range: 2 to 106+wks). Drug-related adverse events (DrAEs) occurred in 71 (67%) pts. The most common DrAEs occurring in ≥5% were fatigue (15%), rash (11%), diarrhea, pneumonitis, pruritus (each 9%), pyrexia (8%), thrombocytopenia, decreased appetite (each 7%), hypocalcemia, lipase increased, leukopenia, lymphopenia (each 6%) and nausea (5%). Serious DrAE in ≥5% of pts included pneumonitis (5%). Efficacy results shown below. The rate of stable disease in MM (n=27) was 63%. Among the 20 responding cHL pts, 10 discontinued NIVO; 6 (1CR and 5 PR) to undergo SCT, 3 for disease progression and 1 for toxicity (MDS, thrombocytopenia) and 10 (7 PR and 3 CR) continue to respond. Among responding B- and T-NHL pts, 1/4 DLBCL, 3/4 FL and 3/4 T-NHL pts remain in response. In this updated analysis, median duration of response has not been reached in cHL, B-NHL and T-NHL.

Tabella 1. Multivariate analyses of PFS by investigator assessment.

Tumor	Objective Response n (%)	Complete Response n (%)	Partial Response n (%)	Stable Disease n (%)	Median Weeks of Response Duration (range)	Ongoing Responders n (%)
cHL (n=23)	**20 (87)**	**4 (17)**	**16 (70)**	**3 (13)**	**NR (2 - 76+)**	**10 (50)**
B-NHL (n=31)	8 (26)	3 (10)	5 (16)	16 (52)	NR (6+ - 81+)	4 (50)
Diffuse Large B-cell Lymphoma (n=11)	4 (36)	2 (18)	2 (18)	3 (27)	22.1 (6 - 73+)	1 (25)
Follicular B-cell Lymphoma (n=10)	4 (40)	1 (10)	3 (30)	6 (60)	NR (23+ - 81+)	3 (75)
Other B-cell Lymphoma (n=10)	0	0	0	7 (70)	—	—
T-NHL (n=23)	4 (17)	0	4 (17)	10 (43)	NR (11 - 63+)	3 (75)
Cutaneous T-cell Lymphoma Mycosis Fungoides (n=13)	2 (15)	0	2 (15)	9 (69)	NR (24+ - 36+)	2 (100)
Peripheral T-cell Lymphoma (n=5)	2 (40)	0	2 (40)	0	NR (11 - 63+)	1 (50)
Other Cutaneous T-cell Lymphoma (n=3)	0	0	0	0	—	—
Other Non-Cutaneous T-cell Lymphoma (n=2)	0	0	0	1 (50)	—	—

Summary and Conclusions: Encouraging, durable objective responses were observed in cHL, DLBCL and FL, including CR and PR. NIVO treatment remains safe and tolerable with a safety profile similar to that in solid tumors, and further analysis is warranted in cHL and selected B-NHLs and T-NHLs.

S809

EFFECT OF BLEOMYCIN HYDROLASE GENE POLYMORPHISM ON LATE PULMONARY COMPLICATIONS OF TREATING HODGKIN LYMPHOMA

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Background: Bleomycin induced pulmonary toxicity occurs in 20-46% of Hodgkin lymphoma (HL) patients, treated with ABVD (doxorubicin, bleomycin, vinblastin, dacarbasine) regimen. Bleomycin hydrolase (BLMH), an enzyme that inactivates bleomycin, may be a potential candidate that influences pulmonary function. Single nucleotide polymorphisms (SNP) of the BLMH gene have been investigated previously in testicular germ-cell cancer (TC) patients in parallel to survival data. Interestingly, homo- and heterozygous variants of BLMH gene SNP A1450G were found to significantly affect survival of TC patients. Yet, there is no available data of BLMH SNPs and bleomycin induced pulmonary toxicity in correlation with HL patients.
Aims: We hypothesized, that BLMH gene SNP A1450G (rs1050565) influences BLMH activity and by an altered metabolism of bleomycin, differences could be seen at the risk of bleomycin induced toxicity appearance.
Methods: Pulmonary functions of previously treated HL patients have been collected by using St. George Respiratory Questionnaire (SGRQ), dynamic inhalation lung scintigraphy and spirometry at the Department of Hematology, University of Debrecen, retrospectively. After informed consent of these patients genomic DNA was isolated from peripheral blood samples and TaqMan genotyping assay was used to determine genotype distribution. Local Research Ethics Committee approved this investigation.
Results: A total of 131 patient samples have been collected of previously treated HL patients, with a median age of 29 years (14-73), median time elapsed since treatment completion was 11 years (1-44). Genotype frequency was found as follows: A/A 55.0%, A/G 33.6%, G/G 11.5%, where A is the wild allele (71.8%) and G is the mutated allele (28.2%). Our results are comparable to the NCBI SNP database for SNP A1450G. Patients were subdivided into subgroups containing the mutated allele: A/G+G/G (45.1%) and those homogenous for the wild allele: A/A (55.0%). All bleomycin treated patients (n=102) had more favorable lung function test result in the wild A/A genotype group, with significant difference in the forced vital capacity results (FVC), p=0.041. When narrowing patients to those who received only ABVD regimen (n=68), significantly more favorable results were seen in the wild A/A genotype group with every investigated test method (SGRQ score: p=0.035, lung scintigraphy results: p=0.045, spirometry results: FVC: p=0.020; forced expiratory volume in 1 s (FEV1): p=0.028). Univariate analysis also confirmed these results. As control group (n=29), patients treated with agents excluding bleomycin were tested with no significant differences between the A/G+G/G and A/A groups. Other factors (smoking, age, bleomycin dose, chest irradiation, kidney function, use of colony stimulating factors) that could potentially affect lung function were equally represented in all investigated subgroups.
Summary and Conclusions: BLMH gene polymorphism significantly distinguished in retrospective pulmonary test results of ABVD treated patients. Based on these results prospective trials could be designed to further evaluate its role

in pulmonary toxicity of treating HL, and thus treatment could be adjusted individually. One of its potential roles could be to identify those cases even in the frontline setting, where brentuximab vedotin should be administered instead of bleomycin, either in the standard care or considering expense issues. Patients with multiple risk factors for lung toxicity should be particularly considered for this test method. Eventually, life expectancy and quality of life of HL patients wouldn't remarkably differ from the untreated healthy population.

CML: Molecular-cytogenetic diagnostics

S810

PREDICTORS OF DEEP MOLECULAR RESPONSE IN CHRONIC MYELOID LEUKEMIA PATIENTS TREATED FRONTLINE WITH IMATINIB MESYLATE: AN ANALYSIS BY THE GIMEMA CML WP

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Background: Imatinib mesylate (IM) has been for many years the standard of care for chronic myeloid leukemia (CML) in early chronic phase (CP). As first-line treatment, IM produce high probability of long-term survival, but few patients are able to achieve a stable deep molecular response ($MR^{4.0}$ or better), that is a pre-requisite to discontinue the TKI treatment. The treatment-free remission (TFR) is an emerging CML treatment goal. The identification of patients with higher probability of achieving a stable deep molecular response is important for the optimization of treatment strategy.
Aims: The aim of the present study was to investigate the predictors of $MR^{4.0}$ in CML patients treated frontline with imatinib.
Methods: We analyzed 559 patients enrolled within 3 multicentric prospective studies conducted by the GIMEMA CML WP (NCT00514488, NCT00510926, observational trial CML023). Definitions: major molecular response (MMR), BCR-ABL^{IS} ratio <0.1%; deep molecular response ($MR^{4.0}$), detectable disease ≤0.01% BCR-ABL^{IS} or undetectable disease with ≥10,000 ABL transcripts; stable $MR^{4.0}$: $MR^{4.0}$ lasting ≥24 months and ≥3 evaluable samples; unstable $MR^{4.0}$: unconfirmed occasional positive samples <0.1% BCR-ABL^{IS} in patients with a stable MR4.0 (Rousselot *et al.* J Clin Oncol 2014). The intention-to-treat population of each study was analyzed and all the 559 enrolled patients were included.
Results: Baseline demographics characteristics: median age, 52 years (extremes 18-84 years); male/female sex, 60%/40%; high Sokal, high Euro and high EUTOS scores, 22%, 7% and 7%, respectively; clonal chromosomal abnormalities (CCA) in Ph+cells, 4% (not evaluable in 32% of patients for insufficient number of metaphases); e13a2 BCR-ABL transcript, 36%. The median follow-up was 82 (60-105) months. The median time to MMR and MR^4 was 8 and 42 months, respectively. The rate of $MR^{4.0}$ at 24, 36, 48 and 60 months was 18%, 27%, 30% and 33%, respectively. Overall, 61% of patients achieved a $MR^{4.0}$ at least once. According to the previously detailed criteria, at the last contact 28% of patients had a stable $MR^{4.0}$ and 12% had an unstable $MR^{4.0}$ (stable+unstable $MR^{4.0}$=40%). The median duration of stable $MR^{4.0}$ was 36 months and the median duration of stable+unstable $MR^{4.0}$ was 40 months. In a multivariate Cox analysis including baseline variables, female sex, smaller spleen size and e14a2 transcript type resulted independent good prognostic factors on the probability to achieve a $MR^{4.0}$. Female sex was the only baseline variable able to predict a stable $MR^{4.0}$. The reduction of BCR-ABL1 transcript levels and the time to molecular response are influenced by baseline variables, so the impact of the dynamics of molecular response on the probability of $MR^{4.0}$ was analyzed separately: the time to MMR strongly predicted both the achievement and the stability of $MR^{4.0}$.
Summary and Conclusions: As suggested by a previous report (Branford *et al.* Blood 2013), in a large nationwide multicentric experience, the female sex was confirmed as a predictor of the achievement of a stable deep molecular response. Moreover, our results support strategies aimed to a rapid reduction of BCR-ABL1 transcript levels to optimize potential suitability for imatinib discontinuation studies.

S811

EAC BCR-ABL1 ASSAY PERFORMANCE VALIDATION ON THE DPCR QS3D PLATFORM AND COMPARISON WITH OTHER DPCR PLATFORMS

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Background: After the clinical success of TKIs in treating patients with CML, the clinical management of these patients became the core of patient care. RT-qPCR is the current gold standard for monitoring patients' response to therapy. Transcript levels are prognostic and capable of stratifying the patients in to risk groups as early as 3 month after therapy induction. Several molecular milestone have been introduced based on the log reduction of the transcript levels in response to therapy. Optimal response has been defined by the recently revised ELN guidelines as *BCR-ABL1* levels to be ≤10% by 3 month, <1% by 6 month, ≤0.1% by 12 month then ≤0.1% at any time. However, these percentages hugely vary from one centre to the other due to inherent limitations introduced by the gold standard method of quantification, unless expressed on the International scale. Recently, dPCR has been introduced as a method of quantification that promises simplified assay standardisation procedures mainly for being a method of absolute quantification as opposed to the relative quantification ascribed to the gold standard. In this study, we sought to optimize the use of such a platform, the QS3D, for our routine practice in addition to a performance comparison study with other dPCR platforms.
Aims: In this study, we sought to optimize the use of the dPCR platform, the QS3D, for our routine practice in addition to a performance comparison study with other widely used dPCR platforms, namely the Bio-Rad QX200 and RainDance.
Methods: For validating the EAC assay on the QS3D dPCR, we used the reference ERM-AD623 plasmid to assess the linearity metric of the platform by running a series of dilutions that fell within, below and above the sweet spot of the chip used. To assess the bias introduced to our measurements of BCR-ABL1 transcript ratios in patient material using our in-house generated Wessex plasmid, we ran a series of dilutions using the Wessex plasmid and compared it against the reference plasmid. To assess the limit of detection, we ran 6 categories of patient material at different disease ranges on the IS: 20%, 10%, 1%, 0.1%, 0.01%, and 0.001% (10 samples per category). These 60 patient material were also ran on the two other platform for comparing their performances.
Results: Linearity was maintained within the sweet spot and down to 3 molecules per chip. Inte and intra experimental variations were insignificant indicating excellent reproducibility (p=0.2). Linearity above the sweet spot was maintained, However a systematic underestimation was observed (p=0.05). There was a systematic bias introduce using out in-hour plasmid (p=0.001), and this bias was factored into the quantification of the cNDA molecules collected form the patients' samples. The limit of detection of the platform was concordant with that of the gold standard down to 0.1% IS (p=0.2); however dPCR demonstrated better precision in quantifying molecules in samples at the 0.01% IS level (p=0.07), and had improved sensitivity and precision in quantifying molecules in samples at the 0.001% IS level (p=0.06). Platform comparison showed reassuring concordance in sensitivity at all levels (p=0.16-0.4).

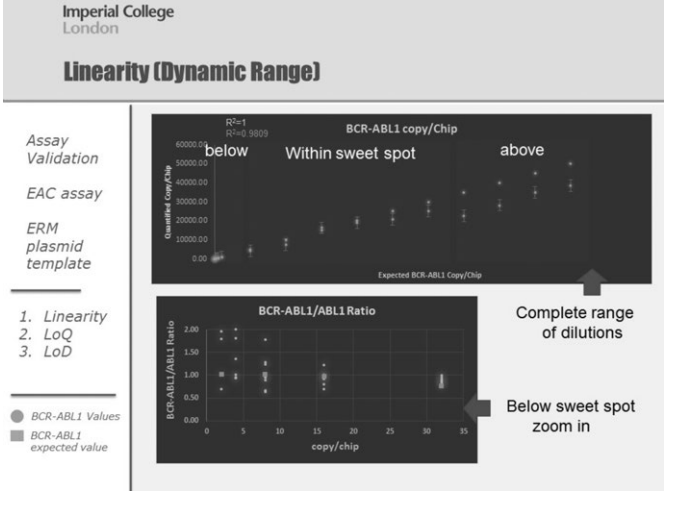

Figure 1.

Summary and Conclusions: In conclusion, our investigations demonstrate that the QS3D digital PCR platform demonstrates performance characteristics to favour its implementation in routine clinical use with a one log increased limit of detection compared to the gold standard.

S812

IMPACT OF MAJOR ROUTE VERSUS UNBALANCED MINOR ROUTE KARYOTYPES AT DIAGNOSIS ON PROGNOSIS OF CML

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Background: Major route additional cytogenetic aberrations (ACA) at diagnosis of chronic myeloid leukemia (CML) indicate an increased risk of progression and shorter survival. Since all major route ACA are unbalanced it is unclear whether other unbalanced ACA at diagnosis also confer an unfavourable prognosis.
Aims: Since major route ACA are unbalanced aberrations and since patients with minor route ACA may also progress, the question arose whether a more generally defined group of chromosomal abnormalities such as unbalanced aberrations rather than only the specific major route ACA indicate progression.
Methods: On the basis of 1348 Philadelphia (Ph) chromosome positive chronic phase patients of the randomized CML-study IV we examined the impact of unbalanced minor route ACA at diagnosis in comparison to major route ACA on prognosis. Cytogenetic analyses of at least 20 Giemsa(G)-banded or Reverse(R)-banded bone marrow metaphases at diagnosis were interpreted according to the International System for Human Cytogenetic Nomenclature (2013). Patients with cytogenetic aberrations in Ph-negative clones at diagnosis were excluded from this analysis. Patients with constitutional changes were assigned to the group with standard translocation t(9;22)(q34;q11) or variant translocation t(v;22). In patients showing a complex aberrant karyotype G- or R-banding analysis was combined with m-FISH analysis. Cytogenetic remission was defined according to the ELN recommendations. Progression-free survival (PFS) was defined as the time from diagnosis until the beginning of accelerated phase, blast crisis, or death from any cause whatever event came first. For overall survival (OS), death from any cause was the only event. Probabilities of PFS and OS were calculated by the Kaplan-Meier method and compared by the log-rank test. Patients were censored at the date of last follow-up. P-values lower than 5% were considered significant. Due to the explorative character of this work, no adjustment of p-values was done and all p-values have to be interpreted descriptively.
Results: At diagnosis, 1175 patients (87%) had a translocation t(9;22)(q34;q11) and 74 (5.5%) a variant translocation t(v;22) only, while a loss of the Y-chromosome (-Y) was present in addition in 44 (3.3%), balanced or unbalanced minor route ACA each in 17 (1.3% each) cases and major route ACA in 21 (1.6%) cases. Patients with unbalanced minor route ACA achieved complete cytogenetic remission, major molecular remission, PFS and OS at similar rates as did patients with t(9;22), t(v;22), -Y, and balanced minor route karyotypes. In contrast, patients with major route ACA had a shorter OS and PFS than all other groups (p<0.005 for all pairwise comparisons with major route). Five year survival probabilities were for t(9;22): 91.4% (95% CI 89.5-93.1), t(v; 22): 87% (77.2-94.3), -Y: 89.0% (76.7-97.0), balanced: 100%, unbalanced minor route: 92.3% (72.4-100), major route: 52.2% (28.2-75.5).
Summary and Conclusions: We conclude that only major route, but not unbalanced minor route ACA at diagnosis have a negative impact on prognosis of CML. This observation may be of relevance also to other cancers.

S813

SECOND GENERATION TYROSINE KINASE INHIBITORS MITIGATE THE HIGH RISK OF DISEASE PROGRESSION IN CHRONIC MYELOID LEUKAEMIA PATIENTS WITH HIGH DIAGNOSTIC LEVELS OF CIP2A

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Background: CML treatment has been significantly improved by the tyrosine kinase inhibitor (TKI) imatinib, but at least one-third of patients will eventually fail imatinib treatment[2,3] and a significant proportion of these will progress towards blast crisis (BC), which is usually rapidly fatal. Suppression of PP2A activity by the inhibitors SET and CIP2A is important in the pathogenesis and progression of chronic myeloid leukaemia (CML). We have previously shown that CML patients with a high diagnostic CIP2A protein level who then receive imatinib have a high risk of progressing to blast crisis.
Aims: The aim of this study was to investigate if high CIP2A also confers a high progression rate in patients receiving a second generation (2G) TKI dasatinib or nilotinib from diagnosis.
Methods: The diagnostic CIP2A protein level was assessed by flow cytometry

in 74 newly diagnosed chronic phase patients, who were subsequently treated with either imatinib or a 2G TKI. ROC analysis was used to define a CIP2A cut off level to stratify cases into high and low CIP2A groups. All have been seen since original diagnosis of chronic phase CML at our centre and have been followed for at least 9 months (median follow-up: 50 months).

Results: For patients with high diagnostic CIP2A level treated with imatinib, the overall and progression-free survival probability at 24 months was 41% and 17% respectively, compared to 100% for patients in any other group ($p<0.001$, Figure 1). Disease progression to blast crisis only occurred in those patients with a high diagnostic level of CIP2A and treated with imatinib. All progressions occurred within 32 months from diagnosis with the median time to progression being 12.5 months and disease progression was not associated with the development of BCR-ABL kinase domain mutations. The cumulative complete cytogenetic response (CCR) rate for low CIP2A level imatinib treated patients was 85% at 18 months. In contrast, only a single patient with a high diagnostic CIP2A level achieved CCR (cumulative CCR rate of 9% at 18 months; $p<0.001$); this patient subsequently progressed. This deleterious effect of high CIP2A was not seen if patients were treated with a 2G TKI from diagnosis, where the estimated cumulative CCR rate at 18 months was 86% and 56% for the low and high CIP2A 2G TKI patients respectively. The cumulative rate of MR4 (*BCR-ABL1/ABL* transcript ratio of ≤0.01%) for low CIP2A imatinib treated patients was 16% at 18 months. No patient with a high diagnostic CIP2A level and treated with imatinib achieved MR4 ($p=0.001$). High CIP2A patients treated with a 2G TKI have a lower rate of MR4 compared to low CIP2A 2G TKI treated patients, 11% and 48% respectively. High diagnostic CIP2A levels in patients treated with a 2G TKI predicts for poor molecular response. Early molecular response (EMR, *BCR-ABL1/ABL* ratio of <10% at 3 months) is an excellent predictor of clinical outcome in imatinib treated patients. 55% of imatinib treated low CIP2A patients achieved an EMR, compared to only 9% of high CIP2A patients ($p=0.01$). 90% of low CIP2A patients treated with a 2G TKI achieved an EMR compared to 44% of those with a high CIP2A level.

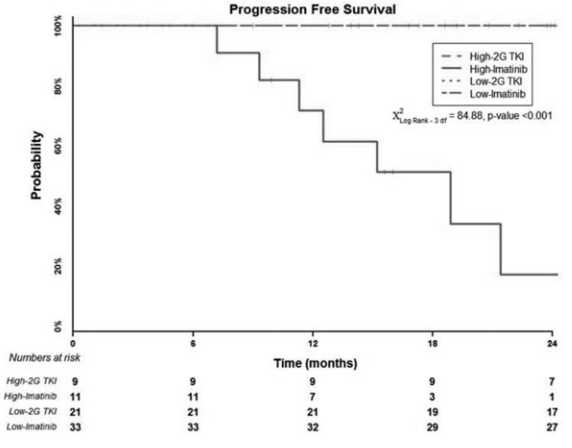

Figure 1. High CIP2A expressing patients do not progress if treated with a second generation TKI. All 74 patients involved in the study are included in the Kaplan-Meier estimate of progression free survival plots. The Log rank was used.

Summary and Conclusions: In summary, patients with high diagnostic CIP2A levels and treated with a 2G TKI at initial diagnosis do not progress to BC, suggesting that if a patient has a high CIP2A level at diagnosis then they should not be treated with imatinib due to the high risk of disease progression. A high diagnostic level of CIP2A in patients treated with a 2G TKI can predict an inferior deep molecular response. There is therefore now a case for routine CIP2A testing at diagnosis. Further study to determine the true incidence of high CIP2A patients in the general CML population is needed.

INTERNATIONAL CONTROL ROUND FOR DEEP SEQUENCING ANALYSIS OF BCR-ABL KINASE DOMAIN MUTATIONS IN 11 LABORATORIES FROM 7 EUROPEAN COUNTRIES

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Background: Point mutations in the BCR-ABL kinase domain are the most frequently identified mechanisms of acquired resistance towards tyrosine kinase inhibitors in chronic myeloid leukemia (CML). Although Sanger sequencing is still regarded as the gold standard technique for routine BCR-ABL mutation screening, next-generation sequencing (NGS) has evolved rapidly and is accessible to an increasing number of diagnostic laboratories. Thus far, data is limited on the technical performance of NGS for BCR-ABL mutation screening in a clinical diagnostic setting.

Aims: As an international consortium of 11 laboratories in 7 countries, we sought to investigate the robustness, precision, and reproducibility of NGS for BCR-ABL mutation analysis. The study was conducted as a subproject within the Interlaboratory Robustness of Next-generation sequencing (IRON)-II study.

Methods: Optimized PCR protocols and preconfigured 96-well plates containing lyophilized primer pairs targeting the entire BCR-ABL kinase domain were generated and distributed to each participating laboratory. To evaluate performance, 22 blinded control samples were sent to each laboratory (a total of 242 samples). Seventeen control samples contained cDNA of Ba/F3$^{BCR\text{-}ABL}$ cell lines harboring 12 different BCR-ABL kinase domain mutations that were mixed with non-mutated Ba/F3$^{BCR\text{-}ABL}$ to produce dilutions ranging from 1% to 100% of mutant alleles. Five control samples contained cDNA of non-mutated Ba/F3$^{BCR\text{-}ABL}$. All control samples were diluted into HL60 cells to simulate a BCR-ABL level of 10% on the International Scale. NGS was performed on 454 GS Junior sequencing instruments using 454 GS Junior Titanium chemistry for amplicon sequencing.

Results: Overall, a median of 104,732 high quality sequencing reads were generated across each of the 11 laboratories, where the number of sequencing reads ranged from 11,307 to 185,775. The mean read length obtained in all runs was 356 bases (range, 346 to 363), indicating a homogenous read length pattern across all centers. A combined number of % mixed and % dots filtered reads of less than 10% on average indicated a robust performance for each emulsion PCR (emPCR) reaction in this study. Concerning mutation analysis, 203 of 242 samples (84%) were evaluated correctly. Hereby, 9 laboratories showed an excellent performance with correct identification of 21 (2 laboratories), 20 (2 laboratories) and 19 (5 laboratories) of the 22 control samples including novel artificial variants (*e.g.* E282K) and five low-level mutations with less than 20% mutant alleles. The concordance of mutation quantification was high in all laboratories. All non-mutated control samples were reported correctly. The 1% T315I mutation dilution was not identified in any laboratory, a 5% M244V mutation was not detected in 7, and a 1% F311L mutation was not detected in 5 laboratories. Two laboratories failed to test 9 of the 22 control samples correctly, thereby reporting both false positive and false negative results.

Summary and Conclusions: This multicenter analysis demonstrated that amplicon-based deep sequencing is technically feasible, achieves a high concordance across multiple laboratories and allows a broad and in-depth characterization of BCR-ABL mutations in CML. However, data also illustrates that expertise in NGS performance and characterization of BCR-ABL mutations is advantageous and necessarily required for the report of accurate results.

Novel insights into the mechanisms involved in MPNs

S815

IDENTIFICATION OF FIP1L1-PDGFRA ASSOCIATING MOLECULE THAT LOCATES IN THE NUCLEUS AND AUGMENTS THE ACTIVITY OF FIP1L1-PDGFRA

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Background: FIP1L1-PDGFRA is a fusion tyrosine kinase that plays a pathogenic role in chronic eosinophilic leukemia. This fusion kinase is constitutively active and its kinase activity is essential for the development of chronic eosinophilic leukemia. Therefore, a tyrosine kinase inhibitor, imatinib, is used to treat FIP1L1-PDGFRA-positive chronic eosinophilic leukemia. Although the C-terminal kinase portion of FIP1L1-PDGFRA is essential for activation of downstream substrates, the N-terminal FIP1L1 portion also plays a crucial role in cellular transformation. We have reported that the FIP1L1 portion directs FIP1L1-PDGFRA into the nucleus and is necessary for the higher proliferating activity (Iwasaki *et al.* Ann Hematol. 93:1473-81, 2014). However, little is known about the transforming pathway mediated by the FIP1L1 portion.
Aims: We tried to isolate a molecule interacting with FIP1L1-PDGFRA to elucidate the leukemogenic role of the FIP1L1 portion.
Methods: Yeast two-hybrid screening was performed, where FIP1L1-PDGFRA was used as bait. One of the positive clones encoded Protein inhibitor of activated STAT1 (PIAS1). Expression vectors of FIP1L1-PDGFRA and PIAS1 were constructed. Immunoprecipitation/immunoblotting analysis was performed to analyze the association of two molecules. Immunostaining was also performed to examine the intracellular localization of these molecules. In addition, a series of biochemical analysis was performed to analyze pathological significance of this association. Moreover, we established cells stably expressing FIP1L1-PDGFRA. Using this cell line, we analyzed whether PIAS1 contributes the FIP1L1-PDGFRA-dependent growth.
Results: PIAS1 was isolated as a FIP1L1-PDGFRA associating molecule by yeast two-hybrid screening. The association between two molecules was confirmed by immunoprecipitation/immunoblotting analysis. In addition, FIP1L1-PDGFRA associated with PIAS1 via the FIP1L1 portion. An immunostaining analysis revealed that FIP1L1-PDGFRA and PIAS1 co-localized in the nucleus. FIP1L1-PDGFRA, as a tyrosine kinase, and PIAS1, as a SUMO E3 ligase, catalyzed each other, and this enzymatic catalysis resulted in the stabilization of each molecule. Therefore, inhibition of PIAS1 activity by PIAS1-specific siRNA or a sumoylation inhibitor, ginkgolic acid, resulted in the destabilization of FIP1L1-PDGFRA. Ginkgolic acid and imatinib synergistically inhibit the kinase activity of FIP1L1-PDGFRA.
Summary and Conclusions: Our results revealed a novel pathway in the pathogenesis of chronic eosinophilic leukemia, where FIP1L1-PDGFRA associates with PIAS1 in the nucleus. The interaction of two molecules seems to be crucial for the pathogenesis of chronic eosinophilic leukemia. Moreover, sumoylation system could be a potential target in the treatment of chronic eosinophilic leukemia.

S816

THE THROMBOPOIETIN RECEPTOR SUPPORTS PROLONGED JAK2 V617F DIMERIZATION AND ACTIVATION AND IS MORE SENSITIVE TO LOW JAK2 V617F LEVELS DUE TO LONG HALF-LIFE WHEN COMPARED TO EPOR AND G-CSFR

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Background: The activating mutation V617F in the pseudokinase domain of JAK2 is prevalent in myeloproliferative neoplasms (MPN) such as Polycythemia Vera (PV), Essential Thrombocytosis (ET) and Myelofibrosis (MF). Low JAK2 V617F allele burdens in ET together with evidence from transgenic and retroviral mouse models established that low expression of mutated kinase specifically associates with an ET phenotype. The mechanism for this effect and how this single mutation promotes three distinct diseases still remains elusive.
Aims: Our aim was to investigate the differences in the interaction between JAK2 V617F and dimeric cytokine receptors for thrombopoietin (Tpo), erythropoietin (Epo) or granulocyte-colony stimulating factor (G-CSF) and to assess biochemical reasons to any possible distinctions in these interactions.
Methods: *Gaussia princeps* luciferase protein-fragment complementation was utilized to study interaction between JAK2 V617F and the cytokine receptors as well as dimerization of JAK2 V617F kinase co-expressed with these receptors. Signaling assays in transiently transfected HEK293 and γ2A cells as well as stably transduced Ba/F3 cells were used to validate the results of protein-fragment complementation assay.
Results: We have shown stronger interaction between JAK2 V617F and TpoR compared to EpoR or GCSFR at gradually decreasing dosages of the JAK2 V617F. Also, dimerization of JAK2 V617F appeared to be higher in presence of TpoR compared to other receptors tested. Interaction of the JAK2 V617F kinase with the cytokine receptors and its dimerization correlated with the half-lives of the receptors. The more stable TpoR supported prolonged JAK2 V617F and STAT5 activation compared to the less stable receptors EpoR or GCSFR. We have identified the intracellular domain in these receptors responsible for the distinction in their half-lives and made a shorter-lived TpoR and a longer-lived EpoR mutant by adding or removing a lysine, which triggers ubiquitination and degradation. As expected, the stability of the receptors was crucial for inducing pathologic signaling from JAK2 V617F with the less stable TpoR mutant losing its sensitivity to the low levels of JAK2 V617F, and, conversely, the more stable EpoR mutant becoming more sensitive to low JAK2 V617F levels.
Summary and Conclusions: Our results suggest that due to its natural long half-life in the cell and at the cell-surface, TpoR could support prolonged JAK2 V617F dimerization and activation even at the lower level of kinase expression explaining the *in vivo* JAK2 V617F gene dosage effect, where low JAK2 V617F levels are associated with thrombocytosis.

S817

CONSTITUTUIONAL GENETIC VARIATION AT HBS1L-MYB INFLUENCES WHETHER JAK2 V617F MUTATED MPN PRESENT WITH PV OR ET

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Background: Polycythemia vera (PV) and essential thrombocythemia (ET) are characterized primarily by an excess production of erythrocytes and platelets, respectively. Whilst it is accepted that somatic mutations in *JAK2* and other genes drive clonal proliferation, less is known about the factors that determine the precise disease phenotype. Compared to ET, PV is characterized by higher levels of the *JAK2* V617F mutation and much more frequent outgrowth of homozygous mutant clones, however mutation burden alone cannot distinguish the two disorders and it is clear that other factors must also be important. The finding that leukocyte counts in murine *JAK2* V617F retroviral-mediated transfection/transplantation models differ in a strain-dependent manner was the first suggestion that host genetic factors may influence the disease phenotype.
Aims: Our aim is to identify inherited genetic factors that influence whether *JAK2* V617F positive MPN patients develop PV or ET.
Methods: We undertook a two stage genome-wide association study. At stage 1,556 ET patients and 556 PV patients that were positive for *JAK2* V617F and from the UK were genotyped. Following standard quality control, allelic chi-square tests were used to compare SNPs between ET or PV cases against controls (WTCCC2 n=5200). SNPs with subtype specific effects were identified by using Breslow-Day tests to assess the homogeneity of effect sizes. SNPs with significantly different effects in ET and PV cases were followed up at stage 2 by additional genotyping in two independent cohorts of *JAK2* V617F negative cases with either ET or primary myelofibrosis from the UK (n=524), Germany (n=187) and Austria (n=99). At stage 2, controls from the UK (WTCCC2 NBS n=2706) and Bavaria (KORA n=1805) were used for comparison. The final effect size and significance of SNPs was determined by a fixed effects meta-analysis of the 3 cohorts. To investigate SNP function, we used RT-qPCR to analyse gene expression in myeloid progenitors cultured *in vitro* from a series of healthy controls and MPN cases.
Results: After quality control, 490,755 SNPs were tested in 499 ET and 505 PV cases, and 5200 WTCCC2 controls at stage 1. Excluding the *JAK2* region due to recurrent aUPD in PV and to a lesser extent in ET, rs9376092 in the intergenic region between *HBS1L* and *MYB* was identified as having one of the strongest subtype specific effects (Breslow-Day test $p=4.4x10^{-5}$) which increases the risk of ET (allelic chi-square $p=6.1x10^{-7}$, OR=1.42) and may reduce the risk of developing PV (allelic chi-square p=0.068, OR=0.87). After testing stage 2 and using a fixed effects meta-analysis to combine evidence across stages, rs9376092 achieved genome-wide significance (meta-analysis $p=1.8x10^{-11}$, OR=1.35) without heterogeneity between cohorts (Cochran's Q test p=0.50). Using RT-qPCR we associated the rs9376092 risk allele with reduced expression of *MYB* (5.8-9.7 fold) and, less prominently, *HBS1L* (1.9-2.4 fold) in healthy individuals. We also found that *MYB* expression in ET was significantly lower in *JAK2* V617F-heterozygous BFU-E colonies compared to *JAK2*-wild type colonies from the same patient (Mann-Whitney test P=0.0009; q=0.01) but this difference was not seen in colonies from PV cases.

Summary and Conclusions: We conclude that constitutional genetic variation in the intergenic region between *HBS1L* and *MYB* influences whether *JAK2* V617F mutant MPN develop ET or PV. In *JAK2* V617F-negative MPN, the same variation predisposes to the development of ET. Our findings thus link constitutional differences in *MYB* expression to both predisposition to MPN and to MPN phenotype.

S818

CALCIUM INFLUX IS SHAPED BY THE MUTATIONAL STATUS IN CIRCULATING CD34+CELLS OF PATIENTS WITH MYELOFIBROSIS

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Background: Mutations of the JAK2, CALR, and MPL are detectable in the hematopoietic cells of about 90% of patients with myelofibrosis. Whereas JAK2 and MPL mutations are represented by point mutations resulting in a gain of function of the gene, CALR mutations are mainly represented by either a 52-bp deletion or a 5 bp insertion, resulting in a novel C-terminal peptide sequence. This domain regulates the Ca2+-binding activity of the protein and the two mutations are predicted to modify the Ca2+storage capacity in the endoplasmic reticulum (ER). The ER-Ca2+content of a cell depends on the balance between the so-called Store Operated Calcium Entry (SOCE) and the ER-dependent Ca2+release and, in turn, it regulates many important cell functions, such as proliferation, apoptosis and gene expression. In physiologic conditions, most of the ER-bound Ca2+is stored in the smooth-ER (s-ER). Little is known about the effects of the mutations on intracellular Ca2+homeostasis in CD34+cells of patients with myelofibrosis.
Aims: We have investigated the intracellular Ca2+release and SOCE in circulating CD34+cells of patients with MF with either the V617FJAK2 mutation (n=7) or a mutation of the CALR gene (n=7), and of healthy subjects (HS, n=4). We have also studied the ultrastructural extension of the s-ER of CD34+cells of the same subjects.
Methods: After loading CD34+cells with 4 mcM fura-2 acetoxymethyl ester, depletion of intracellular Ca2+stores was induced by adding 10 mcM cyclopiazonic acid (CPA) to a 0 Ca2+bathing medium. Ca2+was then added to the extracellular solution eliciting a rise in [Ca2+]i due to Ca2+influx through open store-operated Ca2+channels. The amplitude of the peak Ca2+response to CPA, was measured as the difference between the ratio at the peak and the mean ratio of 1 min baseline before the peak. SOCE amplitude was measured as the difference between the peak and the mean ratio of 1 min baseline recorded before the readdition of Ca2+. Extension of sER was assessed by electron microscopy (EM) followed by analysis by ImageJ software. Data are expressed as mean+/-SD.
Results: Resting levels of Ca2+of CALR-mutated CD34+cells were not statistically different from those of JAK2-mutated cells, but higher compared to those of HS. CALR-mutated CD34+cells released more Ca2+from ER stores compared to JAK2-mutated cells (60.6 nM±30.9 *vs* 26.7 nM±24.9, respectively, P=0.038) and compared to HS (27.6±19.8 nM, P=0.041 compared to CALR-mutated cells). SOCE of CALR-mutated CD34+cells (318.5 nM±206.1) was slightly increased compared to JAK2-mutated patients (242.8±146.8) and lower than SOCE of CD34+cells of HS (104.3 nM±66.5), without reaching a statistical significance. At EM evaluation, the extension of vescicles of the sER was higher in CALR-mutated CD34+cells compared to JAK2-mutated cells (0.82 *vs* 0.24 mcm, respectively, P=0.0001) and to HS-cells (0.101 mcm, P=0.0007).
Summary and Conclusions: Our results show that CALR mutation in CD34+cells of patients with PMF is associated with a rearrangement of intracellular Ca++balance, with a significant difference in Ca++release compared to that observed both in JAK2-mutated- and HS-CD34+cells. In keeping with the higher Ca2+release, we observed an increased extension of s-ER. Considering the pivotal role of Ca2+in regulating many cell functions, including proliferation and differentiation, these results seem to suggest that CALR mutation could be implicated in the myeloproliferative phenotype that characterizes PMF patients carrying CALR mutations by interfering with the signaling role of Ca2+.

S819

ROLE OF TREATMENT ON THE DEVELOPMENT OF SECONDARY MALIGNANCIES IN PATIENTS WITH ESSENTIAL THROMBOCYTEMIA

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Background: Survival of essential thrombocytemia (ET) patients during the first 10 years of their disease is similar to that of the general population. While it is well known that alkylating agents (given alone or sequentially) increase the risk of transformation into an acute myeloid leukemia, the specific role of different therapies on the development of secondary malignancies (SM) in ET patients is still under investigation.
Aims: To retrospectively evaluate the role of different treatments on the development of SM in a large cohort of ET patients followed over a 30-year period.
Methods: Data from a series of 1026 ET patients diagnosed between 1980 and 2000, and followed at 11 Hematology Centers of the Lazio region in Central Italy, were collected. The median age at ET diagnosis was 62 years (range 17-93); there were 392 males, 634 females; the median follow-up of the entire population is 6.2 years (range 0.1–32). Among the whole population, 39% of patients carried the JAK2V617F mutation, while 27% were wild type; for 34% of cases, this datum is missing. With regard to adjunctive risk factors (smoke, diabetes, hypertension, dyslipidemia), 64% of patients presented at least one risk factor (42%, 1; 22% >1); 28% of patients did not present adjunctive risk factors and for 8% of cases this datum is missing. Smoke was present in 25% of cases, hypertension in 43%, diabetes in 7%, dyslipidemia in 15%. Sixty three of the 1026 patients (6%) developed 64 SM during the follow-up, after a median time of 50 months (range 2-158) from diagnosis. The observed SM were grouped as follows: genito-urinary 15, breast 14, non-melanoma skin cancer 5, lung 11, gastro-intestinal 8, hematologic 5, thyroid 3, central nervous system 1, soft tissue 1 and unknown 1. Taking into consideration the different treatment approaches, we divided our population into 5 different groups: group 0, untreated patients; group 1, patients treated with hydroxiurea (HU) alone or in combination/sequentially with interpheron α (IFN)/anagrelide (ANA); group 2, patients treated with alkylating agents (ALK) alone or in combination/sequentially with IFN/ANA; group 3, patients treated with ALK+HU sequentially; group 4, patients treated with ANA and/or IFN only.
Results: With regard to the exposure time to drugs, a statistically significant difference was found between the groups HU *vs* ALK (p=0.036), HU *vs* ALK+HU (p=0.006) and ALK+HU *vs* ANA/IFN (p=0.006). No differences were found for the groups HU *vs* ANA/IFN, ALK *vs* ALK+HU, ALK *vs* ANA/IFN. In univariate analysis, the following variables were considered: gender, age, different therapeutic approaches, exposure time, presence of adjunctive risk factors. A statistically significant difference was found only for gender (M *vs* F: p=0.035) and age (>60 years *vs* <60years: p=0.0001). In multivariate analysis, a statistically significant difference was maintained for both gender and age [gender HR1.7 (CI 95% 1.037-2.818) p 0.035; age HR 4.190 (CI 95% 2.308-7.607) p=0.0001)].

Table 1.

Groups	Number of patients	SM No	SM Yes	Median exposure time to drugs (years)	Median FU (years)
No therapy	220 (21.5%)	209 (95%)	11 (5%)	-	5 (0.1-23)
HU	641 (62.5)	599 (93%)	42 (7%)	4 (0.1-30)	6 (0.1-32)
ALK	26 (2.5%)	24 (92%)	2 (8%)	8 (0.4-23)	12 (1-30)
ALK+HU	86 (8.5%)	79 (92%)	7 (8%)	9 (0.6-24)	10 (2-26)
ANA/IFN	53 (5%)	51 (96%)	2 (4%)	4 (0.1-20)	8 (2-21)

Summary and Conclusions: In our series of 1026 ET patients followed over a 30-year period, the different therapies administered, comprising HU and ALK, do not appear to have impacted on the development of SM. A similar prevalence of SM was observed also in untreated patients. The only two variables which showed a statistical significance were male gender and age >60 years.

Towards targeted therapy in ALL

S820

A FUNDAMENTAL ROLE OF CXCR4 IN T-ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive malignancy resulting from the leukemic transformation of T-cell progenitors. Despite progress in intensive chemotherapy, 25% of pediatric and over 50% of adult patients with T-ALL show resistance to therapy and relapse. The pathophysiology of T-ALL suggests a significant deregulation of cell migration and tissue infiltration. Previously we showed that the serin-threonin protein phosphatase Calcineurin (Cn) regulates the physical and functional interaction that T-ALL cells establish with other cells in the tumor microenvironment, notably their adhesive and migratory properties, among other phenotypic traits including increased apoptosis, inhibition of cell proliferation and ultimately inhibition of Leukemia Initiating Cell (LIC) activity (Gachet *et al.*, 2013).
Aims: The aim of this study is to dissect the molecular mechanisms underlying the interactions between T-ALL cells and their microenvironment which are perturbed by Cn inactivation, and explore their therapeutic importance.
Methods: NOTCH-induced murine T-ALL in which Cn can be genetically inactivated were used to study the effect of Cn deletion on leukemic cells migration on stromal cells and on CXCR4 recycling. Retroviral mediated transduction of T-ALL cells was used to perform rescue experiments with both CXCR4 and Cortactin (Cttn). *In vivo* syngeneic transplantation studies were performed to analyse LIC activity of T-ALL cells, and two-photon confocal microscopy to study their homing ability. Sh-RNA lentiviral transduction of T-ALL cells was used to study the effect of CXCR4 knock-down on murine and human T-ALL.
Results: We identify CXCL12/CXCR4 as a highly deregulated pathway in T-ALL upon Cn deletion. Indeed, we demonstrate that Cn has a central role in CXCL12 signaling by regulating CXCR4 recycling at the cell surface. Farther, we link regulation of CXCR4 cell surface expression and CXCL12 responsiveness to the Cn-dependent expression of the actin binding protein Cttn in T-ALL. Moreover, we demonstrate that CXCL12/CXCR4 pathway not only regulates T-ALL migratory properties, but also their survival and proliferation *ex vivo*, and their homing and LIC activity *in vivo* both in NOTCH-induced mouse T-ALL and in human T-ALL xenografts.

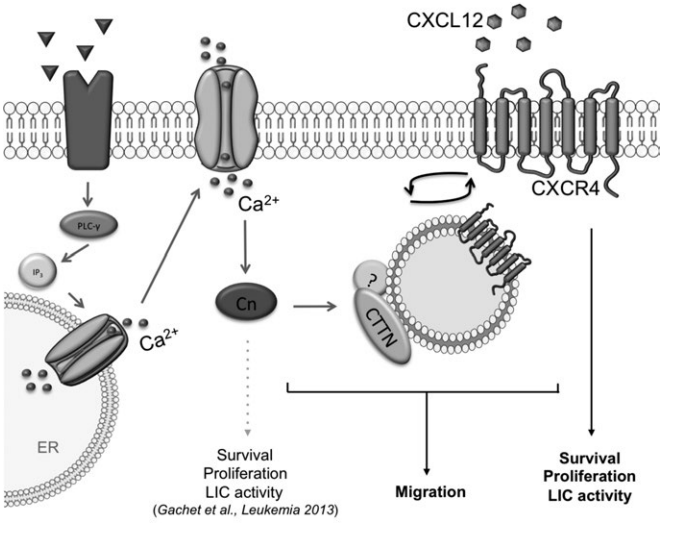

Figure 1.

Summary and Conclusions: Our results reveal a Cn-dependent regulation of CXCR4 surface expression and signaling, which plays a fundamental role in T-ALL migration. Moreover, our findings identify CXCR4 as an important regulator of T-ALL homing and transplantation and call for clinical trials targeting the calcineurin-CXCR4 axis to interfere with leukemogenesis and to improve current chemotherapy regimens in T-ALL.

S821

MEK AND PI3K-AKT INHIBITORS SYNERGISTICALLY TARGET HYPERACTIVATING IL7-RECEPTOR SIGNALING MUTATIONS IN T-CELL ACUTE LEUKEMIA

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Background:
Pediatric T-cell acute lymphoblastic leukemia patients harbor mutations in *IL7Ra* or downstream molecules encoded by *JAK1*, *JAK3*, *N-RAS*, *K-RAS*, *NF1*, *AKT*, and *PTEN*. These mutated signaling molecules can contribute to leukemia by disturbing a multitude of cellular processes such as the cell cycle, epigenetics, apoptosis, or affecting other important signal transduction pathways.
Aims: We aimed to determine the overall incidence of mutations in *IL7Ra* and downstream signaling components in a large cohort of T-ALL patients. Additionally, we were interested to know whether these mutations occur in a mutually exclusive manner. In order to find better treatment options for patients with these mutations, we analyzed the effect of selected IL7Ra-pathway inhibitors—individually and in combinations—on downstream signaling and cytotoxicity for each of the mutations using our expression system in Ba/F3 cells.
Methods: We screened 146 pediatric T-ALL patient samples for mutations in the FERM, pseudokinase and kinase domains of the Janus kinase gene family (*JAK1-3, TYK2*) and hotspot regions of *N-RAS* and *K-RAS*. The three-dimensional JAK1 model was superimposed on the TYK2 pseudokinase-kinase crystallographic structure. We developed a doxycycline-inducible system by adapting the IL3-dependent Ba/F3 cell line to express mutant or wild type genes upon induction by doxycycline. Various IL7Ra-pathway inhibitors were tested using this system, and the synergy of combined inhibitors was determined by comparing the dose-response curve of different ratios of IC_{50}-based inhibitor concentrations to the curves for each of the single inhibitors. The Combination Index was calculated using Calcusyn™ software.
Results: *IL7Ra*, *JAK*, *RAS*, *AKT* and *PTEN* mutations are present in 44% of T-ALL patients and occur in a predominantly mutually exclusive fashion, suggesting they represent a single mutant signal transduction route. We found *JAK1*, *JAK3* and *RAS* mutations as previously reported, but also identified new *JAK1* mutations including V427M, L624YPILKV, E668Q, P815S, and T901G. Our novel three-dimensional model of JAK1 provides insight on how these JAK mutations result in constitutive kinase activity. Amino acid residues that regulate or facilitate direct interactions between the pseudokinase and the kinase domains are frequent targets of *JAK1* and *JAK3* mutations in T-ALL. In our doxycycline-inducible IL3-dependent Ba/F3 system, expression of mutant genes—in contrast to the wild type genes—transforms Ba/F3 cells by supporting IL3-independent growth, and by activating the RAS-MEK-ERK and PI3K-AKT pathways. We used this system to test the sensitivity to pharmacological inhibitors; IL7Ra and JAK mutant Ba/F3 cells are sensitive to JAK inhibition, so JAK inhibitors such as Ruxolitinib may offer therapeutic potential for IL7Ra, JAK1 or most JAK3 mutated T-ALL patients. However, IL7Ra and JAK mutants are relatively resistant to downstream RAS-MEK-ERK or PI3K-AKT-mTOR inhibition, indicating that inhibition of either of these downstream pathways alone is insufficient. Inhibitor combinations of both pathways completely block IL7R signaling irrespective of the level of activation by specific mutations. The RAS and AKT mutants respond to RAS-MEK and PI3K-AKT-mTOR inhibition, respectively, but are insensitive to JAK inhibition.
Summary and Conclusions: We show that the combined inhibition of MEK and PI3K/AKT leads to strong and synergistic cytotoxic effects in the IL7Ra and JAK mutants and efficiently blocks signaling downstream of both pathways. Since leukemia often depends on one or both pathways, the cytotoxic efficacy of synergistic MEK and PI3K inhibition should be further explored in clinical trials for IL7Ra mutant leukemia.

S822

CONCURRENT CREBBP AND KRAS MUTATIONS ARE ASSOCIATED WITH A DISMAL OUTCOME IN CHILDREN WITH HYPERDIPLOID LEUKEMIA IMPLYING A SYNERGISTIC FUNCTION IN RELAPSE EVOLUTION

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Background: High hyperdiploidy (HD) denotes the largest B cell precursor acute lymphoblastic leukemia (BCP ALL) subgroup in children and adolescents. Patients usually present with low risk features and respond well to treatment. The relative proportion of relapses (up to 15%) is small but constitutes the largest genetically homogeneous fraction in the BCP ALL cohort. ≈50% of HD ALL harbor activating mutations in RTK/Ras pathway genes, which, however,

do not qualify as relapse predicting factor based on their high instability at relapse. By contrast, mutations in the transcriptional coactivator *CREBBP* (*CBP*) occur particularly in ALL relapsing cases, attenuate the function of the encoded protein and reduce acetylation of histone and non-histone proteins. Previous studies have focused on conventional sequencing of selected RTK/Ras pathway genes including small numbers of matched diagnosis and relapse samples only.

Aims: Our aim was, therefore, to investigate the mutational landscape of HD ALL using high throughput sequencing in a large cohort of such relapsing cases, in order to infer and compare the clonal composition of diagnostic and relapse samples, and test for clinical association.

Methods: We used whole exome sequencing (WES; n=19) and targeted sequencing in these and additional 81 relapsing cases (including 50 cases with matched diagnosis and relapse samples) as well as 51 non-relapsing ones. Cases were treated according to AIEOP/BFM-ALL95 and 2000, CoALL-08-09, and ALL-Rez BFM 2002 protocols.

Results: We detected at diagnosis 12 (median, range 3-23) and at relapse 25 (range 10-90) non-silent mutations per case. They target most frequently *KRAS*, *NRAS*, *PTPN11* and *FLT3* as well as *CREBBP*. Ras mutations were found in >80% of cases, frequently affecting only small clones with high inter- and intragenic heterogeneity at initial diagnosis; ≈50% were lost at relapse. RTK/Ras mutations were similarly frequent at relapse, when the vast majority of them define the major clone. *CREBBP* mutations were present in 18% of cases at diagnosis and 30% at relapse. They were already part of the major clone in >50% of cases at diagnosis, but were virtually always confined to the predominant relapse clone. Our most salient finding was the coexistence of *CREBBP* and *KRAS* mutations in the major clone of HD ALL relapses (P<0.001). Despite a similar frequency of *KRAS* and *NRAS* mutations, only the *KRAS* ones concurred with *CREBBP* mutations. This observation implies that these two mutations exert an interdependent function and cooperate in a RAS isoform-specific and synergistic fashion under the selective pressure of the applied chemotherapy. Clinically, RTK/Ras pathway and *CREBBP* mutated relapses showed only a slight prevalence of occurring early while *KRAS* mutated relapses usually did develop early; in line, relapses with concurrent *CREBBP* and *KRAS* mutations appeared earlier than those in the remaining cases (*P*=0.012*)*. While cases with *CREBBP* or *KRAS* mutations or double mutant leukemias had a significantly poorer outcome than wt cases, the cumulative incidence of relapses of the double mutant cases tended to be associated with an increased incidence of subsequent relapses.

Summary and Conclusions: *KRAS* and *CREBBP* double mutant HD ALL relapse cases are amongst those with the highest risk and are therefore suitable candidates for novel therapeutic approaches using histone deacetylase inhibitors and Ras pathway inhibitors. Since somatic *CREBBP* mutations are also present in a small number of non-relapsing HD ALL cases, they cannot be used as prognostic markers.

S823

MEK INHIBITION IS A PROMISING THERAPEUTIC STRATEGY FOR MLL-REARRANGED INFANT ALL PATIENTS CARRYING RAS MUTATIONS

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Background: Acute Lymphoblastic Leukemia (ALL) in infants is characterized by a high incidence (~80%) of chromosomal rearrangements of the Mixed Lineage Leukemia (*MLL*) gene. *MLL*-rearranged (*MLL*-r) infant ALL patients are challenged by a very poor prognosis (*i.e.* 30-40% 5-year EFS), hence the need for better risk stratification and improved therapeutic solutions is evident. We recently screened a relatively large cohort (n=109) infant ALL patients (all enrolled in INTERFANT treatment protocols) for the presence of *KRAS* and *NRAS* mutations and found that the incidence of such mutations ranges between 14-24%, depending on the type of *MLL* translocation. Moreover, these mutations were found to represent independent predictors of exceedingly poor prognosis; all *RAS* mutation-positive *MLL*-r infant ALL patients deceased within 3 years from diagnosis.

Aims: We aimed to identify a therapeutic strategy to improve the prognosis of *MLL*-r infant ALL patients carrying *RAS* mutations.

Methods: 8 small molecule inhibitors against different RAS-pathway components were selected and tested for anti-leukemic activity against the *MLL*-r ALL cell lines SEM and RS4;11 (*RAS*^{wt}) and KOPN8 (*RAS*^{mut}) using MTS cell viability assays. Next, primary infant ALL samples (n=20) all carrying *MLL* translocation t(4;11) (giving rise to the MLL-AF4 fusion protein) either with (n=6) or without (n=14) *RAS* mutations were exposed to these inhibitors in MTT cytotoxicity assays. In addition, we assessed the RAS activity in *RAS* mutated and wild-type *MLL*-r infant ALL cells, and analysed downstream MEK and ERK activation both in the absence and presence of the MEK inhibitors. Furthermore, combination treatment of MEK inhibitors and prednisolone, the spearhead drug for ALL treatment regimes, was investigated in SEM and KOPN8 using the MTS assay.

Results: We found that the MEK inhibitors MEK162, Selumetinib, and Trametinib effectively reduced the viability of KOPN8 cells (*RAS*^{mut}), whereas SEM and RS4;11 cells (*RAS*^{wt}) largely remained unaffected. In line with this, MLL-AF4+infant ALL patient samples carrying *RAS* mutations were significantly more sensitive to these MEK inhibitors when compared with patients carrying wild-type *RAS* genes: LC_{50} values for MEK162 were 0.04 *vs.* 26.9 μM (p<0.01), for Selumetinib 0.04 and 23.7 μM (p<0.01), and for Trametinib 0.01 *vs.* 26.5 μM (p<0.01), respectively. Furthermore, the presence of *RAS* mutations in primary *MLL*-r infant ALL samples was associated with significantly increased RAS activity, as determined by immunoprecipitation of GTP-bound RAS. Remarkably, however, enhanced RAS activation was not demonstrated by increased downstream ERK phosphorylation, while a slight increase of MEK phosphorylation was observed. Yet, MEK inhibitor exposure in both KOPN8 and SEM cells resulted in nearly complete abrogation of ERK phosphorylation, without affecting total ERK protein levels, suggesting that the loss of ERK activation plays an important role in the observed anti-leukemic effects. Furthermore, MEK162 and Selumetinib, seemed to induce accumulation of phosphorylated MEK. Additionally, MEK inhibitor treatment in both SEM and KOPN8 synergistically enhanced sensitivity towards prednisolone. Interestingly, a subgroup of patient samples (n=5) with wild-type *RAS* also showed sensitivity towards MEK inhibition, similar to the primary cells with *RAS* mutations. However, this observation could not be explained by increased RAS activation, nor by the phosphorylation levels of either MEK or ERK.

Summary and Conclusions: Our data show that MEK inhibition represents a promising therapeutic approach for *MLL*-r infant ALL patients carrying additional *RAS* mutations. Furthermore, the mechanism of action provoked by these MEK inhibitors seems to involve abrogation of ERK phosphorylation, but the initial levels of ERK phosphorylation did not correlate with MEK inhibitor sensitivity, and has no predictive value. Moreover, the prednisolone sensitization induced by MEK inhibitor exposure, further illustrates the potential value of MEK inhibition for the treatment of *MLL*-r infant ALL. Currently we are in the process of testing the efficacy of the above mentioned MEK inhibitors *in vivo* using a xenograft mouse model, while further elucidation of the molecular mechanisms underlying the anti-leukemic effects of these inhibitors in *MLL*-r ALL cells are in progress.

S824

SMAC MIMETICS KILL REFRACTORY PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA THROUGH PARALLEL INDUCTION OF APOPTOSIS AND NECROPTOSIS

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Background: Acute lymphoblastic leukemia (ALL) is the most common childhood cancer, and although current drug treatment is effective in most cases there are few effective second-line drugs available for drug-resistant disease. SMAC mimetics (SMs) are an emerging class of novel chemotherapeutics, which activate cell death by inhibiting key anti-apoptotic cellular inhibitors of apoptosis proteins (cIAP1, cIAP2, and xIAP).

Aims: To elucidate the potency and mechanisms of cell death induction by SMAC mimetics in pediatric ALL.

Methods: Using an *in vitro* screening approach, we assessed the sensitivity of 51 patient derived ALL xenografts to a preclinical SM compound (Birinapant). We applied specific inhibitors of apoptotic and necroptotic cell death as well as a CRISPR based genetic approach to map the mechanisms of cell death induction by SM treatment in patient derived ALL samples as well as ALL cell lines. Finally, we examined the ability of SM to selectively kill ALL cells in an *in vivo* xenograft model of refractory ALL.

Results: We show that a significant proportion of patient-derived ALL samples are highly sensitive to SM-induced death. SM sensitivity does not correlate with patient risk grouping and includes both relapsed and refractory samples. Pharmacological inhibition of distinct cell death pathways indicates that SM treatment induces both apoptotic and necroptotic cell death. While a minority of ALL samples showed either apoptotic or necroptotic features, a majority of cases had simultaneous induction of both cell death phenotypes. Examination of distinct cell death parameters such as DNA-fragmentation and loss of plasma membrane integrity by FACS and microscopy suggests that both apoptosis and necroptosis occur simultaneously within a given cell population after SM treatment. Lenti-CRISPR mediated gene disruption in ALL cell lines demonstrates that irrespective of the cell death phenotype, induction of death requires Rip1-kinase in all cases. Rip1 in turn activates Rip3-dependent necroptosis and/or Caspase-8-dependent apoptosis. Blockade of both death pathways through gene disruption or inhibitor treatment was required to restore cell viability after SM treatment. Strong anti-leukemic activity of SM was confirmed *in vivo* in a xenograft mouse model of refractory ALL. Treatment of transplanted

mice with SM not only delayed leukemia progression, but also completely eliminated leukemia burden in established disease for several weeks following treatment.

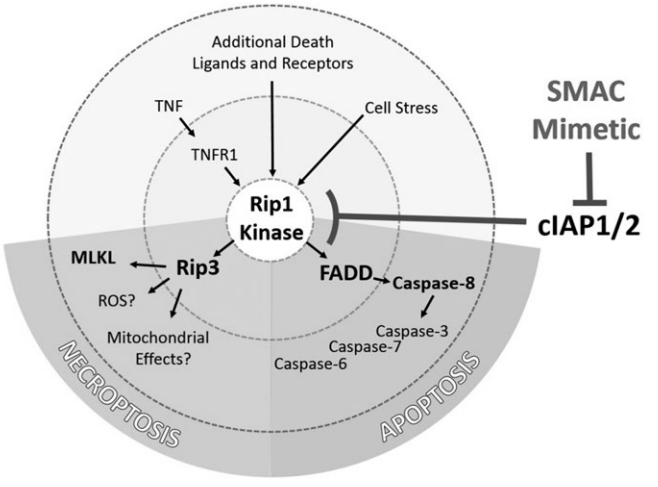

Figure 1.

Summary and Conclusions: This data shows that apoptotic and necroptotic cell death pathways are not mutually exclusive phenomena, but can be activated simultaneously in chemotherapy-resistant ALL. Because SM can induce both pathways of cell death in parallel, these agents have a strong potential for anti-leukemic therapy in refractory childhood ALL.

Biology and clinics of bone marrow failure syndromes and PNH

S825

CELLULAR AND MOLECULAR CHARACTERIZATION OF ERYTHROID DEFECTS IN DIAMOND BLACKFAN ANEMIA

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Background: Diamond-Blackfan anemia (DBA) is a rare, congenital bone marrow failure syndrome with severe anemia, congenital anomalies, predisposition to cancer and variable severity. Approximately 65% of DBA patients have heterozygous autosomal dominant mutations or deletions in one of >15 ribosomal protein (RP) genes encoding both large and small subunit proteins. In addition, recent studies have identified mutations in the *GATA1* gene in three DBA families. Despite targeted sequencing of all 80 RP genes, the causal abnormalities in the remaining ~35% of DBA patients are unknown as is the mechanism by which RP defects cause erythroid failure.
Aims: 1. To develop an *in vitro* system to culture erythroid cells from DBA patient peripheral blood CD34+cells. 2. To use transcriptional profiling of DBA erythroid cells to determine the disrupted pathways. 3. To perform whole exome sequencing on DBA patients without RP mutations to determine the genetic defects.
Methods: We developed a 2-step culture system that expands CD34+cells (7 days), then induces differentiation of erythroid cells (7 days). This system yields >10^7 CD235+erythroid cells from ~10^4 CD34+cells collected from 10 ml of healthy control peripheral blood. RNA isolated from CD235+erythroid cells was profiled on Affymetrix GeneChip Human Gene ST Arrays and by RNASeq. Whole exome sequencing (WES; Illumina HySeq; 80X coverage) was performed on DBA probands, an unaffected sibling and their parents. After filtering out common variants in the 1,000 Genomes and ClinSeq databases we used the VarSifter program to evaluate candidate causal variants for further study.
Results: In contrast to control cells, cells from 9 different DBA patients (5 RP mutations, 3 unknown, 1 with a *GATA1* mutation) exhibited a significantly reduced growth rate, generating ~100-fold fewer CD235+erythroid cells, with an ~2-day delay in the acquisition of the CD235 marker. Using flow cytometry, we isolated populations of CD235+erythroid cells from both control and patient cell cultures for RNA profiling. RNA from DBA patients showed a distinct transcriptional profile compared to control samples and the sample from the *GATA1* patient. Gene ontogeny analysis showed increased expression of genes associated with cellular stress and decreased expressed expression of genes associated with cell cycle progression and protein translation in the RP patients. The GATA1 patient's profile identified decreased expression of red cell structural genes as well as genes in the heme biosynthesis pathway. In one DBA family, we identified candidate autosomal recessive mutations in the *MCM2* and *POLR3B* genes. Normal CD34+progenitor cells expressing shRNAs targeting *MCM2* and *POLR3B* mRNA demonstrated 60-90% knockdown of each. *POLR3B* knockdown cells showed no inhibition of erythroid differentiation, indicating *POLR3B* is an unlikely DBA candidate gene. In contrast, knockdown of *MCM2* ddemonstrated a significant reduction in BFU-E (but not CFU-GM) colony formation. Similar to DBA patient cells, the proliferation of *MCM2* knockdown cells was reduced and the differentiation of CD235+erythroid cells was delayed. *MCM2* knockdown cells were arrested at the G_1/S checkpoint in the cell cycle. As confirmation, mice deficient in *Mcm2* have a severe bone marrow failure syndrome with erythroid defects.
Summary and Conclusions: Our *in vitro* differentiation system is a powerful tool to study erythropoiesis in DBA patients using small volumes of peripheral blood. We have used this system to demonstrate that the common forms of DBA, associated with defects in ribosomal genes, have defects in cell cycle and protein translation that differ significantly from the defects observed in the *GATA1* DBA patient. Finally, we have shown that mutation in the *MCM2* gene is associated with DBA in at least one family, which represents the first autosomal recessive mutation identified in DBA patients.

S826

ELTROMBOPAG ADDED TO STANDARD IMMUNOSUPPRESSION AS FIRST TREATMENT IN SEVERE APLASTIC ANEMIA

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Background: Immunosuppressive treatment (IST) for severe aplastic anemia (SAA) has a 60-65% rate of overall hematologic response (OR), of which only about 10% are complete (CR). Eltrombopag, a synthetic thrombopoietin receptor agonist (TPO-RA), has activity in refractory SAA, producing multilineage hematologic responses in about 40% of patients.
Aims: We conducted an investigator-initiated phase II, single-center trial to test the efficacy of adding eltrombopag to h-ATG/CsA (clinicaltrials.gov, NCT01623167).
Methods: Patients with treatment-naïve SAA were enrolled from July 2012 to November 2014. All subjects received standard h-ATG and CsA. To determine the optimum regimen, eltrombopag was administered at 150 mg daily to two consecutively enrolling cohorts (see Table). The primary endpoint was CR at 6 months. Both cohorts were powered to detect a ≥30% CR rate at 6 months for comparison with our historical rate of 10%. Our historical data suggest that CR is a surrogate for late events since it is associated with a low rate of evolution to monosomy 7/acute leukemia and correlates with excellent long-term survival.
Results: Thirty subjects enrolled in the first cohort had a median age of 39 years (12-72); thirteen (43%) had very severe disease (ANC<200/µl), and 8 (27%) had PNH clones ≥1%. Eltrombopag was well tolerated when combined with CsA, and only three patients discontinued treatment before 6 months. CR at 6 months was 33% (95% CI, 15-51%), significantly higher than our historical rate of 10% (p<0.001). OR at 6 months was 80% (CI, 65-95%), exceeding the historical rate of 60% (p=0.040). Median increases in blood counts among responders at 6 months (n=24) were: absolute neutrophil count (ANC) +1240/µl; hemoglobin +3.8 gm/dL; and platelets +87,000/µl. Median time to achieve transfusion independence was 32 days for platelets and 39 days for red cells. Serial BM biopsies showed improved cellularity in 25 cases without increased fibrosis. Median increase in BM CD34+cells, as measured by flow cytometry, was 34-fold from baseline to 3 months, and 23-fold from baseline to 6 months (p<0.0001). Primitive hematopoietic progenitors were serially measured in 4 patients, detecting HSC (CD34+CD38-CD45RA-CD90+CD49f+Rho^{lo}) and MPP (CD34+CD38-CD45RA-CD90-CD49f-) by flow cytometry. Undetectable at baseline, these cells constituted 4-48 HSC and 1-27 MPP per 100,000 CD34+cells 6 months after therapy. The first cohort has been followed for median 24 months (range 15-31m); there have been 3 clonal evolution events, all detected in responders: one patient had complex cytogenetics (t(3;3)(q21;q26)) with BM dysplasia followed by increased blasts, and deletion 13q in one subject and trisomy 15 in another, neither with BM dysplasia. Enrollment to the second cohort, in which eltrombopag is stopped at 3 months, is complete and 23 subjects are currently evaluable at 6 months. OR at 6 months is 87% (20/23) and CR rate at 6 months is 22% (5/23) confirming results observed in the first cohort. One clonal evolution event (deletion 7p) occurred at 6 months in a responder and relapse of pancytopenia soon followed.

Tabella 1. Hematologic Response.

	3 months *N (%)*	6 months *N (%)*
Cohort 1	30	30
OR	23 (77)	**24 (80)**
PR	18 (60)	14 (47)
CR	5 (17)	**10 (33)**
Cohort 2	31	23
OR	24 (77)	**20 (87)**
PR	16 (52)	15 (65)
CR	8 (26)	**5 (22)**

Cohort 1: eltrombopag d14 - 6mos.
Cohort 2: eltrombopag d14 - 3mos.

Overall response (OR) = blood counts no longer meeting criteria for SAA
Partial response (PR) = blood counts not meeting criteria for SAA or CR
Complete response (CR) = ANC ≥1,000/µl, hemoglobin ≥10 gm/dL, and platelets ≥100,000/µl.

Summary and Conclusions: The addition of eltrombopag to IST increases overall and complete hematologic response rates. Marked increase in CD34+cells, the appearance of multipotent progenitor cells, and rapid blood count recovery support a mechanism of action of direct stimulation of stem and progenitor cells by eltrombopag.

S827

COMBINATION OF MINOR PAROXYSMAL NOCTURNAL HEMOGLOBINURIA CLONES AND LYMPHOCYTE TELOMERE LENGTH PREDICT RESPONSIVENESS TO IMMUNOSUPPRESSIVE THERAPY IN PEDIATRIC APLASTIC ANEMIA

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Background: Acquired aplastic anemia (AA) is characterised by a hypo-cellular marrow and peripheral blood cytopenias. Unfortunately, allogeneic bone marrow transplantation is limited by the availability of human leukocyte antigen (HLA)-matched donors. Most AA patients receive immunosuppressive therapy (IST) combining anti-thymocyte globulin (ATG) and cyclosporine A (CyA). The presence of minor paroxysmal nocturnal hemoglobinuria (PNH) clones and a short telomere length (TL) were identified as predictive biomarkers of IST responsiveness.
Aims: To assess whether combining minor PNH clones and TL could predict IST response with greater accuracy.
Methods: We enrolled 118 children (67 boys and 51 girls) diagnosed with acquired AA in Japan between July 2001 and November 2013. Fifty-three patients received horse ATG (Lymphoglobulin, 15mg/kg/day for 5 days) and 65 received rabbit ATG (*Thymoglobulin*, 2.5−3.75 mg/kg/day for 5 days). All patients received CyA (6 mg/kg/day) starting on day 1 for at least 180 days. The dose of CyA was adjusted to maintain trough levels of 100–200 ng/ml. Written informed consent was obtained from the parents of all patients. This study was approved by the ethics committee of Nagoya University Graduate School of Medicine. A flow cytometry assay was used to detect PNH-type granulocytes and red blood cells (RBCs). The presence of >0.005% $CD13^+CD55^-CD59^-$ granulocytes and/or >0.010% glycophorin $A^+CD55^-CD59^-$ RBCs was defined as PNH clones positive (PNH^+). Cut-off values were determined using receiver operator characteristic (ROC) analysis. The telomere length of peripheral lymphocytes was measured by flow-fluorescence *in situ* hybridization (flow-FISH). According to ROC analysis, we defined −1.21 SD of age-adjusted controls as the cut-off value.
Results: Patients' median age when IST was administered was 9.7 years (range, 0.9–16.0 years), disease was moderate-to-very severe, and all three categories of disease severity were well represented. The causes of AA were idiopathic in 103 patients and hepatitis-associated in 15 patients. Forty-eight patients (41%) carried PNH^+clones. The median TL of AA patients was −1.08 standard deviation (SD) (range, −4.01–+3.01 SD). Overall, 63 patients (53%) responded to IST after 6 months. Multivariate logistic regression analysis identified WBC ≥2.0 × 10^6/L (OR, 2.82; 95% CI, 1.13–7.03; p=0.026), absence of PNH^+clones (OR, 4.66; 95% CI, 1.81–12.00; p=0.001), and a shorter TL (OR, 4.10; 95% CI, 1.66–10.10; p=0.002) as independent unfavorable predictors of response to IST at 6 months. Accordingly, the cohort was stratified into unfavorable group (PNH^- and a shorter TL, n=41) and favorable group ($PNH^+$and/or a longer TL, n=77). The response rate at 6 months of the unfavorable group was significantly lower (22%) compared with that of the favorable group (70%) (p <0.001). Five-year transplantation free survival (TFS) and failure free survival (FFS) were significantly lower in the unfavorable group compared with those in the favorable group [TFS, 53% (95% CI, 34%–68%) *vs.* 73% (95% CI, 60%–82%); p=0.010; FFS, 24% (95% CI, 11%–39%) *vs.* 53% (95% CI, 40%–65%); *p* <0.001]. However, no difference was observed in 5-year overall survival (OS) between groups [unfavorable group, 98% (95% CI, 84%–100%) *vs.* favorable group, 96% (96% CI, 88%–99%); p=0.743], possibly because of effective salvage hematopoietic stem cell transplantation (HSCT).
Summary and Conclusions: The current study reveals that PNH^- patients with shorter TL are less likely to respond to IST. Our findings suggest that combining these two markers may help to provide the appropriate treatment option to the patients and prevent adverse events due to unnecessary treatment. Despite the risk of complications, unrelated or mismatched related donor transplantation may be considered as a front-line therapy for patients in the unfavorable group lacking HLA-matched sibling donors.

S828

A SUBCUTANEOUSLY ADMINISTERED RNAI THERAPEUTIC (ALN-CC5) TARGETING COMPLEMENT C5 FOR TREATMENT OF PNH AND COMPLEMENT MEDIATED DISEASES: INTERIM PHASE 1 STUDY RESULTS IN HEALTHY VOLUNTEERS

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Background: Uncontrolled complement activation plays a pivotal role in a variety of disorders such as PNH and aHUS.
Aims: ALN-CC5 is a subcutaneous (S.C.) investigational RNAi therapeutic targeting complement C5 (C5) with the purpose of decreasing terminal complement activity and thereby protects against intravascular hemolysis and complement mediated tissue damage.
Methods: Preclinical studies in rat and non-human primate (NHP) models were used to investigate the ability of ALN-CC5 to reduce complement C5, inhibit complement-mediated hemolytic activity and complement alternative- and complement classic pathway (CAP and CCP). Furthermore, ALN-CC5 was investigated in a rat membranous nephropathy model, the anti-collagen antibody induced arthritis model and in a passive myasthenia gravis model. A placebo controlled double blind phase 1 clinical study in healthy volunteers and patients with PNH is ongoing. Several cohorts in part A, a single ascending dose study have been completed and Part B, a multiple ascending dose study is currently ongoing. Part C will be a multiple dose study in patients with PNH. Primary endpoints are safety and tolerability. Secondary endpoints are pharmacokinetics, reduction of circulating C5, reduction in hemolytic and CAP as well as CCP activity.
Results: Pre-clinical studies demonstrated that ALN-CC5 resulted in mean 98.4±0.7% reduction of C5 levels in NHPs, mean 88±6.1%% reduction in hemolysis and mean 95.1±0.93% reduction in CAP with every-other week or monthly S.C. dosing. Furthermore, ALN-CC5 significantly reduced proteinuria in a rat membranous nephropathy model, reduced disease activity in the mouse arthritis model and reduced clinical disease score and improved grip strength in the myasthenia gravis model. ALN-CC5 reduced disease manifestation comparable to treatment with C5 monoclonal antibody. In Part A of the phase 1 study, human volunteer subjects were randomized (3:1) to placebo or a starting single subcutaneous dose of 50 mg ALN-CC5 and followed for at least 70 days. Following safety review, additional single ascending dose cohorts were authorized. Part B multiple ascending cohorts are planned to start in parallel to Part A. Up-to-date results on safety, tolerability and C5 knockdown, changes in CAP, CCP and hemolytic activity from study will be presented.
Summary and Conclusions: Collectively, these data suggest that the use of a novel RNAi therapeutic targeting C5 is a promising approach for inhibiting complement in PNH, aHUS and other complement mediated diseases. The subcutaneous route of administration and infrequent dosing make this a particularly encouraging potential therapy.

S829

ACUTE AND CHRONIC RENAL FAILURE IN A PATIENT COHORT FROM THE SPANISH PNH REGISTRY

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Background: Paroxysmal Nocturnal Hemoglobinuria (PNH) is a rare, genetic, and life-threatening blood disease. This illness is characterized by the destruction of red blood cells and hemolytic anemia [1]. The consequent intravascular hemolysis, hypercoagulability, and bone marrow failure may result in several clinical sequels as thrombosis or renal failure [2]. Although its treatment was usually symptomatic, Eculizumab has recently shown efficient results for this disease world-wide [3] and specifically in Spanish cohorts [4].
Aims: This study was planned to describe Acute and Chronic Renal Failure (ARF and CRF) related values in PNH-patients and to assess how Eculizumab affects these parameters.
Methods: From 128 registered patients (PNH Spanish Registry; April 2014), 60 were diagnosed with classic PNH (47%). 27 of these cases (45%) also suffered ARF or CRF and were included in this retrospective and descriptive study.
Results: PNH studied patients were 12 males and 15 females with a mean age of 48.5 (±16.2) y/o whom were diagnosed with PNH when they had a mean age of 38.6 (±13.8) y/o. PNH clone values for analyzed patients was 71.7 % (±23.4) granulocytes, 76.6 % (±28.1) monocytes and 42.3 % (±23.6) erythrocytes. All 27 patients presented renal failure. Specifically, 5 (18.5%) patients were diagnosed with CRF without any previous ARF, 8 (29.6%) were diagnosed with CRF and suffered ARF and 14 (51.9%) only presented ARF. In 20 cases, ARF diagnosis was coincident with a hemolytic crisis. In addition, a patient presented 5 ARF episodes and 4 patients presented 2 episodes. ARF patients showed these mean values at diagnosis: 3880.3 (±2253) UI/L for LDH, 8.1 (±1.9) g/dL for Hemoglobin (Hb), 7.6% (±7.2) Reticulocytes, 117.3 (±46.7) mg/dL for Urea, 4.4 (±2.3) for Creatinine, 26.8 (±17.3) ml/min/1.73 m2 for Glomerular filtrate and 534.8 (±492.1) mg/24 h for Proteinuria. In addition, 21 out of 22 patients showed Hemoglobinuria, 15 out of 16 Hemosideruria and 9 out of 15 Urinary casts. Finally, 6 ARF patients required dialyses and there were 18 complete recoveries and 4 partial recoveries, with a mean time of 13.3 (±7.6) days. Values for CRF-diagnosed patients were 41.7 (±27.2) mg/dL for Urea, 1.3 (±0.4) mg/dL for Creatinine, 56.8 (±14.0) ml/min/1.73 m2 for Glomerular filtrate and 814.4 (±614.0) mg/24 h for Proteinuria. After PNH diagnosis, estimated probability to evade ARF at 5 years was 56.5% (IC 95%; 35.1, 73.3) and median time without an ARF episode was estimated to be 7.3 (IC 95%; 2.2, 14.9) years. Likewise, estimated probability to avoid CRF at 5 years was 60.9% (IC 95%; 37.8, 77.7) and median time without CRF diagnosis was estimated to be 14.5 (IC 95%; 3.8, ND) years. Finally, from 23 Eculizumab treated patients, 20 were still continuing with a mean extent of 3.9 (±2.2) years. Creatinine and Glomerular filtrate values for treated CRF patients were still very preliminary to statistical evaluation. However, recorded data showed a trend towards improvement after 18 months. Considering all treated patients, only 1 (5.3%) suffered a septic shock coincident ARF episode after treatment. All patients were alive at the end of the study.
Summary and Conclusions: This study described ARF and CRF values of Spanish PNH patients and estimated the probabilities to present these diseases after PNH diagnosis. Also, preliminary results suggested that there might be a low ARF incidence in Eculizumab treated patients and CRF related clinical values might have a positive development in time.
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Late breaking simultaneous session 1

LB2067

APL OF ALL RISK GROUPS IS HIGHLY CURABLE WITH A CHEMO-FREE COMBINATION OF ATTENUATED ARSENIC TRIOXIDE AND ATRA

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Background: Survival for patients with APL has improved in the last 20 years. The MRC AML15 trial delivered 80% 5 year survival using anthracycline and All-trans retinoic acid (ATRA) and the exclusion of cytarabine and maintenance which has become standard of care. Arsenic Trioxide (ATO) and Gemtuzumab Ozogamicin (GO) are effective as single agents with the former approved for relapsed disease. The next stage in de-intensification in this disease is the "chemo-free" combination of ATO+ATRA. In low risk disease (WBC<10x10^9/L) the GIMEMA-AMLSG-SAL trial, using a daily schedule of ATO+ATRA, indicated this was at least as effective as chemotherapy containing protocols (Lo Coco *et al*., NEJM 2013;369: 111-21).
Aims: The NCRI AML17 Trial compared Anthracycline/ATRA (AIDA) *vs* the chemo-free combination of ATO (in an attenuated schedule) +ATRA. Importantly high risk patients (presenting WBC>10x10^9/L) were also included with the option to add a single dose of GO (6mg/m^2) in induction.
Methods: From May 2009 to October 2013, 235 patients aged>16 who entered the AML17 trial with molecularly confirmed APL were randomised to either **ATRA+ATO** (8 week induction 0.3mg/kg d1-5 w1, 0.25mg/kgx2/w w2-8, followed by 4 consolidation courses of 0.3mg/kgx2 w1, 0.25mg/kgx2/w w2-4 (63 ATO doses) **OR AIDA** schedule: Idarubicin (Ida)12mg/m^2 d2,4,6,8 +ATRA to d60) (induction) then Ida 5mg/m^2 d1-4 +ATRA d1-15 (Course 2); Mitox. 10mg/m^{2}d1-4 +ATRA d1-15 (course 3); Ida 12mg/m^2 d1 +ATRA d1-15 (Course 4). ATRA was 45mg/m^2/d**.** Follow-up is complete to 1/1/2014.
Results: The median age was 47y (16-77); 57 had WBC>10x10^9/L (27 AIDA, 30 ATRA+ATO) and 49 (24 AIDA, 25 ATRA+ATO) were >60y. 90% entered morphological CR; overall day 30, 60 mortality was 5%, 7%. When a landmark analysis is performed from day 30, 4-year OS is 96%. 20 patients have relapsed. Survival at 4 years is 91%. For patients with a high WBC OS was 86%, and for patients >60 years OS was 77%. There was no significant difference in CR rate between the arms (Chemo 89%, Chemo-free 94%; OR 0.54 (0.21-1.34), p=0.18). The day 30, 60 mortalities were 6% *vs* 4% (p=0.6) and 9% *vs* 5% (p=0.2). One patient on ATRA+ATO experienced frank relapse compared with 12 on AIDA plus a further 7 molecular relapses on this arm (cumulative incidence of frank relapse 13% *vs* 1%, HR 0.16 (0.05-0.48) p=0.001). Among patients who achieved molecular negativity cumulative incidence of molecular relapse was 27% *vs* 0%. The OS at 4 years is 89% (Chemo) *vs* 93% (Chemo-free), HR 0.60 (0.26-1.42) p=0.2, but EFS was significantly superior with ATRA+ATO (91% *vs* 74%, HR 0.36 (0.19-0.70) p=0.003) due to the lack of relapse after CR. There was significantly less alopecia, grade 3,4 liver and GI toxicity on the chemo-free arm. Significantly fewer blood and platelet transfusions were used and there were fewer days on antibiotics and in hospital. The QoL and HADS assessments did not differ significantly. There was no heterogeneity of treatment effect by subgroup. Importantly, when considered by WBC risk, for low risk patients (WBC<10x10^9/l) cumulative incidence of frank relapse was 5% *vs* 1% (p=0.03) with 4-year survival of 90% *vs* 95% (p=0.17). In high risk patients (WBC>10^9/L) CIR was 26% *vs* 0% (p=0.008), and OS 87% *vs* 84% (p=0.8). Of 30 high risk patients allocated to the chemo-free arm, 28 received GO as per protocol. The OS at 4 years for high risk patients given GO was 89%. Of the 2 patients not treated with GO, one died on d12. Compared with the GIMEMA-AMLSG-SAL trial, results were similar, but with less liver toxicity (<10% *vs* 63%); less frequent dosing of ATO (63 *vs* 140); less ATO required (151 vials *vs* 280 vials for a 70kg patient, which at an acquisition cost of £350/vial represents a cost reduction of £46k/patient).
Summary and Conclusions: The combination of ATO and ATRA represents a new standard of care resulting in a very low risk of relapse resulting in significantly better EFS and excellent survival. The attenuated dosing approach is safe and effective in high risk patients particularly if combined with a single dose of GO, is less toxic and less costly.

LB2068

IMPACT OF A GAIN-OF-FUNCTION MUTATION OF CXCR4 ON HAEMATOPOIESIS IN MICE

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Background: The Warts, Hypogammaglobulinemia, Infections and Myelokathexis Syndrome (WS) is a rare immuno-haematological disorder characterized notably by a chronic pan-leukopenia. It is mostly caused by inherited heterozygous autosomal dominant gain-of-function mutations in *CXCR4*, which engender a distal truncation in the carboxyl-terminal tail (C-tail) domain and lead to a desensitization-resistant receptor. CXCR4 is widely expressed on non-haematopoietic cells and virtually all leukocytes. Thus, we hypothesized that WS-associated circulating lymphopenia arises from altered CXCR4-mediated signalling that skews tissue distribution and differentiation of lymphocytes. We recently produced major breakthroughs in the pathophysiology of the WS by generating a knock-in mouse strain (*Cxcr4$^{+/1013}$*) harbouring a WS-linked heterozygous *Cxcr4* mutation. Mutant mice displayed *in vitro* lymphocytes with enhanced migration to Cxcl12, the sole ligand of Cxcr4, and phenocopied severe circulating pan-lymphopenia. Analyses of primary lymphoid organs (*i.e.* bone marrow [BM] and thymus) have unveiled defects in early stages of B- and T-cell development, *i.e.* B-cell progenitors and Early T-cell progenitors. The mechanisms underlying deregulation of lymphopoiesis process, and the resulting chronic blood lymphopenia, are still unknown.
Aims: Here we explore the biological impact of a gain-of-Cxcr4-function on haematopoiesis in both BM and extra-medullary sites.
Methods: We took advantage of our original knock-in model to investigate BM and splenic haematopoietic stem and progenitor cells (HSPC) distribution, clonogenicity, quiescence and *in vivo* migration by flow cytometry and clonogenic assays. We further examined the capacity of mutant HSC to repopulate the BM in long-term BM reconstitution experiments. To exclude any interference of the *Cxcr4$^+$*(WT) allele, we produced and investigated homozygous *Cxcr4$^{1013/1013}$* mice in parallel.
Results: We showed that the total number and homing properties of HSC, as well as the clonogenicity of progenitors, were not altered in the BM of mutant mice as compared to WT littermates. The quiescence status of LSK SLAM was increased, whereas the total number of multipotent and common lymphoid progenitors was decreased in an allele dose-dependant manner. Long-term BM reconstitution experiments indicated that these defects were cell-autonomous and further revealed a decreased capacity of *Cxcr4$^{1013/1013}$* HSC to repopulate the BM. Based on clonogenic assays, we reported a decrease in the total number of HSPC in the blood of mutant mice, an anomaly also found in four WS patient carrying a heterozygous *CXCR4* mutation. This suggested an abnormal HSPC circulation that was strengthened by the increased extramedullary haematopoiesis observed in the spleen of mutant mice. This phenomenon was allele dose-dependent, cell autonomous and associated with an increased expression of Cxcl12 in the red pulp area.
Summary and Conclusions: These results provide new details about haematopoiesis by establishing the C-tail in Cxcr4-mediated signalling as a pivotal regulator of such process. Our current activities consist in investigating *in vitro* the lymphoid differentiation of mouse and human HSPC and characterizing BM and spleen HSC niches.

LB2069

ASSESSMENT OF INTRAVENOUS FERRIC CARBOXYMALTOSE VERSUS ORAL IRON SULPHATE IN THE MANAGEMENT OF PREOPERATIVE ANAEMIA: A PROSPECTIVE RANDOMIZED CONTROLLED TRIAL

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Background: Both pre and postoperative anaemia are highly prevalent in surgical patients. Depending on the type of surgery, underlying disease and the definitions of anaemia, about 14% up to 76% of surgical patients may present with pre-operative anaemia according to different studies. Recently, there has

been increasing evidence to suggest that pre-operative anaemia can lead to an increased risk of peri-operative adverse outcomes such an increase in the requirement for blood transfusion, increase of hospital Length of Stay (LoS) and increased overall morbidity and mortality. In addition, evidence has shown that pre-operative anaemia increases the chance of receiving unnecessary allogeneic blood transfusion with its consequences when compared to patients with a normal haemoglobin level, preoperatively.

Aims: Assessment of safety of a single intravenous iron carboxymaltose dose versus standard oral iron sulphate and the subsequent effect on the perceived quality of life (QoL) in both treatment groups.

Methods: We conducted a prospective randomised controlled trial with two different iron therapies for the treatment of iron deficiency anaemia (IDA) patients who were undergoing elective surgery. During the period between January 2012 and August 2014 at our tertiary referral Hospital for Northern Tasmania, Australia, we recruited 125 patients 2-4 weeks preoperatively who were randomized to a single intravenous ferric carboxymaltose (FCM) infusion (1000 mg standard dose) versus oral daily iron sulphate (325 mg, equivalent to 105 elemental iron) until the time of the surgery. Median age was 71 years (range, 38-94) with a male to female ratio of 67:58. The vast majority of patients were Caucasian. All patients gave an informed consent according to Helsinki guidelines. There were 70 patients who underwent major orthopaedic surgery, 23 abdominal surgery, 27 genitourinary surgery and 5 patients underwent general surgery.

Results: Average Hb level at recruitment was 113 g/L in the FCM intervention group versus 120 g/L in the oral iron group that increased respectively to an average of 122 g/L versus 124 g/L immediately prior to surgery and was maintained at 126 g/L versus 123 g/L at 3 months after treatment respectively. The median baseline ferritin in the FCM group was 32 mcg/L versus 76 mcg/L that increased immediately prior to surgery to 544 mcg/L in the FCM versus 107 mcg/L in the oral iron group and was maintained for 3 months at 176 mcg/L versus 90 mcg/L respectively (p<0.001). Average LoS in the hospital was reduced by 1 day in the FCM group as compared to the oral iron group. FCM was well tolerated by all patients without serious adverse events.

QoL assessment using SF36 at baseline, immediately preoperative, and 3 months post operatively showed improvement in physical function, energy and general health in favour of the FCM group.

Summary and Conclusions: Our data suggest that IV FCM was well tolerated and was associated with improvement of both immediate preoperative Hb and ferritin levels compared to oral iron. Accordingly, there was a reduction of length of hospital stay and improvement of perceived QoL after surgery. Further trials that aim to improve and optimize preoperative and postoperative Hb are warranted.

Trial registration: This study was registered prospectively in the Australian New Zealand Clinical Trials Registry:
(http://www.ANZCTR.org.au/ACTRN12611001256965) and the World Health Organization:
(http://apps.who.int/trialsearch/trial.aspx?trialid=ACTRN12611001256965*).*

Late breaking simultaneous session 2

LB2070

WHOLE EXOME SEQUENCING OF 278 CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS FROM THE CLL8 TRIAL UNCOVERS DRIVER MUTATIONS WITH CLINICAL IMPACT AND THEIR EVOLUTIONARY HISTORY IN CANCER PATHOGENESIS

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Background: Massively parallel whole-exome sequencing (WES) studies have uncovered cancer drivers in CLL and have provided new insights in disease pathogenesis, evolution and prognostication.

Aims: To overcome limitations in size and the clinical heterogeneity of previous cohorts, we performed WES on pretreatment tumor and matched germline samples of 278 CLLs enrolled in the CLL8 trial of the GCLLSG treated with FC/FCR and with full clinical annotation and mature follow up time. We enhanced the power to detect putative drivers through integration with previously published WES data for a total discovery cohort of 538 CLL exomes.

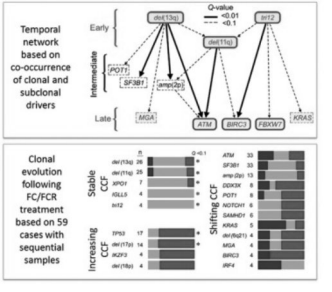

Figure 1.

Results: This resulted in detection of 55 putative CLL driver events (44 sSNVs, 11 sCNVs), many of which have not been previously associated with CLL. At least one potential driver event was found in 91.1% of samples, with 65.4% harboring 2 or more. These putative drivers appeared to affect 8 major cellular processes, including the MAP-ERK pathway (KRAS, NRAS, BRAF, MAP2K mutations; affecting 5.6% of cases), and the MYC pathway (MGA, FUBP1, PTPN1, IRF4 mutations; 8% of cases). For the majority of samples, two driver events were found within the same sample, one at clonal and the other at subclonal frequency. Such cases enabled us to infer a temporal sequence of acquisition and built a network based on significantly recurrent temporally directed events. Thus we found distinct points of origin restricted to few events such as del13q and tri12, with early convergence to del11q and subsequent divergence in diverse late-occurring driver events including mutated TP53 and KRAS. We assessed the clinical impact of driver events in 278 patients from CLL8 in the context of front line therapy. Mutated TP53 and SF3B1 cases had significantly shorter PFS and OS. XPO1 (HR 2.02, *P* =0.02), BRAF (HR 2.05, *P* =0.05) and EGR2 (HR 2.14, *P* =0.05) mutations were associated with reduced OS and trend towards shorter PFS. ATM and BIRC3 mutations had no significant impact on outcome. Both the presence of a pre-treatment subclonal driver (66.5% of subjects) and clonal driver (84.2%) were associated with a significantly shorter PFS (HR 1.64, p=0.003 and HR 1.78 p=0.009) with only subclonal drivers achieving significance in the FCR arm. To directly observe clonal evolution following FC/FCR, we compared the cancer cell fractions (CCFs) of the putative driver alterations in 59 of 278 samples for which a matched relapse samples was available. We observed clonal shifts in 57 of 59 cases of which 21 showed linear and 36 branched evolution with the latter significantly associated with

FCR treatment. Neither type of evolution nor presence of the relapsed clone at baseline (n=18) had an impact on outcome. Notably events inferred as early events by our temporal network were stably clonal over time. Late events demonstrated either increasing (*i.e.* in TP53) or shifting (*i.e.* in SF3B1, ATM) CCFs after FC/FCR suggesting evolution in relation to therapy (figure). In addition, this temporal map suggests that the copy number loss in 11q precedes point mutations in ATM or BIRC3 in cases with biallelic loss. By contrast, TP53 and 17p show a concordant rise of both events suggesting that biallelic inactivation of TP53 is typically required for fitness advantage.

Summary and Conclusions: WES of a large, uniform cohort revealed several novel putative drivers in CLL, enabled their categorization into 8 major functional pathways and investigation of their prognostic impact within the CLL8 trial cohort. Furthermore we developed a network model of CLL evolution with earlier initiating and late accelerating events.

LB2071

CARFILZOMIB AND DEXAMETHASONE IMPROVES PROGRESSION-FREE SURVIVAL AND RESPONSE RATES VS BORTEZOMIB AND DEXAMETHASONE IN PATIENTS (PTS) WITH RELAPSED MULTIPLE MYELOMA (RMM): THE PHASE 3 STUDY ENDEAVOR

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Background: Bortezomib and dexamethasone (Vd) is a standard-of-care regimen for RMM. Carfilzomib (20/27 mg/m^2; 2–10 min intravenous [IV] infusion) is approved in Argentina, Israel, Mexico, and the United States for relapsed and refractory multiple myeloma and significantly improved progression-free survival (PFS) when given with lenalidomide and dexamethasone for RMM in the phase 3 study ASPIRE (NCT01080391; Stewart et al, *N Engl J Med*, 2015). In study PX-171-007 (NCT00531284), carfilzomib (20/56 mg/m^2; 30-minute infusion) and dexamethasone (Kd) had promising activity in pts with RMM (Papadopoulos et al, *J Clin Oncol*, 2015).

Aims: ENDEAVOR (NCT01568866) compares Kd with Vd in pts with RMM. Results from a prespecified interim analysis are presented.

Methods: Adults with RMM (1–3 prior treatments) were eligible. Pts were randomized 1:1 and stratified by prior K or V (yes *vs* no), prior lines of treatment (1 *vs* 2–3), International Staging System stage (1 *vs* 2–3), and intended route of V (IV *vs* subcutaneous [SC]). The Kd arm received K (30-min IV infusion) on days (D) 1, 2, 8, 9, 15, 16 (20 mg/m^2 on D1, 2 [cycle 1]; 56 mg/m^2 thereafter) and dexamethasone (20 mg) on D1, 2, 8, 9, 15, 16, 22, 23 of a 28-day cycle. The Vd arm received V (1.3 mg/m^2; IV or SC) on D1, 4, 8, 11 and dexamethasone (20 mg) on D1, 2, 4, 5, 8, 9, 11, 12 of a 21-day cycle. Cycles were repeated until disease progression or unacceptable toxicity. The primary end point was PFS assessed by an independent review committee. Secondary end points include overall survival (OS), overall response rate (ORR), duration of response (DOR), rate of peripheral neuropathy (PN), and safety.

Results: In total, 929 pts (Kd: 464; Vd: 465) were randomized. In the Vd arm, 83.6% of pts received SC bortezomib. Median treatment exposure was 39.9 weeks (Kd) and 26.8 weeks (Vd). Kd led to a 47% decrease in the risk of progression or death (hazard ratio, 0.53; 95% confidence interval [CI], 0.44–0.65; *P*<.0001), with a median PFS of 18.7 months (95% CI, 15.6–not estimable) in the Kd arm *vs* 9.4 months (95% CI, 8.4–10.4) in the Vd arm. OS data were immature (deaths: Kd=75; Vd=88); pts continue to be followed. Best overall responses are presented in the table. The median DOR was 21.3 months (Kd) and 10.4 months (Vd). Treatment discontinuation due to an adverse event (AE) occurred in 14.0% (Kd) and 15.7% (Vd) of pts. An AE led to dose reductions of K or V in 22.9% and 47.8% of pts, respectively; 61.9% of dose reductions in the Vd arm were due to neuropathy-related AEs *vs* 6.6% in the Kd arm. Rates of grade ≥2 PN (grouped term) were 6.0% in the Kd arm vs 32.0% in the Vd arm (P<0001). The most common hematologic AEs (preferred terms; all grades) in the Kd and Vd arms, respectively, included anemia (39.3% *vs* 27.0%) and thrombocytopenia (20.5% *vs* 17.1%); the most common nonhematologic AEs (preferred terms; all grades) included diarrhea (30.9% *vs* 38.4%), fatigue (29.4% *vs* 28.5%), and dyspnea (28.5% *vs* 13.2%). Grade ≥3 AEs of interest in the Kd and Vd arms, respectively, included hypertension (preferred term; 8.9% *vs* 2.6%), dyspnea (preferred term; 5.4% *vs* 2.2%), cardiac failure (grouped term; 4.8% *vs* 1.8%), and acute renal failure (grouped term; 4.1% *vs* 2.6%). A total of 3.9% of pts in the Kd arm and 3.4% of pts in the Vd arm died on study owing to AEs.

Tabella 1. Best overall responses.

	Kd (N=464)	Vd (N=465)
Best overall response, n (%)[a]		
Stringent complete response	8 (1.7)	9 (1.9)
Complete response	50 (10.8)	20 (4.3)
Very good partial response	194 (41.8)	104 (22.4)
Partial response	104 (22.4)	157 (33.8)
ORR[a]		
n	357	291
ORR, % (95% CI)[b]	76.9 (72.8–80.7)	62.6 (58.0–67.0)
Odds ratio (Kd/Rd) (95% CI)[c]	2.03 (1.52–2.72)	
P value (1-sided)[c]	<.0001	

[a]Determined by independent review committee according to International Myeloma Working Group Uniform Response Criteria. ORR includes patients with a best overall response of partial response or better. One patient in each arm was not evaluable for best overall response owing to censoring but was included in the ORR calculation since they achieved a partial response prior to being censored.
[b]Clopper–Pearson interval.
[c]The odds ratio and 95% CI and *P* values were calculated using the stratified Cochran–Mantel–Haenszel method.

Summary and Conclusions: Carfilzomib and dexamethasone demonstrated statistically significant and clinically meaningful superiority over bortezomib and dexamethasone in RMM, with a 2-fold improvement in median PFS and a favorable benefit–risk profile. These data suggest that carfilzomib could be a best-in-class agent for RMM.

LB2072

PATIENT-REPORTED OUTCOMES (PROS) IN PERSIST-1: A RANDOMIZED, MULTI-COUNTRY PHASE III TRIAL OF THE JAK2 INHIBITOR PACRITINIB (PAC) VS. BEST AVAILABLE THERAPY (BAT) IN MYELOFIBROSIS (MF)

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Background: MF is a serious, life-threatening disease characterized by splenomegaly and debilitating symptoms such as fatigue, night sweats, pruritus, weight loss, and abdominal pain. MPN-SAF TSS is a PRO tool designed to measure the burden of MF-related symptoms. The instrument was developed and validated at Mayo Clinic for utilization in clinical practice. In agreement with the FDA, the instrument was modified (MPN-SAF TSS 2.0) for use in PERSIST-1 and PERSIST-2, both, international, phase 3 randomized clinical trials to support a PRO endpoint.

Aims: Compare symptom reduction of PAC *vs* BAT in the open-label PERSIST-1 (NCT01773187) trial in primary myelofibrosis (PMF), post-polycythemia vera (PV-MF), and post-essential thrombocythemia (PET-MF).

Methods: JAK inhibitor naïve patients (pts) were randomized 2:1 to oral PAC 400 mg once daily or BAT stratified by DIPSS risk (Int-1/Int-2 *vs* High) and platelet (plt) count (<50,000/μL *vs* 50,000 to <100,000/μL *vs* ≥100,000/μL). Pts had baseline total symptom score (TSS) ≥13 using MPN-SAF TSS. Each symptom is rated on a scale from 0 (absent) to 10 (worst imaginable) using MPN-SAF TSS and MPN-SAF TSS 2.0 with core symptoms tiredness, night sweats, early satiety, itchiness, bone pain, and abdominal pain. Other PROs included Patient Global Impression of Change (PGIC), EORTC QLQ-C30, and EQ-5D-5L.

Results: 327 pts were enrolled (PAC: 220, BAT: 107), 62% with PMF; 32% had plt counts <100,000/μL and 16% had plt counts <50,000/μL. 24.5% of pts treated with PAC and 6.5% treated with BAT achieved a ≥50% decrease in TSS at Week 24 (WK24; Figure; p<0.0001) in the intent-to-treat population. In pts with baseline plt count <100,000/uL, 25.0% of pts treated with PAC and 8.8% treated with BAT achieved a ≥50% decrease in TSS at WK24 (p=0.0677). There were significant reductions from baseline to WK24 in fatigue, early satiety, abdominal discomfort, inactivity, night sweats, itching, and bone pain (MPN-

SAF TSS), and tiredness, early satiety, abdominal discomfort, night sweats, and pain under ribs on left side (MPN-SAF TSS 2.0). In the PAC group, 81% of pts rated the change in their overall condition as very much improved, much improved, or minimally improved by PGIC in pts who completed questions, *vs* 21% for pts in the BAT group (p<0.001). Among those with improvement in PGIC, 40% had ≥50% reduction of TSS at WK24. On the EQ-5D-5L, overall health scores were higher on average in patients treated with pacritinib and a greater proportion reported improvement in the usual activities, pain/discomfort, and anxiety/depression dimensions. Pacritinib-treated patients also reported higher scores on functioning (physical, role and social) scales and lower scores on symptom scales including fatigue, pain, insomnia, and loss of appetite on the EORTC QLQ-C30.

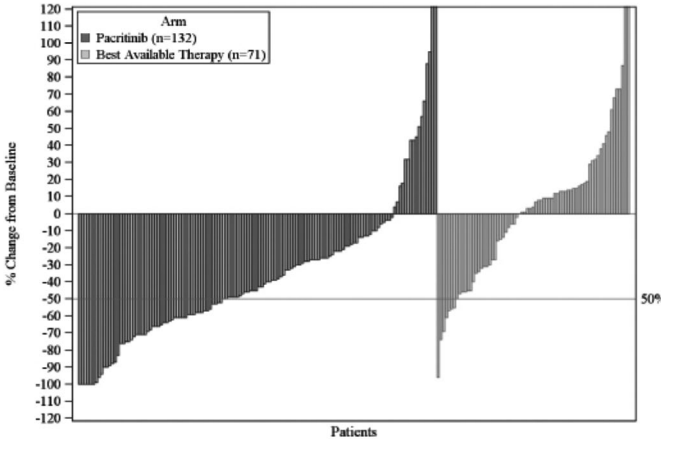

Figure 1.

Summary and Conclusions: PAC treatment improved PROs across several measures. Specifically, it resulted in significantly higher response rates based on TSS which were associated with better PGIC and QoL.

LB2073

EFFICACY AND SAFETY OF INOTUZUMAB OZOGAMICIN (INO) VS STANDARD OF CARE (SOC) IN SALVAGE 1 OR 2 PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA (ALL): AN ONGOING GLOBAL PHASE 3 STUDY

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Background: The majority of cases of B-cell ALL express CD22. InO, a humanized anti-CD22 antibody conjugated to calicheamicin, a potent cytotoxic antibiotic, showed initial efficacy and safety in ALL (Kantarjian. *Cancer.* 2013;119:2728).
Aims: To assess antitumor activity and safety of InO *vs* SOC (intensive chemotherapy) in adults with relapsed/refractory ALL
Methods: In this phase 3 trial (NCT01564784), patients (pts) were randomized to InO (starting dose 1.8 mg/m^2/cycle [0.8 mg/m^2 on d1; 0.5 mg/m^2 on d8 and 15 of a 21–28 d cycle [≤6 cycles]) or SOC (either fludarabine/cytarabine [ara-C]/granulocyte colony-stimulating factor [FLAG], ara-C plus mitoxantrone, or high-dose ara-C). A split-α design was used for 2 primary endpoints: 1) complete remission (CR) or CR with incomplete hematologic recovery (CRi) assessed in 218 pts; and 2) overall survival (OS) to be assessed in all 326 pts enrolled. Secondary endpoints: duration of remission (DOR), minimal residual disease negativity (MRD-neg; <0.01% by central flow cytometry) in pts with CR/CRi, stem cell transplant (SCT) rate. Data as of October 2, 2014 are presented, except for survival (not mature at this date).
Results: Per protocol, the first 218 of 326 pts randomized were included in the primary intent-to-treat (ITT) analysis (for InO *vs* SOC [n=109 each]: CR1 duration ≥12 mo, 43% *vs* 35%; age <55 y, 61% *vs* 63%; Salvage 1, 67% *vs* 63%); CR/CRi rate was significantly better in the InO *vs* SOC arm (see **Table** for efficacy). Safety was assessed in 259 pts who received ≥1 dose of study drug; pts received InO (n=139) for median 8.3 (range, 0.1–26.4) wks *vs* SOC (n=120) for median 0.9 (0.4–15.1) wks; 83% *vs* 89% discontinued, primarily due to CR in InO (35%) *vs* resistant ALL in SOC (40%) arms. Most common grade (Gr) ≥3 AEs were hematologic cytopenias. For InO *vs* SOC, Gr≥3 hepatobiliary AEs occurred in 9% *vs* 3% pts; any grade veno-occlusive liver disease (VOD) occurred in 15 *vs* 1 pts (Gr≥3, 13 *vs* 1 pts). More patients proceeded to allogeneic SCT with InO (n=48) *vs* SOC (n=20); in the InO arm, 5 VOD cases (2 in pts with prior SCT) occurred during treatment and 10 after subsequent SCT (2 fatal).

Tabella 1. Best overall responses.

% (95% CI)	InO (n=109)	SOC (n=109)	1-sided *P*-Value
CR/CRi[a]	80.7 (72–88)	33.3 (24–44)	<0.0001
S1	87.7	31.3	<0.0001
S2	66.7	37.9	0.0104
Median DOR, mo	4.6 (3.9–5.4)	3.1 (1.4–4.9)	0.0169
MRD-neg in pts with CR/CRi	78.4 (68–87)	28.1 (14–47)	<0.0001

[a]Modified ITT analysis excluding 13 untreated SOC pts; assessed per independent endpoint adjudication committee

Summary and Conclusions: CR/CRi, DOR, and MRD-neg rates were significantly greater with InO *vs* SOC. InO safety profile is consistent with previous studies; liver toxicities and VOD were more common with InO; additional VOD analyses characterizing risk factors and management considerations will be presented. Trial is ongoing to allow survival data to mature.

E-POSTERS

Acute lymphoblastic leukemia - Biology

E849

FISH-BASED SCREENING FOR TYROSINE KINASE-ACTIVATING FUSION GENES IN CHILDHOOD HIGH-RISK B-CELL PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA (BCP-ALL)

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Background: Tyrosine kinase (TK)-activating gene fusions define a biologically and clinically distinct class of apparently high-risk BCP-ALL in children and adolescents (Roberts et al, New Engl J Med 371:1005;2014). Because they provide unique targets for therapeutic interventions with various fusion type-specific tyrosine-kinase inhibitors, these abnormalities attain considerable interest. Consequently, there is also a pressing need to identify all the respective lesions in a fast and cost efficient way.
Aims: In consideration of these demands, we devised a hierarchical FISH-based screening algorithm that enables the detection of the vast majority of all relevant alterations. We used this approach to examine a careful selected group of high-risk patients that were enrolled in the Austrian BFM-ALL 2000 treatment study with the aim to identify the respective TK-activating fusion genes in order to determine their type, frequency and clinical relevance.
Methods: Our strategy consists of several hierarchical steps of FISH screening, the first of which is required to define and exclude the preponderant proportion (>60%) of cases with all known specific abnormalities, such as those with an *ETV6/RUNX1, BCR1/ABL1, KMT2A, TCF3* and *IGH* rearrangement as well as those with a hyper- or hypodiploid karyotype, iAMP21 and dic(9;20), because cases with the TK fusions of interest are almost exclusively found in the remaining so-called "B-others" cohort. Next, we exploit the fact that one of the respective fusion partners is always one of a few TK-activating hub genes, namely *ABL1, ABL2, CRLF2, CSF1R, JAK2, PDGFRB* or *EPOR.* The potential involvement of the one or the other of these genes is then assessed with the respective 3'-5' dual-color split apart FISH probes.
Results: So far, we have analyzed 50 high-risk "B-others" patients that were selected according to the above criteria. These cases were screened for the presence of TK-activating gene fusions with all currently available commercially *PDGFRB/CSF1R, JAK2, CRLF2* and (the non-TK) *PAX5* split-apart FISH probes. We identified specific rearrangements in 13/50 (26%) cases. Three had a *PDGFRB/CSF1R,* two a *JAK2,* seven a *CRLF2* and one an *ABL1* gene rearrangement, the latter of which became apparent during screening with a *BCR/ABL1* FISH probe. Moreover, with the help of metaphase FISH, CGH array and RT-PCR analyses we were also already able to identify the fusion partner in 11/13 of these cases. Six of the 13 patients were transplanted, two of them in second remission, five experienced a late relapse and three died, two because of septicemia and one after experiencing a third relapse after transplantation.
Summary and Conclusions: The results of our analyses confirm the efficiency and validity of our FISH screening approach, which currently is supplemented also with additional assays, in particular those identifying *ABL1* and *ABL2* gene rearrangements. The presented strategy can be easily adopted in an analogous fashion to identify such TK-activating gene fusions also in other forms of hematologic malignancies. Their unambiguous identification is an essential requirement for choosing a suitable TK inhibitor, an option that is increasingly required for the appropriate disease-specific treatment.

E850

CUSTOMIZED NEXT-GENERATION SEQUENCING PANEL FOR IMPROVED CLINICAL DIAGNOSTICS OF HIGH-RISK B-CELL PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA OF CHILDHOOD

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Background: Next generation sequencing (NGS) applications have recently identified various recurrent kinase and cytokine receptor mutations in a subgroup of B-cell precursor acute lymphoblastic leukemia (BCP-ALL) that is characterized by a Ph-like gene expression signature. Combined genome, exome or transcriptome analyses have identified recurrent genetic alterations of kinases and cytokine-receptors/adaptors, which often result in ligand-independent, constitutive activation of downstream signalling pathways. The diversity of mutation types including complex genomic rearrangements demands new strategies for cost-effective, time-saving routine clinical diagnostics.
Aims: We report a feasible approach for the identification and characterization of kinase and kinase-dependent pathway aberrations in BCP-ALL utilizing a customized sequence enrichment capture panel for NGS.
Methods: Thirteen BCP-ALL patients with a high minimal residual disease (MRD) level (≥1x10^{-2}) after induction therapy from COALL studies 07-03 or 08-09 were applied to the panel. Four positive controls with known rearrangements (*BCR-ABL1* and *ID4-JH*) were included to test the general applicability of the method. Genomic capture probes were specific for exonic and intronic regions of *ABL1, JAK2, PDGFRB, CRLF2* and for the *IgH-JH* region on chromosome 14. These predefined genomic regions from recurrently mutated or rearranged kinase and cytokine receptor genes were enriched from 50 ng of initial genomic DNA without gene specific PCR amplification steps, followed by paired end sequencing of the captured genomic fragments (Nextera Rapid Capture Custom enrichment protocol with paired end sequencing on a MiSeq benchtop sequencing platform; Illumina).
Results: All breakpoints from 4 positive controls were successfully identified and verified by patient specific amplification of the genomic fusion sites. *ABL1* and *JH* specific capture probes allowed the "fishing" of the genomic fusion partner gene, here *BCR* and *ID4*, by paired end sequencing of overlapping, breakpoint spanning reads. This approach also allowed the identification and exact mapping of the genomic breakpoint for *CRLF2 (P2RY8-CRLF2; CRLF2-JH*), *PDGFRB (EBF1-PDGFRB)* or *EPOR (IgH-EPOR)* in 7/13 BCP-ALL with high level of post-induction MRD.
Summary and Conclusions: Rapid identification of kinase- or cytokine receptor rearrangements in BCP-ALL offers new therapeutic options for the treatment with target specific tyrosin kinase inhibitors (TKI's). Our approach is applicable for routine clinical applications with short turnaround times of less than 2 weeks. Up to 12 samples can be processed in parallel on one MiSeq run. Bioinformatical analysis follows a standardized, automated workflow. Despite limitations in target gene selection, the method allows the identification of unknown fusions partners from frequently rearranged kinase genes, providing the exact breakpoint location. MRD guided NGS panel analysis is a feasible strategy for identification of patients suitable for targeted TKI intervention, which may reduce the high risk for therapeutic failure/relapse.

E851

THE LNCRNAS MEG3 AND LINC-PINT REGULATE THE EXPRESSION OF HMOX1 IN ALL, SHOWING A POTENTIAL AS A THERAPEUTIC TARGETS FOR THIS DISEASE

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Background: Long non-coding RNAs (lncRNAs) are functional RNAs longer than 200 nucleotides in length. Compared to mRNAs, most lncRNAs are localized preferentially to the nucleus, are more cell type specific and are expressed at lower levels. The function of lncRNAs in the cell is primarily regulatory and they have a clear impact in cell behavior. Furthermore, several lncRNAs are deregulated in human diseases including several cancers.
Aims: we aimed to analyze the expression and function of lncRNAs in ALL.
Methods: In order to identify aberrantly expressed lncRNAs in ALL, we used the SurePrint G3 Human Gene Expression array that detects 27956 coding genes and 7419 lncRNAs. The transcriptomes of primary ALL cells, ALL cell lines and control samples were compared.
Results: In this analysis, we detected 37 lncRNAs differentially expressed, 24 of them down-regulated and 13 up-regulated in ALL cells compared to healthy controls. We analyzed the expression of 21 deregulated lncRNAs by quantitative RT-PCR (q-PCR) and we validated the data obtained by expression arrays in 18 out of the 21 genes tested. We focused our study in *MEG3* and *linc-PINT*, and by q-PCR, we observed a decreased expression of these 2 lncRNAs in 10 ALL-derived cell lines compared with B and T cells obtained from healthy donors. Also, we detected a direct correlation between an inappropriate DNA methylation and the decreased expression of *MEG3* in ALL cell lines. Interestingly, we observed that re-expression of *MEG3* or *linc-PINT* in ALL cells led to a decrease in cell proliferation, due to cell cycle arrest in G2 phase and induction of apoptosis. The transcriptome analysis of ALL cell lines after overexpression of *MEG3* or *linc-PINT*, showed an increase expression of *HMOX1*. Interestingly, the overexpression of *HMOX1* on its own also decreased cell proliferation, suggesting that HMOX1 could be a mediator of the phenotype observed after re-expression of *MEG3* or *linc-PINT*. Finally, we observed that different drugs that affect proliferation of ALL cells, such as curcumin or panobinostat, induced drastically the expression of *linc-PINT* and *HMOX* in these cell lines. These results indicate that *linc-PINT* and *HMOX1* could be interesting therapeutic targets for improving the treatment of ALL patients.
Summary and Conclusions: The expression of several lncRNAs is altered in primary samples and cell lines of ALL. In some cases, altered expression is due to inappropriate DNA methylation. *MEG3* and *linc-PINT* are decreased in ALL cells and their re-expression affects cell cycle progression and induces apoptosis in these cells. These effects could be mediated by HMOX. Curcumin and panobinostat also induce *linc-PINT* and *HMOX,* which could drive their effects in cell proliferation. Therefore, *linc-PINT* and *HMOX1* could be therapeutic targets for improving the treatment of patients with ALL.

E852

THE ROLE OF NRARP IN T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA PATHOGENESIS AND CHEMOTHERAPY SENSITIVITY

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Background: T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive hematological malignancy. Although the outcome of T-ALL patients has improved over recent years, the poor prognosis of patients with resistant or relapsed disease is still a major concern. Even though NOTCH is a known driver in T-ALL, its inhibition cannot be efficiently achieved with the drugs currently available, due to their weak therapeutic effects and severe toxicity. Thus, a better understanding of the mechanisms downstream of NOTCH oncogenic signaling in T-ALL is necessary in order to develop NOTCH targeting therapies more effective and less toxic. We have shown that loss of *mir-181ab1* blocks Notch-induced T-ALL development partly by de-repressing the expression of NRARP (NOTCH regulated ankyrin repeat protein)(Fragoso R., PLOS Genetics 2012), a transcriptional target of NOTCH and a negative regulator of NOTCH signaling. Interestingly, NRARP over-expression in murine hematopoietic stem cells impairs T-cell differentiation. Since T-ALL originates from a block during T-cell development, these results suggest that de-regulation of NRARP expression can contribute to the pathogenesis of T-ALL. Importantly, over-expression of NRARP in thymoma cells displaying NOTCH-induced glucocorticoid resistance was shown to sensitize the cells to this drug. This suggests that targeting NRARP may further tackle chemotherapy-resistance in T-ALL.
Aims: To investigate the role of NRARP in human T-ALL cell growth and survival and its potential to sensitize T-ALL cells to commonly used chemotherapeutic agents.
Methods: NRARP mRNA and protein expression was determined by real time-PCR and western blot analyses. *in vitro* functional evaluation of NRARP in T-ALL cell lines was performed by flow cytometry analysis of proliferation and viability upon NRARP over-expression. To determine the effects of NRARP in chemotherapy sensitivity and resistance, T-ALL cells were exposed to Dexamethasone (Dex), Asparaginase (APR) and Methotrexate (MTX).
Results: We started by quantifying *NRARP* expression in human T-ALL cell lines (n=10) and compared it with the expression of *NRARP* in human thymocytes (n=10). We observed that *NRARP* expression is significantly decreased in T-ALL cells (p=0.05). Moreover, quantification of NOTCH1 in the same cells showed that *NRARP* expression is inversely correlated with *NOTCH1* levels. Of note, although NRARP is a downstream target of NOTCH signaling, which is constitutively active in T-ALL patients, it is also a target of *mir-181ab1*, which is highly expressed in T-ALL cells. Next, we over-expressed NRARP in human T-ALL cell lines. Remarkably, NRARP over-expression results in a block in leukemia cell expansion (4 out of 5 cell lines). Proliferation and viability analyses revealed that NRARP over-expression affects T-ALL cell proliferation (CEM and Jurkat cells) and survival (Molt4 and DND41 cells). Importantly, NRARP overexpression sensitizes leukemia cells to APR (CEM cells), MTX (CEM and DND41 cells) and Dex (CEM and DND41). Moreover, over-expression of NRARP partly re-sensitizes Molt4 Dex-resistant cells to this drug.
Summary and Conclusions: Taken together our preliminary results suggest that NRARP plays an important role in T-ALL pathogenesis and chemotherapy resistance.

E853

THE INHIBITION OF CHECKPOINT KINASE 1 INCREASES THE EFFECTIVENESS OF CONVENTIONAL CHEMOTHERAPY IN B-/T-ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: During the last few years the efficacy of many Checkpoint Kinase (Chk)1/Chk2 inhibitors has been assessed for the treatment of different type of cancers but only few studies have been performed to evaluate their effectiveness on hematological diseases.
Aims: In this study we evaluated the *in vitro* efficacy of the Chk1 inhibitor, LY2606368, as single agent and in combination with other tyrosine kinase inhibitors (imatinib and dasatinib) or chemotherapy drugs (clofarabine) in B-/T-ALL cell line and in primary blasts.
Methods: Human B (BCR-ABL1-positive: BV-173, SUPB-15; BCR-ABL1-negative: NALM-6, NALM-19, REH) and T (MOLT-4, RPMI-8402, CEM) leukemia cell lines were incubated with increasing concentrations of drug (1-100 nM) for 24 and 48 hours and the cell viability was evaluated using WST-1 reagent. The effect of the inhibition of Chk1 on cell cycle profile was analyzed using Propidium Iodide (PI) staining. To deeply understand the effect of the compound on the pathway of Chk1, different western blots analyses were performed on specific downstream targets of Chk1. The Chk1 inhibitor LY2606368 was purchased by ApexBio Technology.
Results: LY2606368 deeply reduced the cell viability in a dose and time dependent manner in all the cell lines treated, with the BV-173 (6.33 nM IC50 24hrs) and RPMI-8402 (8.07 nM IC50 24hrs) cell lines being the most sensitive while SUP-B15 (61.4 nM IC50 24hrs) and REH (96.7 nM IC50 24hrs) cell lines being the less sensitive. In addiction the sensitivity was no correlated with the different sub-type of ALL or with the mutational status of p53. The cytotoxic activity was confirmed by the significant increment of the number of apoptosis cells (Annexin V/Propidium Iodide), by the increment of γH2AX foci and by the activation of different apoptotic markers (Parp-1 and pro-Caspase3 cleavage). To better understand the relationship between the activation of apoptosis and the effect on cell cycle, different cell cycle analyses were performed. The inhibition of Chk1, even with a high heterogeneity among the different cell lines, deeply changed the cell cycle profile. In all the cell lines the percentage of cells in G1 phase and in G2/M phase were reduced by the treatment while the numbers of cells in sub-G1 and S phase were increased. Furthermore the cytotoxicity of LY2606368 was evaluated in combination with imatinib, dasatinib or the purine nucleoside antimetabolite clofarabine. The combination strongly reduced the cell viability when compared to the cytotoxic effect of the single drugs. Moreover the combination showed an additive efficacy in term of induction of DNA damages as showed by the increase number of γH2AX foci and the activation of phospho-Chk1 (ser 317).
Summary and Conclusions: LY2606368 as single agents showed a strong cytotoxic activity on B-/T-All cell lines. Furthermore the compound was able to increase the cytotoxic effect of imatinib, dasatinib and clofarabine in all the cell lines treated. In our opinion this data are the basis for a future clinical evaluation of this compound in the treatment of leukemia. Supported by ELN, AIL, AIRC, progetto Regione-Università 2010-12 (L. Bolondi), FP7 NGS-PTL project.

E854

LEUKEMOGENICITY OF RCSD1-ABL1 OF PH-LIKE ACUTE LYMPHOBLASTIC LEUKEMIA VARIES BETWEEN FUSION SITES

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Background: The RCSD1 gene, located at 1q23 involved in t(1;9)(q23;q34) translocation in acute lymphoblastic leukemia (ALL)ALL is known as a novel gene fusion partner of ABL1 gene. RCSD1-ABL1-positive ALL is included in Philadelphia chromosome-like (Ph-like) ALL. The leukemogenicity of RCSD1-ABL1 are undefined. We found two types of fusion gene in a case of RCSD1-ABL1-positive ALL—the first involved fusion of exon 2 of RCSD1/exon 4 of ABL1 (R2A4; 2952 bp) and the other involved fusion of exon 3 of RCSD1/exon 4 of ABL1 (R3A4; 3042 bp).
Aims: The aim of this study is examine the leukemogenicity of two kinds of fusion genes of RCSD1-ABL1.
Methods: We generated retroviral vectors expressing R2A4 or R3A4, which we used to transduce Ba/F3 cells, and examined the IL-3 dependency of each transduced Ba/F3 cell line. Secondly we examined the multiplication activity and the IL-3 dependency of each Ba/F3. Thirdly we injected $2x10^8$ of each Ba/F3 to SCID mice and examined the overall survivals (OS) and histopathology of each mice. Fourthly we examined phosphorylation receptor tyrosine kinase activity of each Ba/F3 under no IL3 by Phosphorylation Antibody Array. Based on the result of Phosphorylation Antibody Array, we examined the mechanisms of leukemogenicity of RCSD1-ABL1 by Western Blotting. Finally we examined the response of leukemogenicity of RCSD1-ABL1 to Tyrosine Kinase Inhibitors
Results: Only R3A4-Ba/F3 showed significant independence from IL3 (Control *vs.* R3A4, $P = 0.001$; Control *vs.* R2A4, $P = 0.90$). *In vivo*, R3A4-Ba/F3-injected mice showed significantly shorter survival times than Control or R2A4-Ba/F3-injected mice (42 *vs.* > 100 days, respectively, $P = 0.0009$). Leukemic changes were observed only in R3A4-Ba/F3-injected mice. Phosphorylation Antibody Array showed that Tyk2 were activated only in R3A4-Ba/F3. Western Blotting showed that the leukemogenicity of R3A4 consists of Tyk2-STAT2 pathway. R3A4-Ba/F3 was resistant to Imatinib and Dasatinib but sensitive to pan-JAK family including Tyk2 inhibitor.
Summary and Conclusions: Previous study reported that another R3A4 fusion activated STAT5, which was inhibited by Dasatinib. Our findings, together with this previous report, suggest that leukemogenicity of RCSD1-ABL1 of Ph-like ALLvaries between fusion sites.

E855

TRD/TRA REARRANGEMENTS OCCUR INFREQUENTLY IN EARLY THYMIC PRECURSOR SUBTYPE OF T LYMPHOBLASTIC LEUKEMIA BY HIGH-THROUGHPUT SEQUENCING

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Background: High-throughput sequencing (HTS) of T-cell receptor genes may be useful for detecting minimal residual disease (MRD) in acute lymphoblastic leukemia. We previously demonstrated application of high-throughput sequencing for the detection of minimal residual disease in T-lineage acute lymphoblastic leukemia (T-ALL) (Sci. Transl. Med. 4(134):134ra63. 2012), and showed that early thymic precusor T-ALL and a subtype which we termed "near-ETP" with variable low CD5 expression frequently lacked a complete clonal rearrangement of *TRB*. The absence of a pre-treatment clonal rearrangement of *TRB* limits the potential for molecular monitoring by next-generation sequencing of this T-cell receptor gene rearrangement for minimal residual disease in these subtypes of leukemias. *TRD* gene rearrangements are expected to occur earlier in T cell development than *TRB*, suggesting they are an attractive marker.
Aims: Given TRD is the first T-cell receptor to rearrange during T lymphocyte development, we evaluated the feasibility of amplifying and sequencing of *TRD* gene rearrangements to monitor MRD in ETP and "near-ETP" subtypes of T-ALL.
Methods: We sequenced the *TRD* and *TRA* of residual samples from 33 patients enrolled in Children's Oncology Group AALL0434 using an amplification bias-controlled multiplex PCR. These 33 samples included 11 cases of early thymic precursor (ETP) subtype, 12 so-called near-ETP sample (which have similar immunophenotype with ETP but differences in CD5 expression) and 10 non-ETP samples. We defined high-frequency clones as probable neoplastic if they exceeded 15% of the repertoire.
Results: We find that clonal *TRD* and *TRA* rearrangements can be identified in only 2 of 11 of pre-treatment samples of ETP-TALL, using a cut-off of 15% for defining clonality. By comparison, in a cohort of 12 "near-ETP" samples, samples with leukemic blasts that have some but not all of the immunophenotypic features of ETP, we found 5 of 12 cases with *TRD* gene rearrangement. Lastly, typical, "non-ETP" T-ALL subtypes showed evidence of clonal rearrangement in 7 of 10 cases.
Summary and Conclusions: Our data suggest that clonal rearrangements in ETP subtype of T-ALL are infrequent, suggesting that molecular monitoring of *TRD* and *TRA* T-cell receptor gene rearrangements does not substantially enhance the ability to follow ETP- clones through treatment for post-therapy MRD detection.

E856

PROGNOSTIC VALUE OF DELETIONS AND PROMOTER METHYLATION OF CDKN2A AND CDKN2B IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Alterations in *CDKN2A* and *CDKN2B* are the most common molecular patterns in pediatric acute lymphoblastic leukemia (ALL). Deletions in these genes occur in 30% of cases and are the leading mechanism in inactivation of these genes. The available data for promoter methylation of *CDKN2A* and *CDKN2B* is discrepant and the importance of this mechanism needs to be answered.
Aims: To assess the prevalence and size of deletions and promoter methylation of *CDKN2A* and *CDKN2B* and to ascertain the impact of these aberrations on clinical and biological features in children with ALL.
Methods: We analyzed copy number variations in selected genomic loci using MLPA (Multiplex Ligation dependent Probe Amplification; P202, P335, ME028 kits) on DNA extracted from bone marrow samples collected from patients at diagnosis of childhood ALL. Promoter methylation of *CDKN2A* and *CDKN2B* was examined by using Methylation-specific MLPA (MS-MLPA) method. Data mining analysis technique was used for evaluation of coexistence of non-random patterns of factors. We analyzed overall survival and event-free survival with respect to genetic alterations as well as clinical features of these patients.
Results: Six hundred fifty two samples were analyzed. In 185 (28.4%) samples deletions in 9p21 region were detected: 29 (4.4%) were positive for deletions of *CDKN2A*, 151 (23.2%) of *CDKN2A* and *CDKN2B* and 5 (0.8%) of *CDKN2B*. In 85 (13%) patients deletions of *CDKN2A* were homozygous. Methylation status was determined in 208 samples. P15 promoter was methylated in 49 (23.5%) samples, p14 and p16 promoters were methylated in 9 (4.3%) and 11 (5.3%) cases, respectively. The deletion of *MIR31* was significantly associated with the deletion of both *CDKN2A* and *CDKN2B* (p<0.001). We detected associations between deletions in 9p21 region and *ETV6, IKZF1* and *PAX5*: concurrent deletions in 9p21 and *ETV6* were found in 42 (7.0%) cases (p=0.04), in 9p21 region and *IKZF1* were found in 41 (6.3%) cases (p=0.008) and in 9p21 region and *PAX5* were found in 44 (7.0%) cases. Homozygous deletions in *CDKN2A* and *CDKN2B* genes were associated with worse event free survival (p=0.03), but there was no significant impact on overall survival. Methylation of p15, p14 and p16 promoters were not associated with other molecular alterations. Methylation of p15 worsened overall survival (p=0.003) and the tendency was seen regarding shortening event free survival.
Summary and Conclusions: Deletions in *CDKN2A* and *CDKN2B* are commonly associated with deletions of *ETV6*, *PAX5* and *IKZF1* genes. The co-occurrence with deletions in *IKZF1* may explain the fact of worse prognosis in terms of both EFS and OS in patients with homozygous deletions of *CDKN2A*. We found that the deletion of *MIR31* is a valid indicator of large deletions in 9p21. Methylation of p15 promoter seems to be a frequent and biological important occurrence in childhood ALL.

E857

MAJOR IMPACT OF AN EARLY CHECKPOINT FOR MINIMAL RESIDUAL DISEASE IN FLOW CYTOMETRY IN CHILDHOOD ALL: A BICENTRIC STUDY

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Background: Although childhood acute lymphoblastic leukemia (ALL) is of good prognosis, about 15 to 25% of the patients relapse. The early persistence of positive minimal residual disease (MRD) is considered as a poor prognostic factor indicative of chemoresistance.
Aims: We report a study on the level of MRD assessed by multiparameter flow cytometry (MFC) in the bone marrow samples used for morphologic assessment of chemosensitivity at day 21 (D21) post induction.
Methods: This study enrolled 123 children (median age 5 years, range 6 months-21 years), treated between 2006 and 2014 in Nantes and Marseilles University Hospitals with a median follow up of 30 months. On D8, 85 children were corticosensitive and 21 were unassessable (<1G/L blasts at diagnosis). On D21, 109 patients with less than 5% bone marrow blasts were considered chemosensitive. Sixteen relapses were observed and 5 patients died.MRD was investigated at D21 (MRD0) in MFC and at day 35 by RT-qPCR for IGH/TCR rearrangements (MRD1). Antibody combinations (5 to 10) corresponding to the specific Leukemia Associated ImmunoPhenotypes of the blasts at diagnosis was used. A minimum of 10 clustered events allowed to assess a positive result. Acquisition of at least 100,000 events were required for a negative result. A median of 537 816 events were analyzed per patient.Event-free survival (EFS) and overall survival (OS) were studied according to the log-rank test. A Chi2 test was performed to compare MRD0 to MRD1.
Results: Early MRD defines three risk groups: three risk groups were established according to the level of MRD0 by MFC at D21: Level 1 (high risk, n=25)>10^{-2}, Level 2 (intermediate risk, n=46) 10^{-2} to 10^{-4} and Level 3 (low risk, n=50) <10^{-4}.OS (p=0.048) and EFS (p=0.00017, Figure 1) were significantly different between the three MRD0 risk groups. These subgroups also allowed to reclassify patients at high cytogenetic risk, those reaching level 3 presenting no relapse (p=0.02). Early MRD levels are more discriminating than classically defined early responses to therapy: evolution of the 14 corticoresistant patients appeared to strongly depend on their MRD0 level (p=0.004), all low-risk level 3 patients still being in complete remission 1 (CR1) at 4 years while all high-risk level 1patients had relapsed. Similarly, chemosensitive patients had a significantly different evolution according to their level of MRD0, both for EFS (p=0.004) and OS (p=0.02). MRD0 has more prognostic value than MRD1: MRD0 and MRD1 levels were compared for 112 patients. Consistent results (-/- or +/+) were observed in 57.2 % of the cases. Four patients had a low level of MRD1 but undetectable MRD0. By contrast, the 44 MRD0+/MRD1- patients had a significantly worse EFS (p=0.004) than the 33 with undetectable MRD at both MRD0 and MRD1.Multivariate analysis for EFS, using the Cox model analysis with age, corticosensitivity, chemosensitivity, MRD0 and MRD1, only retained a significant prognostic value for age (p=0.01) and MRD0 (p=0.0001).

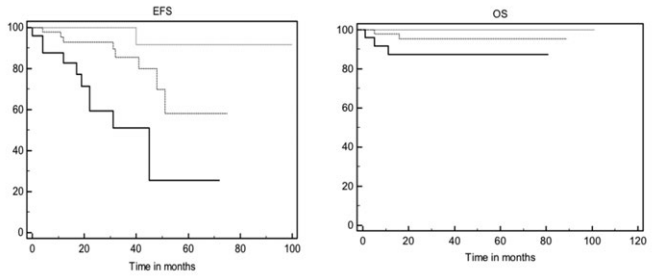

Figure 1. Comparative EFS and OS according to MRD levels in MFC at day Level 1, solid line, level 2 dashed line, level 3 dotten line.

Summary and Conclusions: This study confirms the usefulness and superiority of MRD detection by MFC in day21 bone marrow in order to better discriminate patients with less than 5% blasts in cytomorphology at this time point. In addition, MFC MRD0 identifies a subgroup of patients with poorer prognosis (MRD0+/MRD1-) who would be missed with MRD only performed on D35. MFC thus provides valuable early information on the actual chemosensitivity of these children.

E858

TARGETING THE P53–MDM2 INTERACTION BY THE SMALL-MOLECULE MDM2 ANTAGONIST NUTLIN-3A IN ADULT PHILADELPHIA POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS

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Background: The human p53 tumor suppressor is functionally impaired in nearly 50% of human cancers. Although its alterations are relatively infrequent in B-ALL, wild-type *TP53* remains dysfunctional due to overexpression of MDM2 and also to deletion of *CDKN2A* gene.
Aims: In testing novel therapeutic approaches activating p53, we investigated the preclinical activity of the MDM2 antagonist, Nutlin-3a, in Philadelphia positive (Ph^+) and negative (Ph^-) leukemic cell line models, and primary Ph^+B-Acute lymphoblastic leukemia (ALL) patient samples.
Methods: Ph^+(BV-173 and SUP-B15) and Ph^- (NALM-6, NALM-19 and REH) cell lines and primary Ph^+ALL cells were incubated with increasing concentrations of Nutlin-3 for different times. Methanethiosulfonate test was used to evaluate cell viability and flow cytometry was performed to analyze the apoptosis. Gene expression profile (GEP) was performed using Affymetrix GeneChip Human Gene 1.0 ST platform. p53, BMI-1, p21, caspase-3 and caspase-7 protein expression was evaluated by Western Blot (WB). *BMI-1* mRNA expression was measured by quantitative real time PCR in ALL patient samples.
Results: In order to investigate the effects of Nutlin-3a treatment, we examined cell viability of $Ph^+$and Ph^- cell lines and primary Ph^+ALL cells. Nutlin-3a efficiently reduced cell viability of Ph^+(BV-173 and SUP-B15) and Ph^- (NALM-6 and NALM-19) ALL cells with wild-type p53, in dose-dependent manner. In contrast, no significant changes in cell viability were observed in chronic myeloid leukemia p53-null cell line K562 and in Ph^- ALL p53-mutated cell line REH after incubation with MDM2 inhibitor. Next, we observed that Nutlin-3a treatment, at 5 μM concentration for 24 hours, induced a reduction of cell viability, ranging from 25% to 40%, on primary ALL cells compared to their untreated counterpart. Notably, the dose-dependent reduction in cell viability was confirmed in primary blast cells from two Ph^+ALL patients with the T315I BCR-ABL kinase domain mutation. MDM2 inhibitor treatment increased p53 protein expression in wild-type p53 cell lines activating p53 pathway, as demonstrated by increasing expression of p53 downstream target, p21, and activation of the cleaved caspase-7 apoptotic marker. Nutlin-3a also induced a dose- and time-dependent apoptosis in BV-173, SUP-B15 and NALM-6 cell lines, with wild-type p53. By contrast, REH cells were not sensitive to Nutlin-3a. In order to better elucidate the implications of p53 activation and to identify biomarkers of clinical activity, GEP analysis in sensitive cell lines showed 621 genes (48% down-regulated and 52% up-regulated) differentially expressed ($p<0.05$) upon treatment. We found a strong down-regulation of *BMI-1*, repressor of INK4/ARF and p21, after treatment as compared to control cells (fold-change -1.11; p=0.03). This was validated in BV-173 and SUP-B15 cells after Nutlin-3a treatment. Noteworthy, *BMI-1* mRNA expression was down-regulated in Ph^+ALL patients after treatment ($p<0.05$), if compared with their untreated counterparts.
Summary and Conclusions: Inhibition of MDM2 efficiently reduces cell viability and induces p53-mediated apoptosis in ALL cells. Therefore, targeting p53-MDM2 could be able to overcome or synergize with conventional therapies in Ph^+ALL patients with wild-type p53. Our results suggest a new possible therapeutic strategy in resistant T315I mutated ALL patients. These findings provide a strong rational for further clinical investigation of Nutlin-3a in $Ph^+$and Ph^-ALL.
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E859

CHARACTERIZATION OF IMMUNOPHENOTYPING AND MOLECULAR PROFILE OF FLT3 MUTATIONS IN CHILDHOOD T-CELL LEUKEMIA

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Background: T-cell Acute Lymphoblastic Leukaemia (T-ALL) in childhood is an aggressive and biological heterogeneous malignancy. T-cell immature immunophenotype might identify settings of T-ALL with gene mutations. The FMS-like kinase-3 (FLT3/CD135) and the stem cell factor receptor (cKit/CD117) are members of the receptor tyrosine kinase family that contribute to normal differentiation and proliferation of hematopoietic cells. Mutations in *FLT3* -internal tandem duplication (ITDs) and single mutations in the tyrosine kinase domain (TKD), results in constitutive activation of the FLT3 receptor in the absence of ligand.
Aims: To identify *FLT3* mutations in a cohort of childhood T-ALL at diagnosis at any point in time and in relapses; to test the possible correlation between *FLT3* mutation with cellular expression of CD135 and CD117
Methods: A cohort of 178 T-ALL (≤18 years) was evaluated throughout flow cytometry and molecular techniques. Diagnostic samples (DS, n=157) prior to any oncotreatment, and relapsed samples (RS, n=23) were selected. The levels of cellular surface of CD135 and CD117 were quantified by multiparametric flow cytometry (M-FCM). The monoclonal antibodies anti-CD135; CD117; CD135;CD34; CD127 were included in four or 6 colours to M-FCM analysis. These variables were categorized by percentage of cell positivity and median intensity fluorescence. Positivity was evaluated in CD45 intermediate/low gate in≥20% of blasts cells. DNA and RNA were extracted from BM aspirate or PB blast cells to perform molecular tests (*SIL-TAL1, HOX11L2, IL7R* and *FLT3* mutations); *FLT3*-D835 mutations were tested in exon 17 by restriction fragment length polymorphism PCR assay, whereas, *FLT3*-ITD was examined by the amplification of the juxtamembrane domain in exons 11 and 12. To compare the distribution of categorical variables Fisher`s exact test was used and Mann-Whitney U test comparing two groups (positive/negative) and Kruskal Wallis test comparing more than two groups to test differences in continuous variable. P-values of <0.05 were considered statistically significant.
Results: Fifteen out of 176 (8.5%) cases presented mixed phenotype- T / Myeloid lineage. Early T cell precursors (ETP-ALL) were identified in 20 cases (11.4%). Amongst the remaining 141 T-ALL cases, 30 T-II (18.6%), 78 T-III (48.4%) and 33 T-IV (20.5 %) cases were found. CD127 was expressed in 55 out of 70 (78.6%) cases tested; whereas CD117 was expressed in 19 out of 86 (22%) and CD135 found in 10 out of 85 (11.7%) cases tested. *FLT3*-ITD were founds in 8 out of 157 (5%) of DS and in 2 out of 23 (8.7%) in RS. There were no statistical differences regarding demography (gender and age at the diagnosis), and clinical features (mediastinal mass, high white blood cell count or SNC involvement) in T-ALL with *FLT3-ITD.* The frequencies of somatic aberrations of *SIL-TAL1, HOX11L2, IL7R* and *FLT3* mutations were 24.8%, 12%, 10% and 7%, respectively. Besides *FLT3* and *IL-7R* mutation were also mutually exclusive. No association was observed in CD127 and CD135 cellular expression and T-ALL subtypes, nor association with *IL7R* mutation (p=0.64). The percentage of CD117 positive cells was greater in the ETP-ALL than other subtypes (T II, T-III and T-IV) (p <0.001); The variation of CD117 expression according to *FLT3* and/or *IL7R* mutations demonstrated that high expression level of CD117 were associated with FLT3 mutation (p=0.02), whereas CD117 lower expression with *IL7R* mutation (p=0.03). The higher expression of CD135 was associated with *HOX11L2* alteration (p=0.012) and with *FLT3* mutation (p=0.03). The CD127 expression was higher in cases with *SIL-TAL1* rearrangement (p=0.04) and in cases with mutated *FLT3* had a lower expression (p=0.04).
Summary and Conclusions: *FLT3-ITD* is important in the pathogenesis of T-ALL and it seems to contribute to disease progression in cooperation with other alterations. Preliminary results demonstrated that CD117 and CD127 could predictive molecular aberrations in some T-ALL settings.

E860

IMPACT OF SMALL MOLECULE INHIBITORS OF SKP2 IN T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: T-cell acute lymphoblastic leukemia (T-ALL) accounts for 10-

15% of pediatric leukemias. NOTCH1 activating mutations are found in more than 50% of T-ALLs and have been shown to induce transcription of the S phase kinase-associated protein 2 (*SKP2*), which in turn decreases CDK inhibitor $p27^{KIP1}$ levels, thereby promoting cell cycle progression. During the last years, efforts have been directed towards therapeutically targeting the ubiquitin-proteasome system in order to reduce protein degradation. Recently, small molecule inhibitors of SKP2, which showed different specificities, have been developed. Compound C1 has been described to specifically target the interface between SKP2 and CKS1, an accessory protein responsible for the recognition of Thr187-phosphorylated $p27^{KIP1}$ (Wu *et al.*, 2012). A second compound, C25, interacts with SKP2 and prevents its binding to SKP1, thus inhibiting SKP2-SCF E3 ligase activity (Chan *et al.*, 2013)

Aims: The purpose of this study was to evaluate the effects of these new small molecule inhibitors of SKP2 in T-ALL.

Methods: To assess the impact of SKP2 inhibitors, we treated four different T-ALL cell lines (Jurkat, DND-41, TALL-1, and Loucy) with the compounds C1 or C25 for 24 and 48 hours. Trypan blue exclusion assay was used to determine the number of viable cells. Apoptosis (Annexin V/PI double staining) and cell cycle distribution were analyzed by flow cytometry. Equal amounts of cell lysates were subjected to immunoblot analysis in order to evaluate the protein levels of the main cell cycle regulators.

Results: As has been previously shown by others, we, too, observed that SKP2 is up regulated in T-ALL, suggesting that SKP2 inhibitors may hold promise for therapeutic intervention. Applying compound C1 resulted in a decrease of cell proliferation in a dose- and time-dependent manner in all cell lines. In contrast, when using C25, only TALL-1 showed a slight reduction in cell growth. To evaluate whether the observed growth inhibition was related to cell death, we performed Annexin-V/propidium iodide staining and flow cytometry analysis. Depending on the cell line used, compound C1 showed different effects with a tendency towards a faster cell death in Loucy, as compared to Jurkat, and no impact at all on the other cell lines. This observation was confirmed by western blotting for cleaved PARP. In marked contrast, cells treated with the C25 inhibitor did not show any induction of apoptosis and cell death. Notably, in TALL-1 and DND-41 cells, neither C1 nor C25 induced major changes in viability, which - together with the observed growth inhibition - suggests an effect on the cell cycle as the main impact of the inhibitors. To further substantiate this notion, we assessed the influence of the two compounds on cell cycle progression. On the one hand, in TALL-1 and DND-41 C25 treatment resulted in an accumulation of G0/G1-phase cells, reflected by an increase in $p27^{KIP1}$ protein levels, which inversely correlated with SKP2 expression. However, no effects at all were observed in Loucy or Jurkat. On the other hand, treatment with C1 induced a significant accumulation of G2/M-phase in a dose- and time-dependent fashion in all cell lines. Quite unexpectedly, also the cell line Loucy, which lacks $p27^{KIP1}$ expression, showed a decrease in cell proliferation, increased cell death and achieved a G2 arrest, suggesting that cell cycle arrest occurs independent of $p27^{KIP1}$.

Summary and Conclusions: Our results indicate that T-ALL cell lines show different sensitivities towards SKP2 inhibitors and that the two compounds tested have distinct effects on the cell cycle, apoptosis, and cell death. Furthermore, they raise the question whether the compounds C1 and C25 may have so far unrecognized (off-target) effects.

E861

ANTI-CD47 AND METHOTREXATE INDUCE CELL DEATH BY PHAGOCYTOSIS – PHAGOPTOSIS – OF LEUKAEMIA CELLS, SUGGESTING NOVEL WAYS OF TARGETING CANCER

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Background: Antibodies to CD47 have been shown to increase clearance of many types of cancer cells *in vitro* and *in vivo*, but the means by which they cause cell death and clearance is not yet clear.

Aims: To explore the mechanism of action of anti-CD47 in a model of B-ALL/B lymphoblastic lymphoma.

Methods: U937 cells were treated with 10 ng/mL PMA for 24 hours, then allowed to mature for a further 48 hours. Phagocytosis was assayed with a 1:1 target:effector ratio, using either flow cytometry (2 hour co-incubation), or fluorescent microscopy for live/dead cell phagocytosis of propidium iodide stained target cells (1 hour co-incubation). Live cell imaging of the mitochondrial membrane potential measured with JC-1 stained target cells was performed over 3 hours. The blocking CD47 antibody clone B6H12 was used at 2 µg/mL, with $F(ab')_2$ blocking in a 1:5 molar ratio. CD19 at 2-10 µg/mL, 4N1K at 0.12 nM-30 µM, thrombospondin at 1.2-12 nM, methotrexate at 0.01 µM and 6-mercaptopurine (6MP) 5 µM (24 hours for phagocytosis assays, up to 72 hours for direct cell death).

Results: Anti-CD47 strongly increased the phagocytosis of 697 cells, a pre-B ALL cell line, by U937 macrophages. The leukaemia cells were alive when phagocytosed by macrophages as indicated by their exclusion of propidium iodide and their intact mitochondrial membrane potential. However, death rapidly followed as shown by mitochondrial depolarisation of leukaemia cells after their engulfment. 2 µg/mL anti CD47 was sufficient to clear all leukaemic cells within 3 hours. Inhibition of phagocytosis prevented clearance and death of leukaemic cells, indicating that cell death was by phagoptosis. Phagocytosis required the Fc domain of the anti CD47 antibody as blocking this domain with $F(ab')_2$ prevented the phagocytosis. However, an antibody against CD19, a B cell marker, did not increase phagocytosis, indicating that phagocytosis required both antibody binding with an available Fc domain and blocking/activation of CD47. Alternative ligands for CD47, low dose 4N1K and thrombospondin, had little effect on phagocytosis, suggesting that activation of CD47 is not sufficient for phagocytosis. Finally, low doses of the maintenance phase treatment methotrexate increased the phagocytosis and subsequent death of live 697 cells. The same doses induced little or no death of the lymphoblasts in the absence of macrophages.

Summary and Conclusions: CD47 is an exciting treatment for lymphoid malignancies, and does induce death of live cells by phagocytosis (phagoptosis). This finding may explain the mechanism of action of the little understood requirement for prolonged low dose maintenance phases, and this could potentially be shortened by utilising the more effective pro-phagocytic treatment anti-CD47.

E862

CD22 AS A TARGET FOR ELIMINATION OF THE LEUKEMIC BLASTS IN B-CELL PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Epratuzumab is a humanized anti-CD22 monoclonal antibody (mAb) derived from LL2 clone. Its B-cell suppressive effect was demonstrated in autoimmune diseases (systemic lupus erythematosus), and some hematological malignancies (non-Hodgkin lymphoma). In current IntReALL study of iBFM group, epratuzumab is included in post-induction treatment phase in relapsed B-cell precursor acute lymphoblastic leukemias (BCP-ALL). CD22 function in mature B cells is partly known, the exact mechanism of action of epratuzumab is not completely understood. Furthermore, the effect of the concomitant and proceeding chemotherapy on CD22 expression remains unknown.

Aims: Optimize the detection of CD22 before and during epratuzumab treatment. Assessment of the level of CD22 expression on leukemic blasts and its correlation with BCP-ALL genetic subtype. Monitor CD22 expression on leukemic blasts in BCP-ALL patients during treatment. Determine the effect of epratuzumab to leukemic cell proliferation, cell cycle, apoptosis, and intracellular signaling.

Methods: CD22 was detected with 2 different antibody clones (S-HCL-1 and epratuzumab) by flow cytometry. Proliferation was determined by cellular counting. Changes in cell cycle were monitored with Pyronine Y/Hoechst 33342 staining. Apoptosis was checked with Annexin V/propidium iodide or DAPI staining. Intracellular signaling (phosphorylation of Syk, Akt and Erk1/2) was measured by single cell phospho-flow cytometry.

Results: For optimal detection of CD22 three temperatures (4, 25, and 37°C) and two antibody clones were tested. We observed reduction of epratuzumab binding to cells with decreasing temperature (n=3): 41% at 25°C and 2% at 4°C. In contrast, S-HCL-1 binding did not differ at low temperature (34% at 25°C and 40% at 4°C). This reduction of LL2 affinity at 4°C was previously described (Mattes et al, 1997). Moreover epratuzumab and S-HCL-1 partially competed for CD22 binding but we were able to detect CD22 by S-HCL-1 at 25°C well when the cells were pre-incubated with epratuzumab. *In vitro* experiments did not show any anti-proliferative effect of epratuzumab on leukemia cell lines (n=5) probably due to their partial growing independence on external stimuli. In contrast, we detected reduced numbers of viable B-cells isolated from healthy peripheral blood after epratuzumab treatment (n=3, $p<0.05$). In addition, cell cycle transition from G0 to G1 phase was inhibited after 24h (n=2, $p<0.05$) and intracellular signaling led via phosphorylation of Syk and Erk1/2 in healthy B-cells (n=4). Deeper proteomic profiling is in progress. In our so far limited cohort of patients with BCP-ALL where the CD22 expression was quantified with the two mAb clones (n=16), the CD22 levels varied greatly. In contrast, CD22 expression on mature B cells was comparable among healthy individuals (n=8). The only cases without detectable CD22 expression were found among patients with pro-B BCP-ALL.

Summary and Conclusions: CD22 is detected by both clones of antibodies at 25°C at diagnosis and relapse. During the epratuzumab treatment, CD22 can be monitored by S-HCL-1 clone. CD22 expression varies on leukemic blasts among individuals. CD22 negativity is found within pro-B BCP-ALLs. BCP-ALL cell lines are frequently resistant to epratuzumab treatment and cannot be used as *in vitro* model. In healthy B-cells, epratuzumab partially inhibits cell cycle and proliferation *in vitro*, the signaling occurs via B-cell receptor associated kinase Syk and via kinase Erk1/2. *Supported by FP7 IntReALL, IGA NT13462, UNCE 204012.*

E863

BONE MARROW STROMAL PRECURSOR CELLS OF THE ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS TREATED ACCORDING TO THE ALL-2009 PROTOCOL RESTORE THEIR FUNCTIONAL AND BIOLOGICAL POTENTIAL DAMAGED BY THE DISEASE

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Background: Stromal microenvironment essential for normal hematopoiesis, as it forms niches for hematopoietic precursors, suffers during leukemia development. High dose chemotherapy affects not only hematopoietic cells but also stromal precursor cells.
Aims: The goal of this study was to analyze the alterations in the characteristics of human multipotent mesenchymal stromal cells (MSC) and their more differentiated progeny – fibroblastic colony forming units (CFU-F), derived from the bone marrow (BM) of acute lymphoblastic leukemia (ALL) patients at the moment of diagnosis and during treatment according to the protocol ALL-2009.
Methods: Fourteen newly diagnosed cases (8 B-ALL and 6 T-ALL) were involved in the study after informed consent. BM was aspirated prior to any treatment and at days 37, 100 and 180 since the beginning of the protocol. MSC were cultured in aMEM with 10% fetal calf serum. Cumulative MSC production was counted after 3 passages. CFU-F concentration was analyzed in standard conditions. The ability of MSC to maintain hematopoietic precursor cell was assessed by cobble-stone area forming cells (CACF-1-2 wk) method. As a reference control MS5 cell line was used. The relative expression level (REL) of different genes was measured by TaqMan RQ-PCR. As a control MSC and CFU-F from 88 healthy donors of BM for alloHSCT were used.
Results: The CFU-F concentration in the BM of patients at the moment of ALL diagnostics was about 1/7 of the donor's (table, p<0.0001). BM blasts count did not correlate reversely with CFU-F concentration in the BM of each patient, thus the dramatic decrease in CFU-F concentration is caused by the damage of this stromal precursor cells by leukemic cells. The treatment of patients resulted in the restoration of CFU-F concentration in as little as 37 days. Cumulative MSC production at the disease manifestation did not differ from donor's, while their ability to maintain normal hematopoietic cells had the tendency to decrease. At the same moment in MSC the REL of PDGFRA and PDGFRB increased in 3 and 2 fold correspondingly (p=0.01 and 0.03), while REL of TGFB2 decreased 3 fold (p=0.002). Chemotherapy did not affect MSC production and during treatment their ability to maintain CAFC 1-2wk normalized. Table Main characteristic of stromal precursor cells from the BM of ALL patients.

Tabella 1.

	CFU-F per 106 BM cells	Cumulative MSC production per 3 passages, x106	Frequency of CAFC 1-2wk, proportion to MS5
Donor	25.4±3.0	7.1±1.0	74.9±9.5
Before treatment	3.8±1.6	7.9±2.4	57.5±11.4
37 days of ALL-2009	30.3±6.8	9.7±2.5	62.1±8.2
100 days of ALL-2009	31.5±1.0	11.1±2.4	74.4±16.6

Summary and Conclusions: It was demonstrated that Protocol ALL-2009 was highly effective for treatment adult ALL patients. Such treatment restores the functional and biological characteristics of stromal microenvironment initially damaged by leukemic cells.

E864

THE PAN-BCL-2-TARGETING DRUG OBATOCLAX (GX15-070) AND THE PI3-KINASE/MTOR-INHIBITOR BEZ235 PRODUCE SYNERGISTIC GROWTH-INHIBITORY EFFECTS ON LEUKEMIC CELLS IN PATIENTS WITH PH+AND PH- ALL

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Background: Acute lymphoblastic leukemia (ALL) is a life-threatening stem cell disease defined by abnormal expansion of lymphoblasts in hematopoietic tissues. Despite improved therapy only a subfraction of patients can be cured. Therefore, current research is attempting to identify new drug targets and novel targeted drugs to improve therapy in these patients. A number of previous and more recent data have shown that members of the BCL-2 family and components of the PI3-Kinase/mTOR pathway are critically involved in the regulation of growth and survival of leukemic cells in ALL.
Aims: We examined the effects of the pan-BCL-2-targeting drug obatoclax (GX15-070) and the dual PI3-Kinase/mTOR blocker BEZ235 on growth and survival in ALL cells.
Methods: The effects of obatoclax and BEZ235 on proliferation were examined by ^{3}H-thymidine-uptake, and drug-induced apoptosis was determined by flow cytometry and Western blotting.
Results: Both drugs were found to suppress the *in vitro* proliferation of leukemic cells obtained from patients with Ph+ALL (n=4) and Ph- ALL (n=9) in a dose-dependent manner (obatoclax $IC_{50:}$ 0.05-0.1 µM; BEZ235, IC_{50}: 0.01-0.5 µM). In addition, obatoclax and BEZ235 suppressed the proliferation in the Ph+ALL cell lines BV-173 (obatoclax IC_{50}: 0.08-0.3 µM; BEZ235, IC_{50}: 0.01-0.03 µM), NALM-1 (obatoclax IC_{50}: 0.1-0.5 µM; BEZ235, IC_{50}: 0.05-0.1 µM), TOM-1 (obatoclax IC_{50}: 0.1-0.3 µM; BEZ235, IC_{50}: 0.01-0.03 µM), and Z119 (obatoclax IC50: 0.05-0.1 µM; BEZ235, IC50: 0.03-0.05 µM) as well as in the Ph- cell lines RAJI, RAMOS, REH, and BL-41 (IC_{50}: <1 µM). The growth-inhibitory effects of both drugs were accompanied by apoptosis in all cell lines examined, as evidence by Western blotting using an antibody against cleaved caspase 3 as well as by AnnexinV/PI-staining and staining for active-caspase 3 by flow cytometry. We also confirmed that BEZ235 counteracts phosphorylation of pAkt and pS6 in all cell lines tested. Since obatoclax and BEZ235 interact with a different spectrum of molecular targets, we also determined drug-combination effects. In these experiments, obatoclax was found to cooperate with BEZ235 in inhibiting the growth of all Ph+ALL cell lines and all Ph- cell lines tested. Finally, we were able to show that primary ALL cells, CD34+/CD38- leukemic stem cells and all cell lines tested express major drug targets, including members of the BCL-2 family (BCL-2, MCL-1, BCL-xL), the PI3-Kinase and mTOR.
Summary and Conclusions: Obatoclax and BEZ235 effectively counteract growth and survival in ALL cells. Both agents exert synergistic anti-leukemic effects on ALL cells which may have clinical implications.

E865

GDF15 MODULATES HEPCIDIN-FERROPORTIN AXIS IN LYMPHOBLASTIC LEUKEMIC CELLS

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Background: Being a novel pleotrophic member of TGF-beta family, growth differentiation factor 15(GDF15) is intimately involved in cancer development and prognosis, and has been documented recently as an upstream negative regulator of the iron regulatory hormone hepcidin, with its expression upregulated remarkably in a variety of anemias characterized by ineffective erythropoiesis.
Aims: To investigate the expressions of GDF15, key iron regulators and transporters, and oxygen sensing molecule hypoxic inducible factor 1 alpha (Hif1alpha) in lymphoblastic leukemic cells, exploring roles of GDF15 on modulation of iron homeostasis in lymphoblastic leukemic cells.
Methods: Sixty newly diagnosed children with ALL and 19 age-and-sex matched control children were enrolled in this study, together with 5 types of leukemic cell lines. mRNA expressions of target genes were assayed by Eva-Green real-time RT-PCR in triplicates. Relative gene expression was represented by the mean of target gene against housekeeping gene by delatdeltaC$_t$ method. suppressed in ALL group as against controls (P=0.000) and was positively correlated both to hepcidin and GDF15. (4) There was no significant difference in terms of Hif1alpha expression in the two groups.
Results: (1) Hepcidin was expressed in both leukemic cell lines and clinical sample of primary lymphoblastic cells. GDF15 expression in ALL group was significantly increased as compared to controls(p=0.026), with median of expression being 0.5 and 0.005 in ALL and control groups respectively; (2) Expression of GDF15 was significantly higher in ALL group than that in controls (p=0.003) and was positively correlated to hepcidin(P=0.000); (3) Expression of ferroportin was remarkably suppressed in ALL group as against controls (P=0.000) and was positively correlated both to hepcidin and GDF15. (4) There was no significant difference in terms of Hif1alpha expression in the two groups.
Summary and Conclusions: We demonstrated that GDF15 is remarkably upregulated in ALL cells independent of Hif1alpha signaling pathway. More importantly, we found that hepcidin is expressed by both leukemic cell lines and primary cells. In addition, ferroportin, the only cellular iron exporter, is significantly downregulated in lymphoblastic leukemic cell, which is consistent with reduced expression in breast cancer cells (cell lines). And GDF15 expression is positively correlated both to hepcidin and ferroportin. Taken together, our study strongly indicates that upregulation of GDF15 stimulates induction of hepcidin by leukemic cells themselves, with in turn mediates enhanced ferroportin internalization and degradation, resulting in reduced iron efflux from leukemic cells, providing more iron available for leukemic cell proliferation. Further studies are urgently needed to explore interrelationship between regulations of cellular and systemic iron homeostasis in patient with lymphoblastic leukemia.

E866

PROGNOSTIC SIGNIFICANCE OF THE CYTOGENETIC EVOLUTION AFTER THE ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN PEDIATRIC ACUTE LEUKEMIA

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Background: Cytogenetic abnormalities at diagnosis have significant impact on disease outcome and clonal evolution at cytogenetic level is considered to be associated with relapse and refractoriness to chemotherapy. Relapse of acute leukemia was reported to be associated with cytogenetic clonal evolution in 40% of patients who have received chemotherapy. The cytogenetic clonal evolution in post-transplant relapse has been recently studied in adult acute myeloid leukemia (AML) by Yuasa *et al.*,2013. They showed that the cytogenetic clonal evolution is an important predictor of resistance to therapy after allogeneic hematopoietic stem cell transplantation (alloHSCT).
Aims: To study karyotypic changes in post-transplant relapses of pediatric acute leukemia in order evaluate a prognostic impact of the cytogenetic clonal evolution on survival.
Methods: We retrospectively reviewed children and adolescents with diagnosis of AML or ALL who underwent allo-HSCT at our institute from 2009 to 2014 and analyzed the cytogenetic evolution patterns in patients (pts) at relapse of acute leukemia after HSCT.
Results: Thirty children and fifteen adolescents with post-transplant relapse were included in this study. The bone marrow karyotypic changes were analyzed before and after allo-HSCT. Twenty six (58 %) pts were male, and median age at alloHSCT was 10 years (range, 1,2-21). Seventeen (38 %) pts are still alive. Thirteen (29 %) pts received related transplantation, 15 (33 %) did unrelated HSCT, and 17 (38 %) did haploidentical HSCT. Four (9 %) pts had normal karyotype, 22 (49 %) had one chromosomal abnormality, 4 (9 %) had two abnormalities, and 15 (33 %) had more than three abnormalities before HSCT. Median follow-up after HSCT was 500 days (40-861). Twenty-eight (62 %) pts showed gain or loss of chromosomal abnormalities from original one (group 1), 16 (36 %) showed no karyotypic change between HSCT, and 1 (2 %) showed totally different karyotype. We defined the cytogenetic evolution group as group 1, and others as group 2. Median time from transplantation to relapse was not different between group 1 and 2. Shorter survival after relapse was observed for group 1 (8,9 % *vs* 51,5 % at 2-year post-relapse survival, P=0,041)(Figure1). Moreover, survival after relapse was inferior for pts with 2 or 3 cytogenetic subclones (n=10) compared to pts with 1 cytogenetic clone (n=35) (0% *vs* 32,5 % at 2 year post-relapse survival, P=0,016).

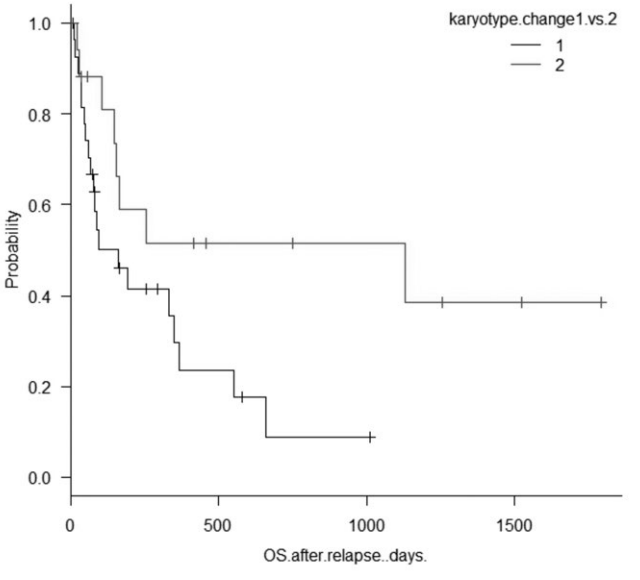

Figure 1.

Summary and Conclusions: Survival time of pediatric acute leukemia pts was similar with those of the above-mentioned adult AML being significantly shorter for group 1 than group 2. It supports that cytogenetic clonal evolution may be an important predictor of resistance to therapy after allo-HSCT, and needs novel therapeutic approaches.

E867

SEQUENCE ANALYSIS OF CLONAL IMMUNOGLOBULIN REARRANGEMENTS IN ACUTE LYMPHOBLASTIC LEUKEMIA: PATHEGENESIS AND MECHANISM

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Background: Clonal immunoglobulin rearrangements are assembled from the joining of (V) variable, (D) diversity and (J) joining gene segments (VDJ rearrangement). This process takes place during the maturation of B-cells.
Aims: Aim of the present study was to evaluate the clonal immunoglobulin rearrangements in children with B-cell acute lymphoblastic leukemia (ALL), in order to describe the underlying mechanism and ascertain whether it is influenced by the stage of leukemogenesis and the clonal evolution. Furthermore, characteristics of the VDJ rearrangement were evaluated as novel prognostic factors.
Methods: DNA extracted from 80 bone marrow samples at diagnosis of ALL patients was amplified by Polymerase Chain Reaction. Direct sequencing for PCR products was carried out on a MJ Research PTC-225 Peltier Thermal Cycler. The V, D and J segments were identified using the ImMunoGeneTics Information System (IMGT, European Bioinformatics Institute, Montpellier, France) and the software algorithm JOINSOLVER in order to characterize the closest matching human germline counterparts. The rearranged IGH V-D-J sequences were characterized as productive or non-productive.
Results: V region was identified in all VDJ rearrangements, involving 21 different gene segments, while 45% were in the 3' terminus of chromosome 14. V3 family (V3-13, V3-15, V6-1, V3-11) was the most common (55%). In pre-B-ALL patients, V3-11 and V6-1 regions were used frequently (67%). D identification was feasible at 84% rearrangements, distinguishing totally 24 different sections. In 73% sequences, segments of the 3' end of region D were associated with gene segments of the 5' end of J region. In 9.7% cases, D irregular recombination (DIR) signal sequences were recognized. In patients with pre-B-ALL only J4 and J6 areas were observed. In 90% of the rearranged VDJ sequences the CDR3 region was short. Insertion of N regions was recorded in 95% of rearrangements with a greater number of nucleotides in the region between V and D. Most sequences (75.5%) were non-productive and were associated with better prognosis, which means 97% of relapse-free events. On the other hand, productive rearrangements counted for 24.5% of cases and were linked to worse outcome that is 75% of relapse-free episodes.
Summary and Conclusions: The selective involvement of the V gene fragments depending on their position on chromosome 14 reinforces the theory of D-proximal model. The association of D and J for the creation of DJ complex is selective depending on the stage of maturation of B-cells. The superiority of V3-11 and V6-1 rearrangements in immature B-lymphocytes suggests that possibly the leukemogenesis occurs at the initial stages of B-lymphocytes maturation. Furthermore, the small size of the CDR3 region shows that malignant transformation occurred in the early stages of differentiation of B-cell. The increased risk of relapse in patients with productive VDJ rearrangements is based on the fact that they are completed at the advanced phases of B-cell maturation, which means they are more prone to mutations. Concluding, gene rearrangements are subject to the influence of molecular mechanisms, the discovery of which might give more information regarding leukemogenesis and subsequently serve as a therapeutic target in acute lymphoblastic leukemia of childhood.

E868

NUDT15 GENE POLYMORPHISM RELATED TO MERCAPTOPURINE INTOLERANCE IN TAIWAN CHINESE CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: It is well known that the European descent have higher risk allele frequency of thiopurine methyltransferase (*TPMT*) gene than that of East Asian. However, this can not explain why the East Asian are more intolerable to mer-

captopurine than the European descent. A recent study identified a variant of the *NUDT15* gene (rs116855232 C>T) associated with intolerance to thiopurine in Korean patients with Crohn's disease.
Aims: We aimed to determine the prevalence of *NUDT15* variant in a Taiwan Chinese population and sought to clarify the reason why the East Asian tolerate mercaptopurine poorly.
Methods: Four hundred and four Taiwan Chinese children with acute lymphoblastic leukemia (ALL) at achieving complete remission and 100 adult patients with normal bone marrow (BM) were examined. Pyrosequencing for *NUDT15* (rs116855232) c.415 C>T (NM_018283.1) and genotyping for *TPMT* (rs1142345) c.719 A>G (NM_0003673) by use of TaqMan assay were performed.
Results: Of 404 ALL patients, 224 were boys and 180 girls. Five (3 boys and 2 girls), were homozygous for *NUDT15* rs116855232 (TT), 84 (42 boys and 42 girls), were heterozygous (TC), and the remaining 315 were wild-type (CC), with a risk allele frequency of 11.6 %. In 100 normal adult BM, one was homozygous, 29 were heterozygous, and the remaining 70 were wild-type *NUDT15*, with an overall risk allele frequency of 15.5%. There was no statistical difference between pediatric ALL patients and normal adult BM in *NUDT15* variant (*P*=0.154). By contrast, of the 404 ALL patients, none was homozygous for *TPMT* (GG), 13 were heterozygous (AG), and the remaining 391 were wild-type (AA), with an overall risk allele frequency of 1.6%. In the 100 normal adult BM samples, none was homozygous, one was heterozygous, and the remaining 99 were wild-type *TPMT*, with an overall risk allele frequency of 0.5%. No difference in the variant frequency of *TPMT* genotype was observed between children with ALL and adults with normal BM (*P*=0.325).The high risk *NUDT15* variant was closely associated with the intolerance to mercaptopurine in children with ALL. The maximal tolerable daily doses of mercaptopurine, in which a patient could maximally tolerated for at least several months, in *NUDT15* TT, TC and CC groups were 9.4±3.7 mg/m^2 (mean±SD), 30.7±11.7 mg/m^2, and 44.1±15.3 mg/m^2, respectively (*P*<0.0001). The maximal tolerable dose of mercaptopurine in *TPMT* AG and AA were 31.4±10.2 mg/m^{2}and 41.2± 15.8 mg/m^2, respectively (*P*=0.034). The maximal tolerable daily dose of mercaptopurine in double heterozygous for both *NUDT15* and *TPMT* (n=4) was 32.7±14.3 mg/m^2/day compared with single heterozygous for *NUDT15* or *TPMT* of 30.0±11.7 mg/m^2/day *(P* =0.655*)*, the latter was significantly different from the dose 44.6±15.3 mg/m^2/day in wild-type for both *NUDT15* and *TPMT* (*P*<0.0001). The 5-years event-free survival (EFS) was 87.6%, 90.2%, and 50.0%, for *NUDT15* CC, TC, and TT genotype, respectively (*P*=0.0118). The patients with homozygous for *NUDT15* (TT) had inferior EFS compared with heterozygous TC (*P*=0.034). Neither heterozygous *NUDT15* nor *TPMT* variant alone affected EFS of children with ALL.
Summary and Conclusions: The high frequency of *NUDT15* risk variant, but not the low frequency of *TPMT* risk variant, was closely associated with the intolerance to mercaptopurine in children with ALL in Taiwan.
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E869

HTS COUPLED WITH A BIOINFORMATICS ANALYSIS IN ROUTINE HOSPITAL PRACTICE FOR MULTICLONAL DIAGNOSIS AND MRD FOLLOW-UP IN ALL

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Background: The molecular diagnosis in ALL allows, by the research of V(D)J rearrangements on lymphoblast DNA, to find clonal markers in 95% of the cases. These markers are also used to quantify the minimal residual disease (MRD) by real time Q-PCR to adapt treatments. This strategy fails in some cases: Absence of initial marker, failure of sequencing or emergence at relapse time of a clone which was not observed at diagnosis time or in minority. Several studies have asserted the usefulness of high-throughput sequencing (HTS). It enables deep sequencing of a whole lymphoid population, bypassing some of these problems. However, the huge amount of data raises two challenges. First, hospitals must be able to store and process terabytes of data per year. Second, the data must be nicely synthesized to ease clinician interpretation.
Aims: Here we report the use of HTS in a hematology lab for diagnosis and follow-up of ALL, combined with a bioinformatics analysis and visualization of the results with the new dedicated Vidjil software (Giraud, Salson, et al, BMC Genomics 2014, http://www.vidjil.org).
Methods: In a first work we retrospectively studied the clonality of 8 pediatric patients (5 B-ALL and 3 T-ALL, 2w-6m, 2-14 years) at diagnosis and follow-up (37 follow-up time points). Since January 2015 we lead a prospective study of pediatric ALL clonality at diagnosis in a routine hospital practice (15 patients in mid-February: 11 B-ALL and 4 T-ALL, 2-22 and 10-25 years). 500ng of bone marrow DNA are extracted on Qiagen® Kit and amplified by PCR systems for TRG, TRD, IGKB and IGH targets (compared to only TRG and IGH for the retrospective study). These systems are described or derived from the BIOMED-2 works. The libraries are done with Ion Fragment Plus® Kit and sequenced with an Ion Torrent® 318 or 316-V2 Chip system on PGM™. The Vidjil platform clusters reads in clones, names the clones based on their V(D)J rearrangement and tracks them along the time. Sequences can be aligned, merged and sent to IMGT/V-QUEST or IgBlast. It exports medical results tailored to general laboratory computer system or to a printable file.
Results: We identified a number of clones in the diagnosis samples and observed their evolution at different follow-up time points. All the clones detected by classic methods were also found by Vidjil. Moreover the software allows us to look deeper at other clones appearing at lower concentrations. This way we could easily notice emergence of minority clones or clones that were not detected at diagnosis: Relapses were detected, and for one patient two emerging clones were observed. (See FIGURE 1 below.). No sequencing failure occurred in the 15 prospective analyses compared to one Sanger failure in the usual protocol. The whole analysis from the receipt of the sample to the end of the validation by the clinician was several days faster than the current protocol, with equal resources.

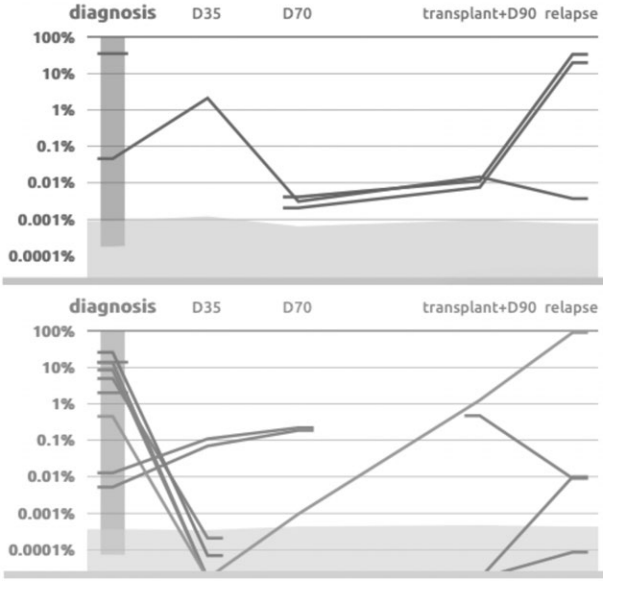

Figure 1.

Summary and Conclusions: Vidjil has been designed and tested for two years in Lille and is now being tested in other French hospitals involved in ALL-MRD. It opens the floodgates to the use of HTS in routine hospital practice. Now all diagnosis samples received in Lille are analyzed both with the usual and the HTS protocols.

LB2074

Abstract withdrawn

LB2076

A NOVEL HOTSPOT FOR IKZF1 WHOLE GENE DELETIONS IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: B-cell precursor acute lymphoblastic leukemia (BCP-ALL) is characterized by chromosomal translocations. Genome-wide studies have revealed additional alterations, such as *IKZF1* deletions (*IKZF1*del). The gene is located at 7p12 and encodes the transcription factor Ikaros, which is involved in the development of the B cell lineage. *IKZF1*del is independently associated with relapse and dismal outcome in BCP-ALL. They are mainly characterized by absence of exons 2-3, 2-8, 4-7, and 4-8. In a recent study, a multiplex PCR

approach was able to detect the most frequent *IKZF1*del on chromosome 7p, which could be used as markers for minimal residual disease monitoring. However, precise data concerning *IKZF1* whole-gene Δ1-8 deletions, which account for approximately 30% of all *IKZF1del*, are still lacking.
Aims: We aimed at characterizing *IKZF1* whole-gene deletions at the genomic level in order to improve the current PCR methods for the analysis of the most frequent *IKZF1* deletions in pediatric patients with BCP-ALL.
Methods: Samples of BCP-ALL with *IKZF1* whole-gene deletions (n=30) were included. Deletions of exons 1-8 were first identified by the multiplex ligation-dependent probe amplification (MLPA) kit (P335-A4), and confirmed using specific *IKZF1* MLPA kit (P202). Six samples were analyzed with CytoScan HD array (Affymetrix. Inc., Santa Clara, CA, USA), and the breakpoints were confirmed by multiplex PCR. Afterwards, 24 samples were screened for the hotspot identified by the array (located within *COBL* gene) with a long distance PCR (LDI-PCR) and sequencing approach.
Results: According to the comparative genome analysis, 2 out of 6 samples with BCP-ALL (33.3%) and *IKZF1* whole-gene deletions had a breakpoint on *COBL* intron 5. They presented a ~1.7 Mb and ~18.8 Mb deletion (7p12.2-*COBL* and 7p14.3-*COBL*). Further screening of 24 samples within *COBL* intron 5 led to the verification of three molecular alterations: a 1.1 Mb inversion of *COBL* that also compromised *IKZF1*; and two chromosomal translocations (Xp21.3-*COBL* and C20orf194-*COBL*). In sum, a hotspot on *COBL* intron 5 was found in 16.7% of the samples (five of thirty samples) with *IKZF1* Δ1-8.
Summary and Conclusions: This study identified chromosomal translocations, deletions, and inversions involving *COBL* intron 5 on samples with *IKZF1* whole-gene deletions. This clearly indicates that *COBL* intron 5 is a new hotspot for *IKZF1* whole-gene deletions. Genetic alterations starting on *COBL* intron 5 also promote *IKZF1* deletions due to the proximity between *IKZF1* and *COBL* (~800 Mb). Further analyses covering a larger genomic area within *COBL* are ongoing in order to unravel novel regions associated with DNA breaks and *IKZF1* deletions.
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LB2082

EXPRESSION ANALYSIS OF GENES LOCATED IN THE COMMON REGION OF AMPLIFICATION IN PEDIATRIC B CELL PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA WITH INTRACROMOSSOMAL AMPLIFICATION OF CHROMOSOME 21

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Background: B-cell precursor acute lymphoblastic leukemia (BCP-ALL) cases frequently present high hyperdiploidy (>50 chromosomes) or chromosomal translocations resulting in chimeric gene fusions (*ETV6-RUNX1, BCR-ABL1, TCF3-PBX1*, and *KMT2A-AFF1*). Recently, molecular cytogenetic studies defined a subgroup among BCP-ALL with cryptic intrachromosomal amplification features on chromosome 21 (iAMP21). The iAMP21 has been described in 1.5-3% of BCP-ALL and is associated with a higher relapse risk when patients are treated under standard regimens. Genomic analysis demonstrated the presence of a common region of amplification (CRA), including genes such as *IFNGR2, GART, SON DSCR1, RUNX, DYRK1A, ERG*, and *ETS*. These genes are described to be involved in the leukemogenesis and/or in the therapeutic response of some leukemia subtypes. Our hypothesis is that aberrant expression of these genes could also contribute to the particularities of iAMP21-BCP-ALL.
Aims: We aimed to identify genes that could be potentially relevant in the leukemogenesis of iAMP21-BCP-ALL by evaluating the expression of genes with functions previously described in leukemia and located in the CRA.
Methods: From 368 bone marrow (BM) samples of patients with BCP-ALL diagnosed between 2002 and 2012, 74 were suggestive for chromosome 21 copy number alterations by *Multiplex Ligation-dependent Probe Amplification* (MLPA). In 9 patients an iAMP21 was confirmed by Fluorescence *in situ* hibridization (FISH). Gene expression analysis of these samples was performed by reverse transcriptase quantitative PCR (qPCR) and CT values of the selected genes among BCP-ALL samples either with or without an iAMP21 were calculated. The non-iAMP21 subgroups were: *ETV6-RUNX1* fusion (n=9), hyperdiploid karyotypes (n=3), no recurrent gene fusions (n=7), and non-leukemic controls (n=7). Statistical analysis was performed using *Student's t-tests,* p values <0.05 were considered statistically significant (*Unpaired t-test*), using the GraphPad Prism 1.5 software.
Results: Patients with iAMP21-BCP-ALL had higher expression of *IFNGR2* (*p=0.0331; **p=0.0147; ***p=0.0023), *ERG* (*p=0.0284; ***p=0.0115), *ETS2* (*p=0.0343; **p=0.0058; ***p=0.0021) and *DYRK1A* (***p=0.0008) genes when compared with *ETV6-RUNX1* (*p), no recurrent gene fusions (**p), or with hyperdiploid karyotypes (***p) groups.

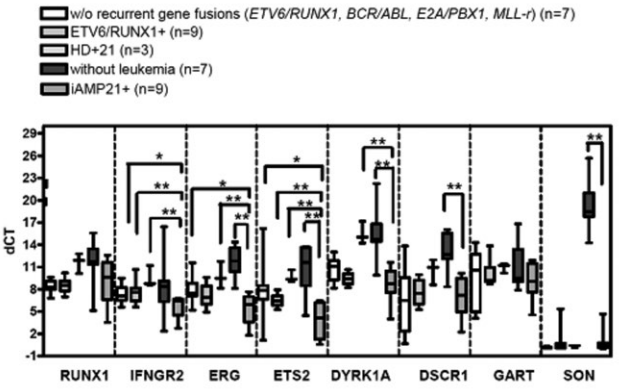

Figure 1.

Summary and Conclusions: Our results suggest that there is a differential expression of some genes in the CRA of samples from patients with iAMP21-BCP-ALL. Thus, these data allow further studies in order to evaluate the contribution of these genes in leukemogenesis and therapeutic response of iAMP21-BCP-ALL.

LB2083

AKT REGULATION OF ONCOGENIC NOTCH PATHWAY IN T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Notch and PI3K/AKT signaling are two key oncogenic pathways closely associated in T-cell acute lymphoblastic leukemia (T-ALL). These pathways collaborate in controlling proliferation, survival and migration of T-ALL cells and are deregulated in ~60% (Notch pathway) and 48% (PI3K/AKT) of T-ALL patients. Recent evidences indicate that in T-ALL cells, Notch and PI3K/AKT pathways collaborate through a reciprocal control.
Aims: of this work was to investigate the molecular mechanisms of PI3K/AKT-dependent Notch1 activity regulation.
Methods: T-ALL cell line, Molt4, and HEK293T cell line were grown in RPMI-1640 and DMEM respectively, supplemented with 10% heat-inactivated FBS. 1 mg RNA isolated from cells was retro-transcribed in 20 ml by M-MuLV reverse transcriptase using random hexamer primers. RT-PCR analysis was performed using primers for Notch1, HES1, preTCRa, GAPDH. Apoptotic cells were identified by Annexin-V and propidium iodide staining. Protein expression was detected by Western blot analysis of whole cell lysates. HEK293T cells were transfected with expression plasmid encoding the dominant negative mutant form of AKT (DN-AKT) and with plasmid encoding full length Flag-tagged NOTCH1 using the DMRIE-C reagent (Invitrogen), according to manufacturer's instructions. Molt4 cells were electroporated with 20 μg of pcDNA3.1 DN-AKT or mock pcDNA3.1 using the Neon Transfection System (Invitrogen) according to the manufacturer's guidelines. After 72 h cells were harvested to prepare protein lysates. Immunoprecipitation of ubiquitin-conjugated proteins was performed using the UbiQapture-Q Kit (Biomol, Exeter, UK), as described by the manufacturer. Co-immunoprecipitation analysis was performed using Protein G Agarose beads, eluted immunoprecipitates were analyzed by Western blot. Immunofluorescent staining was done on HEK293T cells incubated with anti-Flag or anti-c-Cbl primary antibodies and the appropriate AlexaFluor-conjugated secondary antibodies. Images were acquired with a Leica TCS SP2 confocal microscope. A colocalization area was determined based on a 2D cytofluorogram and density analysis performed by Multicolor Analysis Leica Confocal software.
Results: Both LY294002-mediated chemical inhibition of PI3K/AKT signaling and transient transfection of a DN-AKT mutant strongly reduced Notch1 protein level and activity, without affecting Notch1 transcription. We showed that downstream AKT regulator, GSK-3β, did not mediate the effect of PI3K/AKT withdrawal on Notch1 protein. We demonstrated that Notch1 protein decrease upon PI3K/AKT inhibition was due to lysosomal degradation of the Notch1 membrane-bound form. IP and Co-IP analyses revealed that PI3K/AKT withdrawal in Molt4 cells resulted in an increased tyrosine phosphorylation of Notch1 and monoubiquitination of Notch1 as detected by ubiquitin capture assay. Co-immunoprecipitation assay and co-localization analysis further showed that E3 ubiquitin ligase, c-Cbl, interacted with Notch1 in order to direct it into the lysosome for degradation.
Summary and Conclusions: To our knowledge, our results provide the first evidence of mechanism by which AKT pathway controls Notch1 activity reduc-

ing the amount of protein undergoing to lysosomal degradation. Given the crucial role of Notch1 in T-ALL, our findings suggest that hyperactive AKT signaling in T-ALL may contribute to increase the oncogenic Notch signaling in T-ALL independently from mutations in Notch1. Therefore a therapeutic strategy directed to PI3K/AKT signaling in T-ALL could provide advantages to inhibit the dysregulated NOTCH signaling.

Acute lymphoblastic leukemia - Clinical

E870

EVALUATION OF INOSINE TRIPHOSPHATE PYROPHOSPHOHYDROLASE 94 C>A, IVS2 +21 A>C POLYMORPHYSMS IN ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS TREATED WITH 6-MP AND PREDICTION OF ITS MYELOSUPPRESSIVE EFFECTS

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Background: 6- Mercaptopurine (6-MP) plays an importante role in treatment of ALL. ITPA is one of the enzymes which is involved in 6-MP metabolism pathway, catalyzes the pyrophosphohydrolysis of inosine triphosphate (ITP) to inosine monophosphate (IMP) and prevents accumulation of toxic ITP metabolits.
Aims: Our objective was to evaluate the *ITPA 94 C >A, IVS2 +21 A >C* gene polymorphisms in patients with ALL under treatment with 6-MP and prediction of its myelosuppressive effects.
Methods: In this case series study the population consisted of 70 patients diagnosed with ALL in the Division of Hematology-Oncology of Tehran Mofid Hospital. PCR was carried out to amplify exon 2, exon 3, intron 2 and intron 3 of *ITPA* gene. All the amplified fragments were subjected to directional sequencing, then association between genotype and 6-MP toxicity was studied.
Results: In this study two exonic variants including *94 C>A* and *138 G>A* showed a prevalence of 8.5% and 36.4% respectively and two intronic variants, *IVS2 +21 A>C* and *IVS3 +60 G>A* were found in 13.5% and 7% of the samples respectively. The rate of myelosuppression in the presence of mutant homozygote and heterozygote alleles (*94 C>A, 138 G>A, IVS2 +21 A>C* and *IVS3 +60 G>A*) was higher compared to wild type (CC, AA and GG) alleles during the use of 6-MP (P<0.05). Hepatotoxicity in individuals with mutant homozygote and heterozygote *94 C>A* and *IVS3 +60 G>A* alleles was higher than individuals with wild type alleles (CC and GG) during the use of 6-MP (P<0.05).
Summary and Conclusions: Our results indicate that individuals who have aberrant ITPase genotype (mutant homozygous or heterozygous), more likely to have myelosuppression and hepatotoxicity during the use of 6-MP. This results also suggest that pre-therapeutic screening of *ITPA 94 C>A*, *IVS2 +21 A>C* and *IVS3 +60 G>A* may help in minimizing the myelosuppression and hepatotoxicity induced by 6-MP in ALL patients.

E871

COMPARISON OF THREE DIFFERENT PRIMING REGIMENS BASED ON IDARUBICIN, ACLACINOMYCIN OR PIRARUBICIN FOR REFRACTORY/ RELAPSE ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Refractory/relapse acute lymphoblastic leukemia (ALL) has very poor clinical outcome for the resistance to routine chemical agents. Priming regimen was a promissing choice for refractory/relapse ALL, especially for those eldly patients.
Aims: We were interested and analyzed three different priming regimens based on idarubicin, aclacinomycin or pirarubicin for refractory/relapse ALL. Both the effectiveness and safety were investigated.
Methods: A total of 43 refractory and/or relapsed ALL patients were enrolled in this study. These patients composed of 31 male and 12 female, with the medial age of 26(8~ 85) years old. The salvage regimen was idarubicin, aclacinomycin or pirarubicin combined with cytarabine (Ara-C) and granulocyte colony-stimulating factor (G-CSF). The detailed three regimens were performed as, IAG (idarubicin 10mg/d, d1,4,8,11; Ara-C 10 mg/m^2 twice daily, d1-14; G-CSF 200 mg /m^2 d0~14), CAG(Acla 10mg/d, d1-8; Ara-C 10 mg/m^2 twice daily, d1-14; G-CSF 200 mg /m^2 d0~14) and TAG(pirarubicin 10mg/d, d1,4,8, 11; Ara-C 10 mg/m^2 twice daily, d1-14; G-CSF 200 mg /m^2 d0~14). There were 13 cases in IAG group, 18 cases in CAG group and 12 cases were assigned to TAG group. Effectiveness was measured using objective response and safety was measured using the NCI classification system of common toxicity criteria for adverse events. Some parameters which may influence the clinical outcome, such as age, immunophenotype and white blood cell (WBC) count were also explored.
Results: The overall complete remission (CR) rate for all patients in three groups was 46.5% (20 cases), partial remission (PR) rate was 16.2% (7 cases), and 16 cases (37.2%) got no remission (NR). The overall response rate (ORR) was 62.8%. In IAG group, the ORR was 69.2%, and the CR rate as well as PR rate was 53.8% and 15.4%, respectively. There were 8 patients (44.4%) got CR, 3 patients (16.6%) got PR, and ORR was 61% in CAG group. In group TAG, a total of 5 cases (41.7%) got CR, and 2 cases (16.6%) got PR, with ORR 58.3%. The response rate in three groups had no statistical difference (P=0.837). Compared with patients □35 years old (ORR 71.4%), the ORR for ≤35 years old group was 54.5%, and no statistical difference was found between two groups (P=0.347). There was also no significance about ORR between T-

ALL and B-ALL groups (T-ALL group 68.4% *Vs* B-ALL group 54.1%, P=0.542). No significance could be found between WBC decreased group and WBC normal or increased group (ORR, WBC decreased group 65% *Vs* WBC normal or increased group 60.9%, P=1.000). ORR in >60 years old group and ≤60 years old group did not show any significant difference (ORR, >60 years old group 60% *Vs* ≤60 years old group 54.3%, P=0.272).If we define WBC≥100×10^9/L in T-ALL or WBC≥30×10^9/L in B-ALL as high WBC group, there was also no significance between high WBC group and non high WBC group (ORR, 50% *Vs* 64.9%, P=0.808). Further more, it was analyzed in every two chemotherapy regimen groups, and no significance was found for response rate. Some common adverse events occurred during and after chemotherapy, including fever, bone marrow suppression, gastrointestinal tract reaction and liver function damage. These events were all well tolerated.
Summary and Conclusions: Three different priming regimen based on idarubicin, aclacinomycin or pirarubicin for refractory/relapse ALL showed high remission rate, good tolerance, and the non hematological toxicity was mild and could be well tolerated. These regimens did not show any significance in both response rate and side effects.

E872

INVESTIGATING THE PROGNOSTIC SIGNIFICANCE OF MICRORNA-335 EXPRESSION IN ADULT ACUTE LYMPHOBLASTIC LEUKAEMIA

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Background: Acute lymphoblastic leukaemia (ALL) accounts for approximately 15% of all leukemias in adults. Unlike paediatric ALL, which has a 90% five-year event-free survival, adult ALL tend to fare poorly, with a 20-30% five-year event-free survival rate. While some of the issues related to poor outcome may be due to host conditions, most patients die of refractory disease rather than treatment-related complications. Prognostic factors and the treatment regimen for adult ALL have not evolved in more than 15 years; it is therefore critical that we try to identify better prognostic factors that not only identify high-risk patients, but may also provide insight into the use of therapeutic agents that can improve prognosis. MicroRNA has been shown to be dysregulated in cancer cells, and to be more powerful prognostic factors in leukaemia. Low levels of miR335 have previously been found to result in higher MAPK1-mediated survival of paediatric ALL and is associated with prednisolone resistance. The use of MAPK inhibitors could overcome this resistance. miR335 has not been studied in adult samples.
Aims: We aimed to determine the level of miR335 in samples of newly diagnosed Adult ALL patients. Our hypothesis is that low expression of miR335 in adult ALL is associated with a poorer outcome. We also hoped to determine miR335 association with known poor prognostic factors as well as the need for Stem Cell Transplants.
Methods: We determined the level of miR335 in 37 newly diagnosed Adult ALL patients who were treated uniformly on our institutional protocol over the past 5 years. Mononuclear cells were separated and harvested at diagnosis using Ficoll-Paque density gradient centrifugation. Total RNA was extracted using TRIzol Reagent and subsequently purified. cDNAs was then synthesized from total RNA with miR335 specific primers and qRT-PCR quantification of miR335 was performed of the samples. Using the median expression of miR335 as a cut off, we compared a number of parameters between the 2 populations, including complete response rates, survival, known poor prognostic factors and need for stem cell transplantation. The t-test is used for comparison of continuous values, chi-square test for categorical values and log-rank test for survival.
Results: Those with a high level of miR335 were noted to have more poor prognostic factors (94%) as compared to the group with a low level of miR335 (71%, p=0.04). Despite this, those with a high level of miR335 had a higher rate of remission after induction chemotherapy (71% *vs* 41%, p=0.046). Although fewer patients with low miR335 had poor prognostic factors, they required more stem cell transplants (47% *vs* 11%, p=0.03) compared to the high miR335 group. This was mainly due to poor response to induction chemotherapy with 78% of them not achieving remission then. Although the overall survival is the same in both groups (High miR335 39%, Low miR335 42%, p=0.66), it is largely confounded by the high rate of stem cell transplant in the low miR335 group.
Summary and Conclusions: This data supports the hypothesis that miR335 in adult ALL confers a poorer outcome which is independent of traditional poor prognostic factors. This may allow us to identify poor risk patients for which we can tailor treatment using MAPK1 inhibition to overcome therapeutic resistance as we have shown previously, at the beginning of treatment rather than at a later stage based on minimal residual disease measurement.

E873

IDENTIFICATION OF DISTINCT PROGNOSTIC SUBGROUPS IN HIGH-HYPERDIPLOID PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA BY MULTI-TARGET INTERPHASE FISH

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Background: Acute lymphoblastic leukemia (ALL) is the major malignancy in childhood. Patients with high hyperdiploid (HeH) chromosome constellation represent the largest genetic subgroup. Despite its generally favorable prognosis, patients show heterogeneous clinical outcome.
Aims: The eight most commonly gained chromosomes (4, 6, 10, 14, 17, 18, 21, X) were investigated by correlated way at single-cell level using multicolor interphase fluorescence *in situ* hybridization (iFISH) in order to explore genetic heterogeneity and identify subgroups of patients with distinct prognostic features.
Methods: After exclusion of children with t(9;22)(q34;q11.2), t(v;11q23), t(12;21)(p13;q22) or t(1;19)(q23;p13.3) a complete multi-target iFISH analysis was successfully performed on bone marrow samples of 168 patients with pre-B ALL. A minimum of 300 nuclei were aimed to be fully evaluated in each sample. Modal number (iMN_8), defined as the chromosome number of the largest subclone by iFISH, has been identified for each leukemia.
Results: iMN_8 indicated a HeH ≥51 chromosome set in 28.5% (48/168) of cases. As compared to non-HeH patients higher DNA index (p<0.001), lower WBC at presentation, higher proportion of patients in the standard risk group, somewhat younger age and longer survival were all indicators of better prognosis in the iMN_8 ≥51 group. A subgroup of patients (n=20) with the highest modal number, iMN_8 55-56, had an excellent 5-year pOS (95.2%) which was not only higher compared to all other patients involved in the study (p=0.019) but also considering HeH patients exclusively (p=0.046). iMN_8 55-56 patients had significantly lower WBC at diagnosis (p=0.010), more patients belonged to the standard risk arm (p=0.007) and none of them had central nervous system involvement at diagnosis. We scrutinized whether the pattern of specific numerical chromosome aberrations has an association with the outcome heterogeneity observed in the HeH group. Improved survival was achieved by patients gaining chromosome(s) 4, 4-6, 4-10, 4-17, 4-18 and 4-10-17. and 4-10-17. Patients with 4-10-17 triple trisomy, a feature previously reported to be associated with the best prognosis, showed a better pEFS but not pOS. The multi-target iFISH approach allowed us to determine the copy number status of 8 chromosomes in single nuclei. Cells sharing identical alterations were grouped during clonality identification and subclonal composition was then analyzed to assess chromosomal heterogeneity. Amongst patients with iMN_8 55-56 on average 23.0 (SE 9.24) subclones were detected, which was significantly lower (P=0.028) compared that in the iMN_8 51-54 group.
Summary and Conclusions: We successfully performed a screening for whole chromosome copy number changes at single-cell level using an extensive panel of FISH probes on unselected leukemic cells of patients with childhood pre-B ALL. We were able to identify patients with HeH ALL with high reliability as well as to explore chromosomal heterogeneity, reveal cytogenetic patterns as predictors of outcome and classify HeH ALL patients into prognostically distinct subgroups.

E874

EARLY CLEARING OF PERIPHERAL BLOOD BLASTS IS AN INDEPENDENT PROGNOSTIC FACTOR IN ADULTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Although therapeutic regimens for acute lymphoblastic leukemia (ALL) in use for adult patients achieve complete remission (CR) rates over 80%, most patients eventually relapse and long-term mortality is high. Steroid resistance is known to be associated with aggressive disease in children, but it has not been routinely evaluated as a prognostic factor in adults.
Aims: In order to evaluate the impact of steroid sensitivity at diagnosis, we retrospectively analyzed the day 7 peripheral blood blast count (d7BC) in adult patients treated in our department over a period of 11 years with the hyperCVAD regimen, which includes a 7-day pre-induction phase with dexamethasone.
Methods: We evaluated 131 consecutive patients treated from January 2003 to December 2013 for the influence of the following variables on disease-free survival (DFS) and overall survival (OS): age, sex, immunophenotype, hyperleukocytosis (defined as >30,000/ul for B and >100,000 for T subtypes), central nervous system or testicular involvement at diagnosis, unfavorable cytogenetics [t(9;22) or *MLL*rearrangements] and d7BC.
Results: Median age was 40 years (15-69) with 18% (23 patients) >60 years; 53% were male. The phenotype was B in 79% (104) patients and T in 21% (27). Hyperleukocytosis was present at diagnosis in 22% of B and 30% of T-ALL; median leukocyte count at diagnosis was 13300/ul. The CR rate was 88%. With a median follow-up of 60 months, DFS was 25,7 months and OS

was 30 months. Among the 108 evaluable patients for circulating blasts, 36% (39) had persisting d7BC. This subgroup of patients had a shorter OS (17,1 *vs* 38,8 months, P <0,01). In multivariate analysis, the Hazard Ratio was 2,0 for persisting d7BC.

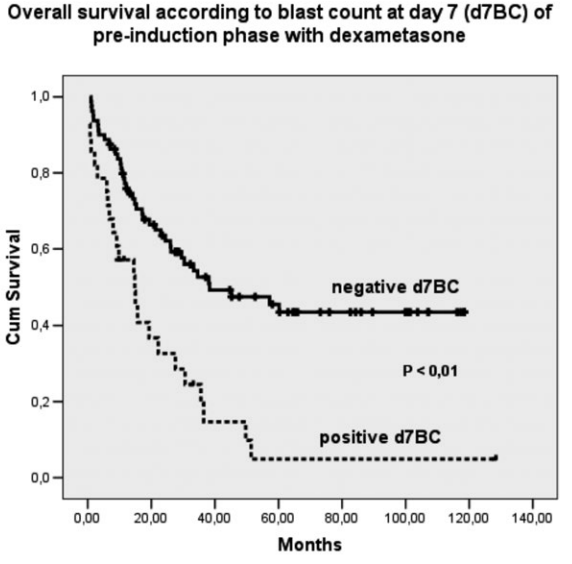

Figure 1.

Summary and Conclusions: Our results show that d7BC is an independent risk factor, suggesting that early clearance of blasts has a major impact on the outcome of ALL in adults. The potential of emerging novel therapies should be explored in this particular group of patients.

E875

PHILADELPHIA CHROMOSOME MAY NOT BE AN ADVERSE PROGNOSTIC FACTOR IN THE OLD-AGE B-CELL PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS IN THE ERA OF TYROSINE KINASE INHIBITOR

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Background: Philadelphia chromosome-positive (Ph+) acute lymphoblastic leukemia (ALL) is approximately comprised of 25% of adult ALL, and has been characterized by poor clinical outcomes, especially in the elderly patients. However, the prognosis in these patients might be improved with the advent of tyrosine kinase inhibitors (TKIs) as combination therapy to various chemotherapeutic regimens.
Aims: The aim of this study is to analyze the prognostic significance of Ph+B-cell precursor (BCP) old-age ALL treated with TKIs and compare its clinical outcomes with Ph- BCP old-age ALL.
Methods: The clinical outcomes of 41 elderly patients with BCP, who were treated at 5 university hospitals in Korea from Sep 2005 to Nov 2013, were retrospectively analyzed.
Results: In this study, the median age of the patients was 65 (range, 60 to 82) and median follow up duration was 58.3 months (range, 1.5 to 115.4 months). Twenty-one patients (51%) were Ph+and treated with TKI combined chemotherapy. Patients with Ph- ALL were treated with conventional chemotherapy identical to those for the Ph+patients. There were no differences in the patient characteristics including age, white blood cell count, immunophenotype, extramedullary involvement, and lactic dehydrogenase level according to the presence of Philadelphia chromosome. Complete remission (CR) rate was significantly higher in the Ph+ALL compared to the Ph- ALL elderly patients (80.0% *vs* 50.0%, *P*=0.047). There was no difference in the treatment-related mortality rate between two groups. Median overall survival was not different between the Ph- and Ph+ALL (5.4 months *vs* 7.5 months). Among Ph+patients, Bcr/abl subtypes at diagnosis and quantitative PCR analysis after induction therapy did not show any prognostic values. Multivariate regression analysis demonstrated that Charlson comorbidity score of less than 4 at diagnosis (Hazard ratio (HR) 0.186, *P*=0.05), CR achievement after the first induction chemotherapy (HR 0.188, *P*=0.001) and negative CD20 expression (HR 0.271, *P*=0.007) remained as the independent prognostic factors predicting higher overall survival rate. In contrast to Ph- ALL patients (HR 1.462, *P*=0.481), CD20 expression level on the leukemic blasts was significantly associated with low overall survival rate in the Ph+old-age ALL (HR 5.338, *P*=0.008).

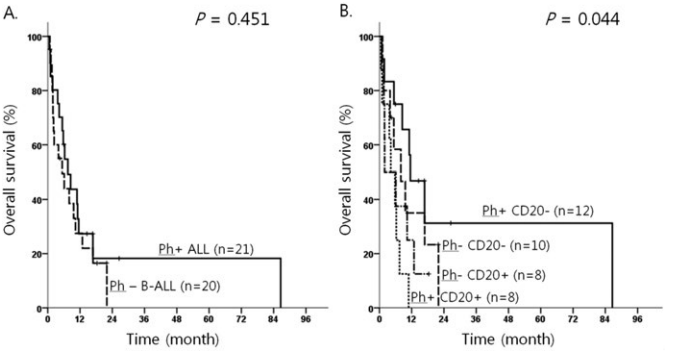

Figure 1.

Summary and Conclusions: In this study, the survival rates of elderly BCP ALL patients were very low as expected. However, no additive poor prognostic value of the presence of Philadelphia chromosome was observed in the elderly ALL patients treated with TKI-combined chemotherapy. In addition, the prognostic relevance of CD20 antigen expression should be further evaluated for the large number of elderly Ph+ALL patients.

E876

NELARABINE IN CHILDREN WITH REFRACTORY AND RELAPSED T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Nelarabine is a pro-drug of the deoxyguanosine analogue ara-G. Accumulation of ara-GTP in leukemic blasts allows for incorporation into deoxyribonucleic acid (DNA), leading to inhibition of DNA synthesis and cell death. It is indicated for patients with T-cell acute lymphoblastic leukemia (T-ALL) and T-cell lymphoblastic lymphoma whose disease has not responded to or has relapsed following treatment with at least two chemotherapy regimens. This use is based on the induction of complete responses by Nelarabine alone. We used Nelarabine in combination with other agents in treatment of children with refractory T-ALL.
Aims: To assess rational use of Nelarabine in children with T-ALL
Methods: In multicenter studies ALL-MB from 1991 to 2014 yy there were registered 506 pts with T-ALL, 81 of them (16%) didn't respond to induction therapy and were classified as high-risk group (HRG). There were no significant differences between responders and non-responders in terms of initial characteristics, except immunophenotype – significantly more patients with CD1a expression <25% were refractory (p,0,01). Nelarabine was used in 26 cases – for 21 patients (80,8%) as postinduction therapy and for 5 pts (19,2%) with early relapses as second-line treatment. For both groups we used two 5-days courses of Nelarabine 650mg/m^2, combined with Dexa, Cph, PEG-asp in the 1st block and with Dexa, VP-16 and Ara-C in second.
Results: Among high-risk patients 16 (76,2%) responded after one or two blocks. 13 of them were transplanted from MUD, MSD or haploidentical donor. Among 5 relapsed T-ALL patients nobody responded to therapy. We also compared those, who were treated by standard chemotherapy for HRG (12 pts) with those with Nelarabine. There was no difference in initial data, and also in remission achievement rate (47% *vs* 34%, p=0,2). Patients with T3-ALL responded significantly worse in Nelarabine group(p<0,0001). Severe reversible neuropathy was observed in 2 patients, who achieved remission.
Summary and Conclusions: Nelarabine didnt show an advantage over standard HR-blocks in our non-randomized study. But the groups were small, and we need further research to establish optimal dose regimen, possible drug combinations and reasons for refractoriness in T3-variant of ALL.

E877

DAUNORUBICIN INDUCED EARLY CARDIAC DYSFUNCTION IN ACUTE LYMPHOBLASTIC LEUKEMIA - A PROSPECTIVE STUDY

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Background: Anthracyclines are effective agents used in the treatment of hematological malignancies and form important part of induction chemotherapy in Acute Lymphoblastic leukemia (ALL). However, they cause progressive chronic cardiotoxicity resulting in heart failure and acute toxicity manifesting as arrhythmia and diastolic dysfunction. This is due to irreversible cardiac myocyte damage caused by topoisomerase II inhibition and iron free radical induced DNA damage. The early detection of cardiotoxicity is important as it may be useful in the prevention of heart failure. Left ventricular ejection fraction (LVEF) has been the main indicator of cardiac dysfunction and a powerful predictor of mortality. However, impairment in LVEF is often detected only after considerable myocyte loss has taken place. However, Doppler parameters and tissue Doppler parameters can detect early cardiac changes and may help predict later overt LVEF decline.
Aims: To study the acute cardiac toxicity of anthracycline infusion in ALL patients by serial echocardiographic parameters during induction chemotherapy.
Methods: The study was conducted on newly diagnosed acute lymphoblastic leukemia without any history of previous anthracycline exposure for any reason. Patients were treated as per Augmented BFM protocol and during induction, they were administered daunorubicin 25 mg/m^2/week for 4 weeks, vincristine 1.4 mg/m^2/week for 4 weeks, Prednisone 60 mg/m^2/day for 28 days and L- asparaginase 6000 U/m^2 on alternate day for 9 doses. Echocardiography was done in all the patients before start of chemotherapy (baseline) and at end of induction chemotherapy (post). One more echo was done after 2 weekly doses of anthracycline (mid echo). Various parameters on 2D mode, Doppler and tissue Doppler were recorded and ejection fraction and myocardial performance index calculated.
Results: A total of 20 patients of acute leukemia were enrolled in the study. Median age was 16.5 year (4-42 year), with 17 males (85%). Baseline leucocyte count was 6910/ul (range: 600-788,250), with 3 patients having high risk cytogenetics and two having CNS 3 status. Changes in echocardiographic parameters occurring during baseline, mid chemotherapy and post chemotherapy are shown in table. 2 D Parameters: LVIDes/BSA showed a decreasing trend from baseline to mid echo (p value 0.07). It returned to near baseline value in post echo. LA dimensions, IVS thickness was not significantly altered at any point of time as was the case with LVPW. Doppler Parameters: E velocity was showing overall significant changes in ALL patients (p value 0.031). A velocity showed trends towards overall changes during induction (p value 0.07), attaining significant difference in mid and post echo (p value 0.048). E/A ratio showed statistical significant difference in post chemotherapy echo (p value 0.049). Deceleration time showed a non significant dip after chemotherapy cycle and reverted back to some extent in ALL patients. Tissue Doppler Parameters: Sm lateral was insignificant during chemotherapy. Sm septal showed a significant dip from baseline to mid echo (p value 0.02). Em lateral had significant decrease (p value 0.045). Em septal had non-significant decreasing trend (p value 0.08). Am septal and lateral showed non- significant changes. Iso-volumetric relaxation and contraction time did not show significant variation during chemotherapy at any time. Left ventricular ejection fraction did not show significant changes. Myocardial performance index was also almost unchanged during induction.

Tabella 1. Changes in echocardiographic parameters during ALL induction.

Parameter	Baseline (B) Mean ± SD (n=20)	Mid chemo(M) Mean ± SD (n=18)	Post chemo(P) Mean ± SD (n=17)	P value B vs M	B vs P	M vs P
LVIDes /BSA(mm)	2.1±0.4	1.9±0.3	2.1±0.5	0.07	0.4	0.2
LVIDed /BSA(mm)	3.3±0.7	3.0±0.5	3.2±0.6	0.1	0.4	0.2
LA (cm)	2.7±0.5	2.8±0.5	2.8±0.4	0.9	0.9	0.9
Aorta (cm)	2.3±0.6	2.4±0.5	2.5±0.6	0.9	0.1	0.4
IVS (cm)	0.8±0.1	0.8±0.1	0.8±0.1	0.8	0.9	0.5
LVPW (cm)	0.8±0.1	0.8±0.1	0.8±0.1	0.9	0.9	0.2
E vel (cm/s)	94.0±23.0	82.0±17.7	84.0±18.7	0.09	0.2	0.9
A vel (cm/s)	52.0±14.3	49.5±12.2	60.8±18.9	0.5	0.8	0.048
E/A	1.9±0.7	1.7±0.4	1.5±0.4	0.9	0.2	0.3
Deceleration time (msec)	175.1±66.0	146.8±38.1	156.5±35.9	0.2	0.8	0.9
Sm(lateral) (cm/s)	10.1±1.8	10.3±1.9	10.0±2.2	0.9	0.9	0.9
Sm (septal) (cm/s)	9.3±1.9	8.3±1.4	8.7±1.7	0.02	0.4	0.9
Em(lateral) (cm/s)	15.4±3.4	14.1±3.8	13.9±3.7	0.2	0.09	0.9
Em(septal) (cm/s)	11.3±2.6	10.1±1.6	10.1±2.6	0.2	0.3	0.9
Isovolumetric contraction time(msec)	60.1±17.9	55.9±10.6	51.4±10.0	0.9	0.3	0.6
Isovolumetric relaxation time(msec)	68.4±15.0	70.2±15.8	64.1±15.9	0.9	0.9	0.3
Am(latera) (cm/s)	7.6±2.1	7.6±2.6	8.7±1.6	0.9	0.6	0.8
Am(septal) (cm/s)	7.9±2.6	8.5±2.7	9.0±1.8	0.9	0.7	0.9
Ejection time(msec)	267.8±36.6	252.9±34.7	249.3±33.7	0.8	0.2	0.9
LVEF (%)	60.3±15.9	58.9±16.8	60.4±16.5	0.5	0.9	0.9
MPI	0.49±0.1	0.48±0.1	0.48±0.08	0.9	0.9	0.9

Summary and Conclusions: Daunorubicin induced early cardiac toxicity causes predominantly diastolic dysfunction, as is reflected by changes in various Doppler and tissue Doppler indices. Systolic function of the heart as assessed by LVEF is relatively preserved. Myocardial performance index (Tei index), proposed as sensitive indicator of cardiac dysfunction, does not change either during induction. These changes in echo parameters remain sub- clinical during induction. Patients with changes in echocardiographic parameters need to be followed up with repeated echo to find improvement or worsening of these changes and their relation to clinical symptoms due to heart failure.

E878

APPLICATION OF SYSTEMS PHARMACOLOGY MODELING FOR EVALUATION OF THERAPIES EFFECT ON BLAST DYNAMICS IN ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Acute lymphoblastic leukemia (ALL) is an acute form of cancer of the white blood cells, characterized by the overproduction of cancerous, immature white blood cells. ALL is most common in childhood with a peak incidence at 2–5 years of age, and another peak in old age. About 80% of ALL patients will have complete remission, but the question about the most effective therapy remains open.
Aims: The aim of this work is to reproduce dynamics of ALL progression and compare efficacy of several clinically accepted and potential treatments.
Methods: Systems pharmacology model of ALL progression and treatment was developed. The model is a system of ordinary differential equations. It includes description of B-lymphoblasts proliferation and distribution between cell cycle phases with and without treatment, B-lymphoblasts and other blood cells dynamics during ALL progression, pharmacokinetics of several compounds (vorinostat, sorafenib) and its effect on B-lymphoblasts dynamics in ALL patients. Parameters of the model were calculated on the basis of the literature data or fitted against published experimental data. Treatment was simulated for two types of virtual patients: with slow and high rate of disease progression.
Results: Model satisfactory reproduces *in vitro* data on B-lymphoblasts cell lines proliferation and distribution between cell cycle phases with and without drug treatment and *in vivo* blasts levels in blood measured during ALL progression. The model was validated against blast dynamics during treatment with sorafenib. Model shows that for virtual patients with slow ALL progression vorinostat is more effective than sorafenib. For virtual patient with fast ALL progression both compounds are ineffective.

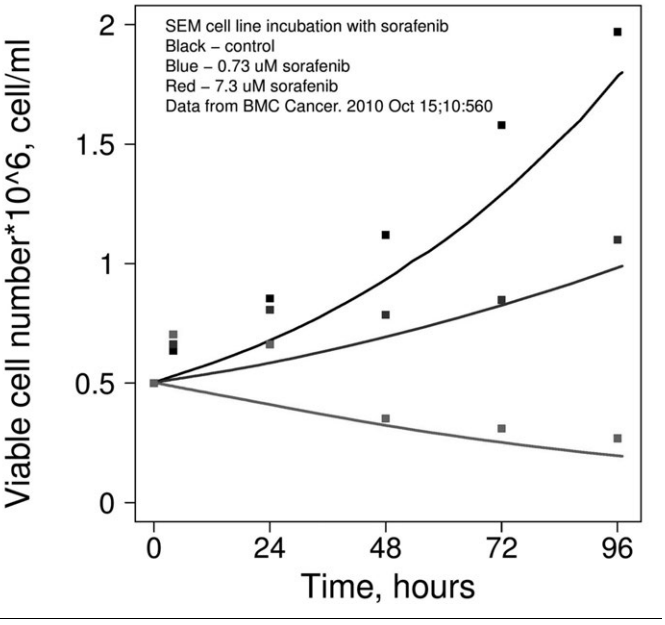

Figure 1.

Summary and Conclusions: Model predicts that slow progression rate patients have better respond to the therapy. Systems pharmacology model of ALL progression and treatment could be used as a tool to compare different clinically accepted and potential treatments.

E879

LACK OF PROGNOSTIC IMPACT OF ABERRANT MYELOID ANTIGEN EXPRESSION IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: A subset of adult acute lymphoblastic leukemia (ALL) patients have blast cells which coexpress myeloidassociated markers. The expression

of aberrant myeloid antigen (MyAg) in ALL differs considerably in independent studies and its prognostic value is still controversial.
Aims: To analyze the incidence of MyAg in a group of adult ALL and its correlation with clinical features, prognostic factors and outcome.
Methods: We retrospectively analyzed the expression of MyAg CD13 and/or CD33 of 79 adult patients with *de novo* ALL in a single center, between 2006 and 2014.
Results: The median age at diagnosis was 44 years [17-70]. The median white blood count (WBC) was 9.6 x10^9/L [0.2-254] and 24 (30.4%) patients had high WBC (>30x10^9/L in B-ALL and >100x10^9/L in T-ALL). Physical examination revealed hepatomegaly in 30 (38%) and splenomegaly in 27 (34.2%) patients. The central nervous system (SNC) was involved in 9 (11.4%) and 31 (39.2%) had serum lactate dehydrogenase (LDH) >4xULN. Sixty-nine (87.3%) patients had a B-lineage ALL (common-B ALL 63.8%, pre-B ALL 29%, pro-B ALL 7.2%), while only 10 (12.7%) patients had T-lineage ALL (Thymic ALL 60%, mature-T ALL 40%). MyAg expression (CD13 and/or CD33) was documented in 25 (31.6%) patients. No differences were found between the MyAg +and MyAg- groups with regard to median age, median WBC, high WBC, high LDH (>4xULN), hepatomegaly and/or splenomegaly or involvement of SNC at diagnosis. MyAg expression was only found in B-ALL and none of the 10 cases of T-ALL showed aberrant phenotype (31.6% vs. 0%; p=0.017). According to immunophenotypic subtype B, no difference was observed (p=0.275). Karyotype was evaluated in 71 of 79 patients and 34 (47.9%) had an unfavorable karyotype: t(9,22) in 32 (45.1%) patients, t(4,11) in 1 (1,4%) and hypodiploidy in 1 (1.6%) patient. Fifty-one (64.6%) patients fulfilled criteria for High Risk (HR) group, while the remaining 28 (35.4%) were classified as Standard Risk (SR) group. In B-lineage ALL, the MyAg+group was more frequently associated with unfavorable karyotype (47.1% *vs* 25%; p= 0.074) and with HR group (41.3% *vs* 26.1%; p=0.215) but these differences were not statistically significant. Patients were treated with the Hyper-CVAD protocol or HOVON 100 ALL/EORTC 06083 protocol (nonexperimental arm). Seventy-seven (97.5%) patients achieved complete remission (CR) after induction therapy. The two patients with resistant disease had MyAg+expression but this difference was not statistically significant (p=0.097). The 5-year overall survival rates for MyAg+group *vs* MyAg- group were 26.7% *vs* 39.7%, respectively, but again, the difference was not statistically significant (p=0.555). The 5-year disease free survival (DFS) rates were 29.5% for MyAg+group and 43.5% for MyAg- group, difference not significant (p=0.567). We separately analyzed B-lineage ALL cases and we found no differences in DFS (p=0.460) and OS (p=0.464) between MyAg+and MyAg- cases. Additionally when cases with unfavorable karyotype were analyzed as a separate group, we failed to observe any statistical difference between the two groups in terms of DFS (p=0.978) and OS (p=0.888). Again, when the HR group was evaluated as a separate group, no differences were recorded between the two groups (DFS p=0.587; OS p=0.583).
Summary and Conclusions: In our study we have found that there was no prognostic impact of aberrant myeloid antigen expression in adult acute lymphoblastic leukemia. However, the evaluation of the expression of these antigens remains valuable, as for example, in the monitoring of minimal residual disease.

E880

OUTCOME OF OLDER ADULTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA TREATED WITH HYPERCVAD

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Background: Older patients with acute lymphoblastic leukemia (ALL) have a poorer outcome. Due to assumptions about a higher risk of complications and toxic death, they are often denied potentially curative treatment approaches.
Aims: To address the impact of known prognostic factors in older fit patients offered intensive chemotherapy, we retrospectively analyzed the outcome of patients ≥55 years of age who received HyperCVAD as a first-line treatment.
Methods: We compared the outcome of 35 consecutive older patients (age 55-69) who were treated in our center from January 2003 to December 2013, and of the 96 younger patients (age 15-54) treated in the same period with the same regimen. We evaluated the complete remission (CR) rate, the disease-free survival (DFS) and the overall survival (OS) of these 2 groups, and we analyzed the influence of age, performance status, immunophenotype, leukocyte count at diagnosis (≤ or >50000/uL), presence of hyperleukocytosis (defined as >30000/uL for B and >100000/uL for T subtypes) and unfavorable cytogenetics (*BCR-ABL1* or *MLL* rearrangements).
Results: Disease characteristics were similar in older and younger patients (*BCR-ABL1* being non-significantly more frequent in the former, 32% *vs* 20%). The CR rate was lower in older patients (82% *vs* 90%). At a median follow-up of 60 months (m), DFS was worse in older patients (7,8m *vs* 32,8m), as was OS (9,8m *vs* 36,4m, p<0,01). In older patients, a T phenotype had a strong negative impact on OS (1,8m *vs* 17m, p<0,05). In multivariate analysis, the 2 independent prognostic parameters for OS were a T phenotype (Hazard Ratio 2,1) and older age (Hazard Ratio 2,25).

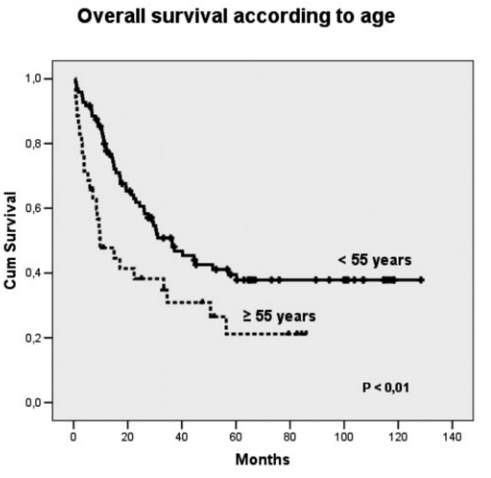

Figure 1.

Summary and Conclusions: Older patients easily achieve CR, but CR duration and OS remain highly unsatisfying (in particular for T-ALL) even with an intensive regimen such as HyperCVAD, underscoring the need for novel strategies.

Acute myeloid leukemia - Biology

E881

INHIBITION OF AXL KINASE SUPPRESSES RETINOIC ACID METABOLISM THROUGH REGULATION OF CYP26

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Background: Retinoic acid (RA), a metabolite of Vitamin A (retinol), is a known regulator of cell growth and differentiation, inducing expression of multiple genes such as retinoic acid receptor responder 1 (RARRES1/TIG1). RARRES1, and its associated binding partners such as AXL kinase, have been shown to promote tumor growth and drug resistance in multiple tumor types. RA has been used as a single-agent treatment in patients with acute promyelocytic leukemia (APL) with roughly 90% of patients attaining a complete remission. However, remissions can be transient and resistance arises within a few months following treatment. This resistance is thought to develop through increased RA metabolism through upregulation of CYP26, a cytochrome P450 enzyme that plays a key role in the metabolism of RA.
Aims: We have sought to understand the role of AXL inhibition as a means of restoring sensitivity to RA treatment. We hypothesize that treatment with our AXL inhibitor, TP-0903, would disrupt RA metabolism by CYP26.
Methods: RT-PCR was used to measure mRNA expression of CYP26 in cells induced with RA and treated with TP-0903. Following treatment, changes in CYP26 expression was assessed at the protein level, using standard western blotting techniques. Endogenous RA levels were measured using a competitive ELISA technique. To determine the effect of TP-0903 on tumor growth in an *in vivo* model, we tested TP-0903 treatment in a MV4-11 xenograft mouse model.
Results: Consistent with prior reports, we observe a robust induction in mRNA expression levels of CYP26 following 1 mM RA treatment in MV4-11 leukemia cells, reaching nearly 4.3-fold after 6 hours of treatment. However, treatment of cells with TP-0903 at levels as low as 100 nM inhibited RA-mediated induction of CYP26 mRNA levels by 88.9% in MV4-11 cells at 6 hours. Interestingly, TP-0903 also inhibited basal mRNA levels of CYP26 by 94.1% at 6 hours. Similar trends are observed in additional cell lines (HL60, A549, and H1650), and with an alternative AXL inhibitor, R428, although only at higher concentrations. At time points greater than 24 hours, and without re-treatment with TP-0903, CYP26 expression exceeded levels observed in induced samples. In TP-0903-treated cells, we observed that RA levels are maintained at time points where CYP26 expression is suppressed. TP-0903 strongly inhibited tumor volumes by up to 100% at multiple dose levels and treatment schedules. Analysis of CYP26 expression in fixed tissues, and RA levels in plasma will be assessed to determine the effects of TP-0903 on physiological levels of CYP26 and RA.
Summary and Conclusions: Our observations support our hypothesis that inhibition of AXL kinase by TP-0903 disrupts RA metabolism by suppressing CYP26. Taken together, our data suggest that AXL may serve as a suitable therapeutic target for addressing cellular responses mediating RA resistance.

E882

AN EFFICIENT COMPUTATIONAL APPROACH TO EVALUATE THE EXPRESSION PROFILE OF INDIVIDUAL ACUTE LEUKEMIA PATIENTS

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Background: Microarray and sequencing studies have been instrumental in the mapping of genome wide associations in hematological diseases. Thus, the MILE Study (Haferlach *et al*, J Clin Oncol 2010) has taught us that it is possible to robustly classify and predict leukemia subgroups. However, several factors have impeded the general use of such data and methods in the everyday clinical setting: *(i)* technical expertise may not be available and, *(ii)* inter-study variations have hampered direct comparison of results between centers. As a consequence, *(iii)* the practical implications of analyzing single patient samples have to some extent been overlooked. Tools, which enable *the singular patient omics* to be addressed in a clinically relevant fashion, are thus much needed. We hypothesized that combining data from MILE Study and data mining at the single patient level by novel scripts could delineate the unique expression signature.
Aims: *(1)* To develop an algorithm for implementing results from large profiling studies (microarray or RNA-seq) without the need for normalization between sample sets *(2)* to predict leukemia subtype of single samples in a timely fashion, thus *(3)* enabling informative exploration within the private expression profile of genes of interest.
Methods: We accessed the MILE Study data (2096 samples from Stage I study), to generate a single compressed dataset for individual leukemia prediction of 21 separately run samples (Aarhus, DK) derived from microarray analysis (HG-U133A arrays, Affymetrix Inc., CA, USA) of bone marrow aspirates (Figure 1A). This resulted in a reference matrix, which covered 2181 predictor genes in total and 15 leukemia subtypes, denoting genes as upregulated, unaltered or downregulated (discrete values of 1, 0, -1), evaluated via the Leukemia Gene Atlas (Hebestreit *et al*, PLoS One 2012). The patient samples were comprised of three clinically defined subtypes (6 AML inv(16), 8 t(8;21) and 7 ALL t(12;21)). The evaluation of individual molecular subtypes was subsequently performed by entry-wise matrix multiplication followed by scoring. Data mining was performed by coupling external sources and results, utilizing Wolfram Mathematica as computational platform (Hansen *et al*, Br J Haematol 2015, in press).
Results: Prediction of leukemia subtype matched clinical diagnoses in all cases (21/21). Consecutive data mining enabled us to evaluate and emphasize individual expression patterns, which were otherwise masked by attempts to draw parallels between samples. Setting an arbitrary threshold of *e.g. eightfold* expression difference, the *common* gene set for each subgroups was low compared to the total number of genes observed (Figure 1B). In contrast, evaluating combinations of samples in each subtype showed a higher number of common differentially expressed genes in-between patients. At the individual level a substantial part of the genes with increased or decreased expression was leukemia associated (median values of 17–62 in sets of 74–230 genes, evaluated through DisGeNET database, Bauer-Mehren *et al*, PLoS One 2011). Of these sets each genes was found to have two or more MeSH term associated references in the PubMed database (within the three clinically defined subtypes: $^{74}/_{138}$, $^{97}/_{178}$, $^{181}/_{376}$). It was also observed that the vast majority of biological gene functions matched potentially malignant functions, *i.e.* cell cycle, migration, defense response and signal transduction.

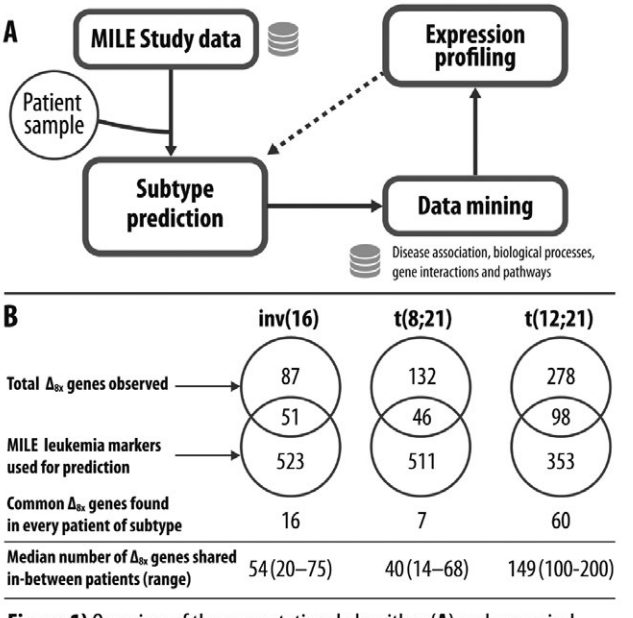

Figure 1) Overview of the computational algorithm (**A**) and numerical evaluation of the 8-fold differentially expressed genes (Δ_{8x}) shared in subtypes and with MILE Study markers (**B**) and a low number of common genes

Figure 1.

Summary and Conclusions: We have recently described how genomic analysis on the single patient level can be utilized in the setting of exome sequenced samples (Hansen *et al*, Br J Haematol 2015, in press). Here we show that this data mining approach can also be utilized for expression profiling of single patients. Hopefully, with shortened lab turn-over time for *omic* studies, the present approach, which involves minimal computational effort, taken together with standard molecular screening in acute leukemia patients, should result in speedy information flow from research to the clinic with much more detailed characterization of the single patient. Ultimately, this should in turn enable more focused personalized medicine.

E883

MOLECULAR CORRELATES AND DRUG RE-PURPOSING SCREENING IN HYPOMETHYLATING AGENT FAILURE IN AML AND MDS

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Background: After initial response to the hypomethylating agents (HMA)/DNMT inhibitors 5-Azacitidine (AZA) and Decitabine, ultimately all patients with Myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) progress/fail treatment. Subsequent therapies and outcomes are dismal. Developing novel therapeutic approaches in HMA failure MDS and AML patients is one of the largest clinical needs in this field.
Aims: The mechanisms of HMA failure are still poorly understood and effective novel regimens are lacking. To address these deficiencies, we pursued several approaches in parallel including extensive genomic characterization of AZA resistant cells as well as *in vitro* drug re-purposing screens of FDA approved small molecule libraries with the goal to define the molecular landscape and novel therapeutic targets in HMA failure MDS/AML, as well as to identify novel drugs that could be immediately translated into clinical use.
Methods: Over a 2-year period AZA resistant (RES) cell lines (TF-1, HEL) were generated including non-clonal (NC, requiring continuous AZA exposure) and clonal (C) sub-lines with 20-50 fold AZA resistance. Lines were extensively profiled by various next generation assays including exome sequencing, RNA-seq, Illumina 450K methylome and miRNA arrays; to identify differences in molecular changes in resistant vs. parental (PAR) cells mimicking clinical scenarios cells were either AZA treated or left untreated prior to performing assays. A 1,489 RNAi gene silencing screen for molecular vulnerabilities is available as well. A ~ 2,216 drug library (*i.e.* Prestwick, Mayo customized selected approved drugs) was run on RES and PAR cells. Lastly, we performed targeted sequencing and RNA-seq in HMA failure MDS/AML patients to assess clonal evolution and correlate findings with *in vitro* models.
Results: In RES cells no actionable new mutation were identified by exome sequencing. RNA-seq showed several genes and regulators of the MAPK and specifically RAS pathway activated in RES cells. Methylation analysis showed shared genes with loss of methylation following AZA in PAR and RES cells. Further, genes with methylation changes only in RES or PAR cells as well as genes that were unmethylated at baseline in RES vs. PAR cells but became methylated following AZA treatment, indicate a loss of response to epigenetic regulation upon chronic HMA exposure. These genes represent novel genomic therapeutic vulnerabilities and depict resistance mechanism. From the 2,216 compound screen, only 4 compounds were overlapping in >3 of 6 cell lines, these 4 drugs are formally being validated now including primary HAM resistant patient samples. In parallel we tested putative HMA/DNMT inhibitors based on structural bioinformatics knowledge mining in the HMA resistance context. Several drugs, including known drug entities such as antihypertensives, showed similar activity in sensitive and resistant lines. Lastly, we have generated a mutational landscape of HMA resistant clinical samples (Foundation Medicine Heme Panel) and RNA-seq has been performed.
Summary and Conclusions: At the meeting our efforts and data will be presented to give an overview of our translational research platform and our approach at HMA failure. We present and highlight results and data of our combined integrated platforms of elucidating molecular mechanisms of HMA resistance while providing drugs and therapeutic avenues for MDS and AML patients who have failed AZA or Decitabine.

E884

ABSOLUTE QUANTIFICATION OF EVI1 OVEREXPRESSION IN ACUTE MYELOID LEUKEMIA BY RQ-PCR ANALYSIS: A STUDY OF THE ALFA GROUP

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Background: The *Ecotropic viral integration site 1* (*EVI1*) gene is located on chromosome band 3q26.2, and is composed of 16 exons. At diagnosis, *EVI1* overexpression has been implicated in approximately 8% of patients harboring acute myeloid leukemia (AML) and confers a poor prognosis. It is often associated with adverse cytogenetic abnormalities, but can be found in normal karyotypes. Quantification of *EVI1* expression is complex due to its various splice variants and its 5 alternative mRNA 5'-ends. *EVI1* mRNA expression has been mainly assessed by real-time quantitative PCR (RQ-PCR) using the housekeeping genes *Abelson* (*ABL*) and the ΔCt method. The technique currently used in our laboratory is based on the relative quantification of the *EVI1-1D* alternative splice form. Although this assay provides good results at AML diagnosis, it is not powerful enough to monitor MRD.
Aims: In the present study, we developed a RQ-PCR assay to perform the absolute quantification of *EVI1* expression covering the different *EVI1* splice variants. This assay was applied in control samples to define threshold values in normal bone marrow (BM) and peripheral blood (PB), and in AML diagnostic and follow-up samples.
Methods: This study focuses on 64 patients with *EVI1* overexpression included in the ALFA-0701 and ALFA-0702 trials. BM and PB samples were collected at AML diagnosis (n=67) and during follow-up (n=152). Using relative quantification, *EVI1* was considered as overexpressed at AML diagnosis if the ΔCt value between *EVI1* and the housekeeping gene *ABL* was less than 10. Reference values of normalized *EVI1* levels in normal PB and BM were established by analyzing a total of 31 PB and 17 BM samples collected from healthy volunteers. To achieve absolute quantification, we cloned a sequence of *EVI1* localized on exons 14 and 15, framing primers and TaqMan probe previously described by Gröschel *et al.* The sequence was integrated into plasmids that were used to determine the standard curve for the RQ-PCR. In parallel, the expression of the housekeeping gene *ABL* was evaluated by RQ-PCR for each sample. The results were expressed as a percentage of *EVI1* copies by *ABL* copies.
Results: The median value of *EVI1* expression in normal PB samples was 0.11% (range, 0.01 to 0.49%) and was 0.36% (0.01 to 0.96%) in normal BM samples. The overexpression threshold was set at 0.5% for PB and at 1% for BM. At diagnosis, we confirmed *EVI1* overexpression for 62 samples out of 67 selected samples. We observed a good correlation between absolute and relative quantification, only 5 PB samples considered as low positive in relative quantification were discordant. The median *EVI1* expression was 23.3% (4.1 to 614.1%) in BM and 3.6% (0.53 to 80.7%) in PB samples. Patients with unfavorable cytogenetics (n= 42) had a significantly higher *EVI1* expression level compared to patients with intermediate cytogenetics (n= 20) (*P=.004*), with an average *EVI1* expression of 43.1% *versus* 6.8%, respectively. The highest *EVI1* overexpression was found in the AML subgroup with *MLL* rearrangement (n= 11), with an average *EVI1* expression of 91.4% *versus* 18.3% in the absence of *MLL* rearrangement (*P=.006*). MRD monitoring by *EVI1* mRNA level quantification was performed in diagnostic and follow-up samples from 48 patients. *EVI1* expression in BM varied from 21.6% in diagnostic samples to 3.56% in post-induction samples. *EVI1* expression in PB varied from 4.0% at diagnosis to 0.22% in post-induction. Although reduction was significant (*P <.001*), it remained insufficient for MRD follow-up on this parameter. Finally, among the 44 patients who achieved CR1, we observed a trend of higher *EVI1* expression in relapse group with an average expression of 163.1% in BM (n=6) and 11.5% in PB (n=12) against 26.8% in BM (n=12) and 4.4% in PB (n=15) in relapse free group (*P=0.1*).
Summary and Conclusions: We developed a model of absolute quantification of *EVI1* expression correlating with routine relative quantification. Nevertheless, the basal expression of *EVI1* is high and variations are weak between diagnosis and follow-up. However at diagnosis, we observed higher expression in patients with *MLL* rearrangements, and thus, a trend of higher *EVI1* expression in patients who relapsed after having achieved CR1.

E885

THE D816V C-KIT MUTATION HAS HIGHER ABILITY OF JAK-STAT AND SRC FAMILY KINASE THAN N822K C-KIT MUTATION, AND THEREFORE PROLIFERATION ACTIVITY IS HIGH IN CORE-BINDING FACTOR ACUTE MYELOID LEUKEMIA

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Background: Acute myeloid leukemia (AML) with the t(8;21) or inv(16)/t(16;16) is defined as core-binding factor AML (CBF-AML) and has been considered an AML group with favorable prognosis. In recent years, it has been reported that CBF-AML with *c-kit* mutations show poor prognosis. Among these mutations, D816V in exon 17 is the most frequent mutation in CBF-AML and confers higher tumor growth and anti-apoptotic potential compared to other mutations of exon 8, 10 and 11. Interestingly N822K in exon 17 is reported at frequency similar to D816V mutation in Asia. Our previous report (Leukemia. 2011;25(9):1423) suggested the possibility that the prognosis of CBF-AML with the N822K mutation was different from D816V and that D816V and N822K may differ functionally even if this mutation is also in the same A-loop.
Aims: The aim of this study is to clarify a difference of the molecular biologic function for leukemogenesis between D816V and N822K.
Methods: To analyze the molecular function of D816V and N822K, we generated amphotropic retroviral vector expressing *c-kit* (wild type, D816V, and N822K). After transduction with human Interleukin 3 (IL-3) dependent TF-1 cells, we established *c-kit* expressed TF-1 cells (TF-1^{WT},TF-1^{D816V}, and TF-1^{N822K}). In these three cells, we analyzed the proliferation rate by cell growth assay and the erythropoietin (EPO)-related induction of differentiation by fluorescence activated cell sorting. We also analyzed the signal pathways by Western blotting, and examined responsiveness to protein kinase (PTK) inhibitors which were Imatinib, Dasatinib, and CP690550 (JAK inhibitor).
Results: Cell growth assay showed IL-3 independent proliferation in TF-1^{D816V} and TF-1^{N822K} but not TF-1^{WT}. The proliferation rate of TF-1^{D816V} was significantly higher than that of TF-1^{N822K} (18610±6269 cells *vs* 4354±1109 cells in day 10, p=0.026). The erythroid differentiation assay showed TF-1^{D816V} inhibited differentiation to erythroblast as compared with TF-1^{N822K} and TF-1^{WT} with or without EPO. (The rates of $CD71^{+}CD235a^{+}$cells 72 hours after EPO addition:

TF-1^{D816V} 22.1%, TF-1^{N822K} 84.6%, TF-1^{WT} 82.8%) Subsequently, we investigated the difference of signal pathway for cell proliferation by Western blotting. Under the condition starved from IL-3, the same levels of constitutive phosphorylation of c-kit protein were observed in the TF-1^{D816V} and TF-1^{N822K}, but the TF-1^{WT} hardly induced phosphorylation of it. We investigated about the downstream of phosphorylated c-kit protein in the TF-1^{D816V} and TF-1^{N822K}. We observed a strong phosphorylation of STAT3 and Src proteins in the TF-1^{D816V}, but phosphorylation of ERK protein in the TF-1^{N822K}. Finally, we examined responsiveness to PTK inhibitors. Imatinib could inhibit cell proliferation of TF-1^{N822K}, but not inhibit it of TF-1^{D816V}. In contrast, Dastinib could inhibit cell proliferation of both TF-1^{N822K} and TF-1^{D816V}. We could demonstrate these findings by Western blotting that half maximal inhibitory concentration (IC50) of Dasatinib more strongly inhibited phosphorylation of c-kit and Src proteins as compared with Imatinib in the TF-1^{D816V}. CP690550 could also inhibit cell proliferation of both TF-1^{N822K} and TF-1^{D816V}. We could also demonstrate these findings by Western blotting that IC50 of CP690550 inhibited phosphorylation of STAT3.

Summary and Conclusions: We conclude that the D816V *c-kit* mutation confers higher proliferation activity by JAK-STAT and Src family kinase compared to N822K *c-kit* mutation. These findings suggest that biological functions of *c-kit* mutations differ depending on the mutation site, which may affect responsiveness to treatments.

E886

FREQUENCY AND PROGNOSTIC RELEVANCE OF HTERT SPLICED VARIANTS IN AML

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Background: Telomeres shortening, a pro-apototic cellular event that occurs at every round of DNA replication is prevented by human telomerase. This last consists of two components, hTERT, a telomere specific reverse transcriptase that catalyzes the addition of telomeric repeats, and hTERC, the RNA component used as a template. Thus, telomerase activity mostly relies on hTERT which activity depends on the spliced variant produced by an alternative splicing process. This last can generate not only the full-length hTERT transcripts (+α+β), but also transcripts carrying α and/or β deletions (transdominant negative isoform -α+β, inactive products +α-β and -α-β). Recent data provided evidence that in AML hTERT over-expression may be associated with a poor clinical outcome, but up to now no study has examined the frequency and prognostic significance of spliced variant transcripts.

Aims: Our objectives were to estimate the frequency of hTERT isoforms in a series of de novo AML patients (pts) and to establish whether the expression of these isoforms varied between pts with a normal and a complex karyotype and had any influence on clinical outcome.

Methods: The one-hundred and nine de novo AML patients included in the present study came to our observation in the period January 2010 and January 2014. They were forty-three females and sixty-six males; their median age was 59 years (range 18-84). According to WHO classification 8 (7.3%) patients were classified as M0/M1, 51 (46.7%) as M2, 42 (38.5%) as M4, 7 (6.4%) as M5 and one as M6. Cytogenetic studies revealed a normal karyotype (NK) in sixty-two (56.8%) pts, whose median age was 56 years (range 24-84), and a complex chromosomal pattern (CK) with ≥3 defects in forty-seven (43.2%), whose median age was 60 years (range 33-84). The two patients groups were comparable by age. An internal tandem duplication (ITD) of the FLT3 gene and a NPM1 mutation were revealed in 15 and 2 chromosomally normal patients. No CK presented a FLT3 ITD. All patients received standard induction chemotherapy followed by two courses of consolidation treatment. At the time of the study 41 patients achieved a complete remission (CR) and 39 died. Median follow-up was 22.7 months (range: 13.5-60.2). hTERT isoforms expression was determined in bone marrow samples by real-time reverse transcriptase polymerase chain reaction, using SYBR Green I. hTERT transcript (+α+β) primers design was made using BLAST (http://www.ncbi.nlm.nih.gov/BLAST/), while for the other primers we referred to Capraro *et al.* 2011. Specific amplifications were confirmed by sequences analysis. In order, to estimate hTERT isoforms expression levels in normal mononuclear cells, twenty-three umbilical cord bloods (UCB) were examined (control group).

Results: At first, the expression of hTERT isoforms of NK pts was compared to that of CK pts by applying the Kruskal-Wallis rank test, but no significant difference was observed (P=0.39, P=0.77; +α+β, -α+β respectively). Instead, Cox univariate analysis revealed that independently from karyotype response to treatment was significantly better in pts presenting an over-expression of the trans-dominant negative isoform (-α+β) (P=0.03, HR=2.11; 95% CI:1,13-3,93). In addition, leukemia-free survival was significantly worse in pts who over-expressed inactive products (-α-β) (first group) than in those who presented a low expression of these same products (second group) (P=0,003, HR=3,26; 95% CI:1,33-7,96). Pts who over-expressed the full-length hTERT transcript (+α+β) and the trans-dominant negative isoform (-α+β) presented only a trend towards significance. Moreover, the first group of pts presented a worse overall survival (HR=1,66; 95% CI:0.94-2,91), whereas the second group a better overall survival (HR=0,58; 95% CI:0,33-1,02).

Summary and Conclusions: Thus, in our study the over-expression of the hTERT trans-dominant negative isoform identified low-risk AML pts, whereas the over-expression of the hTERT inactive product (-α-β) identified high-risk AML pts. Interestingly, this last isoform might control the expression of its trans-dominant negative isoform. In conclusion, our results suggest an intriguing link between the control of hTERT isoforms expression and AML outcome.

E887

COMPLEMENTARY DYNAMIC BH3 PROFILES PREDICT INDUCTION OF APOPTOSIS BY THE MULTI-KINASE INHIBITOR TG02 IN COMBINATION WITH THE BH3 MIMETIC ABT-199 IN AML CELLS

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Background: The heterogeneity of MCL1 and BCL2 expression in primary AML samples, and likely even within individual patient isolates, suggests that it would be difficult to establish cut-off points that would enable the clinician to select patients for either MCL1 or BCL2-directed targeting, so the use of complementary agents may offer an ideal solution.TG02 is a novel CDK9-selective multi-kinase inhibitor that down-regulates RNA Polymerase II causing the depletion of the labile BCL2-family pro-survival protein MCL1, but not its stable paralogs BCL2 or BCLX_L. ABT-737 and ABT-199 are BH3 mimetics that inhibit BCL2.

Aims: We investigated whether ABT-737 and/or ABT-199 would co-operate with TG02 to kill AML cells, and we studied the mechanisms of their interactions.

Methods: Cytotoxicity was measured by alamar blue in AML lines and flow cytometry in patient samples. Apoptosis was determined using flow cytometric assays for Annexin V binding, and active epitopes of BAX, BAK and caspase 3. In dynamic BH3 profiling assays, cells were treated with TG02 or ABT-199 for 4 hours and probed with BH3 peptide mimetics for a further hour. Mitochondrial outer membrane permeabilisation was then determined by Cytochrome C release assays.

Results: In CD34+CD38- KG1-a cells, TG02 was strongly synergistic with ABT-737 or ABT-199 with combination indices as low as 0.13. In a cohort of 18 patient samples treated *in vitro*, 50 nM TG02 +50 nM ABT-199 or ABT-737 was significantly more toxic than 100 nM of each agent individually. Whereas the agents were synergistic in some samples, others showed enhanced apoptotic responses to TG02 but not to ABT-199/737 and vice versa. Normal CD34+cells from stem cell harvests were not sensitive. In AML cells all three agents exposed activation-specific epitopes of BAX, BAK and caspase 3. The sensitiser protein BAD binds to BCL2, which can then release the apoptosis-activator protein BIM, whereas the sensitiser NOXA releases BIM from MCL1. Following treatment of AML cells with TG02 or ABT-199, the resultant dynamic BH3 profiles were found to be complementary: both agents sensitised cells to BIM, TG02 sensitised to BCL2-targeting BAD and ABT-199 sensitised to MCL1-targeting NOXA. Drug priming altered patient cell BH3 peptide sensitivity in a manner that corresponded closely to the cells' *in vitro* demise.

Summary and Conclusions: We conclude that this combinatorial approach broadens the therapeutic potential of anti-survival signalling drugs in AML and that dynamic BH3 profiling is a sensitive methodology for investigating complementary mechanisms of chemoresponsiveness.

E888

COMBINED INHIBITION OF HEDGEHOG AND FLT3 SIGNALLING LEADS TO EFFECTIVE ANTI-LEUKEMIC EFFECTS IN ACUTE MYELOID LEUKEMIA

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Background: The interaction of leukemic stem cells with the bone marrow niche is dependent on stem cell activators implicated in normal stem cell biology including the Hedgehog pathway. Recently, we revealed that expression of the Hedgehog downstream transcription factors GLI1 and GLI2 represents a negative prognostic marker for patients with acute myeloid leukemia (AML) and that targeted inhibition of GLI1/2 mediates anti-leukemic effects *in vitro* and *in vivo*. Furthermore, the expression of GLI2 was correlated to the occurrence of FLT3 mutations in AML patients. Activating FLT3 mutations represent one of the most frequent aberrations in AML resulting in a constitutively activated downstream signalling including the PI3K cascade. In addition to the Hedgehog ligand-mediated, a non-canonical GLI activation via PI3K, TGF-β or Ras has been described. Therefore, we propose also FLT3 as a non-canonical inducer of GLI transcription in AML.

Aims: The aim of our study was to analyze the therapeutic potential of combined GLI and FLT3 signaling inhibition in AML.

Methods: FLT3 mutated AML cell lines MV4-11 and MOLM-13 as well as the non-mutated cell line OCI-AML5 were treated with combinations of the GLI inhibitor GANT61, the FLT3 inhibitor sunitinib or the PI3K inhibitor PF-04691502

and analyzed for cell proliferation. MV4-11 cells were also analyzed in colony formation and apoptosis induction assays. Specific shRNA knockdown of all targets have been performed to confirm the results.
Results: In proliferation assays, single agent treatment with sunitinib, PF-04691502 or GANT61 was significantly less effective than double and especially triple combinations. Growth inhibition of FLT3-mutated MV4-11 and MOLM-13 cells occurred at lower concentrations compared to non-mutated OCI-AML5 underlining the potential role of a non-canonical activation of GLI via FLT3-PI3K. Investigating the induction of apoptosis in MV4-11 cells, combinations of two inhibitors were more effective compared to the single agent treatments while the triple combination of GANT61, sunitinib and PF-04691502 induced the highest apoptosis rate after 48h. Similar effects were observed in colony formation assays of MV4-11 cells. The double combinations induced a slightly stronger inhibition compared to the single agent treatments while the triple combination resulted in most drastically reduced colony numbers. The impact of combined treatment on colony numbers was also analyzed in CD34 positive hematopoietic progenitor cells isolated from healthy donors. Interestingly, the reduction of colonies upon double or triple inhibitor combinations was only slightly, but not significantly increased compared to the single agent treatments possibly opening up a therapeutic window for combination therapy. Targeted knockdown of GLI, FLT3 and PI3K in MV4-11 cells using specific shRNA lentiviral constructs could confirm our observations. The cells transduced with all shRNAs showed the poorest proliferation rates and colony formation capacities. Upon transduction with the FLT3 shRNA alone the mRNA expression of GLI2 was also reduced supporting our hypothesis of GLI2 activation by FLT3 signaling in AML.
Summary and Conclusions: We propose that the Hedgehog pathway members GLI1/2 are non-canonically activated by FLT3. Therefore, the combined inhibition of FLT3, PI3K and GLI could represent a more effective, therapeutic approach than targeting FLT3 alone in patients with FLT3-mutated AML.

E889

MATURATION-ASSOCIATED IMMUNOPHENOTYPIC CLASSIFICATION OF BLASTIC PLASMACYTOID DENDRITIC CELL NEOPLASMS

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Background: Blastic plasmacytoid dendritic cell neoplasm (BPDCN) is a rare subtype of leukaemia/lymphoma, whose diagnosis can be difficult to achieve due to its clinical and biological heterogeneity, as well as its overlapping features with other hematologic malignancies.
Aims: In this study we investigated whether the maturational stage of tumour cells could be associated with particular clinico-biological and prognostic features of the disease.
Methods: 46 BPDCN cases were analysed and classified into three maturation-associated subgroups according to the expression of CD34 and CD117: 1) immature BPDCN (expression of CD34 in at least a fraction of blasts); 2) intermediate BPDCN (partial positivity for CD117 in the absence of CD34), and; 3) mature cases ($CD34^-$ $CD117^-$ blasts).
Results: Blasts from cases with an immature plasmacytoid dendritic cell (pDC) phenotype exhibited an uncommon $CD56^-$ phenotype, coexisting with $CD34^+$ non-pDC tumour cells, typically in the absence of extramedullary (*e.g.* skin) disease at presentation. Conversely, patients with a more mature blast cell phenotype more frequently displayed skin/extramedullary involvement and spread into secondary lymphoid tissues. Despite the dismal outcome, acute lymphoblastic leukaemia-type therapy (with central nervous system prophylaxis) and/or allogeneic stem cell transplantation appeared to be the only effective therapies.
Summary and Conclusions: Overall, our findings indicate that the maturational profile of pDC blasts in BPDCN is highly heterogeneous and translates into a wide clinical spectrum -from acute leukaemia to mature lymphoma-like behaviour-, which may also lead to variable diagnosis and treatment.

E890

INVOLVEMENT OF HISTONE ACETYLTRANSFERASES IN DEVELOPMENT OF ACUTE PROMYELOCYTIC LEUKEMIA AND RESPONSE TO DIFFERENTIATION-INDUCING COMPOUND

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Background: In a model of acute promyelocytic leukemia (APL) development, PML-RARA fusion protein suppresses transcription of RARA regulated genes through recruitment of histone dedyacetylase (HDAC), which blocks granulocytic differentiation. Differentiation-inducing compound all-*trans* retinoic acid (ATRA) that is used for APL treatment induces granulocytic differentiation in APL cells by modulating PML-RARA structure and subsequently dissociating HDAC from PML-RARA. Although the HDAC involvement in APL development and ATRA response is well documented, roles of histone acetyltransferases (HAT) remain elusive.
Aims: This study aims to uncover involvement of HAT in APL development and ATRA-induced granulocytic differentiation in APL cells.
Methods: mRNA expression levels of major HAT such as PCAF, GCN5, and p300 were assessed in APL patients' bone marrow by quantitative PCR (q-PCR) at onset and complete remission of APL. RNA expression profiles in APL and other hematologic malignancies were further analyzed with public databases. Differential expression of HAT upon ATRA treatment was further examined *in vitro* with cell lines such as HL-60 and NB4, and primary cells from APL patients. The protein levels of HAT were finally examined by immunoblot analysis. For functional characterization, a loss-of-function assay was performed using lentivirus vectors system with 3 independent shRNA sequences against PCAF or GCN5, and a non-target shRNA. Granulocytic differentiation was defined by CD11b expression.
Results: PCAF and GCN5 but not CBP/p300 showed differential expression between onset and complete remission of APL. PCAF expression was suppressed in APL bone marrow, while GCN5 expression was significantly elevated. When leukemic cells were induced for cellular differentiation by ATRA, dynamic change of PCAF and GCN5 mRNA expression was observed in culture cells NB4 and HL-60, and primary cells from APL patients. In cultured cells, levels of PCAF and GCN5 proteins followed those of mRNA. In public databases, reduced level of PCAF-expression in patients' bone marrow compared to healthy individuals was observed in not only APL but also other acute leukemia such as acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) (healthy individuals; n=74, AML; n=505, ALL; n=750). While, the reduction of PCAF-expression was not evident in chronic myeloid leukemia (CML) and myelodysplastic syndrome (MDS). In Contrast, GCN5-expression in AML and ALL was increased compared to that in the healthy individuals, and even in chronic hematologic malignancies. These data suggest that in malignant cells GCN5 expression was generally upregulated whereas PCAF expression is coupled with normal differentiation, which was preferentially blocked in acute leukemia cells. Potential of contradictory roles between PCAF and GCN5 in leukemia cells were assessed by a loss-of-function assay. Knocking down GCN5 induced massive apoptosis in HL-60 cells, suggesting that GCN5 harbors oncogenic property. In contrast, when knocking down PCAF, cells failed to differentiate into granulocytes after ATRA treatment. Furthermore, primary APL blasts of which PCAF was knocked down became resistant to ATRA-induced granulocytic differentiation, which implies that PCAF possesses a tumor suppressor function.
Summary and Conclusions: Despite that functional redundancy between PCAF and GCN5 in normal development was proposed, our data strongly suggest that PCAF and GCN5 have opposing roles in the APL development and ATRA-induced granulocytic differentiation in APL cells. More detailed understanding of HAT involvement in leukemogenesis and ATRA-induced cell differentiation in APL may lead to a development of novel treatment strategy against not only APL but also other AML.

E891

EFFECTS OF ARSENIC TRIOXIDE ON EVI-1 IN ZEBRAFISH AND IN LEUKEMIA CELLS

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Background: The *ecotropic viral integration site-1* (Evi-1) was first identified as the integration site of the ecotropic retrovirus leading to myeloid leukemia in murine model systems. In humans, Evi-1 is located on chromosome 3q26, and rearrangements on chromosome 3q26 often activate EVI-1 expression in myeloid malignancies. Arsenic trioxide (ATO), used in some traditional Chinese remedies for a very long history, has been found to be an effective treatment for acute promyelocytic leukemia (APL) and is being tested for treating other malignancies. Therefore, in our study we chose Tg(*EVI*-1:HSE:EGFP) zebrafish model (zebrafish embryos) and leukemia cells (both primary leukemia cells and leukemia cell line THP-1) to examine the effect of ATO on Evi-1 gene expression and clarify the mechanisms.
Aims: High expression of oncogene Evi-1 is an independent negative prognostic indicator of survival in leukemia patients. The present study investigates the effects of ATO on Evi-1 in zebrafish and in leukemia cells via apoptosis.
Methods: Embryos of Evi-1 transgenic zebrafish, tagged with Enhanced green fluorescent protein (EGFP),were treated with different concentrations of ATO

(50, 100 and 150μM) *in vivo* from 24 hours post fertilisation (hpf) to 72 hpf prior Evi-1 mRNA quantification by RT-PCR. Meanwhile, we measured the Evi-1 expression in bone marrow mononuclear cells treated with 1 μM ATO for 24h, 72h or 120h *in vitro*, the specimen was donated by a patient diagnosed with acute monocytic leukemia with high Evi-1 expression. Furthermore, THP-1 cell line, the highest expression of Evi-1 out of four leukemia cell lines (K562, HL-60, U937, THP-1),were treated with different concentrations of ATO (1, 3 and 5μM), or with a novel selective inhibitor of c-Jun N-terminal kinase (JNK), named as SP600125 (10μM),for 24h, 48h or 72h, tested for cell viability by CCK-8 kit, cell morphology by cytospin smear, cell apoptosis by flow cytometry, Evi-1 mRNA expression by RT-PCR, protein quantity by western blot.
Results: ATO downregulated Evi-1 mRNA in zebrafish *in vivo* and in primary leukemia cells *in vitro*. ATO treatment was shown to inhibit proliferation, induce apoptosis, downregulate both Evi-1 mRNA and Evi-1 oncoprotein expression, increase the expression of pro-apoptosis protein JNK, p-JNK, p-P53, PUMA, Bax, caspase-9 and caspase-3, and decrease the expression of anti-apoptotic protein Bcl-2 and Bcl-xL in THP-1 cell line *in vitro*. The pro-apoptotic activity of ATO in THP-1 cells could be inhibited by SP600125 (a specific JNK inhibitor), whereas SP600125 had little effect on the expression of EVI-1 protein.

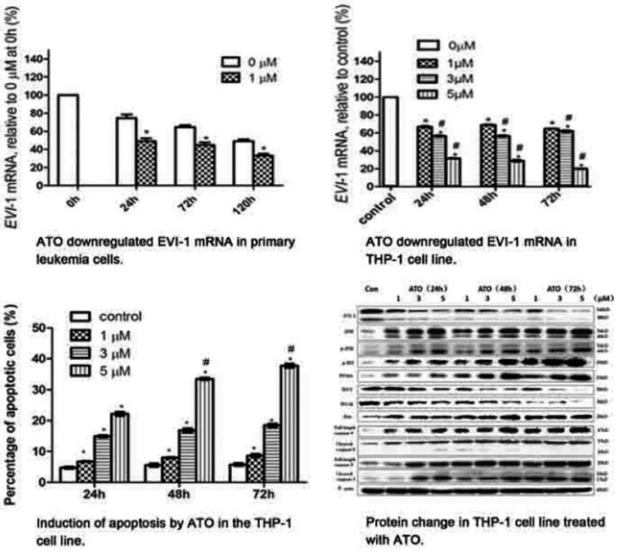

Figure 1.

Summary and Conclusions: Together, the study results reveal that ATO can downregulate Evi-1 mRNA as well as oncoprotein and block the repression of Evi-1 to JNK pathway, so the JNK apoptotic pathway is activated, giving rise to apoptosis of Evi-1 positive cell line THP-1. These novel findings provide theoretical basis for developing personalized medicine strategy to improve the therapeutic efficacy of the treatment of Evi-1 positive leukemia.

E892

CLINICAL CHARACTERISTICS AND PROGNOSTIC IMPLICATIONS OF DNMT3A MUTATIONS IN ACUTE MYELOID LEUKEMIA

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Background: Gene mutations and epigenetic changes have been shown to play a significant role in the pathogenesis of acute myeloid leukemia (AML). The components of epigenetic machinery such as DNA methylation plays an essential role in development, differentiation, genomic instability, specific regulation of gene expression and may contribute to leukemogenesis. Recently, researchers have been interested in investigating *DNMT3A*, because of the high frequency of mutations of this gene in AML. Also, *DNMT3A* mutations are considered to be independently associated with an unfavourable prognosis in adults with *de novo* AML, however, there are still ongoing debates on this topic.
Aims: The aim of the research was to investigate the frequency and prognostic impact of *DNMT3A* mutations in AML patients and to analyze its interaction with other prognostic markers.
Methods: This retrospective study was performed in 143 previously un-treated adult patients (135 pts with de novo and 8 pts with secondary AML). There were 65 males and 78 females with a median age of 55 years (18-86). According to the results of cytogenetic analyses pts were separated in the following groups: with favourable (9.8%), unfavourable (14.0%) prognosis, with normal karyotype (NK) (49.0%) and other aberrations (27.2%). Mutation analysis of *DNMT3A* R882 was performed by high-resolution melting curve analysis. Mutations in *FLT3* and *NPM1* were analysed by PCR and in *NRAS* by sequencing. Metaphase chromosomes were banded with the trypsin-Giemsa technique.
Results: Mutations of *DNMT3A* R882 were found in 23 (16.1%) de novo AML patients: R882H (16 pts), R882C (6 pts) and R882S (1 pts). All but one pts (with mutation R882S) were heterozygous and retained a wild-type allele. *DNMT3A* mutations were detected in all morphologic variants (except M0 and M3, extremely rare in M2 (p=0.006)) and were frequently noted in patients with M5 (7/17, p=0.003) and M4 (11/38, p=0.012). It was revealed, that none of the patients with favourable karyotype harboured *DNMT3A* R882 and only 1/20 pts with adverse karyotype had *DNMT3A* mutation. 17/23 pts (24.3%) with $DNMT3A_{mut}$ had tumors with normal cytogenetic profiles (of a total of 70 NK samples) (p=0.009). Patients with isolated *DNMT3A* mutations were seen in 5 cases; all 3 patients with genotype $DNMT3A_{mut}/NRAS_{mut}$ had the R882C variant of the mutation; 6 pts had genotype $DNMT3A_{mut}/FLT3\text{-}ITD_{mut}/ NPM1_{mut}$. *DNTM3A* mutations were significantly more prevalent in *NPM1* positive cases when compared to *NPM1* wild type cases (p=0.005). *DNTM3A* mutations were also more dominant in *FLT3*-ITD positive pts than wild type (p=0.001). Patients who harbored the mutations in *DNMT3A* had a higher white blood cell count (p=0.001) and platelets count (p=0.020) in peripheral blood, and blasts in bone marrow (p=0.099) at diagnosis. There was no statistical correlation with other parameters, including sex, age and hemoglobin between patients with and without *DNMT3A* mutations (p>0.05). There was statistically significantly correlation between overall survival (OS) and relapse-free survival (RFS) of AML pts with $DNMT3A_{mut}$ and $DNMT3A_{wt}$ (p=0.031 and p=0.045, respectively). Medians of OS and RFS were: 5.2 and13.0 months; 4.8 and10.0 months, respectively.
Summary and Conclusions: AML with *DNMT3A* mutations represent a group, which is homogeneous on a number of clinical and laboratory parameters, associated with adverse prognosis and a high risk of relapse. Therefore, the discovery of highly recurrent mutations in *DNMT3A* may provide a new tool for the classification of intermediate-risk AML.

E893

CRITICAL ROLE OF SOX12 IN LEUKEMOGENESIS IN AML

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Background: The SOX (Sry-related HMG box) genes belong to a family of transcription factors containing a High-Mobility-Group box domain. In an initial screen of SOX genes in human leukemias, SOX12 is uniquely up-regulated in acute myeloid leukemia, myelodysplastic syndrome and chronic myelogenous leukemia but down-regulated in most cases of normal individuals.
Aims: The study is to examine the expression and function of SOX12 in acute myeloid leukemia (AML).
Methods: Mononuclear cells (MNCs) were isolated from AML and fractionated by immunomagnetic selection and fluorescence activated cell sorting (FACS). SOX12 expression in different leukemia subsets was evaluated by RT-PCR. To examine its function, SOX12 siRNA was nucleofected to K562 cells and gene knock-down was confirmed by RT-PCR and Western Blot. Apoptosis and cell cycle were analyzed by Annexin V/7AAD assay and flow cytometry. To examine the leukemia stem cell (LSC) function in AML, SOX12 was knocked down with siRNA, and K562 cells transduced with siRNA were subjected to *in vitro* colony forming assay, and injected into NOD/SCID mice as well. 2 weeks later, the number of colonies were enumerated, and 8 to 12 weeks later, the mice were euthanized and bone marrow cells from the mice were analyzed.
Results: SOX12 was preferentially expressed in $CD34^+$cells ($CD34^+$*vs* $CD34^-$: P<0.015) but not $CD34^+CD38^-$ cells which were considered stem/progenitor cells in normal hematopoiesis. SOX12 knockdown in K562 cells with siRNA could reduce the cell proliferation (scramble *vs* SOX12 at day 3, p=0.025), induce the increase of cells in G1 phase (SOX12 *vs* scramble at day 3, p=0.03), but had no effect on apoptosis. *in vitro* colony number was reduced with siRNA transduced K562 cells compared to control group (P=0.032). Engraftment of transduced K562 cells in NOD/SCID mice were significantly reduced 8 to12 weeks after transplantation (p=0.04).
Summary and Conclusions: SOX12 is preferentially expressed in human $CD34^+$cells of AML. SOX12 knock-down reduced cellular proliferation by arresting the AML cells in G1 phase of the cell cycle. SOX12 knock-down could also reduce the repopulating ability of AML in NOD/SCID mice, indicating SOX12 was involved in the regulation of leukemogenesis in AML. Its mechanism of action would have to be further evaluated.

E894

THE SYK INHIBITORS R788, R406 AND P505-15 COUNTERACT NEOPLASTIC CELL SURVIVAL AND SYNERGIZE WITH MIDOSTAURIN IN PRODUCING GROWTH ARREST IN AML CELL LINES AND PRIMARY AML BLASTS

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Background: In acute myeloid leukemia (AML), relapse after high-dose chemotherapy is frequent and the overall outcome is poor. Therefore, new therapeutic strategies are warranted. Currently, the effects on leukemic cells of novel targeted drugs, including signal-transduction inhibitors, have been examined. Syk is a tyrosine-kinase that has been shown to play a key role in oncogenic signaling in AML cells.
Aims: In this study, we evaluated the effects of the Syk-inhibitors Fostamatinib (R788), its active metabolite R406 and the more specific inhibitor P505-15 on proliferation and survival of AML cells.
Methods: The inhibitors R788 and R406 were purchased from Selleck (Houston, TX), and P505-15 from Exon Medchem (Groningen, NL). The multikinase inhibitor PKC412 (midostaurin; active against Flt-3) was purchased from Chemietek (Indianapolis, IN). In this study, the Flt-3-ITD positive AML cell lines MOLM-13 and MV4-11 were used. Primary AML blasts were isolated from the peripheral blood or bone marrow from 13 AML patients. Neoplastic cells were exposed to various concentrations of Syk-inhibitors alone or in combination with PKC412. Thereafter, cell proliferation was measured by ^{3}H-thymidine-uptake. Cell cycle progression and induction of apoptosis were analyzed by flow cytometry. Apoptosis was confirmed by light microscopy and by Western blot analysis of cleaved caspase-3. Drug interactions (additive, synergistic) were qualified with Calcusyn software. The enzymatic activity of Syk was evaluated by detection of autophosphorylated tyrosine on the position 348 by flow cytometry.
Results: In a first step, MOLM-13 and MV4-11 cells were exposed to the three Syk-inhibitors. As assessed by ^{3}H-thymidine-uptake, all tested drugs were found to inhibit proliferation in both cell lines in a dose-dependent manner (IC_{50}: 0.1-5 μM). The underlying mechanisms of growth-inhibition were induction of cell-cycle arrest in G0/G1 phase and induction of apoptosis, as assessed by light microscopy, flow cytometry and Western blot analysis of cleaved caspase-3. These effects were accompanied by a dose dependent decrease of the enzymatic activity of Syk. We next incubated primary AML-blasts (n=13 patients) with R406, R788 and P505-15. Again, all compounds tested were found to inhibit cell-proliferation with IC_{50} -values ranged between 0.1 μM and 5 μM in all tested samples, including in primary cells expressing Flt-3-ITD. No difference in IC_{50} -values were seen between samples isolated from newly diagnosed patients and heavily pretreated patients, suggesting, that Syk-inhibition may exert antileukemic effects even in relapsed patients. Finally, we combined the Syk-inhibitors with the Flt-3-inhibitor PKC412 at suboptimal concentrations and were able to demonstrate synergistic effects of these targeted drugs in producing growth-inhibition in Flt-3-ITD+AML cell lines.
Summary and Conclusions: Altogether, Syk-inhibitors act antineoplastic in AML cell lines and primary AML blasts, including in samples isolated from patients with relapsed AML. Further, Syk-inhibitors synergize with the Flt-3-inhibitor PKC412 in blocking leukemic cell expansion. Our data suggest that Syk-inhibitors may be implicated in the therapy of relapsed AML. However, the therapeutic value of these agents alone or in combination with other drugs remains to be investigated within the frame of clinical trials.

E895

ACCUMULATION OF THE NPM-1 SPLICE VARIANT R2 ACCOMPANIES AML DEVELOPMENT

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Background: The relevance of differential splicing in human cancer is an evolving area of cancer biology. The recent findings of frequent mutations of the splicing pathways in MDS provide insight into the mechanism of alternative splicing, which has been long associated with the development of cancer. The genome-wide microarray analysis using Exon arrays discovered significantly differentially spliced genes in AML. Moreover, the AML specific "splicing profile" was normalized in remission and reappears with patient relapse, thereby supporting a role of deregulated splice variants in the process of leukemogenesis. A close cytogenetic and molecular deregulation between *de novo* MDS and AML of elderly people suggests a common pathogenic mechanism for these conditions. Although the full spectrum of leukemic progression has not yet been completely clarified, recent clinical and biological studies indicate that MDS and AML could be considered as part of the same continuous disease spectrum rather than as distinct disorders. Recently, we found that high expression of the *NPM-1* splicing variant R2 may provide prognostic value for CN-AML patients. As the R2 splicing variant represents a truncated form of *NPM1* gene this isoform mostly localizes in the nucleoplasm, and thus might also have a biological impact in the malignant cells.
Aims: Assuming the common origin of MDS and AML we aimed to characterize the *NPM1* R2 splice variant expression as well as the influence of R2 expression on NPM1 localization in groups with MDS, sAML and AML patient samples.
Methods: Since we found prognostic significance of the expression level of R2 for the AML cohort of patients, therefore we decided to evaluate its significance for MDS and sAML cases. For 61 samples (25 AML, 30 MDS and 6 samples with sAML) qRT-PCR was performed. Expression level of *NPM-1* R2 was assessed. To investigate whether R2 might disrupt localization of the NPM1 wild type protein, immunohistochemistry analysis for NPM1 in 23 AML bone marrow smears was performed.
Results: We found significant differences in *NPM-1* R2 expression levels between cytogenetically normal (CN-AML), sAML, MDS and healthy volunteer (HV) groups. Interestingly, expression of *NPM-1* R2 tended to be elevated in CN-AML compared to MDS (median 0.022 *vs* 0.015, p=0.061). The expression of R2 was significantly increased in the CN-AML, sAML and MDS groups compared to HVs (median 0.022 *vs* 0.005, p<0.001, 0.015 *vs* 0.005, p<0.001, and 0.022 *vs* 0.005, p<0.001, respectively). Moreover, we stratified MDS and AML patients according to IPSS and genetic risk classification, respectively and found significant differences in *NPM-1* R2 expression between those groups. The IHC stainings for AML samples revealed that in cases with high *NPM-1* R2 expression we were able to determine a cytoplasmic localization of NPM1 even in the absence of a concomitant *NPM1* mutation. Therefore, we provide further evidence that the cytoplasmic localization of NPM1 might depend not only on its mutational status, but might also be influenced by the distribution of its splice variants.
Summary and Conclusions: In our study we found that the expression levels of *NPM-1* R2 were elevated in AML, sAML and MDS groups compared to HVs suggesting that *NPM-1* R2 might play some role in the process of the tumorigenesis not only in AML cases but also in early stages of development of this disease. As the *NPM-1* R2 splice variant represents a truncated form of *NPM1* gene this isoform mostly localizes in the nucleoplasm, and thus might also have a biological impact in the malignant cells. The *NPM-1* R2 might interact with cellular proteins affecting signal pathways and by this have influence on outcome or modulate treatment response. In summary, the expression of *NPM-1* R2 might be of biological importance for CN-AML as well as for sAML and MDS patients. This work was supported by National Centre for Science Grant HARMONIA (UMO-2013/10/M/NZ5/00313).

E896

Abstract withdrawn

E897

NEXT GENERATION SEQUENCING OF MYELOID MALIGNANCIES IDENTIFIED A SPECTRUM OF NOVEL MUTATIONS IN EPIGENETIC REGULATORS – CENTER FOR INDIVIDUALIZED MEDICINE CLINIC EXPERIENCE

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Background: Next generation sequencing (NGS) studies have been instrumental in identifying novel and recurrent mutations in hematologic malignancies. FoundationOne Heme© is a comprehensive NGS platform designed to provide targeted assessment of the genomic landscape of hematologic neoplasms. It interrogates the entire coding sequence of many genes, described to be somatically altered.
Aims: We wished to report the frequency of somatic mutations and variants of unknown significance (VUS) in myeloid neoplasms and their impact on clinical care including timing of testing.
Methods: After due IRB approval, patients with myeloid malignancies who underwent FoundationOne Heme© analysis at Mayo Clinic Rochester were identified. The complete coding DNA sequence of 405 genes, selected introns of 31 genes as well as the RNA sequence (cDNA) of 265 genes was interrogated. Mutations identified were stratified as having prognostic or actionable clinical impact. Actionable mutations were defined as potentially leading to a treatment decision with clinical trials or off label therapies, whereas prognostic mutations were those that helped with risk stratification. All data was retrospectively abstracted.
Results: A total of 45 patients were included in this analysis. The distribution of underlying conditions was: AML (27), ALL (5), acute undifferentiated leukemia (2), MDS (4), MPN (3), MDS/MPN overlap syndrome (3) and others (3). At last follow up, 19 (42%) patients were alive. A. Somatic mutations: A total of 164 somatic mutations were identified with a median of 4 per patient (range: 0-8). Of therapeutic relevance, the following mutations were most common: *DNMT3A* (8%), *RUNX1* (6%), *TET2* (5%), *FLT3* and *KRAS* (4% each), whereas *TP53* (10%) and *ASXL1* (8%) were the most common prognostic mutations. A total of 58 (35%) aberrations were actionable; 53 via clinical trials and 38 through off label therapeutics. Median number of potential therapies and clinical trials

identified after testing were 2 (0-6) and 3 (0-7) per patient, respectively. Among patients who succumbed to their disease, median time to death from sample acquisition was 68 days (6-375), and 7 patients (16%) died within 1 month of testing, 5 of whom died before results were available. In Patients surviving beyond 2 weeks of available results, testing changed clinical management in 7/38 (18%); 5 received off label therapy and 2 were enrolled in phase I/II clinical trials. B. Variants of unknown significance (VUS): A total of 286 somatic variants were identified; 215 in AML and 71 in other myeloid neoplasms, with 29 overlapping variants. Most common variants observed were in the following genes; *MLL2* (3%), *DNMT3A, EP300, FLT1, LRP1B, LRRK2, MAP2K6, PDCD11* and *ROS1* (2% each) in AML and *HDAC7* (4%), *ATRX, CRKL, DOT1L, EP300, FLT1, FOXO3, MLL2, RICTOR, SPEN* and *STAT6* (3% each) in other myeloid neoplasms.

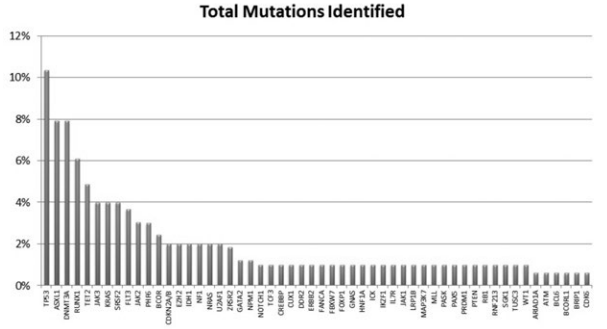

Figure 1.

Summary and Conclusions: Targeted use of NGS potentially yields prognostic and therapeutic information about somatic mutations in Hematologic malignancies. This permitted the use of novel targeted therapies including clinical trials in some patients. However, delayed testing until exhaustion of therapeutic options limits the utility of this tool. Utilizing a larger cohort with functional studies is required to comprehensively investigate the potential pathologic nature of recurrent VUS.

E898

A COMPREHENSIVE ANALYSIS OF ACCESSIBLE BONE MARROW PROTEINS FOR ANTIBODY-BASED THERAPY OF ACUTE MYELOID LEUKEMIA

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Background: Immunoconjugates are targeted agents that combine the tumor homing properties of monoclonal antibodies with the cancer killing capacity of effector molecules such as drugs, cytokines, toxins or radionuclides. There is no therapeutic antibody approved for the treatment of AML to date, but several are under clinical investigation. Well characterized antigens like CD33 and CD123 are leukemia-selective, but lack true leukemia-specificity. New targets are usually identified by comparison of the expression of membrane-bound proteins in leukemic cells and their normal hematopoietic counterparts.
Aims: Here, we used a general chemical proteomics approach based on *in vivo* protein biotinylation to identify bloodstream-accessible proteins as they exist natively in the bone marrow in AML, a disease that is, despite therapeutic improvements of the last decades, still lethal to the majority of patients.
Methods: Brown Norway (BN) rats bearing the syngeneic BNML acute myeloid leukemia model and healthy control rats were subjected to *in vivo* protein biotinylation by vascular perfusion with an aqueous solution of sulfo-NHS-LC-biotin, a reactive ester derivative of biotin, that enables the covalent labeling of proteins readily accessible from the blood-stream. Biotinylated proteins from the perfused bone marrow were purified on streptavidin-resin, trypsinized and subjected to LC-MALDI-TOF/TOF mass spectrometry and bioinformatic processing for identification and relative quantification. The most promising AML-associated targets were validated by APAAP-immunohistochemistry (IHC) on rat and human AML bone marrow specimens.
Results: In total, almost 1500 bloodstream-accessible proteins were identified in healthy and leukemic bone marrow. Among them, 181 proteins were more than 100-fold up-regulated in AML as compared to normal bone marrow. Fifteen of the most differentially expressed proteins were selected for further validation by IHC. Of these, ECM1, Nucleolin, COL6A1 and AP3B2 showed the most differential expression in rat and human AML as compared to normal bone marrow.
Summary and Conclusions: We provide an atlas of circulation-accessible AML-associated bone marrow proteins that could serve as potential targets for a site-specific antibody-mediated pharmacodelivery of cytotoxic drugs, cytokines or radionuclides to the leukemia-infiltrated bone marrow in AML.

E899

IMPACT OF DEVELOPMENTAL STAGE AND AGE ON GENE EXPRESSION IN CHILDHOOD AND ADULT ACUTE MYELOID LEUKAEMIA

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Background: Acute myeloid leukaemia (AML) is a heterogeneous disease with a sharp rise in incidence after the 3rd decade. A strong correlation between specific genetic changes and gene expression patterns is well established, and individual cytogenetic subtypes of AML, in particular t(8;21), t(15;17) and inv(16) display distinct gene expression patterns[1]. Chronological age of the patient is one of the most important markers for prognosis and clinical outcome[2]. The impact of age on leukaemia prognosis is not understood in detail. Differences in gene expression patterns between AML in younger and older patients have been described, and suggests differential oncogenic pathway activation in older patients (>55 years of age)[3]. We have recently defined distinct gene expression patterns associated with the developmental stages in childhood; infancy, early and late childhood, and puberty. These gene expression patterns discriminate stages of childhood development and imply that developmental stage impacts on gene transcription in a tissue-independent manner[4]. We propose that age will affect gene expression patterns independent and dependent on cytogenetic subtype in children and adults.
Aims: I) Identify genes and gene expression patterns that are age dependently regulated in childhood and adult AML independent of cytogenetic subtype. **II)** Delineate expression patterns that are regulated by age according to cytogenetic subtype.
Methods: We have used transcriptomic data from the age annotated dataset of childhood AML (0 – 19 years of age n=237)[5,6], normal children (0 – 19 years of age, n=87)[4] and from adult patients (16-84 years of age, n=436)[7]. We identified genes that showed age-related regulation in expression both dependently and independently of cytogenetic subtype [focussing on t(8;21), inv(16), t(15;17)]. The Isomap dimensional reduction approach to principal component analysis (PCA) was used to define age and cytogenetic relationships within AML patient data (Qlucore Omics Explorer software) using unsupervised time-series clustering. Cross-validation of the PCA by sample exclusion was used to identify robust relationships. Rank regression was used to define age-related gene expression.
Results: The *TPM1* gene, producing an actin binding protein involved in calcium signalling, had differentially phased variation during childhood in AML subtypes and normal children, but displayed age dependent variation only in AML with t(15;17) (p<0.01). *SPIRE2*, a protein kinase involved in cytoskeleton organisation, transcripts showed age dependent variation in normal children and childhood AML (p<0.01) with patterns that suggest differential phasing in adult AML; age-dependent variation was independent of karyotypic subtype. *TSPAN2*, a component of the integrin signalling pathway, transcripts showed different levels of expression in different phases of childhood in normal children (p<0.01), however in AML age dependent regulation was differentially phased dependent on cytogenetic subtype.
Summary and Conclusions: We have identified three examples of individual transcripts where age and karyotype display very different effects. These data imply that some age dependent changes in AML are karyotype specific.

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E900

NEXT-GENERATION SEQUENCING AS A TOOL FOR ASSESSING MINIMAL RESIDUAL DISEASE IN AML PATIENTS WITH CEBPA MUTATIONS

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Background: Mutations in the CCAAT/enhancer binding protein alpha (*CEB-*

PA) gene occur approximately in 5 – 10 % of acute myeloid leukemia (AML) patients and can be used as molecular markers for monitoring of minimal residual disease (MRD). However, detection of MRD using quantitative real-time PCR (qPCR) is complicated due to GC-rich regions and thus validation of leukemia-specific and sensitive MRD assay can be technically difficult.
Aims: The goal of our work was the application of next-generation amplicon-based deep sequencing (NGS) as a quantitative detection method for MRD monitoring.
Methods: Since 2010, we have performed mutational analysis of the *CEBPA* gene coding region in 411 AML patients at the time of diagnosis using Sanger sequencing. In patients with the *CEBPA* mutation/s as the only detected mutation/s, *i.e.* the examined patients did not have other available MRD markers after standard molecular analysis of fusion transcripts and mutations in hematological prognostic genes, we designed leukemia-specific assays using qPCR. However, in 5 patients the assay did not provide sufficiently sensitive and specific detection of residual leukemic cells. In these cases MRD was monitored by NGS technology (GS Junior System, Roche). For MRD analysis starting at first diagnosis and following the course of the treatment we analyzed bone marrow (n=36) or peripheral blood (n=2) samples. The result of the MRD assessment was evaluated as a percentage of the mutated reads from all reads (reads with mutation/all reads). 20 000 reads per sample was considered as a minimum for valid MRD analysis.
Results: From January 2013 to February 2015 we examined 38 samples from 5 AML patients. *CEBPA* mutations found in these patients and used as a marker for MRD analysis were short insertions or deletions. The median of reads per sample was 58 376 (mean 58 076, range 27 829 - 110 564). The assay detection sensitivity achieved the threshold of 10^{-4} to 10^{-5} (1 leukemic cell in 10 000 cells to 1 leukemic cell in 100 000 cells). The MRD levels of residual leukemic cells correlated with clinical outcome. In patients with relapse (three out of five patients), the occurrence of the *CEBPA* mutation/s was also confirmed by conventional Sanger sequencing.
Summary and Conclusions: Quantitative assessment of *CEBPA* mutations using next-generation amplicon-based deep sequencing enabled MRD monitoring in AML patients, where the design of quantitative real-time PCR assay didn not achieve sensitivity of at least four orders of magnitude. NGS technology represents another approach for MRD assessment of the mutated *CEBPA* gene.

E901

GRANULOCYTE COLONY-STIMULATING FACTOR INHIBITS CXCR4/SDF-1A SIGNALING AND OVERCOMES STROMAL-MEDIATED DRUG RESISTANCE IN HL-60 CELL LINE

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Background: The outcome of acute myeloid leukemia in older and relapsed/refractory patients is dismal, which is partially due to drug resistance. The cytarabine, aclarubicin and granulocyte colony-stimulating factor (G-CSF) protocol has been widely used in these patients and has been achieved dramatic therapeutic efficacy, while the underlying mechanisms are still not very clear. Interaction with stromal cells may protect leukemia cells from spontaneous and drug-induced apoptosis.
Aims: We tried to clarify the potential ability of G-CSF to overcome stromal-mediated drug resistance through CXCR4/SDF-1α axis regulated by miR-146a expression in the leukemia cells.

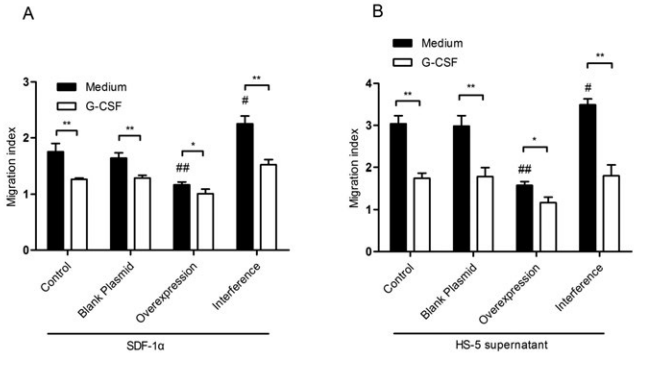

Figure 1.

Methods: HS-5/HL-60 co-culture model was established to imitate the interactions between stromal and leukemia cells. The ability of G-CSF sensitize HL-60 cells to chemotherapy through G-CSF→microRNA-146a (miR-146a)→CXCR4 pathway was investigated. For all experiments, HL-60 cells were divided into four groups: control group, blank plasmid, miR-146a over-expression group and miR-146a interference group.
Results: An obviously inverse correlation between miR-146a expression and CXCR4 expression was observed. In over-expression group, miR-146a was significantly up-regulated and CXCR4 mRNA was decreased. In interference group, miR-146a was down-regulated and CXCR4 mRNA was increased. The levels of surface and total CXCR4 protein were also reduced in over-expression group, while increased in interference group. Then we verified that G-CSF clearly up-regulated the levels of miR-146a in all groups compared with medium, along with down-regulated CXCR4 mRNA and protein expression. In function assays, we firstly found that HL-60 cells can be attracted and adhered to HS-5 cells. The migration index and adhesion was decreased in over-expression group, but increased in interference group compared with control group. Then we also observed that G-CSF disturbed HL-60 cell migration and adhesion. Lastly, we observed that HL-60 cells were protected from apoptosis (both spontaneous apoptosis and drug-induced apoptosis) when co-cultured with HS-5 cells in all groups, while G-CSF partially inhibited this protective effect. We also observed that the protective effect was more apparent in interference group and less apparent in over-expression group than control group.
Summary and Conclusions: Our data suggest that interfering with the CXCR4/SDF-1α signaling axis by using G-CSF inhibited HL-60 cell migration, adhesion and stromal-mediated protective effects. Our study also indicate that G-CSF reduced CXCR4 expression by miR-146a up-regulation, that is to say, there may be exist a G-CSF→miR-146a→CXCR4 pathway to explain how G-CSF inhibits CXCR4/SDF-1α signaling and overcomes stromal cell-mediated drug resistance in acute myeloid leukemia.

E902

THE ROLE OF CYBB IN MAINTENANCE OF MLL-REARRANGED LEUKEMIA

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Background: *MLL* (*mixed lineage leukemia*)-rearranged leukemia is caused by the chromosomal translocation of the *MLL* and partner genes. Previous studies demonstrated that the *Cytochrome b beta chain* (*Cybb*) gene is severely down-regulated in *Mll*-rearranged leukemia. *Cybb* encodes the catalytic subunit of NAPDH oxidase 2 (Nox2) complex and its expression is closely associated with the reactive oxygen species (ROS) level. ROS has been shown to influence stem cells maintenance and progenitors differentiation in hematopoietic system. The down-regulation of *Cybb* may suppress intracellular ROS level and therefore enhance stemness of *Mll*-rearranged leukemia.
Aims: To investigate the function of *Cybb* and Nox2-generated ROS in the maintenance of *MLL*-rearranged leukemic cells.
Methods: We have established a novel Mll-Een cell line from an Mll-Een knock-in mouse model. We compared the expression level of *Cybb* in the Mll-Een cells and wild-type bone marrow cells isolated from C57BL/6 mice by qPCR analysis. Intracellular ROS level was quantified by CellRox staining. Overexpression of *Cybb* in Mll-Een cells was done by lentiviral transduction. The ROS level and leukemic features of *Cybb* overexpressing Mll-Een cells were assessed by CellRox staining, colony forming assay and proliferation assay.
Results: We found that *Cybb* expression was severe down-regulated in the Mll-Een cell line when compared with the wild-type bone marrow cells. Besides, the intracellular ROS level of Mll-Een cells was also reduced. Overexpression of *Cybb* in Mll-Een leukemia cell increased the intracellular ROS level by over 30%. The colony forming number of *Cybb* overexpressing cells was sharping decreased by 89%, indicating that *Cybb* expression attenuates the colony forming potential of Mll-Een cells. Importantly, the colony morphology of *Cybb* overexpressing cells consists of mainly terminally differentiated cell colonies, which are 5 times higher in number than the control sample (57% *vs* 11%). These results suggested *Cybb* reduces self-renewal potential and promotes myeloid differentiation of Mll-Een leukemia. Furthermore, proliferation ability of *Cybb* overexpressing Mll-Een cells was greatly reduced by 87%, implying an impaired proliferative potential.
Summary and Conclusions: Overexpression of *Cybb* in Mll-Een cells increases ROS level and attenuates leukemic feature, such as the self-renewal potential and proliferative ability. *Cybb* also promotes myeloid differentiation in *Mll*-rearranged leukemia cells.

E903

ANALYSIS OF ASXL1 MUTATIONS AND CORRELATION WITH CHROMOSOMAL ALTERATIONS IN ACUTE MYELOID LEUKEMIA

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Background: Additional sex comb-like 1 (*ASXL1*) is an enhancer of Trithorax and Polycomb family, which is necessary for the maintenance of stable repression of homeotic and other loci. Recently, mutations of the *ASXL1* gene were identified in the hematopoietic cells from patients with a variety of myeloid malignancies, including acute myeloid leukemia (AML). The vast majority of these mutations, which are frameshift and nonsense, have been associated with an aggressive phenotype and detrimental effects on overall survival, especially in patients with a normal karyotype.

Aims: The purpose of this study is the identification of the *ASXL1* mutations in *de novo* and secondary AML (s-AML) patients and their correlation with the cytogenetic findings.
Methods: Our study included 275 AML patients (184 *de novo* AML and 91 s-AML) and 10 healthy donors. Conventional cytogenetic analysis was performed on unstimulated bone marrow cells. In order to detect *ASXL1* exon 12 mutations, molecular analysis was performed by PCR and subsequent direct Sanger sequencing in all patients' and controls' samples.
Results: A successful karyotypic analysis was performed in all AML samples at diagnosis. Among them, 201 patients (73.1%) exhibited clonal karyotypic aberrations and 74 (26.9%) showed a normal karyotype. *ASXL1* mutations were detected in 50 out of 275 patients (18.2%) while none *ASXL1* mutation was observed in the control group. A significantly higher frequency of *ASXL1* mutations was found in s-AML compared to *de novo* AML patients (26.4% *vs* 14.1%, respectively; p=0.013). The most frequent *ASXL1* mutation was the c.1934dupG (43/50, 86.0%). *ASXL1* mutations were more frequently found in males than in females (19.8% *vs* 15.8%, respectively), while stratification of patients according to age revealed a higher frequency of *ASXL1* mutations in patients ≥61years than in patients <60years (*p*=0.003). Among the 50 AML patients with *ASXL1* mutations, 28% exhibited a normal karyotype, 22% had trisomy 8 either as a sole abnormality or in combination with others, 4% t(8;21), 4% trisomy 13, 4% -7/del(7q), 2% t(15;17), 2% t(9;22) and 26% other chromosomal abnormalities. Moreover, 8% of *ASXL1* mutated patients revealed complex karyotypes.
Summary and Conclusions: These results suggest that *ASXL1* mutations are frequent in AML, especially in s-AML and are highly correlated with older age, male sex and specific karyotypic aberrations. The most common chromosomal abnormality associated with *ASXL1* mutations is trisomy 8, followed by t(8;21) and trisomy 13. *ASXL1* mutations are present in all AML cytogenetic risk groups with good, intermediate and poor prognosis.

E904

TARGETED NEXT GENERATION SEQUENCING (NGS) IN PARALLEL ANALYSES OF CHILDHOOD (CAML) AND ADULT ACUTE MYELOID LEUKEMIA (AAML) PATIENTS

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Background: The existence of age-specific differences in the genetic mechanisms of myeloid leukemogenesis has long been observed and has been the subject of numerous studies. However, only the introduction of new NGS technology has allowed us to obtain, in a relative simple manner, a large amounts of mutation data.
Aims: The aim of this study was to facilitate our understanding of the similarities and differences in molecular pathogenesis between cAML and aAML by application of parallel targeted NGS technology.
Methods: We analyzed DNA isolated from the bone marrow mononuclear cells from 20 childhood (cAML) and 20 adult AML (aAML) unselected patients, using TruSeq Cancer Panel with the MiSeq platform (Illumina) for the detection of somatic mutations, consisting of 212 amplicons targeting mutational hotspots in 48 cancer-related genes. Filtering and variant calling were performed using GATK UnifiedGenotyper and VariantFiltration tools. Resulting data were mapped against human genome b37. The average coverage of high-quality sequences was 2981 × per amplicon. Nine genes were discarded due to insufficient coverage, therefore a total of 188 amplicons from 39 genes was used for subsequent analysis. FLT3/ITD and IDH2 mutations were screened using PCR followed by direct sequencing.
Results: We identified 939 (467 cAML, 472 aAML) different mutations in both coding and non-coding targeted regions, with substantial variation from patient to patient. A total of 527 (260 cAML, 267 aAML) mutations in the non-coding regions and 412 (207 cAML, 205 aAML) mutations in the coding regions was detected, out of which only 122 (62 cAML, ranging from 0 –7 mutations per patient; and 60 aAML, ranging from 1 –5 mutations per patient) were potentially protein-changing, *i.e.* nonsense (N), frameshift (F) and missense (M) mutations. Seven genes were found to have more than 5 NFM mutations, namely *NRAS*, *KIT*, *KDR*, *NPM1*, *MET*, *FLT3*, and *TP53*. Out of these, *TP53* and *KDR* were found to have more than 20 mutations in over 50% of AML patients. Finally, the number of patients who harbored at least one mutation in *TP53* gene was 17 of 20 in cAML and 19 of 20 in aAML. As expected, the prevalence of the most AML associated mutations including *FLT3/ITD*, *NPM1*, *IDH1* and *IDH2* gene, differed in cAML and aAML patient cohorts. *IDH1* (0% cAML, 5% aAML), *IDH2* (0% cAML, 10% aAML), *NPM1* (10% cAML, 35% aAML), *FLT3/ITD* (0% cAML, 10% aAML). This discrepancy in prevalence in leukemia associated genes highlights the differences in the pathogenesis of cAML versus aAML at the genetic level.
Summary and Conclusions: Our results confirm that AML contains relatively small number of genetic alterations, suggesting that for the development of AML fewer genetic alterations are required than for other malignancies. In our study in which we used samples from unselected AML patients, we were not able to determine previously unknown prevalence of some gene alterations. Our results merely confirmed existing prevalence AML specific mutations in aAML. Many of them are well known (*FLT3*, *NPM1*, *CEBPA*) and their detection has already entered standard clinical practice. Given that AML is extremely heterogeneous in its clinical and genetic characteristics, more definite results regarding similarities and differences in pathogenesis of these two entities will be obtained in a parallel NGS analysis of the morphological and cytogenetic homogeneous groups of cAML and aAML patients.

E905

PROGNOSTIC SIGNIFICANCE OF CD34$^+$CD38$^{LOW/-}$CD123$^+$ LEUKEMIC STEM CELLS IN ACUTE LEUKEMIA - A PROSPECTIVE COHORT STUDY

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Background: It is believed that acute leukemia arises from stem cells called Leukemic Stem Cells (LSC) and they are usually resistant to chemotherapy. These are also implicated in relapse of the disease. They can easily be quantified by flow cytometry by the presence of CD34$^+$CD38$^{low/-}$CD123$^+$ clone.
Aims: To study the prognostic significance of CD34$^+$CD38$^{low/-}$CD123$^+$ leukemic stem cells in terms of response to induction therapy and survival at induction.
Methods: Prospective single-centre cohort study which included 76 patients of acute leukemia. Bone marrow samples at diagnosis were analysed by flow cytometry to quantify the LSC clone. Patients of both ALL and AML were given the usual induction chemotherapy. Response was assessed by peripheral blood blast clearance, interim bone marrow blast assessment, CR (complete remission) status and survival at induction.
Results: All enrolled patients of acute leukemia were followed during the induction chemotherapy phase. Of these 43% were ALL (median age 23 years; 70% males) and 57% were AML (median age 32 years; 61% males). Fever (97%) and anemia (97%) were the major symptoms followed by bleeding (40%). Amongst the clinical signs presence of lymphadenopathy (40% versus 5%; p<0.001), hepatomegaly (64% versus 12%; p<0.001) as well as splenomegaly (64% versus 12%; p<0.001) were more commonly seen in patients with ALL as compared to AML. Leucocyte counts at baseline and blasts in the peripheral blood were also higher in the ALL group compared to AML group (median of 29,000/mm^3 versus 8900/mm^3; p=0.06). Mortality was significantly more in the AML group as compared to ALL group (49% versus 24%; p=0.03). There was a significant correlation of LSC clone size with ALL type (p=0.002, r^2=0.512), blast clearance (p=0.02, r^2=0.397) and day 7 BM blasts (p=0.01, r^2=0.557). B-ALL had significantly higher LSC clone size compared to T-ALL (5.2 versus 0.004; p=0.002). In patients with ALL, higher LSC clone size was significantly associated with delayed blast clearance in peripheral blood (p=0.02) and less number of patients having M2 or M3 bone marrow (p=0.013). Patients with high LSC clone had higher days of peripheral blood blast clearance and had lesser number of patients clearing blast within 5 days. Similarly, in patients with AML, there was a significant correlation of LSC clone size with AML cytogenetics risk group (p=0.02; r^2=0.352), blast clearance (p=0.03; r^2=0.328) and mortality (p=0.002; r^2=0.452). High risk AML had higher percentage of LSC clone. Patients with high LSC clone had not only delayed clearance of blasts in the peripheral blood (P=0.03) but also higher risk of death (P=0.002).
Summary and Conclusions: Both ALL and AML had presence of LSC clone expressed as CD34$^+$CD38$^{low/-}$CD123$^+$ leukemic stem cells. Presence of high LSC clone is associated with poor blast clearance in peripheral blood and bone marrow and poor survival in patients with acute leukemia

E906

COOPERATIVE MUTATION STUDY IN KOREAN PATIENTS WITH MLL-REARRANGED ACUTE MYELOID LEUKEMIA USING TARGETED NEXT GENERATION SEQUENCING

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Background: Recent advancements of technologies including next generation sequencing (NGS) have led to discovery of molecular pathogenesis of malignant diseases of hematological malignancies, and these discoveries are now enabling the beginning of molecular targeted therapy. The *MLL*-rearranged fusion gene is among the main leukemogenic mutations which is found in both acute myeloid leukemia (AML) and acute lymphoblastic leukemia with a frequency of about 5-10%, and is associated with a poor clinical prognosis. Systematic studies analyzing cooperative genetic events of *MLL*-rearranged leukemia based on NGS technology has mainly been conducted in western populations, and has not yet been carried out in Asian ethnicity, such as the Korean population.
Aims: The aims of this study are to evaluate the cooperative mutation profiles of Korean *MLL*-rearranged acute myeloid leukemia patients using targeted next generation sequencing and to determine the ethnic difference of molecular basis of AML.
Methods: This study includes total of 24 *MLL*-rearranged AML patients who visited two separate tertiary care hospitals between the period of January 2009 and May 2014. The patient group included twelve men and twelve women (M:F=12:12), and also twelve children and twelve adult patients (A:C=12:12). The number of each *MLL* fusion genes are as follows; *MLL/MLLT3* n=12; *MLL/MLLT4* n=6; *MLL/ELL* n=2; other *MLL* fusion genes n=4. Mutation profile study for 19 candidate genes for cooperative mutation (*TET2, DNMT3A, IDH1, IDH2, NPM1, FLT3, CEBPA, ASXL1, BRAF, CBL, KIT, KRAS, NRAS, PTPN11, RUNX1, TP53, WT1, SETD2, JAK2*) was carried out using a MiSeq sequencer (Illumina, San Diego, CA).
Results: Among the twenty-four *MLL*-rearranged patients, 17 patients had no additional mutations (~70%) found. Only 7 patients (29.2%) had positive results for more than 1 gene mutations which were analyzed for (Table 2). Positive gene mutations found were in order of their frequency; *ASXL1* (n=4), *FLT3* (n=2), *CEBPA* (n=2), *KRAS* (n=1), *NRAS* (n=1) and *PTPN11* (n=1). Interestingly, 4 out of 6 patients harboring *MLL/MLLT4* were found to have additional gene mutations. Two patients had multiple gene mutations while one patient had four concurrently detected gene mutations (Table).

Tabella 1. Results of detected cooperative mutations by targeted sequencing in this study.

Patient	RT PCR	NGS	Chromosome	Exon	Position	reference>variant	mutation
P6	*MLL-MLLT4*	*KRAS*	12	2	25398284	C>T	G12D
		PTPN11	12	3	112888211	A>T	E76V
P13	*MLL-MLLT6*						
		FLT3	13	20	28592622	G>T	N841K
p14	*MLL-MLLT3*	*ASXL1*	20	12	31023891	C>T	H1126Y
P17	*MLL-MLLT4*	*ASXL1*	20	12	31022389	G>A	R625Q
P20	*MLL-ELL*	*CEBPA*	19	1	33792795	C>T	A176T
P21	*MLL-MLLT4*	*ASXL1*	20	12	31023733	G>A	R1073H
		NRAS	1	3	115256520	T>G	Y64S
		FLT3	13	20	28592642	C>A	D835Y
P22	*MLL-MLLT4*	*CEBPA*	19	1	33792830	G>T	P164H
		ASXL1	20	12	31023613,	A>T	E1033V
		ASXL1	20	12	31024207	C>T	S1231F

Abbreviations: RT-PCR, reverse transcriptase polymerase chain reaction; NGS, next generation sequencing;

Summary and Conclusions: This is the first cooperative mutation study on *MLL*-rearranged AML patients of Asian ethnicity using targeted NGS technology. Small number of cases was a limitation in assuming the general mutational spectrum of the representing population, however a higher incidence of gene mutation among *MLL/MLLT4* patient group (4/6) and relatively higher incidence of *ASXL1* mutation (4/24) being found could be carefully suspected as an ethnical difference in molecular basis of AML. Further study requires a larger number of *MLL*-rearranged *MLL* patients of equal ethnicity and additional mutation profile study with ethnical comparison.

E907

ASSOCIATION BETWEEN CD117 (C-KIT) AND CD135 (FLT3) RECEPTOR TYROSINE KINASES AND MUTATIONAL STATUS AND CLINICAL FEATURES IN ADULT AML PATIENTS UNDER 60 YEARS

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Background: Proliferation is one of the mechanism for AML pathogenesis. Receptor Tyrosine Kinases have significant contribution in leukemogenesis because of their proliferative role. For this reason we have evaluated CD117 (c-kit receptor) and CD135 (FLT3 receptor) in the setting of AML.
Aims: The purpose of this study was to investigate the association between CD117 and CD135 expressions and FLT3-ITD, NPM1 mutations and clinical features.
Methods: Percentages of expression and Median Fluorescence Intensity (MFI) of CD117 and CD135, tagged with phycoerythrin (PE), were obtained from flow-cytometric AML definition panels acquired on FACSCanto cytometer using BDFACSCanto software. Levels of NMP1 mutation (NPM1m) and internal tandem duplications of FLT3 (FLT3-ITD) were quantified in a RT-PCR assay. Comparison between two groups were performed using the Mann–Whitney *U* (MWW) and Chi-square tests (CSQ) or Fisher exact test (FET) for continuous and dichotomic variables, respectively; for comparison among different groups Kruskal Wallis test (KW) was performed. Measure of association was expressed by Odds Ratio (OR). Cut-off value for the marker was selected using receiver operating curves (ROC). P values lower than 0.05 were considered statistically significant (SPSS 15.0 version).
Results: 106 AML patients (pts) under 60 years with median age of 48 ys (range 15-65; M/F 62/44), diagnosed in our lab from 2007 to 2015, were included in this study. The incidence of FLT3-ITD and NPM1m was 28.9% and 42.0%, respectively. Double FLT3-ITD/NPM1m was present in 18.8% of cases. Pts with FLT3-ITD had higher median WBC (36.4 *vs* 10.5 x 10^9/L, MWW, P=0.004), median percentage of bone marrow blasts (80% *vs* 43%, MWW, P <0.001), peripheral blood blasts (72% *vs* 29%, MWW, P <0.001) and median LDH value (761 *vs* 401 UI/L, MWW, P=0.010) than in FLT3 wild type (FLT3wt) pts. FLT3-ITD cases showed a greater median percentage of CD135 expression (86% *vs* 65%, MWW, P=0.009) than counterpart. NPM1m pts did not exhibit significant differences in clinical features, but showed a lower expression of CD117, both in percentage (61% *vs* 88%, MWW, P=0.001) and MFI (298 *vs* 1458, MWW, P <0.001). By calculating ROC, a MFI cut off value of 1125 provided a best sensitivity (89%) and specificity (63%). Higher median MFI of CD117 were present in FAB M0 (1189), M1 (832), M2 (986) and lower in FAB M4 (394) and M5 (89) (KW, P=0.044). The association between CD117 expression (categorized by ROC) and FAB M5 (FET, P=0.046) showed an OR of 0.14 (95% CI from 0.02 to 0.97) No differences were observed for CD135 expression levels in FAB subtypes. The favorable NPM1m/FLT3wt status (48% of all NPM1m) was associated with FAB M5 (FET, P=0.011), with an OR of 8.33 (95% CI from 1.68 to 41.29). Adverse FLT3-ITD/NPM1 wild type (12% of all FLT3-ITD) correlated with FAB M0 (FET, P=0.006) with an OR of 31.8 (95% CI from 2.77 to 365.53). Any association was found between double NPM1m/FLT3-ITD status and FAB subtypes. Pts with refractory AML were associated with an higher level of CD117 expression (categorized by ROC) (CSQ, P=0.003, OR 4.47, CI 95% from 1.57 to 12.72) and an adverse FLT3-ITD/NPM1wt status (FET, P=0.042, OR 5.87, CI 95% from 1.13 to 30.45).
Summary and Conclusions: High percentage of CD135 expression in pts with the adverse FLT3-ITD status and high level of CD117 expression in refractory AML pts suggested a possible role of this combination for a bad prognosis. In opposite, the strong correlation of CD117 low expression with the presence of the favorable NPM1m can be predictive of a good prognosis.

E908

ROLE OF DNMT3A MUTATIONS IN INTERMEDIATE-RISK ACUTE MYELOID LEUKEMIA PATIENTS: ASSOCIATION WITH PROGNOSIS AND TREATMENT STRATEGIES

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Background: Mutations impacting epigenetic mechanisms, such as *DNMT3A* mutations, are recurrent in acute myeloid leukemia with intermediate-risk karyotype (AML-IR), although their prognostic value remains unclear. The prospective determination of *DNMT3A* mutations may provide an important step forward individualized therapies using hypomethylating agents or high-dose daunorubicin. However, the clinical benefit of allogeneic stem cell transplantation (HSCT) has not been established.
Aims: 1) To evaluate the incidence of *DNMT3A* mutations, their prognostic impact as well as the interaction with other molecular markers in AML-IR patients; 2) To determine if allogeneic HSCT in first remission improves the outcome of patients with somatic mutations in the *DNMT3A* gene.
Methods: A cohort of 74 patients with AML-IR (median age 54 years) (PETHE-

MA AML-99-2010). Analyses of *DNMT3A* mutations (exons 10 to 23) were characterized by direct sequencing in diagnostic bone marrow (BM) samples. Sixty-four patients achieved complete remission (CR) and 30 of them underwent an allogeneic bone marrow HSCT.
Results: Of the 74 patients included in this study, 31% (23/74) had mutations in *DNMT3A* (78% had R882 mutations and 22% other missense mutations). *DNMT3A* mutations were frequently detected in *FLT3*-ITD mutated patients (48% vs. 19%, p=0,013). No significant differences were found in both groups with regard to age, WBC counts or BM blast percentages. The median follow-up time was 543 days (range, 20-2678). AML-IR patients with *DNMT3A* mutations had shorter overall survival (OS) (36% vs. 54%, p=0,024) and relapse-free survival (RFS) (23% vs. 53%, p=0,018) at 5 years than the non-mutated subgroup. These differences were more evident when older patients (>65 y.) were excluded (RFS: 15% vs. 55%, p=0,005) or only *DNMT3A* R882 mutations were considered (RFS: 20% vs. 54%, p=0,012). In multivariate analysis, *DNMT3A* R882 mutations (p=0.039) were independent factors for shorter OS, and only *FLT3*-ITD mutations (p=0.004) had a negative prognostic impact on RFS. We next examined the relapse risk after allogeneic HSCT in first CR in order to elucidate if poor outcome of *DNMT3A*mutations was overcome. Again, *DNMT*3A mutations identified a population of patients with substantially shortened RFS at 5 years (37% vs. 70%, p=0,020).
Summary and Conclusions: Mutations in *DNMT3A*, especially R882 mutations, identify a subgroup with poor prognosis in the intermediate-risk group. Although based on small numbers of patients, these data suggests that mutations in *DNMT3A* identify a significant fraction of HSCT recipients with poor survival, for whom alternatives to standard transplantation options should be considered. **Equal senior contribution. FINANCIAL SUPPORT: Grants PI12/02321 from the Instituto de Salud Carlos III, BIO/SA44/14 from the Consejería de Sanidad (JCYL), RD12/0036/0069 and Asociación Española Contra el Cáncer (AECC).

E909

ACUTE MYELOID LEUKEMIA DISEASE MODELING VIA RNA-GUIDED CRISPR–CAS9 SYSTEM INDUCED SOMATIC MUTATIONS

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Background: Acute myeloid leukemia (AML) is a genetically heterogeneous clonal disorder characterized by the accumulation of acquired somatic genetic alterations in hematopoietic progenitor cells that alter normal mechanisms of self-renewal, proliferation and differentiation. In the majority of AML patients chromosomal aberrations and/or gene mutations that are involved in disease development are detectable in the leukemic blasts. Disease models (incl. retroviral/lentiviral overexpression, transgene mouse models etc.) have largely contributed to reveal key mechanisms of AML leukemogenesis and progression. However, the current models face severe limitations since most of them induce unphysiological gene expression and depend on external promoters for regulation of gene expression/repression. Moreover, a significant proportion of AMLs is based on gene mutations rather than chromosomal rearrangements or up/down regulation of gene expression levels.
Aims: We sought out to develop a new effective methodology to reproduce precise leukemia associated mutations required for improved disease modeling and to gain insight into AML development. IDH2 mutations that occur frequently in AML and are related with poor prognosis for the patients were chosen for acute myeloid leukemia cell genome editing.
Methods: Using the clustered, regularly interspaced, short palindromic repeats (CRISPR)/Cas9 system we induced specific DNA double strand breaks in the IDH2 gene of K562 myeloid leukemia cells and introduced IDH2 R140Q point mutation via homologous recombination introducing a specific DNA template. Thus, integrated IDH2 R140Q mutation was regulated by the endogenous IDH2 gene promoter.
Results: Correct insertion of the mutation was confirmed performing targeted integration PCR as well as genomic DNA sequencing. K562 cells carrying IDH2 R140Q mutation showed significantly enhanced *in vitro* cell proliferation and colony forming capacity as compared with controls carrying wt IDH2 introduced analogously. *in vivo* studies in NSG mouse model are currently underway to confirm differences in human leukemia development upon introduction of IDH2 mutation in leukemia cells.
Summary and Conclusions: Our data present a new strategy for generating leukemia-related gene mutations at defined positions and under endogenous gene promoters. Using this approach for leukemic cells as well as normal hematopoietic progenitor cells will provide a promising new methodology for leukemia disease modeling.

E910

VSTM-V1 IS A POTENTIAL MYELOID DIFFERENTIATION ANTIGEN GENE THAT IS DOWNREGULATED IN BONE MARROW CELLS FROM PATIENTS WITH MYELOID LEUKEMIA

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Background: Myeloid leukemia is a myeloid blood cell malignancy that shows great heterogeneity. Leukocyte differentiation antigens often represent important markers for the diagnosis, classification, prognosis and therapeutic targeting of these malignancies. To find novel leukocyte differentiation antigens, we used an immunogenomics approach and VSTM1 (V-set and transmembrane domain containing 1) was selected as a candidate.
Aims: To find novel leukocyte differentiation antigens and new targets for the diagnosis and treatment of leukemia.
Methods: VSTM1 expression in cell lines, bone marrow samples from healthy donors and myeloid leukemia patients were analyzed by qRT-PCR, western blotting and flow cytometry. The promoter methylation of VSTM1 was assessed by methylation-specific PCR (MSP) and bisulfite genomic sequencing (BGS). All-trans retinoic acid (ATRA) was used to induce APL cell line NB4 and bone marrow cells from APL patients to investigate the relationship between VSTM1 expression and cell differentiation *in vitro*. To explore the effect of VSTM1-v1 on leukemic cell growth, VSTM1-v1 was overexpressed in K562 and MEG-01 cells and performed viable cell counting assays. Additionally, it was tested for correlations between VSTM1 expression and patient characteristics.
Results: Herein, we report that the expression level of VSTM1 is downregulated by qRT-PCR in bone marrow cells from leukemia patients (Table 1) and showed a high degree of promoter methylation. The predominant splice variant encoded by the gene, VSTM1-v1, encodes an ITIM-bearing type I membrane molecule. By flow cytometry, we could divide bone marrow cells into subpopulations by immunophenotyping and showed that VSTM1-v1 expression levels were positively correlated with the maturation state of the myeloid cells (Table 2). Additionally, *in vitro* ATRA treatment could restore VSTM1-v1 expression in both bone marrow cells from APL patients and NB4 cells. The overexpression of VSTM1-v1 in K562 or MEG-01 leukemia cells led to an inhibitory effect on cell growth. When analyzing correlations between VSTM1 expression and the clinical features of AML patients, we detected a higher expression level of VSTM1 in the AML1-ETO-positive group.

Tabella 1. The expression level of VSTM1 in bone marrow cells from leukemia patients and healthy donors.

Groups	MIC[a]	Sample size	Mean ratio, *VSTM1/ABL*	*P*-value[b]
HD[c]		36	17.358±17.904	
Untreated-AML		145	4.333±7.895	<0.001
	M1	3	0.374±0.619	n.a.[d]
	M2	72	5.292±10.171	<0.001
	M3	29	2.884±3.960	<0.001
	M4	24	4.814±5.293	0.001
	M5	14	3.052±4.867	<0.001
	M6	3	1.417±2.205	n.a.[e]
Untreated-ALL[d]		40	0.381±0.755	<0.001
Untreated-CML		57	5.479±8.266	<0.001
	CP	38	7.743±9.312	0.001
	AP/BC	19	0.950±1.367	<0.001

[a] MIC denotes morphological, immunological and cytogenetic classifications.
[b] The P-value was calculated using Wilcoxon signed ranks test as compared to the HD grou
[c] HD denotes healthy donors.
[d] ALL denotes acute lymphoic leukemia patients.
[e] n.a. indicates not available due to a small sample size.

Table 2. The expression of VSTM1-v1 at various stages of myelocytic differentiation.

	Myeloblasts	Promyelocytes	Myelocytes	Metamylocytes
CD markers	CD34+	CD34-CD117+CD16-	CD34-CD117-CD16-CD13+	CD16+
VSTM1-v1-positive cells (%)	5.69±2.69	11.48±4.58	35.02±11.58	61.63±8.53
P-valuea	<0.001	<0.001	<0.001	

[a] *The P-value was calculated using Wilcoxon signed ranks test as compared with the next stage.*
Summary and Conclusions: VSTM1-v1 could represent an important myeloid leukocyte differentiation antigen and might provide a potential target for the diagnosis and treatment of leukemia.

E911

THE PI3K GAMMA/DELTA INHIBITOR, IPI-145 (DUVELISIB) INHIBITS ACUTE MYELOID LEUKEMIA BLAST PROLIFERATION AND ADHESION

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Background: A number of non-receptor tyrosine kinases have been identified as functionally important in the biology of acute myeloid leukemia including protein kinase B (AKT), phosphatidylinositol 3-kinase (PI3K), signal transducer and activator of transcription 5 (STAT5), mitogen-activated protein kinase (MAPK) and Bruton's tyrosine kinase (BTK), have all been shown to be constitutively activated in AML. Tyrosine kinase inhibition is clinically effective and well tolerated in chronic myeloid leukemia, chronic lymphocytic leukemia and mantle cell lymphoma. Inhibition of selected tyrosine kinases seems an attractive strategy to assess in AML. PI3K is an enzyme group that generates phosphatidylinositol 3, 4, 5-triphosphate which provides membrane docking site for the tyrosine kinase AKT. AKT itself is responsible for activation of the mammalian target of rampamycin (mTOR) pathway which plays an essential role in the growth of malignant cells, stimulating glycolysis, driving cells to consume glucose and promoting cell survival and cell-cycle progression. PI3K is known to be constitutionally active in patients with AML. Recently PI3K/mTOR inhibition has been shown to delay tumor progression, reduce tumor load and prolong survival in MLL-AF9$^+$/FLT3-ITD$^+$xenograft mouse models. PI3K has different isoforms, α β δ and γ.
Aims: To determine the functional effects of PI3K δ/γ inhibition on AML signalling, survival and adhesion.
Methods: To investigate the role of PI3K p110δ and p110γ isoforms in the regulation of AML survival, proliferation, migration and adhesion we inhibited PI3K using different doses of IPI-145 (Duvelisib) (δ/γ inhibitor), CAL-101 (Idelalisib) (δ inhibitor), and LY294002 (pan PI3K inhibitor). We used AML cell lines and AML primary cells as well as primary bone marrow stromal cells derived from AML patients to determine the role of PI3K inhibition in AML proliferation and adhesion. We assessed survival using CellTiter-Glo and adhesion by Calcein AM staining of AML cultured on fibronectin or bone marrow stromal cell (BMSC) coated plates. Western blot analysis was used to assess phosphoAKT and phosphoMAPK expression. The concentration range of IPI-145 used covers the plasma steady-state concentration of 0.9uM for patient treated at a dose of 25mg twice a day.
Results: PI3K inhibition with IPI-145, CAL-101 and LY294002 inhibited proliferation in 5/6 AML cell lines. We also observed similar anti-proliferative activity in primary AML cells. Furthermore, we show that inhibition of PI3K with IPI-145, CAL-101 and LY294002 results in a decrease in the activity of phospho-AKT, but has no effect of the activity of phosphoMAPK, indicating that MAPK is activated via a separate signalling pathway. Finally we report that pre-treatment of AML cells with IPI-145 inhibits adhesion of both AML cell lines and primary AML cells to primary BMSC at nanomolar concentrations.
Summary and Conclusions: Here we report that IPI-145 (Duvelisib) inhibits AML proliferation and adhesion through effects on downstream survival signals including inhibition of AKT phosphorylation at concentrations achievable in patients. Our results provide biological rationale for further evaluation of PI3K δ/γ inhibitors in AML.

E912

IDENTIFICATION OF CYTOGENETIC AND MOLECULAR SUBGROUPS OF ACUTE MYELOID LEUKEMIAS SHOWING L-ASPARAGINASE IN VITRO SENSITIVITY

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Background: L-Asparaginase catalyzes the hydrolysis of L-Asparagine to L-aspartic acid and ammonia and sustains reduction in serum asparagine, which is required for rapid proliferation of leukemic cells. The gene coding for L-Asparagine synthetase (*ASNS*) is located in 7q21.3 and its increased expression is correlated with resistance to L-Asparaginase in Acute Lymphoblastic Leukemia (ALL). Monosomy of Chromosome 7, or partial deletion involving its long arm [del(7q)], are recurrent aberrations in myeloid disorders, and it was shown that these events lead to a significant down-regulation of the genes located in the deleted regions, including *ASNS*. Moreover, methylation of CpG islands in the promoter region is one of the epigenetic mechanisms for silencing of genes, and some studies demonstrate that *ASNS* gene is often methylated in ALL cells, thus explaining why ALL cells may be sensitive to L-Asparaginase.
Aims: The aim of the study is to analyze the *in vitro* sensitivity to L-Asparaginase of human AML cell lines with different *ASNS* gene expression levels and to identify cytogenetic and/or molecular events involved in the *in vitro* response to L-Asparaginase. In particular this study investigates if the cytogenetic status of chromosome 7q or, more generally the expression level of *ASNS* gene, could predict the efficacy of L-Asparaginase in Acute Myeloid Leukemia.
Methods: FISH (Fluorescent *in situ* Hybridization) of Chromosome 7 was performed with specific probes D7S522/CEP7 (Abbott Molecular) located at chromosome 7q31 and 7p11.1-q11.1. In order to measure drug sensitivity we used the WST-1 assay (Roche Applied Science, Monza, IT), testing eight concentrations of L-Asparaginase (Erwinia chrysanthemi, Jazz Pharmaceuticals). The expression level of *ASNS* gene was evaluated with quantitative RT-PCR (Roche LightCycler 480 instrument). For methylation analysis DNA was modified with bisulfite, amplified by PCR and sequenced with specific primers.
Results: FISH analysis identified 3 out of 10 AML cell lines displaying Chr 7monosomy (UCSD, FKH-1, OCI-AML6). AML cell Lines carrying Chr 7 monosomy were more sensitive than the other AML cell lines to L-Asparaginase, with P value <0.01 (Figure A, B). Moreover AML cell lines with Chr 7 monosomy showed a statistically significant lower expression of ASNS gene when compared to the other AML cell lines. The four ALL cell lines (DND41, HPB-ALL, MOLT-4 and RPMI-8402) displaying by WST-1 assay the highest sensitivity to the drug, also showed a lower expression of *ASNS* gene with respect to the other three ALL cell lines, that however did not display Chr. 7 abnormalities. We therefore analyzed the methylation status of the promoter region of *ASNS* gene, as an alternative mechanism for gene silencing. ALL cell lines DND41, HPB-ALL, MOLT-4 and RPMI-8402, that had a low expression of *ASNS* gene indeed show hyper-methylation of the *ASNS* gene promoter region. On the contrary none of the AML cell lines showed hyper-methylation of the *ASNS* gene.

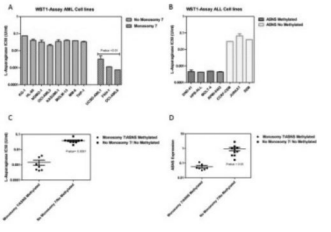

Figure 1.

Summary and Conclusions: The low expression of *ASNS* gene, found in AML cell lines carrying Chr 7 monosomy and in ALL cell lines with hyper-methylation of *ASNS* CpG island, significantly correlates with higher sensitivity to L-Asparaginase *in vitro* (Figure C, D). The study was supported by a research grant from EUSAPHARMA (Europe) Ltd.

E913

AZACITIDINE FOR THE TREATMENT OF MDS, AML AND CMML: THE SOUTH WALES (UK) EXPERIENCE

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Background: The management of elderly patients with myelodysplastic syndrome (MDS) and Acute Myeloid leukaemia (AML) poses significant challenge to the haematologist. Azacitidine (AZA) is a first-in–class hypomethylating agent with activity in MDS and has shown significantly improvement in survival compared with conventional care regimens. AZA was approved by the UK regulatory body, the National Institute for Clinical Excellence (NICE) in 2011. Approval was granted in non-transplant eligible patients with AML with 20-30% marrow blasts, CMML with 10-29% marrow blasts and intermediate-2/ high risk MDS.
Aims: We report the results of the treatment with AZA at our centre in patients with MDS, AML and CMML in a "real life" setting. We investigate the characteristics and outcome of patients receiving AZA including tolerability, efficacy and overall survival.
Methods: We conducted a retrospective analysis of patients receiving AZA between 1st Jan 2011 to 31st Dec 2014 over 4 year period from three hospitals in South Wales, UK within the Abertawe Bro Morgannwg University Health Board (ABM UHB). Patients were included if they received one cycle of AZA. Patients were identified from our electronic prescribing system (Chemocare) and correlated with clinical notes. Disease status was defined by the World Health Organisation (WHO) classification system. Patients with MDS were risk stratified using the IPSS-R. Statistical analysis was conducted using SPSS. Overall survival (OS) was estimated using Kaplan-Meier estimates.
Results: A total of 90 patients with AML and MDS were treated in ABM UHB. Out of this 26 patients received AZA representing 28% of all patients with MDS and AML. A median age 73.9 years (range 66-84 years) was observed. Median follow up was 12.2 months (2-36 months). A male predominance (73.1%) and a range of indications were seen (MDS n=14, 54 %; AML n=9, 35%; CMML n=3, 11%). AZA was used outside NICE indications in 3 (11%) cases. AZA was used as first line therapy in 17 cases (65%) and beyond second line therapy in 9 cases (35%). Out of those receiving 2nd line therapies, 3 (11%) had received previous high dose therapy, 4 (15%) previous low dose therapy and 2 (7%) were on only transfusion support. The median number of cycles received was 6.9 (range 1-26). Most patients tolerated AZA well. Grade I-2 skin toxicity was observed in 9 patients (34%). Grade 1-2 gastrointestinal was common but did

not prompt discontinuation of AZA treatment. At least one hospital admission was required in 9 patients (35%) during treatment. Overall response including stable disease was achieved in 17 patients (65%) out of which complete remission was achieved in 7 patients (26%) and cytogenetic remission in 2 cases (8%). At present 7 patients (26%) are alive and continuing on AZA therapy. Median overall survival (OS) was 8 months. No difference was observed between disease (p=0.92) and IPSS-R classification in MDS patients (p=0.806). Patients receiving >6 cycles (n=14, 54%) obtained transfusion independence and blood count responses with significantly improved overall survival (5 months vs. 20 months, p=0.001).
Summary and Conclusions: AZA is well tolerated and deliverable in our institution. Patients receiving >6 cycles were most likely to achieve transfusion independent, improvement in blood counts and have improved median OS comparable to published data. Persistence with AZA is therefore vital to obtaining a response.

E914

MIRNA SIGNATURES ASSOCIATED WITH MOLECULAR MARKERS IN ACUTE MYELOID LEUKEMIA
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Background: Genomic characterization of normal karyotype acute myeloid leukaemia (NK-AML) has provided in the last years novel molecular markers (such as DNMT3A, IDH1/2 or TET2) that help refine classification and risk assessment. miRNA profiling can also define new biological subtypes and therefore it could complement NK-AML diagnosis and risk stratification.
Aims: Our aim is to identify a miRNA profile in NK-AML, to find miRNA signatures associated with established molecular markers (FLT3, NPM1, DNMT3A and CEBPA), and to determine whether altered miRNA expression has prognostic value in NK-AML.

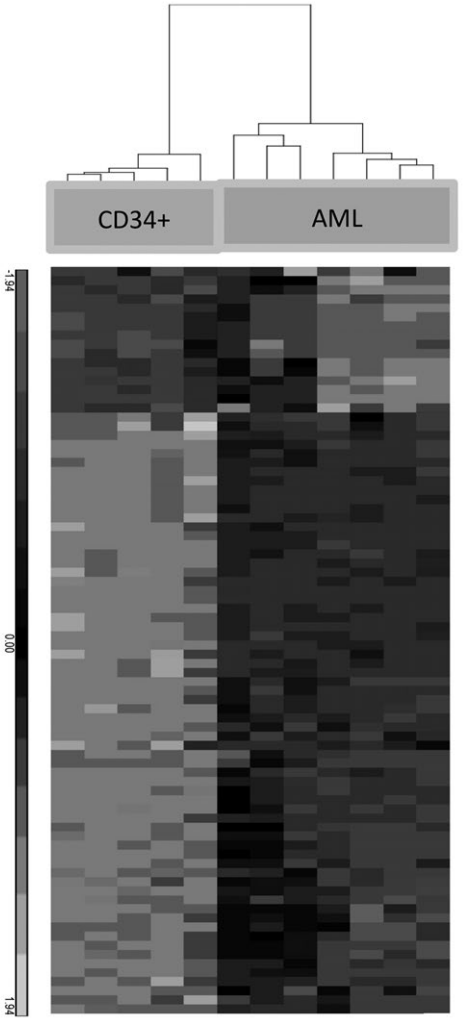

Figure 1.

Methods: Five CD34+samples from cord blood obtained from healthy donors and 7 *de novo* NK-AML samples (>70% blast count, CD34+and no mutations in FLT3, NPM1, DNMT3A or CEBPA) were hybridized onto an array miRNA 3.0 chip (Affymetrix). Deregulated miRNAs in NK-AML were determined with a hierarchical cluster analysis set at 10-fold change (FC) and p<0.001. The most up- and down- regulated miRNAs were validated by qRT-PCR (miScript system) in a cohort of 60 NK-AMLs. Mann-Withney U-test was used to determine differentially expressed miRNAs according to molecular alterations
Results: We found a miRNA signature characterized by 6 up- and 61 downregulated mature miRNAs in NK-AML compared with CD34+controls (Figure 1). By y qRT-PCR we validated 9 miRNAs in a cohort of 60 samples. MiR-20b (p<0.001), miR-99a (p=0.006), miR-126 (p= 0.009), miR-146b and miR-151b (both p=0.012) were downregulated; and miR-4668 and miR-494 were upregulated (p=0.004 and 0.005, respectively). NPM1 and/or FLT3 mutations significantly reduced miR-126, miR-424 and miR-4668 expression. CEBPA and DNMT3a mutations did not modify any of the miRNAs expression. WT1 overexpression was accompanied by a significant reduction of miR-424 expression. We are currently increasing our patient series to establish the prognostic value of the altered miRNAs/miRNA profile. Interestingly, miR-126 (downregulated) targets PI3K (protooncogene), and miR-494 (upregulated) targets PTEN (turmor suppressor gene). These two genes catalyze opposite reactions in cell signalling pathways. We are currently analyzing the involvement of these two miRNAs in PI3K/PTEN expression regulation in NK-AML.
Summary and Conclusions: In conclusion, NK-AML has a defined miRNA profile that is altered by the presence of NPM1 and FLT3 mutations. miR-424 and miR-126 expression is altered in AML and they are predicted to target PTEN and PI3K, respectively, so these miRNAs could be modulating their function in NK-AML

E915

Abstract withdrawn

E916

VPA AND SAHA TREATMENT DIFFERENTIALLY IMPACT PROTEOME AND ACETYLOME OF ACUTE MYELOID LEUKEMIA HL60 CELLS
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Background: Valproate(VPA) and Suberoylanilide hydroxamic acid (SAHA) are both HDAC inhibitors. Previous studies indicated that both inhibitors showed therapeutic effects on acute myeloid leukemia(AML), while the differential impacts of the two different HDACi on AML treatment still remain elusive.
Aims: The aim of this study was to analyze the impact of VPA and SAHA treatment on histone lysine acetylation and proteome in AML HL60 cells and explore the differential impacts of the two different HDACi on AML treatment.
Methods: In AML HL60 cells, by using 3-plex stable isotope labeling for cell culture (SILAC)-based quantitative proteomics, biochemistry assay, and bioinformatic analysis, here we for the first time comprehensively identified and quantified proteome and acetylome profiling on AML HL60 cells in response to VPA or SAHA treatment.
Results: In total, we identified 5,775 proteins with 3,227 quantifiable proteins in AML HL60 cells in response to VPA or SAHA treatment. Taking advantages of anti-acetyllysine antibody based affinity enrichment followed by Nano-HPLC/MS/MS analysis, we identified 1124 Kac sites in 702 Kac proteins in response to VPA or SAHA treatment, among which 1,089 quantifiable Kac sites in 686 Kac substrates were screened. Intensive bioinformatic analysis clearly showed that VPA and SAHA treatment differently induced proteome and acetylome profiling in AML HL60 cells.
Summary and Conclusions: This study therefore for the first time revealed the differential impacts of VPA and SAHA on proteome/acetylome in AML cells, deepening our understanding of HDAC inhibitor mediated AML therapeutics.

E917

PIVOTAL BIOMARKER ALTERATIONS IN CYTARABINE-RESISTANT ACUTE MYELOID LEUKEMIA CELL LINES
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Background: Cytarabine is the backbone of acute myeloid leukemia (AML) therapy. However, the majority of patients succumb to disease relapses. Although several reports tried to unveil the mechanisms of cytarabine resistance, such as diminished cytarabine import, increased cytarabine degradation, decreased formation of active metabolized form of cytarabine, these mechanisms failed to fully explain cytarabine resistance. Recent studies indicated that molecules of signal transduction and apoptosis may be involved.

Aims: To establish cytarabine-resistant leukemia cell lines, and further characterize the pivotal biomarker alterations, on which future treatment may be developed.
Methods: We established a resistant derivative of the human leukemic line MV4-11 by using stepwise dose-escalation method. The parental and resistant MV4-11 cells were named MV4-11-P and MV4-11-R, respectively. Further, pivotal biomarker alterations were characterized by using human phospho-kinase array, human apoptosis array, and human phospho-RTK array. Western blotting was used to confirm those changes. Sanger's sequencing and pyrosequenicng were used to study the mutation status of p53.
Results: MV4-11-P and MV4-11-R showed cytarabine IC_{50} of 0.26μM and 3.37μM, respectively. MV4-11-R cells also showed higher IC_{50} to idarubicin and CI-1040 (an MEK inhibitor). In contrast, they displayed no remarkable difference in response to MK-2206 (an allosteric AKT inhibitor), sorafenib (a multikinase inhibitor inhibits BRAF and VEGFR) and XL-184 (a multikinase inhibitor inhibits c-MET, VEGFR, and FLT-3). There were no detectable differences between MV4-11-P and MV4-11-R cells morphologically and immunophenotypically. By using human phospho-kinase array, there were significant differences between MV4-11-P and MV4-11-R cells in phosphorylated form of several molecules, such as increased phosphorylated ERK1/2 and p53, as well as decreased phosphorylated CREB. Slightly increased phosphorylation in MV4-11-R included p38, JNK, and Akt; on the contrary, slightly decreased phosphorylation of STAT5 was noted in MV4-11-R. By using human apoptosis array, substantial increased phosphorylation of p53 was found in MV4-11-R cells. In addition, slight increase in HSP27 was found; on the contrary, slight decrease in SMAC, survivin, TNF-R and XIAP were found in MV4-11-R cells. By using human phospho-RTK array, increased phosphorylation of EGFR, AXL, HGF-R, Tie-2 and Dtk were found in MV4-11-R cells; on the contrary, decreased phosphorylation was only found in VEGFR. Western blotting confirmed these changes. Exon sequencing revealed that a secondary p53 mutant (D281G) in MV4-11-R cells in addition to the pre-existing p53 mutant (R248W) of MV4-11-P cells. By using pyrosequencer analysis, no significant change of R248W burden was noted, but D281G emerged from 1% to 41%.
Summary and Conclusions: We established a cytarabine-resistance cell line MV4-11-R. This cell line possesses substantial higher phosphorylation of AKT, ERK, MEK, p53 and lower phosphorylation of CREB and STAT5. Of them, an emerging secondary p53 mutant (D281G) was noted. Further characterization of the biologic significance of these molecular alterations may lead to the development of novel therapeutics for AML.

E918

THE EXPRESSION OF THE DNA REPAIR GENE SETMAR IS HIGHLY CORRELATED TO THE LEVEL OF THE MINIMAL RESIDUAL DISEASE IN ACUTE MYELOID LEUKEMIA THROUGHOUT THE DISEASE

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Background: Acute Myeloid Leukemia (AML) is characterized by accumulation of malignant white blood cells in blood and bone marrow. The human fusion protein *SETMAR* is interesting in the context of the disease, as high expression in AML cells has shown inhibitory effects on Topoisomerase IIa (*TOPO2A*) inhibitors that form a cornerstone as drug in this disease entity. *SETMAR* promotes DNA double strand break (DSB) repair in the non-homologous end joining pathway, thereby reducing migration of DNA fragments and suppressing chromosomal translocation. We have previously shown that *SETMAR* is higher expressed in patients without a chromosomal translocation compared to patients with a translocation[1].
Aims: The aim of this study is to explore the correlation of the expression of *SETMAR* and the level of minimal residual disease (MRD) in patients with AML. Furthermore the expression level of *TOPO2A* is correlated to the expression of *SETMAR* to investigate whether a high expression of *SETMAR* has an up regulating effect on the expression level of *TOPO2A*.
Methods: Blood samples from 28 patients diagnosed with AML and treated with *TOPO2A* inhibitors were collected from diagnostic samples and from subsequent follow-up samples (n=82). For 10 patients the follow-up samples were drawn up to seven years from diagnosis (n=28) while samples were drawn shortly after treatment for 18 patients (n=54). The cell line K562 was used as a positive control for the quantification of *SETMAR* and *TOPO2A* expression. A quantitative PCR (qPCR) assay including primers and probes targeting transcript variant 1 of *SETMAR* was used as previously published [1]. Furthermore a qPCR assay targeting *TOPO2A*, established prior to the study, was used for the quantification of *TOPO2A* expression level (forward primer: CCCAAGAGCTTTGGATCAAC, reverse primer: AACTTCACCCAGTTTAGTATGC, TaqMan probe: ATCAAAGCTGCCATTGGCTGTGGT). b-*glucoronidase* (*GUS*) and b-*2-microglobulin* (b2*m*) were used as reference genes. The MRD level was routinely quantified for diagnostics purpose and correlated to the *SETMAR* and *TOPO2A* expression levels. The MRD target was either *WT1*, *inv(16)*, or *t(8;21)*.
Results: We observed a close correlation (Sperman's rho=0.57, P<0.01) between the expression of *SETMAR* and the level of MRD for all 28 patients. This is shown in Figure 1A. Likewise we found a trend towards a positive correlation between *SETMAR* and *TOPO2A* expression (Spearman's rho=0.1753, P=0.0671, Figure 1B). When correlating the expression of *SETMAR* and *TOPO2A* to the level of MRD only for the 18 patients with samples drawn shortly after diagnosis a tighter correlation between the expression of *SETMAR* and MRD level was observed (Sperman´s rho=0.6167, P<0.001). Longitudinally sampling showed excellent correlation between variations in *SETMAR* expression level and disease state from time of diagnosis to follow up. This is depicted in Figure 1C-D where two representative patient courses are shown. The expression of *SETMAR* followed the pattern for MRD at time of relapse suggesting a close relationship between *SETMAR* level and degree of disease in patients with AML. For the 18 patients with samples drawn at three specific time points 15 of these showed expression levels of *TOPO2A* that corresponded to the changes in gene expression levels of *SETMAR* and the MRD level.

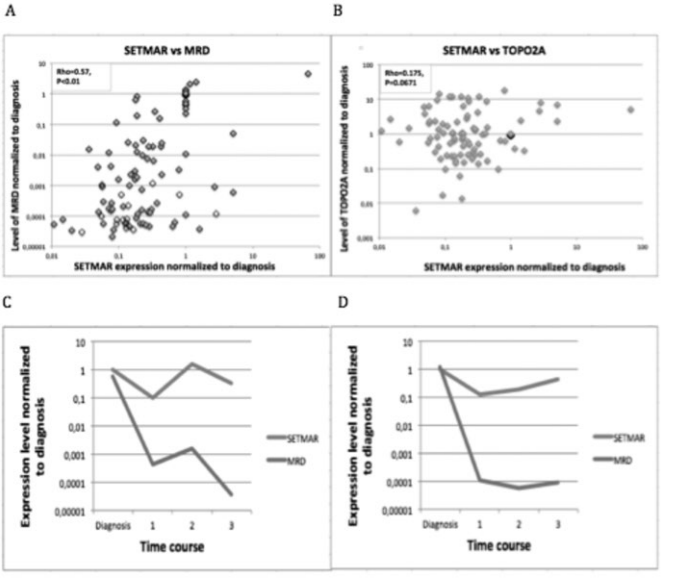

Figure 1. Correlation between *SETMAR*, *TOPO2A*, and *MRD*. A: The expression of *SETMAR* is positively correlated to the *MRD* level (Sperman's rho=0.57, P<0.01). B: The expression of *SETMAR* versus the expression of *TOPO2A* (Spearman's rho=0.1753, P=0.0671). C-D: *SETMAR* and *MRD* during treatment for two representative patient courses. The levels are normalized to the diagnostic samples.

Figure 1.

Summary and Conclusions: We identified a positive correlation between the expression of *SETMAR* and the MRD level as well as a positive correlation between the expression of *SETMAR* and *TOPO2A*. Interestingly there seemed to be very high concordance between the kinetics of *SETMAR* and MRD target during follow-up. This might indicate that *SETMAR* is present in the malignant compartment of the neoplastic cells. The mechanisms behind this remains to be further explored but we speculate that the need for DNA repair in the malignant population might be an issue here. The level of *SETMAR* followed the level of *TOPO2A* over time in the majority of cases indicating that the inhibitory effect of *TOPO2A* inhibitors could be an important subject to be considered in choice of treatment strategy.
Reference 1. Jeyaratnam, D.C., *et al.*, Exp Hematol, 2014. **42**(6): p. 448-56

E919

CORRELATION OF GENOMIC ANALYSIS BY MYAML™ WITH IN VITRO HIGH THROUGHPUT DRUG SENSITIVITY TESTING IN NEW DIAGNOSIS AND RELAPSED ACUTE MYELOID LEUKEMIA

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Background: Whole genome sequencing has demonstrated tremendous heterogeneity in the mutations and chromosomal translocations associated with acute myeloid leukemia (AML), yet we remain quite limited in our ability to predict specific chemotherapy drug sensitivity based on genomics with the exception of a few selected mutations or translocations, such as FLT3-ITD or PML-RARA. The overall survival in AML remains poor, especially for patients who are refractory to ≥2 regimens, or for those with brief duration of first complete remission.
Aims: 1. To categorize the mutations identified by MyAML™ in new diagnosis and relapsed AML, and correlate with clinical features.
2. To determine if specific mutations or patterns of mutations will correlate with results of *in vitro* high throughput drug sensitivity testing in AML.
Methods: MyAML™ uses next generation sequencing to analyze the coding

regions and potential genomic breakpoints within known somatic gene fusion genomic breakpoints of 194 genes known to be associated with AML. Fragmented genomic DNA is captured (3.4Mb) with a customized probe design, and sequenced with 300bp paired end reads on an Illumina MiSeq instrument to an average depth of coverage >1000x. Using a custom bioinformatics pipeline, MyInformatics™, single nucleotide variants (SNVs), indels, inversions and translocations are identified, annotated, characterized, and allelic frequencies calculated. Commonly associated variants in dbSNP and 1000 genomes were eliminated, as well as variants with allele frequencies less than 5%. High throughput drug sensitivity testing was performed against a panel of 160 drugs, of which 56 are FDA approved and 104 are investigational. De-identified samples from 12 patients with de novo AML and 12 patients with relapsed AML were analyzed. For 2 patient samples, Duplex Sequencing was also performed to detect sub-clonal mutations below the detection limit of conventional next-generation DNA sequencing. Statistical analysis was performed to examine relationships between gene mutations and drug sensitivity profiles. Specifically, we computed the Pearson's correlation between all possible pairs of genes containing missense mutations and the *in vitro* cytotoxicity response across the same set of 24 patients.

Results: From the 24 patient samples analyzed to date, an average of 129 missense mutations were identified in each sample with an allelic frequency >5%. Of these, an average of over 21 missense variants were observed in COSMIC and less than 3 were novel (not in dbSNP). These samples also contained an average of over 12 coding indels (~5 frameshift and 7 inframe indels per sample). In addition, MyAML™ identified 3 samples with inv(16) and 6 samples with translocations, including the cryptic NUP98-NSD1 t(5;11) that was not detected by karyotyping. For 2 of the samples, Duplex Sequencing was performed at a depth of at least 6000X, and showed concordance of some of the mutations, with each method identifying additional mutations not observed by the other, an expected finding, as each method targeted distinct regions, and Duplex Sequencing had a greater depth of coverage. The correlative studies between mutations and the results of the high throughput drug sensitivity testing are in progress.

Summary and Conclusions: Data from disease focused genomics and *in vitro* chemotherapy sensitivity testing of individual patient AML samples will likely lead to innovation in treatment, identification of novel targeted agents, and improved outcomes in AML.

E920

ANALYSIS OF GENETIC POLYMORPHISMS IN DNA REPAIR GENES IN ASSOCIATION WITH ACUTE MYELOID LEUKEMIA AND ITS SPECIFIC CHROMOSOMAL ABNORMALITIES

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Background: DNA damage repair mechanisms are vital to maintain the functions of normal cells and genomic integrity. Alterations and a variety of polymorphisms in DNA repair genes have been associated with increased risk of developing acute myeloid leukemia (AML). DNA double-strand break repair pathway (DSB) represents the main pathway in maintaining genome stability and is distinguished into two distinct and complementary pathways homologous recombination (HR) and non-homologous end-joining (NHEJ), while the nucleotide excision repair (NER) pathway constitutes the primary mechanism for removal of bulky adducts from DNA. Essential components of the above pathways represent the XPD23 proteins which participate in the opening of the damaged DNA during NER, the Rad51 proteins in HR and Lig4 proteins which are central components of the NHEJ. The genes that encode the above proteins are subjected to a single-nucleotide polymorphism (SNPs) and have been associated with hematological malignancies.

Aims: The aim of this study was to assess the role of $A^{23927}C$, $G^{135}C$ and $C^{26}T$ germline polymorphisms of *XPD23*, *RAD51* and *LIG4* genes as genetic risk factors for AML and its specific chromosomal abnormalities.

Methods: Genotyping was performed in 83 AML patients and 91 controls by PCR-RFLPs. Cytogenetic analyses were performed on unstimulated bone marrow cells cultured for 24 and 48 hours.

Results: Concerning the polymorphic sites $A^{23927}C$ of *XPD23* gene and $G^{135}C$ of *RAD51* gene, our analysis showed the same allelic and genotypic frequency between patients and controls. However, the genotypic distribution for $C^{26}T$ polymorphism of *LIG4* gene revealed a statistically higher frequency of the variant genotypes in patient's group compared to controls (C/T: 50.6% *vs* 30.8%, T/T: 10.8% *vs* 6.6%, respectively, p=0.006). Allele frequency distribution analysis showed that AML patients exhibited an almost 2-fold increased risk of carrying at least one variant T allele compared to controls (p=0.004, OR [95% CI]=2.100 [1.253-3.224]). After stratification of patients according to karyotype, no statistically significant associations were found between the above polymorphisms and the cytogenetic subgroups. Nevertheless, an increased frequency of the variant genotypes of $C^{26}T$ polymorphism of *LIG4* gene (CT+TT) was observed in patients with +8 (80.0%), -7/del(7q) (76%) and -5/del(5q) (66.7%) compared to healthy donors.

Summary and Conclusions: Our results showed that AML risk is not significantly associated with *RAD51* and *XPD23* polymorphisms. However, our data provide evidence for a possible role of the $C^{26}T$ polymorphism of *LIG4* gene in AML development and its specific chromosomal abnormalities.

E921

EXPOSURE TO ROS INDUCES DNA DAMAGE AND INFLUENCES DNA METHYLATION IN NORMAL AND MALIGNANT HEMATOLOGICAL CELL LINES

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Background: The pathogenesis of myeloid neoplasias is complex and involves multiple genetic and epigenetic events. The oxidative stress and abnormal DNA methylation have been implicated in some types of cancer, namely in myelodysplastic syndromes (MDS), chronic myeloid leukemia (CML) and acute promyelocytic leukemia (APL). Since both mechanisms are observed in some of these patients, we hypothesize that oxidative stress may influence DNA methylation.

Aims: In this context, the present work aimed to analyze the influence of acute and chronic exposure to OS in global and localized methylation, as well as in the gene expression levels of epigenetic modulators and antioxidant enzymes.

Methods: In this study, normal lymphocytes (IMC cell line) and several hematological neoplasia cell lines, the K-562 cells (CML), the F36P cells (MDS), the NB-4 and the HL-60 cells (APL with and without t(15;17), respectively), were exposed acutely (48h) and chronicle (± six months) to oxidative stress inducers (hydrogen peroxide and menadione). Cell proliferation and death were analyzed by trypan blue assay. ROS and GSH levels were analyzed using fluorescent probes (DCFH2DA, DHR123, DHE, DAF, and MO, respectively) by flow cytometry. DNA damage was analyzed through 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels (ELISA). The localized methylation status was analyzed through *p15*, *p16*, *DAPK,* and *KEAP1* methylation profile (MSP). Global methylation status was assessed by 5-methylcytosine (5mC), 5-hydroxymethylcytosine (5hmC) levels, and LINE-1 methylation. Gene expression levels of epigenetic modulators (*DNMT1, DNMT3a, DNMT3b, p300, EZH2, MBD1, MBD2, MECP2*, and *TET2*), oxidative stress genes (*GPX1, GSTM1, NQO1, GSR, HMOX1, SOD1, KEAP1*, and *TXN*), transcription factors (*NF-KB* and *NRF2*), as well as tumor suppressors (*p15, p16, p53*, and *DAPK*), were analyzed by real time PCR. The statistical analysis was carried out by variance analysis ($p<0.05$).

Results: Acute and chronic exposure to hydrogen peroxide (H_2O_2) and menadione (MND) increased intracellular ROS levels in all tested cells lines. Under acute exposure to ROS, GSH content decrease, while in chronic exposure it was increased, probably reflecting an adaptation to OS. Moreover, acute exposure to ROS induces a significant increase of DNA damage, however chronic exposure only significantly increases DNA damage in IMC cells. This oxidative damage is followed by an increase in 5-mC levels and a decrease in LINE-1 methylation. Moreover, OS also conduced to hypermethylation of *p15*, *p16,* and *KEAP1* promoter genes in a cell type- and exposure-dependent manner. In all cells lines, we observed that chronic exposure to H_2O_2 and MND induced an increase in *DNMT1*, *DNMT3a*, *GSR*, *GSMT1,* and *NQO1* gene expression and a decrease in *KEAP1*, *p15* and *p16* genes. In cells, acutely exposed to theses compounds, gene expression levels were cell line-dependent.

Summary and Conclusions: In summary, exposure to hydrogen peroxide and menadione (a superoxide donor), especially chronic exposure, lead to DNA damage, global hypomethylation and localized hypermethylation. These findings suggest a relationship between oxidative stress and aberrant methylation status, two common mechanisms involved in the development of hematological neoplasias. *This work was supported by CIMAGO and R. Alves is supported by the FCT fellowship FRH/BD/51994/2012.*

E922

POLYMORPHISM OF FOLATE AND METHIONINE METABOLISM GENES IN PATIENTS WITH ACUTE MYELOID LEUKEMIA AND MYELODYSPLASTIC SYNDROME

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Background: Abnormal DNA methylation is often seen in patients with acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS). Polymorphism of folate and methionine metabolism (FMM) relating genes can influence the methylation processes and, therefore, modify the risk of AML and MDS development.
Aims: The aim of this study was to assess the features of alleles and genotypes distributions of the FMM genes in MDS and AML patients.
Methods: Forty-eight patients with MDS (21 men and 27 women, mean age 61.3 yrs), 71 patients with AML (25 men and 46 women, mean age 51.2 yrs) and 121 healthy controls (HC) (cancer-free 73 men and 48 women, mean age 54.1 yrs) were genotyped for the MTHFR C677T, MTHFR A1298C, MTR A2756G, MTRR A66G and MTHFD G1958A polymorphisms by PCR-RFLP technique. The differences in allele and genotype frequencies between patients and HC groups were assessed by Fisher's exact test with computation of odds ratios (OR), their 95% confidence intervals (CI) and p-values.
Results: The frequency of the MTHFR 1298AC genotype was decreased in MDS patients and increased in AML patients, when compared to HC group (33.3% vs. 43.8% and 55.7% vs. 43.8%, respectively). The difference between MDS and AML patients was statistically significant (33.3% vs. 55.7%, OR=0.4, 95%CI: 0.2-0.9, p=0.025). At the same time, the MTR 2756 GG variant was present in 2 (4.2%) patients with MDS, none of AML patients (0.0%) and 8 (6.6%) controls. The latter observation also was statistically significant (0.0% *vs* 6.6%, OR=0.09, 95%CI: 0.01-1.6, p=0.027). It is interesting, that the MTR 2756 GG genotype was detected in none of the male patients in MDS and AML groups compared to men in the HC group (0.0% vs. 11.0%, respectively, OR=0.08, 95%CI: 0.01-1.5, p=0.023). The simultaneous presence of the MTHFD 1958GG and MTRR 66GG genotypes was more often detected in MDS group than in HC (14.6% vs. 4.1%, OR=4.0, 95%CI: 1.2-13.2, p=0.04), and in AML group compared to HC group (8.5% vs. 4.1%, OR=0.5, 95%CI: 0.14-1.6, p=0.33).
Summary and Conclusions: We conclude that polymorphism of the FMM genes could have an important role in pathogenesis of both MDS and AML.

E923

SK053, AN INHIBITOR OF ENZYMES INVOLVED IN ALLOSTERIC DISULFIDE BONDS FORMATION, TARGETS EXPRESSION OF HISTONE GENES, SHOWS POTENT ANTI-LEUKEMIC EFFECTS AND INDUCES DIFFERENTIATION OF HUMAN AML CELLS

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Background: *All-trans* retinoic acid and arsenic trioxide have revolutionized management of acute promyelocytic leukemia. Right now targeting epigenetic modifiers, such as histone deacetylases, bromodomain and extraterminal domains or methyltransferases and differentiation-inducing agents are rising hopes for development of effective therapeutic strategies for other types of acute myeloid leukemia (AML). Numerous proteins involved in tumor development have so-called allosteric disulfide bonds amenable to modifications affecting protein structure and function (*Hogg P, Nat Rev Cancer 2013*). Targeting allosteric disulfide bonds becomes a novel approach to cancer treatment. Our interest in this field focuses on proteins with thioredoxin fold such as protein disulfide isomerase (PDI). We have developed SK053, a small molecule inhibitor of thioredoxin/thioredoxin reductase system (*Klossowski S, Muchowicz A et al., J Med Chem 2012*) that is not a target-specific, but mechanism-selective inhibitor of allosteric disulfide bonds formation that blocks the enzymatic activity of PDI
Aims: The aim of this project was to evaluate anti-leukemic and differentiation-inducing effects of SK053 and investigation of the potential mechanisms of its activity.
Methods: We evaluated the biological effects on proliferation and differentiation and the transcriptome changes determined by SK053 in the human myeloid cell lines: HL60, NB4, KG1 and MOLM14
Results: Our results show that SK053 reduces cell proliferation evaluated by trypan blue exclusion. SK053 induces cell differentiation along the monocytic and granulocytic lineages as evidenced with May-Grünwald-Giemsa staining, nitro blue tetrazolium reduction assay and flow cytometry analysis of CD11b, CD14 and CD15 expression. Accordingly relative gene expression calculation and RNA sequencing showed the up-regulation differentiation-related genes. RNA-seq analysis revealed that 5 day incubation of HL60 cells with SK053 down-regulates mRNA for MYC and ID1 oncogenes as well as for histone proteins. Expression of other genes of mature myeloid lineage such as adhesion molecules (collagen type XV, fibronectin I, MAC-1), hydrolytic enzymes (carboxypeptidase, proteinase 3, CA12 anhydrase, ADAM19 metalloprotease), proteoglycan 2 (core of eosinophilic granules) and PGLYRP3 (peptidoglycan recognition protein 3) was significantly up-regulated. Moreover, the transcripts for histone proteins: HIST1H3J, HIST1H2AB, HIST1H1B, HIST1H3H, HIST1H2AH were strongly down-regulated. Drugs that disrupt histone modifiers are in clinical trials with promising results but very little is known about direct targeting of histone proteins. We also plan to analyze transcriptome of HL60 cells incubated for shorter time with SK053 using RNA-seq techniques to estimate an early response to that compound. Figure 1) Differential expression assesed with RNA-seq after 5-day treatment of HL60 cells with SK053. HL60 cells were incubated with 10 μM SK053, next RNA from control and SK053-treated cells was transcribed to cDNA library using Illumina kit. Next-Generation Sequencing was performed with Illumina HiSeq 1500. Transcriptome RNA-seq dataset was analyzed using Spliced Transcripts Alignment to a Reference (STAR) software and HTseq software (short-read counts). The y-axis presents negative log of the p-value from differential gene expression.

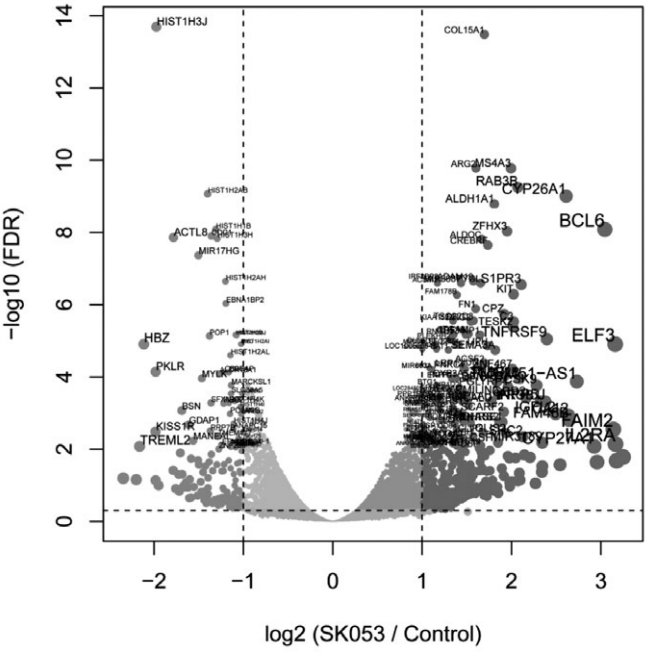

Figure 1.

Summary and Conclusions: We propose a new approach in AML treatment, *i.e.* targeting of enzymes involved in formation of allosteric disulfide bonds which has already been reported as novel promising strategy in other cancer types.

LB2077

PROGNOSTIC IMPACT OF TRANSPORTER GENE POLYMORPHISMS IN NEWLY DIAGNOSED ACUTE MYELOID LEUKEMIA ADULTS

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Background: The intake of anthracyclines in blast cells could be affected by several transporters, including influx transporters (SLC22A16 and SLCO1B1) and efflux pumps of the ABC family.
Aims: Previous studies have suggested that single nucleotide polymorphisms (SNPs) of genes coding for anthracyclines transporters may influence their effectiveness or toxicity, although their impact in acute myeloid leukemia (AML) induction therapy remains undetermined.
Methods: The SNPs of anthracycline transporter genes (ABCB1: rs1128503, rs1045642, rs2032582 and haplotype; ABCC1: rs4148350; ABCC2: rs8187710; ABCG2: rs2231142, rs2231137; SLCO1B1: rs4149056; SLC22A16: rs12210538, rs714368) were evaluated in 149 adult patients at initial diagnosis from AML using a Sequenom (iPLEX) mass spectrometry–based multiplex

genotyping assay (Sequenom, San Diego, CA). All patients received induction chemotherapy consisting of idarubicin plus cytarabine (PETHEMA-LMA 99, 2007 and 2010 trials). Genotypes were grouped as dichotomous variables (dominant and recessive model). Efficacy of first induction cycle was evaluated comparing complete remission (CR) vs. partial remission or resistance. Patients dying during induction were considered as no evaluable for efficacy. Based on the WHO grading scale, toxicities were grouped as binary variables (grade 0-1 vs. grade 2-4). The grade of toxicity assigned to an organ group was the maximum grade of all the specific toxicities within that group. Overall toxicity was grouped with grade 3-4 assessment. Hematologic toxicity was measured with the time to neutropenia and thrombocytopenia recovery since first day of chemotherapy. Categorical and continuous variables were assessed using $\chi 2$ test with Yates correction if needed and Mann–Whitney U test, respectively. Significant values were reanalyzed with logistic regression (covariables: age, gender, ECOG, leukocyte and platelet count).

Results: The median age of patients was 51.7 years (17-78 years). There were lower CR rates among patients harboring ABCB1 G2677T variant allele (rs2032582, GG: 60.0% vs. GT/TT: 78.6%, P=0.03; OR: 2.44; IC95%:1.08-5.55; P=0.033), genotype previously associated with lower activity of ABCB1 pump, and lower anthracycline clearance. Cardiotoxicity was related with wild type ABCB1 haplotype (32.0% vs. 11.3%, P=0.013; OR: 3.42; IC95%:1.19-9.85; P=0.023) and variant allele of ABCG2 (rs2231142, CC: 17.0% vs. CA/AA: 50.0%, P=0.008; OR: 6.87; IC95%:1.89-24.96; P=0.003). Neurotoxicity was associated with wild type ABCB1 haplotype (24.0% vs. 11.4%, P=0.041; OR: 3.00; IC95%:0.97-9.28; P=0.057). Skin toxicity was higher in wild genotypes of ABCB1 G2677T (rs2032582 GG: 44.0% vs. GT/TT: 24.5%, P=0.015; OR: 0.41; IC95%:0.20-0.85; P=0.017) and ABCB1 haplotype (45.7% vs. 18.9%, P=0.007; OR: 2.44; IC95%:1.02-5.88; P=0.046), as well as mucositis with variant allele of ABCC1 (rs4148350 GG: 34.8% vs. GT/TT: 7.1%, P=0.037; OR: 0.14; IC95%:0.02-1.09; P=0.060). Regarding hematologic toxicity, time to neutropenia recovery was delayed with variant allele of SLCO1B1 (TT/CT: 26.8 vs. CC: 29.9 median days, P=0.033).

Summary and Conclusions: This study shows a prognostic impact of transporter genes polymorphisms in adult AML patients regarding induction chemotherapy efficacy and toxicity. Further studies with larger population are needed to validate these associations, which could be useful biomarkers in clinical practice.

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LB2078

NQO1 C609T VARIANTS ARE ASSOCIATED WITH TYPE I AND II MUTATIONS IN PEDIATRIC ACUTE MYELOID LEUKEMIA

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Background: Acute myeloid leukemia (AML) has long been recognized for its biological heterogeneity, as well as the diversity of genetic and epigenetic modifications. The broad range of abnormalities across different subtypes is important to stratify AML cases to different prognostic groups and choosing of an appropriate therapy for the disease. However, there has been no recent comprehensive overview of the type I (*FLT3*, *RAS*) and II [*RUNX1-RUNX1T1*, *CBFb-MYH11*, *MLL* rearrangements (*MLL*-r), *PML-RARa*] genetic alterations in one large cohort of pediatric AML cases and examining the potential contribution of inherited susceptibility to AML development.

Aims: The purpose of this study is to evaluate the association of *NQO1* rs1800566 polymorphism with pediatric AML risk presenting *RAS* and *FLT3* gene mutations as cooperating events with the mainly fusion genes related to AML in children.

Methods: Samples from 601 AML cases aged ≤21 years-old ascertained from 2000-2014 prior to any treatment were included. Variables of reference were type I/II mutations and *NQO1* rs1800566 (C609T). Restriction fragment length polymorphism and/or direct sequencing were used to screen *FLT3* and *K/N-RAS* mutations. RT-PCR and/or FISH were performed to identify the most common partners of *MLL* gene, and the fusion transcripts *RUNX1-RUNX1T1*, *CBFb-MYH11*, and *PML-RARa*. *NQO1* polymorphism was detected by real-time allelic discrimination technique.

Results: Overall, type I mutations were identified in 42.2% of cases and type II mutations in 45.5% (*KRAS*=6.9%; *NRAS*=11.6%; *FLT3*=23.7%; *RUNX1-RUNX1T1*=16.9%; *CBFb-MYH11*=4.5%; *MLL*-r=12.4%; *PML-RARa*=11.7%). Higher rate of *KRAS* mutation was found in AML with *MLL*-r (42.9%) compared with cases without this abnormality, as well *NRAS* in *RUNX1-RUNX1T1* positive cases (50.0%) and *FLT3* with *PML-RARa* (56.7%), suggesting a non-random association among these alterations. Children aged >2-10 years-old with at least one *NQO1* variant allele were at higher risk for developing AML with *PML-RARa* than cases with no type II mutation (odds ratio (OR) 3.67, 95 % confidence interval (CI) 1.01-13.40). Interestingly, we observed a reverse association for those cases *PML-RARa* positives aged >10 years-old (OR 0.18, 95%CI 0.04-0.87). The presence of at least one *NQO1* variant allele also provided an increased risk for cases presenting *RUNX1-RUNX1T1* or *CBFb-MYH11* and concomitant *FLT3* mutations (OR 6.08, 95%CI 1.15-32.29).

Summary and Conclusions: The identification of genetic subgroups in pediatric AML may assist to improve the molecular-epidemiology, risk stratification and etiopathology of AML worldwide. The *NQO1* rs1800566 (C609T) polymorphism modified risk depending on genetic subtype and age range, suggesting that lower NQO1 enzyme activity may play a synergistic effect with genetic alterations and environmental exposures for leukemogenesis.

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Acute myeloid leukemia - Clinical

E924

FAVORABLE PROGNOSTIC IMPACT OF NMP1 MUTATION IN ELDERLY PATIENTS WITH NORMAL KARYOTYPE ACUTE MYELOID LEUKEMIA

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Background: The prognosis of acute myeloid leukemia (AML) in elderly patients is poor. The prognostic impact of molecular mutations in these patients is still unknown. Recent studies showed that NPM1 mutation have a favorable prognostic impact in elderly patients with de novo AML.
Aims: The aim of this study was to investigate the impact of NPM1 and FLT3 internal tandem duplication (ITD) mutations as prognostic factors for overall survival (OS), complete remission (CR) rate and disease-free survival (DFS) in elderly patients with normal karyotype (NK) AML.
Methods: Diagnostics bone marrow samples from 138 *de novo* AML patients aged ≥65years diagnosed between 2009 and 2014 were analyzed for NPM1 and Flt3 mutations by DNA PCR amplification/sequencing. Chemotherapy received 110 patients and 28 patients treated by supportive care only. Co morbidities were evaluated by using the hematopoietic cell transplantation-specific comorbidity index (HCT-CI). Performance status (PS) was evaluated by Eastern Cooperative Oncology Group (ECOG), ranged 0-4. Cytogenetic risk group was assessed by recommendation of European LeukemiaNet. The following parameters were estimated as the risk factors for CR rate, DFS and OS: leukocytes count (<30x10^9/L vs. ≥3010^9/L), level of serum lactate dehydrogenase (LDH) more than 1.5 x upper limit of normal, ECOG PS (<2 vs. ≥2), ELN cytogenetic risk group, HCT-CI (<2 vs. ≥3) and NPM1 and FLT3-ITD mutations. Risk factors were identified using the univariate and multivariate analysis.
Results: NPM1 and/or FLT3-ITD mutations detected in 43.5% (60) of patients. Patients with NPM1 mutations (NPM1+) and without FLT3-ITD mutation (FLT3-ITD-) had higher CR rate than NPM1- patients (p=0.015). Also, NPM+/ FLT3-ITD- patients had longer DFS than NPM- patients (p=0.023). Nevertheless, in NPM1+/FLT3- patients multivariate analysis indicated HCT-CI <3 as the most important risk factor for longer OS: p=0.02, relative risk (RR)=2.486; 95% confidental interval (CI)=1.156-5.346.
Summary and Conclusions: NPM1 mutation was associated with higher CR rate and longer DFS in elderly patients with NK-AML and without FLT3-ITD mutation, but comorbidity remained to be the most important risk factor for OS of its patients.

E925

STUDY OF PLASMA ENDOSTATIN LEVEL IN PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background: Endostatin is a naturally-occurring 20-kDa C-terminal fragment derived from type XVIII collagen. It serves as an anti-angiogenic agent. Endostatin is a broad spectrum angiogenesis inhibitor and may interfere with the pro-angiogenic action of growth factors such as basic fibroblast growth factor (bFGF/FGF-2) and vascular endothelial growth factor (VEGF).
Aims: To evaluate the role and prognostic value of endostatin in AML patients
Methods: Sixty subjects were included in this study, 30 apparently healthy adult subjects as control (group 1) and 30 adult patients with newly diagnosed de novo AML(group2). Estimation of serum endostatin level by ELIZA, at the onset of the disease and after 1 month of induction treatment
Results: Serum endostatin levels varied widely from 2.1 ng/mL to 7.1 ng/mL (median, 4.1ng/mL), and the median level was higher in patients with AML compared with patients in the control group (2.6 ng/mL; *P =0.001*). No significant difference was found between pre-and post-treatment in group 2 (0.23).
Median overall survival of group 2 was 7 months (1 to 16 months), while the disease free survival ranged from 2 to 15 months with a median of 8.5 months. The overall survival of patients who had pre-treatment endostatin level ≤50% percentile was significantly longer than patients with pre-treatment endostatin level >50% percentile (p=0.007). According to change of endosatin levels, the overall survival of patients with increased endostatin level was significantly longer than patients with decreased endostatin (p=0.03). Also there were significant association between change of endosatin level and age, performance status, cytogenetics, response to treatment and the disease outcome.
Summary and Conclusions: Inhibition of the angiogenesis may have a role in remission of AML patients. Levels of endostatin in patients with AML may be effective in predicting the survival

E926

LONG TERM EFFICACY OF CONSOLIDATION TREATMENT WITH COURSES OF HIGH-DOSE CYTARABINE AND LIMITED AUTOLOGOUS PBSC SUPPORT IN A SERIES OF ADULTS WITH ACUTE MYELOID LEUKEMIA NOT AT HIGH BIOLOGICAL RISK

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Background: In adult acute myeloid leukemia (AML) consolidation treatment after complete remission (CR) is mandatory to improve remission duration. Allogeneic stem-cell-transplantation (allo-SCT) is considered standard for young pts but repeated cycles of high-dose cytarabine (HDAraC) or autologous SCT may be as effective in standard risk (SR) pts. In these pts, the AML-00 NILG program (Bassan, ASH 2003) called for 3 consolidation cycles of idarubicin (8 mg/sqm for 2 days) and HDAraC at 2g/sqm/bid for 5 days (A20). Small doses (1-2x10^6/kg) of autologous CD34+peripheral blood stem cells (PBSC) were reinfused after each A20 to minimize toxicity.

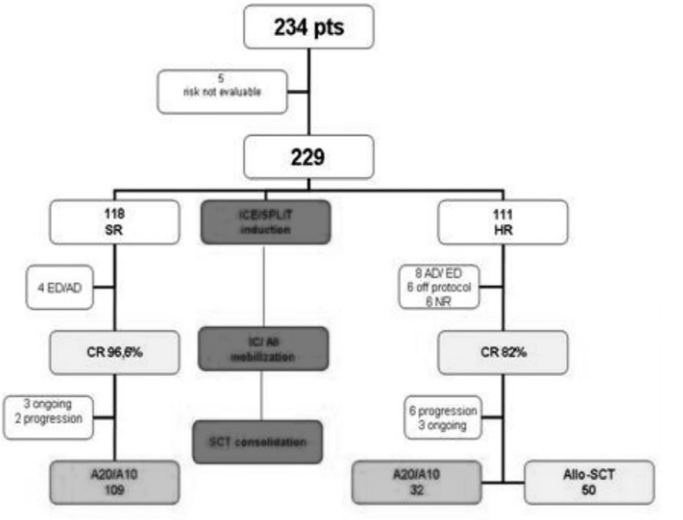

Figure 1.

Aims: To analyze, by intention to treat and according to cytogenetic/molecular characteristics, the very long term outcome of SR pts who received A20 consolidation with PBSC support according to the AML-00 NILG program, adopted as standard at our Institution. High risk (HR) pts, identified by high-risk cytogenetics, secondary AML, late CR or FLT3-ITD positivity, were addressed to allo-SCT and analyzed separately.
Methods: Induction treatment consisted of ICE (3-7-5) followed by IC (2-7) consolidation if CR was achieved, or by SPLIT (HD-AraC and HD-Idarubicin) in patients refractory to ICE. AraC 1g/sqm/bid for 4 days (A8) was then given to all patients achieving CR to allow collection of 3-6x10^6/Kg PBSC in three aliquots. SR pts were treated with 3 cycles of A20 followed by reinfusion of 1-2x10^6/Kg CD34+PBSC, at 1-2 months intervals upon hematologic recovery. Pts with insufficient CD34 +PBSC yield received 2 courses of A10 (HD-AraC 1g/sqm/bid for 5 days +idarubicin 10mg/sqm day 1). HR pts received A20/A10 waiting for donor availability and allo-SCT. From January 2001 to December 2014, 234 consecutive AML pts were diagnosed at our Institution, (median age 53, range 15-65). According to molecular/cytogenetic data, available in 229 (97.8%), 118 were SR (51,5%) and 111 HR (48,5%). The CR rate after ICE induction was 78,6% (180/229) and increased to 89,5% (205/229) after SPLIT (SR 96,6% and HR 82%). Early/aplastic death rate was 3,9%. A8 course was administered to 196/205 pts, and 160 (82%) successfully collected CD34+PBSC (Fig1).
Results: Of 118 SR pts, 109 actually received A20 (86,2%) or A10 (13,7%). Of 111 HR pts, 50 actually received allo-SCT and 32 A20/A10 consolidation only. The TRM at 100-days after A20/A10 was 2/141 (1,4%) (*Ps aeruginosa* sepsis and toxic megacolon). Neutrophil recovery occurred at a median of 10 days after reinfusion. After a median of 20 mo., the median OS of the entire population was 37.8 mo.; 45+/-6% (IC 95%) of pts were alive 5 years and 42+/-7% 8 years after diagnosis. Focusing on SR pts, median OS was 131 months and 62+/-6% were alive after 5 years. According to ELN risk, 5y-OS was 86+/-4% in CBF+, 100% in CEBPA+, 80+/-10% in NPM+, 54+/-15% in ELN Int-1, and 30+/-12% in Int-2 risk groups, respectively. OS at 8 years did not change except in NPM+pts, where it dropped to 53+/-18% because of late relapses (Fig2). In HR pts, 5y-OS was 24+/-5% by intention to treat. It was 45+/-8% in the 50 pts actually receiving allo-SCT and 25+/-9% in 32 patients actually completing A20/A10 because of allo-SCT refusal or lack of donor.

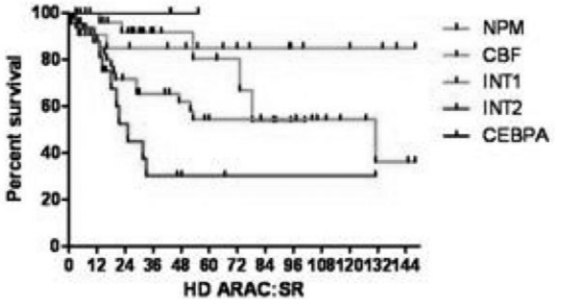

Figure 2.

Summary and Conclusions: Repeated courses of HD-AraC with limited PBSC support as consolidation was feasible, well tolerated and very effective in non HR adult AML. After long term follow up, 86% of CBF+, and more than 50% of NPM+and ELN int-1 risk pts appear to be cured. In NPM+pts late relapses can occur even after 5 years from diagnosis.

E927

HIGH CURE RATE OF YOUNGER AML PATIENTS WITH FLUDARABINE, CYTARABINE AND IDARUBICINE INDUCTION AND RISK ORIENTED CONSOLIDATION: TEN- YEARS REAL LIFE EXPERIENCE

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Background: Conventional induction therapy of acute myeloid leukemia (AML) is still largely based on the combination of cytarabine (ARA-C) and daunorubicin. In the last two decades alternative drug combinations have been tested in order to improve complete remission (CR) rate and quality of remission. Fludarabine has been shown to enhance ara-CTP accumulation in leukemic blasts and to inhibit DNA repair mechanisms, thus providing a rationale for combination with DNA damaging agents.
Aims: In our institution a fludarabine containing induction regimen (FLAI-5) is being used since more than 15 years. The aim of present study was to critically rewiev feasibility and efficacy of our strategy.

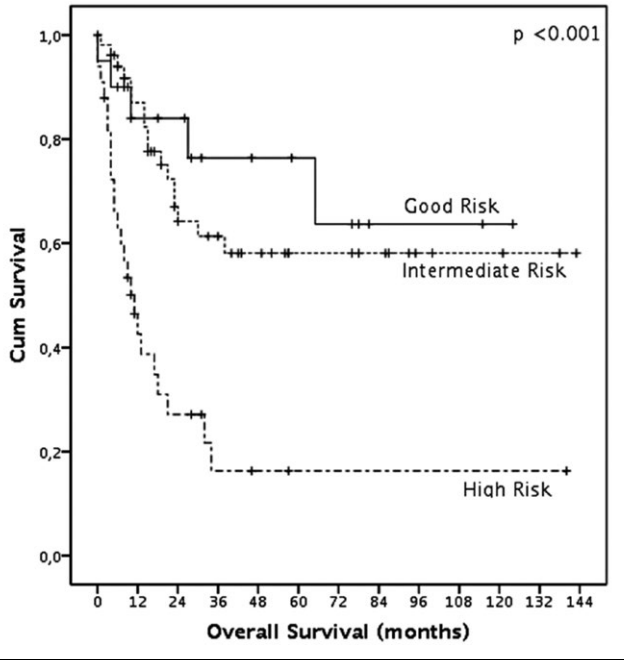

Figure 1. Overall Survival according to risk group.

Methods: One hundred-five consecutive non M3 AML patients (age 17-72 years) treated in our center between 2004 and 2013 were retrospectively analyzed. Median age was 45 yy; 90/105 patients (86%) were de-novo AML while 15 (14%) had secondary disease. Fifteen patients (14%) had high risk karyotype; 35 patients had NPM-1 mutation (33%), and 24/105 (23%) carried FLT3-ITD. Median follow-up was 48 months. Induction regimen included fludarabine 30 mg/sqm and ARA-C 2g/sqm on days 1 to 5 plus idarubicin (IDA) 10 mg/sqm on days 1-3-5, with or without gemtuzumab ozogamicin (3mg/sqm) on day 6. Patients achieving complete remission received a second course including ARA-C 2g/sqm on days 1 to 5 and IDA at an increased dose of 12 mg/sqm on days 1-3-5. Patients were stratified in prognostic risk groups according to a comprehensive score based on karyotype, de-novo or secondary disease, NPM and FLT3 status. High-risk and selected intermediate-risk patients underwent allogeneic bone marrow transplantation (BMT) in first CR if a donor was available. The other patients were scheduled to receive at least 2 and up to 4 courses of consolidation therapy with 4 days of ARA-C (2g/sqm, daily).
Results: Five (4.8%) and nine patients (8.6%) died during the first 30 and 60 days, respectively, mainly because of infective or hemorrhagic events**.** After 1st induction cycle 83 patients achieved CR (79%) and 17 did not respond (16%). After cycle 2, CR rate was 84% (88/105). FLAI-5 was generally well tolerated, with negligible non-hematological toxicity; median time to neutrophil and platelets recovery was 18 and 17 days, respectively. Most patients were able to receive subsequent therapy at full dose and in a timely manner; 29/88 CR patients underwent BMT in first CR whereas 41 out of the 59 not scheduled for an early transplant (70%) received the full programmed therapy. In the whole cohort, 3 years DFS and OS were 42.8% and 48.3% (median 34 and 35 months, respectively). Patients not undergoing early allo-BMT and receiving the full planned dose had a very good outcome, with a 3-year DFS of 56.7% (median 63 months). The outcome was further improved if a third consolidation cycle was given, with a 3-years DFS and OS of 71.2% and 100%, median not reached.
Summary and Conclusions: In a large randomized trial Burnett et al showed that FLAG-Ida favorably compared with other induction schedule. However the longer disease free survival and the lower relapse rate observed in the FLAG-Ida arm did not translate in a higher OS mostly due to the severe hematological toxicities following the second FLAG-Ida course. Our results are comparable to those recently published and show that delivering only one fludarabine containing regimen may significantly reduce hematological toxicity therefore allowing patients to proceed along the risk tailored consolidation program in a good performance status.

E928

IMPACT OF CYTOGENETICS ON CLINICAL OUTCOME IN PATIENTS WITH FIRST RELAPSED OR REFRACTORY ACUTE MYELOID LEUKEMIA TREATED WITH VOSAROXIN PLUS CYTARABINE VS PLACEBO PLUS CYTARABINE: RESULTS FROM VALOR

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Background: Diagnostic karyotype is one of the most important predictors of outcome in acute myeloid leukemia (AML). VALOR, a large phase 3 trial of vosaroxin plus cytarabine (vos/cyt) *vs* placebo plus cytarabine (pla/cyt) in patients with relapsed or refractory (R/R) AML [NCT01191801], provided the opportunity to evaluate the impact of karyotype on clinical outcomes in this difficult-to-treat patient population.
Aims: The aim of this posthoc analysis was to assess efficacy outcomes by cytogenetic risk category and treatment arm in a large cohort of patients with R/R AML.
Methods: Patients were randomized 1:1 to receive cyt (1 g/m^2 IV over 2 hr, d 1-5) plus either vos (90 mg/m^2 IV over 10 min, d 1 and 4; 70 mg/m^2 in subsequent cycles) or placebo. Complete remission (CR) rates, overall survival (OS), and 30-day and 60-day mortality were assessed by treatment arm in R/R AML patients based on cytogenetic risk category (an optional entry in the case report form at screening) per NCCN guidelines.
Results: Overall, cytogenetic data were available for 479 of 711 (67%) of patients on the VALOR study, of whom 133 (28%) had unfavorable risk, 330 (69%) had intermediate risk, and 16 (3%) had favorable risk karyotype as reported at screening. CR rates by cytogenetic group were: 12.0% for unfavorable, 27.0% for intermediate, and 56.3% for favorable risk patients. Median OS was 4.0 mo, 8.1 mo, and 15.6 mo for unfavorable, intermediate, and favorable risk groups, respectively. On the vos/cyt treatment arm, there were 58 patients with unfavorable risk, 175 patients with intermediate risk, and 7 patients with favorable risk. For the pla/cyt arm, there were 75 patients with unfavorable risk, 155 patients with intermediate risk, and 9 patients with favorable risk (test for association, p=0.16). When CR was examined by treatment arm, there were improved rates in the vos/cyt treatment arm compared with the pla/cyt arm for

each cytogenetic risk group: 15.5% *vs* 9.3% for unfavorable, 34.3% *vs* 18.7% for intermediate, and 71.4% *vs* 44.4% for favorable risk. In the intermediate risk group, the difference in CR rate was statistically significant (P=0.0015, chi-square test). Forest plots of OS hazard ratios (HR) reflected treatment benefit for all categories of cytogenetic risk (Figure). When median OS was reported by treatment arm, patients with unfavorable and intermediate cytogenetics who received vos/cyt had improved survival compared with patients who received pla/cyt (for unfavorable group: 5.0 mo *vs* 3.8 mo, respectively [HR 0.79; P=0.20]; for intermediate group: 8.3 mo *vs* 7.1 mo, respectively [HR 0.88; P=0.32]). Between cytogenetic risk groups there were no clinically meaningful differences between treatment arms with respect to 30-day and 60-day mortality.

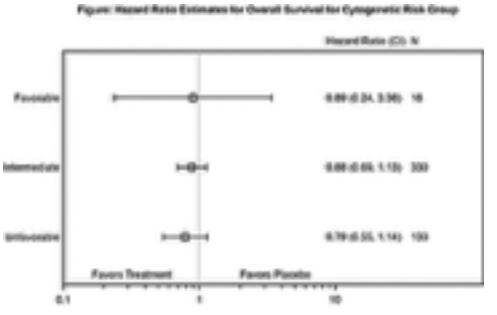

Figure 1.

Summary and Conclusions: These data further validate previous reports of important prognostic value of cytogenetics in R/R AML. Improvement in CR rate and OS benefit was observed for vos/cyt-treated patients with the poorest prognosis, those with intermediate or unfavorable cytogenetics, without an increase in early mortality. Funded by Sunesis Pharmaceuticals, Inc., South San Francisco, CA.

E929

CLOFARABINE AND HIGH-DOSE CYTARABINE ARABINOSIDE (CLARA) FOR PRIMARY REFRACTORY OR RELAPSED ACUTE MYELOID LEUKAEMIA – A PROSPECTIVE FOLLOW-UP STUDY OF 60 PATIENTS

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Background: Relapsed or refractory acute myeloid leukaemia (AML) is associated with a poor prognosis with a remission rate ranging between 10 to 40 percent with conventional salvage regimens. Clofarabine was designed to incorporate the anti-leukaemic properties of fludarabine and cladribine, both of which are active against AML.
Aims: The aim of this study was to prospectively evaluate the efficacy and tolerability of the salvage regimen combining clofarabine and high-dose cytarabine in patients with primary refractory or relapsed AML in the real-world scenario.
Methods: Patients with primary refractory or relapsed AML were prospectively recruited between 1 June 2009 and 31 December 2013. All data was censored on 30 June 2014. Patients were treated with **Cl**ofarabine at 40mg/m^2/day from day 1 to day 5) in combination with cytarabine arabinoside (**Ara**C) at 2g/m^2/day from day 1 to day 5 (**CLARA**). Clinicopathologic features, treatment response and survivals were determined. Prognostic factors for response and survivals were determined using logistic regression and cox regression.
Results: 60 patients with primary refractory or relapsed AML were recruited (primary refractory,N=18; first relapse,N=31, second relapse,N=10, third relapse,N=1). 29 men and 31 women with a median age of 48.5 (range: 18-66) were recruited. They received a median of 2 (range:1-5) prior induction/re-induction regimens for AML. 36 patients (40%) had normal karyotype at diagnosis while 24 patients (60%) had abnormal karyotype. 8 patients had fms-like tyrosine kinase 3-internal tandem duplication (*FLT3*-ITD) and 5 patients had mutated nucleophosmin 1 (*NPM1*). 8 patients achieved complete remission (CR) and 25 patients achieved a complete remission with incomplete haematopoietic recovery (CRi) making the CR/CRi rate of 55%. 17 patients progressed after initial remission. 15 patients (25%) underwent allogeneic haematopoietic stem cell transplantation (HSCT) after remission. Grade 3/4 haematological toxicity was seen in all 60 patients. Febrile neutropenia occurred in 24 patients (40%) and grade 3/4 hepatotoxicity was seen in 5 patients (8%). With a median follow-up of 6.5 months (range: 1-43), the median overall survival was 8 months (95% confidence interval: 5.04-10.96). The overall survival at 12 and 24 months was 38.9% and 29.7% respectively. Patients who were successfully bridged to allogeneic HSCT had a significantly better overall survival (P<0.001). The overall survival at 36 months in patients who underwent HSCT was 62.2%. The median progression-free survival following CLARA was 6 months.
Summary and Conclusions: Clofarabine in combination with high-dose cytarabine was effective in inducing a response in a significant proportion of patients with heavily-pretreated primary refractory or relapsed AML. Successful bridge to subsequent curative allogeneic HSCT was the major determinant of overall survival.

E930

IDH1 (BUT NOT IDH2) MUTATIONS CAUSE INFERIOR OUTCOME IN PATIENTS WITH AML WITH AN INTERMEDIATE-RISK CYTOGENETICS

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Background: IDH1 and IDH2 are enzymes that catalyze the oxidative carboxylation of isocitrate to α-ketoglutarate in the Krebs cycle. Heterozygous mutations at *IDH1* Arg132, *IDH2* Arg140 and *IDH2* Arg172 were found to cause loss of normal enzymatic function. *IDH1* mutations are described in 6-16% of patients with cytogenetically normal AML, incidence of *IDH2* mutations is slightly higher (15-20%). *IDH2* mutations, particularly Asp140 are considered to have an unfavourable prognostic impact.
Aims: Patients: Presence of *IDH1* and *IDH2* mutations was analysed in 296 patients with AML with an intermediate-risk cytogenetics (defined according to Grimwade *et al.*, Blood, 2010) diagnosed between years 1998-2012. Median age at diagnosis was 55.1 years (range 16.0-81.7), the initial median WBC count was 22.3 x 10^9/L (range 0.4-483.7). The male/female ratio was 147/149 and the median of follow-up was 10.8 months.
Methods: Exons 4 of both *IDH1* and *IDH2* genes were amplified by RT-PCR; PCR products were treated by ExoSAP-IT reagent and directly sequenced. Obtained sequences were compared with wild type ones and mutations were identified.
Results: *IDH1/2* mutations were detected in 81 (27.4%) from 296 patients, all but one mutations were heterozygous. *IDH* mutations were more frequent in female patients (P=0.055) and independent of age (P=0.395). *IDH* mutations were associated with mutated *DNMT3A* (P=0.0008), but not with *FLT3*/ITD (P=0.124). *IDH1* mutations were found in 28 (9.5%) patients; 14/28 (50.0%) patients had Arg132His mutation, 9/28 (32.1%) Arg132Cys, 4/28 (14.3%) Arg132Ser and the remaining one had a Arg132Gly change. 6 of *IDH1*-positive patients had also *FLT3*/ITD. 53/296 (17.9%) cases carried mutations in *IDH2* gene; the majority of them (39/53; 73.6%) had Arg140Gln mutation, 13/53 (24.5%) harboured Arg172Lys change and one patient carried a previously undescribed alteration Gly145Trp. 11/53 cases were *FLT3*/ITD positive. None of the *IDH* mutations influenced WBC counts at diagnosis. Neither *IDH1*, nor *IDH2* mutation worsened the chance for reaching complete remission (CR): 15/27 (55.5%) *IDH1*-positive cases receiving standard induction treatment achieved CR as well as 30/47 (63.8%) cases with *IDH2* mutation and 126/208 (60.6%) without any *IDH* alteration. 10/15 (66.7%) patients harbouring *IDH1* mutation suffered a relapse, while only 59/125 (47.2%; P=0.362) cases without any *IDH* mutation and 9/30 (30.0%; P=0.009) carrying *IDH2* change relapsed. The difference was more obvious, when *FLT3*/ITD positive cases were excluded from analysis (66.7% patients with *IDH1* mutation relapsed vs. 37.5% relapsing cases with wt *IDH* and 26.1% of *IDH2*-positive ones). Patients with mutated *IDH1* had significantly shorter overall survival (OS) than cases without this mutation (P=0.048) or cases with *IDH2* mutation (P=0.026). OS 2 years after the diagnosis was 18.8% in cases with mutated *IDH1*, compared with 35.2% and 39.3% in patients without any *IDH* mutation and *IDH2*-positive ones, respectively.
Summary and Conclusions: We detected *IDH* mutations in 27.4% of patients with intermediate-risk cytogenetics. *IDH1*-positive patients had significantly shorter OS in comparison with *IDH* negative ones as well as comparing to *IDH2*-positive cases. This may result from a slightly lower CR rate on one hand and higher incidence of relapses in *IDH1* group on the other. *IDH2* mutations did not influence the prognosis of AML patients whatsoever. Supported by Research project 00023736 of Ministry of Health of Czech Republic and grant RVO VFN64165.

E931

DISTINCT EPIDEMIOLOGIC EXPOSURES AND PROGNOSTIC FACTORS FOR SURVIVAL IN OLDER ADULTS AGE ≥70 YEARS RECEIVING LOW INTENSITY THERAPY FOR ACUTE MYELOID LEUKEMIA (AML)

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Background: Many older adults age ≥70 years with AML were not previously candidates for intensive remission induction therapy and consequently the epidemiology and prognostic factors for overall survival (OS) have not been studied systematically in this population. However many now receive lower intensity treatment strategies, with distinct patterns of OS. We sought to study prognosis, clinical outcome and role of epidemiologic risk factors for AML development in this population.

Aims: The primary study aim was to evaluate prognostic factors for overall survival after AML and the incidence and contribution of epidemiologic exposures in older (≥70yrs) versus younger adults, and following intensive vs. low intensity therapy.
Methods: Epidemiologic exposures associated with AML development [including family history of hematologic malignancy (FHx); prior myelodysplasia (MDS); secondary AML (sAML); obesity (BMI ≥30kg/m^2); smoking; history of farm habitat or toxin exposure; and medical history, medications and prior immunosuppressive therapy (IST)] and clinical risk factors associated with OS were studied in a well characterized and non-selected cohort of n=295 consecutive AML patients diagnosed and treated at Mayo Clinic Florida & Arizona from 2002-12. Patients were treated in accordance with existing institutional algorithms, and central cytogenetics testing was performed at Mayo Clinic in all cases. Single variable Cox proportional hazards regression models, testing interaction p-values between groups (Age ≥70 vs. ≤69 yr.; intensive therapy vs. non-intensive or supportive care), was performed to evaluate differences in associations with OS. Fisher's exact test (or Wilcoxon rank sum test) was used for comparisons of patient characteristics.
Results: Older patients had a lower incidence of favorable risk cytogenetics, and were more likely to have prior MDS (p=0.032); sAML (p=0.026); a comorbidity index score (HCTCI) ≥3 (p<0.001); ECOG performance status (PS) >1 (p<0.001); and history of aspirin (p=0.001), statin (p=0.004) and metformin use (p=0.047) (but not acetaminophen). No other major difference in the incidence of epidemiologic exposures was observed between groups. Older patients were significantly less likely to receive intensive therapy (38% vs. 90%, P<0.001). Significant differences in prognostic factors for OS were observed. sAML was strongly associated with inferior OS in younger (Hazard Ratio (HR) 2.00, 95% Confidence Intervals (CI) 1.32-3.03) but not older adults (HR 1.09, 95%CI 0.75-1.58) (p interaction =0.030). In contrast, ECOG PS >1 was associated with OS in older (HR 2.39, 95%CI 1.67-3.43) but not younger patients (HR 0.97, 95%CI 0.60-1.56) (p interaction =0.003). Intensive therapy had a similar effect on OS in both groups (<70yr, HR 0.17, 95%CI 0.07-0.40; ≥70 yr, HR 0.21, 95%CI 0.13-0.33) (p interaction =0.77). Poor risk cytogenetics was associated with inferior OS in both groups, however cytogenetically normal AML was adverse in older (HR 5.71, 95%CI 1.35-24.12) but not younger (HR 1.69, 95%CI 0.58-4.90) adults. Other epidemiologic exposures (smoking, obesity, acetaminophen, IST, Farm habitat) did not affect OS, with the exceptions of FHx and Toxin exposure both associated with inferior OS after intensive therapy (HR 1.5, 95%CI 0.88-2.54 and HR 2.33, 95%CI 1.30-4.15, respectively) but not after low intensity/supportive care (HR 0.53, 95%CI 0.23-1.22 and HR 0.70, 95%CI 0.22-2.21, respectively) (p interaction =0.047 and =0.085, respectively).
Summary and Conclusions: Patients age ≥70 years and those receiving lower intensity therapy demonstrate a distinct profile of epidemiologic exposures, and prognostic factors for OS differ from younger adults. This suggests that prognostic factors in younger patients receiving intensive therapy cannot be directly extrapolated to this population, and prospective studies are needed to evaluate unique etiologic and prognostic factors for older adults.

E932

CHARACTERISTICS OF ACUTE MYELOGENOUS LEUKEMIA PATIENTS WITH GATA2 MUTATIONS

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Background: The zinc-finger transcription factor GATA2, located on chromosome 3q21, serves an essential role in the development and regulation of hematopoietic stem cells and myeloid differentiation. Acquired and inherited GATA2 mutations leading to decreased GATA2 transcription have been described in patients with myeloid malignancies, often in the setting of a hypocellular marrow with atypical megakaryocyte morphology. In addition to a predisposition to myeloid malignancies, GATA2 deficiency is characterized by deficiencies in B-lymphocyte, monocyte, NK and dendritic cells and a propensity for mycobacterial, fungal and viral infections, as well as lymphedema and pulmonary disease and propensity for venous thromboembolic disease among other described clinical manifestations.
Aims: The aim of our present study was to investigate the frequency, characteristics and outcome of acute myelogenous leukemia (AML) patients with GATA2 mutations.
Methods: We performed a database search for GATA2 mutations in AML patients from 2013 to 2014; conveyed as part of our institutional Next-Generation Sequencing (NGS) platform. All patients signed an informed consent form approved by the University of Texas MD Anderson Cancer Center institutional review board. Medical records were retrospectively reviewed.
Results: Among 233 patients with AML, 16 patients were identified to have GATA2 sequence variations. Six patients with previously reported non-pathogenic population polymorphisms (2 P161A, 1 G237D and 3 P250A) were excluded from further analysis. Data from 10 (4.3%) patients were collected and analyzed; clinicopathologic characteristics of these 10 patients are provided in Table 1. Median age was 53 (range 22-79) years old, with 4 patients under the age of 35 years. No patient had a prior known diagnosis or family history of GATA2 syndrome or clinical history of frequent infections. Complete blood count at diagnosis demonstrated a median WBC 29.3x10^3 (3.3-215.9x10^3)/uL, peripheral blast 37(0-95) %, Hemoglobin 9.25 (5.8-11.30) g/dL and platelet 72.5x10^3 (8-181x10^3)/uL. By bone marrow morphology, 4 patients were classified as AML without differentiation, 3 as AML with differentiation, 1 myelomonocytic leukemia and 2 as AML with MDS-related changes. Patient 4 with dysplastic changes had GATA2 L359V that was previously reported to cause myelomonocytic differentiation in blastic transformation of chronic myelogenous leukemia. Cytogenetics revealed a diploid karyotype in all evaluable patients except one patient with isolated deletion 5(q22q35) in 10 out of 20 metaphases. GATA2 mutations were always found in the presence of other somatic mutations, with a median number of 3 (range 2-6) mutations. Of significant interest, CEBPA mutations were identified in 6 (60%) of patients with double-CEBPA mutations in 5 of the 6 patients. With a median of 9.5 (2-14) months, 7 patients are alive in first complete remission status post high-dose cytarabine based induction chemotherapy; Patient 9 was additionally consolidated with a stem cell transplant in CR1. Patient 4 is alive 10 months into decitabine therapy with stable disease not requiring transfusion. Two patients have died; one of infectious complications after a cord-blood stem cell transplant in remission and the other had multiorgan failure in the setting of relapsed disease.

Tabella 1.

Table 1. Clinical characteristics of the AML patients with GATA2 mutations.

	Age	Sex	Family Cancer History	Presenting event	AML	Follow Up (months)	Outcome	Infectious Episodes	cytogenetics	GATA 2 mutation exon	GATA 2 mutation amino acid	CEBPA	FLT3	DNMT 3A	RAS	NPM 1	WT1	ASXL 1	MLL	TET2	IDH1 IDH2	IDH2 R140
1	52	F	father-kidney	sinusitis	M2	10+	CR	influenza A	diploid	3	S152N					○						
2	22	F		fatigue	M1	7+	CR	perirectal cellulitis	N/A	4	G320C	double										
3	59	F	father: pancreas cousin leukemia	abnormal CBC on annual exam	M1	2+	CR	neutropenic fever	diploid	4	L321H	double								○	IDH1	
4	79	M	daughter-breast mother-breast	bacteremia after vascular procedure	dysplastic	10+	SD	klebsiella, E coli bacteremia stenotrophomonas pneumonia	del5	5	L359V							○				
5	59	F	sister-colon	pharyngitis	M4	10	CR died post SCT	Stomatococcus bacteremia, pneumonia, Pseudomonas cellulitis	diploid	5	R362Q			○	KRAS NRAS	○						
6	33	M		pharyngitis	M2	2+	CR	Streptococcal pharyngitis neutropenic fever	diploid	5	R362Q	double			NRAS							
7	29	M	mother-breast	cellulitis	M2	10+	CR	Corynebacterium bacteremia cellulitis	diploid	5	R362_N363insV	double		ITD		○						
8	54	F		back pain	dysplastic	9	Relapsed, died of sepsis	Staphylococcus bacteremia	diploid	5	A372T	○		ITD	○	NRAS						○
9	58	M	mother-CLL	DVT	M1	14+	CR, SCT	CMV reactivation	diploid	5	A372T			D835			○			○		
10	24	M		pharyngitis	M1	7+	CR	pharyngitis	diploid	5	L375I	double										

Summary and Conclusions: We identify the presence of GATA2 mutations in approximately 4% of newly diagnosed AML patients. GATA2 mutations are associated with younger age at AML diagnosis, diploid cytogenetics, an apparently favorable outcome, and notably a strong correlation was seen with concomitant CEBPA mutations suggesting cooperativity. Evaluation for familial inheritance of the presumed somatic GATA2 and/or CEBPA mutations is ongoing in select families.

E933

DAY-14 LAIP ON BONE MARROW PREDICTS COMPLETE RESPONSE AND DRIVE EARLY SALVAGE CHEMOTHERAPY IN AML: RESULTS OF MULTIPARAMETER FLOW CYTOMETRY AND WT1-RNA QUANTIFICATION

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Background: In acute myeloid leukemia (AML) response to induction chemotherapy (CT) is evaluated by bone marrow (BM) morphology and cytogenetics. Real-time quantitative PCR (RQ-PCR) and multiparametric flow citometry (MFC) are sensitive techniques to assess minimal residual disease (MRD). Early evaluation of response at day14 (d14) predicts the achievement of complete remission (CR). Most studies rely on BM morphology at d14 but this timepoint is often not evaluable for cytological blast count. Few data on BM blast clearance by MFC and/or RQ-PCR are available; the correlation between these two assays is unknown in this setting. Moreover, it is to be established if early intensification with a second CT cycle could increase CR rate or reduce the time to achieve CR in pts with disease persistence at d14 BM.
Aims: Objective of the study were i) to compare the two assays to identify the more sensitive/specific assay; ii) to compare the outcome of pts who received or did not receive an early intensification for d14 disease persistence, if in appropriate clinical conditions.
Methods: We retrospectively evaluated 33 newly diagnosed AML pts who received induction CT at our Center between 02/2009 and 02/2015, and for whom analysis of BM both at d14 and after hematologic recovery was available. We evaluated MFC (d14-LAIP) and WT1 quantification by PCR (d14-WT1) to predict the response to induction at d14, in particular when morphologic blast count is not evaluable.
Results: 25 pts received "3+7" and 8 pts the ICE induction regimen. After d14 BM evaluation, 6 pts received early reinduction CT (FLAG-Ida) starting at d16 (median), 27 pts did not receive further therapy before d28 BM evaluation. Overall CR rate was 64% (21 pts), PR/NR 36% (12 pts), d60 treatment related mortality (TRM) 3% (1 pt). At d14, morphologic blast count was not evaluable in 13 (39%) cases due to marrow aplasia, in 1 case (3%) blasts were <5%, in 5 (16%) <20%, in 14 (42%) ≥20%; by MFC (33 evaluable), in 15 pts (45%)

blasts were ≤2%, in 18 (55%) >2%; by PCR (27 evaluable), in 7 pts (26%) WT1 was <250cp/10^4ABL, in 20 (74%) ≥250cp/10^4ABL. At d28 of the 13 pts with d14 aplastic marrow, 10 (77%) achieved CR and 3 (23%) PR/NR, all the 6 pts with d14 blasts 5-19% achieved CR, of the 14 pts with d14 blasts ≥20%, 5 (36%) achieved CR and 9 (64%) PR/NR. Analysis of the paired results from nadir to recovery revealed that d14-LAIP 2% had a positive predictive value (PPV) of 83% and negative predictive value (NPV) of 93%, with 90% sensitivity and 87% specificity; d14-WT1 250 had a PPV of 53% and NPV of 86%, with 89% sensitivity and 46% specificity. The d14-LAIP was strongly associated with CR after induction (p=0.00007). Considering the 14 pts with d14 blasts ≥20%, 5/6 (83%) who received d14 FLAG-Ida obtained CR after a median time of 43 days, 3 pts did not receive further therapies, 3/5 (60%) who received d30 reinduction CT obtained CR after a median time of 54 days (p=0.23).

Summary and Conclusions: D14-LAIP proved to be superior to morphology and d14-WT1 in terms of feasibility and predictive value of subsequent CR. Even in one-third of pts who had an early morphologic response not evaluable due to marrow aplasia MFC proved to be a useful tool with a 100% applicability. Correlation between the two assays was >90%. Our data confirm the prognostic value of d14 BM evaluation and suggest that MRD detection, also in aplasia, could drive early salvage CT. Our results with early intensification are promising; clinical impact of this strategy will be investigated in larger studies.

E934

IDENTIFICATION OF PROGNOSTIC FACTORS RELATED TO THERAPEUTIC OUTCOMES OF CHEMOTHERAPY AND ALLOGENEIC STEM CELL TRANSPLANTATION IN 120 ACUTE MYELOID LEUKEMIA PATIENTS WITH MONOSOMAL KARYOTYPE IN KOREA

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Background: Monosomal karyotype (MK) was known to be associated with a dismal prognosis in acute myeloid leukemia (AML) and provide additive prognostic value in AML patients with complex karyotype.

Aims: In this study, we evaluated the prognostic significances of clinical characteristics and additive chromosomal abnormalities, which are associated with therapeutic outcomes of chemotherapy and allogeneic stem cell transplantation in ML AML.

Methods: Clinical data on AML in Korea has been collected through AML registry managed by the AML/MDS Working Party of the Korean Society of Hematology, and a total of 3,041 AML patients were registered at the time of analysis. From Jan. 2007 to Dec. 2011, 184 patients who met the definition of MK-AML were selected for this study, and 120 of 184 received induction therapy were retrospectively analyzed.

Results: The MK-AML accounted for 6.1 % of total AML in our registry, and the median age of was 56 years (range, 17-82 years). Median follow up duration for 120 patients was 40.5 months. The most frequent cytogenetic abnormality was -7/7q deletion (46.7%), and -5/5q deletion (40.8%) and 17p abnormality (17.5%) followed. MK defined by one single autosomal monosomy with at least one structural chromosomal abnormality was shown in 45 patients (37.5%), and MK defined by equal or more than two autosomal monosomies in 75 patients (62.5%). Fifty-two patients (46%) achieved complete remission (CR) after 2 cycled of induction chemotherapy, and early mortality rate was 16% (19 patients). Univariate and multivariate analysis revealed that ≥10% cells with normal metaphase was an independent good prognosis factor for CR achievement. Older age (≥60) and 17p abnormality were significant risk factors for early mortality. The 3-years overall survival (OS) and progress-free survival (PFS) for all patients was 20.8% and 7.9%, respectively. Univariate and multivariate analysis revealed that single monosomy, achievement of CR, and ≥10% cells with normal metaphase were independent good prognostic factors for PFS and OS. De novo type of AML and absence of 17p abnormality were another good prognostic factors for PFS and OS, respectively. Total 44 patients were finally received allogeneic hematopoietic stem cell transplantation (alloHSCT). Thirty-three of 44 patients were in CR1 or CR2 status, and 11 in relapsed or refractory status at alloHSCT. The 3-years overall survival (OS) and progress-free survival (PFS) for these transplanted patients was 38.6% and 24.1%, respectively. For 52 patients achieving CR, single monosomy, de novo type of AML, and alloHSCT at CR1 were significant good prognostic factors for relapse-free survival. Interestingly, as shown in Figure 1, MK-AML with one single monosomy receiving alloHSCT at CR1 showed better prognosis (3-yr OS and DFS rate was 64.6% and 48.1 %, respectively)

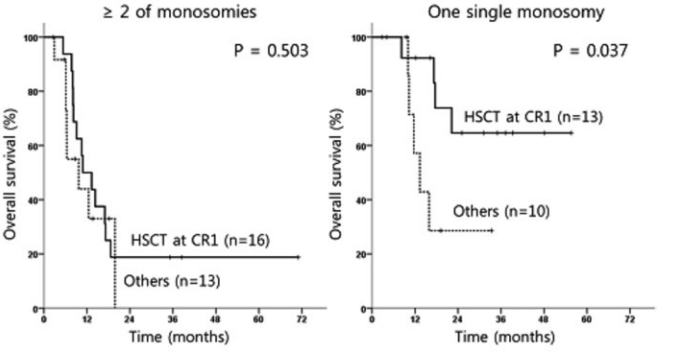

Figure 1. Overall survival according to type of MK and treatment modalities.

Summary and Conclusions: MK-AML, one of subtypes of AML accounting for about 6 % of AML in Korea, showed dismal prognosis with lower CR rate, PFS, and OS. However, compared to patients showing equal or more than 2 monosomy or <10% cells of normal metaphase, MK showing single monosomy or ≥10% cells with normal metaphase showed better prognosis in our analysis. AlloHSCT at the first CR should be strongly recommended to MK-AML patients with better prognostic factors, and for those without, more prudent decision is required. Furthermore, biologic mechanism of these better prognostic cytogenetic features should be investigated.

E935

NO INCREASE IN LEVEL OF FATIGUE AMONG LONG-TERM SURVIVORS WITH ACUTE MYELOID LEUKEMIA

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Background: At least 60% of patients <40 years and about 40% of patients 41-60 years of age can expect to become long-term survivors if diagnosed with acute myeloid leukemia (AML). These survivors are at risk of potential long-term disease- and treatment-related side complications due to intensive chemotherapy treatment and hematopoietic stem-cell-transplantation (HSCT). Most studies on long-term survivors have been performed in the HSCT population. Impaired sexual function, increased fatigue and psychological distress have been described among patients treated with HSCT when compared to patients receiving conventional chemotherapy only. There are few comparisons between AML survivors and the general population.

Aims: The aim of this study was to assess the impact of AML and its treatment on fatigue in long-term survivors in a population-based setting and with a matched control population.

Methods: Adult AML patients diagnosed 1973-2003 within the area of the Leukemia Group of Middle Sweden were identified in the Swedish Cancer Registry and those who died within 5 years of their diagnosis were excluded from the study using theCauses of Death Registry. Survivors were invited to participate by letter. Participants for the comparison group were identified through Statistics Sweden. For each survivor, 3 controls matched for sex, year of birth and residence were identified. Fatigue was measured using the Multi-dimensional Fatigue Inventory (MFI-20), a validated instrument consisting of five subscales of fatigue: general fatigue, physical fatigue, reduced motivation, reduced activity, and mental fatigue.

Results: 161 survivors were invited to participate; 100 accepted and were included (response rate 62%; 55 women). 150 controls accepted participation (response rate 31 %); 96 women. 27 patients had been treated with HSCT. AML survivors did not report a higher level of fatigue than the comparison group. We investigated whether there were any differences in the reported fatigue levels associated with the treatment applied. Radio therapy (*i.e.* total body irradiation as part of conditioning treatment prior to HSCT) was associated with an increased level of reduced motivation (p=0.044). When adjusting for age and co-morbidities using a linear regression analysis, radio therapy was associated with reduced motivation (p=0.010) and mental fatigue (p=0.044). We did not find any association between allogeneic HSCT and increased fatigue level in any dimension, neither in crude analyses or when adjusting for age and co-morbidities. Presence of chronic graft versus host disease (GvHD) or previous recurrence of AML was not associated with a higher level of fatigue. Among patient characteristics, we found a strong association between the presence of co-morbidities and general fatigue, physical fatigue and reduced activity. All five fatigue

dimensions were also associated with anxiety and depressive symptoms. There was no difference between male and female AML survivors.

Table 1. Five dimensions of fatigue among AML survivors and comparison group.

	AML survivors median (range)	Comparison group median (range)	p-value*	Crombach's
General fatigue	11.00 (4-20)	10 .00 (4-20)	0.826	0.84
Physical fatigue	10.33 (4-20)	8.00 (4-20)	0.186	0.90
Reduced activity	9.00 (4-20)	9.00 (4-20)	0.637	0.89
Reduced motivation	7.00 (4-19)	7.00 (4-18)	0.362	0.72
Mental fatigue	8.00 (4-19)	9.00 (4-20)	0.800	0.76

Summary and Conclusions: In this cohort of long-term AML survivors, most patients were treated with chemotherapy only. After more than five years after diagnosis, this group of survivors did not experience increased fatigue when compared to the general population.

E936

LONG-TERM REPORT OF A PILOT STUDY EVALUATING CLOFARABINE COMBINED WITH INTERMEDIATE-DOSE CYTARABINE AS CONSOLIDATION THERAPY IN PATIENTS WITH NON-FAVORABLE ACUTE MYELOID LEUKEMIA IN FIRST REMISSION

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Background: In younger patients with AML in first complete remission (CR), a standard consolidation option relies on repeated cycles of high-dose cytarabine (HDAC), according to the CALGB schedule (Mayer, NEJM, 1994). Nevertheless, relapse incidence remains high, especially in those with non-favorable AML. Combination of 40mg/m^2/d clofarabine d2-6 with 1000 mg/m^2/d cytarabine d1-5 has been associated with promising response rate in high-risk AML patients (Faderl, Blood 2005 & 2006).
Aims: We conducted a pilot study to assess the feasibility of this "CLARA" combination as first CR consolidation in younger patients with non-favorable AML and no identified donor for allogeneic stem cell transplantation (SCT). We report here on long-term outcome and safety of this experimental approach.
Methods: Between 2004 and 2007, 23 patients aged 22 to 63 years (median, 50) with non-favorable AML in first CR (10 intermediaire I, 2 intermediaire II, and 11 adverse risk, according to ELN classification) received 3 CLARA cycles as consolidation, without further maintenance. All had previously received remission induction according to ALFA-9802 protocol (Thomas, Blood). All received oral antibacterial prophylaxis with levofloxacin or amoxicillin, but none received anti-fungal prophylaxis.
Results: Eight patients received the planned 3 CLARA cycles; 13 patients received only 1 or 2 cycles because either they became eligible for SCT (5 patients) or presented severe complications (8 patients). Two patients received a reduced clofarabine dose (30 mg/m^2/d) from the first cycle. Six patients are still in CR1 (26%) with a median follow up of 59 months, including 3 non-transplanted patients. In addition, 2 patients are still in CR2 with a median follow-up of 51 months. Infectious adverse events were frequently reported: 10 non documented infections, 7 sepsis documented with Gram-negative bacteria, 12 sepsis documented with coagulase-negative staphylococcus, 2 clostridium colitis, 3 viral reactivation (CMV), 7 pulmonary invasive aspergillosis (4 possible and 3 probable, according to EORTC classification) and 1 candidemia. Two patients required ICU hospitalization and 1 patient died from infection after the second CLARA cycle. Liver cytolysis was also frequently reported (7 Grade III and 7 Grade IV episodes) in 12 patients.
Summary and Conclusions: Administration of repeated CLARA consolidation cycles is a promising approach in younger patients with non-favorable AML in first CR. Nevertheless high rate of infections (especially pulmonary aspergillosis) led us to reduced the clofarabine dose from 40mg/m^2/d to 30mg/m^2/d and to introduce systematic anti-fungal prophylaxis in the following and not yet reported randomised ALFA-0702 trial, which compared prospectively CLARA to HDAC consolidation in similar patients.

E937

RETRO-TRANSCRIPTION LOOP MEDIATED ISOTHERMAL AMPLIFICATION (RT-Q-LAMP) AS A RAPID AND ROBUST MOLECULAR ASSAY FOR THE DIAGNOSTIC DETECTION OF PML-RARA FUSION TRANSCRIPTS

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Background: The accurate and timely molecular identification of the Acute Promyelocytic Leukemia (APL) associated PML-RARa fusion gene is mandatory to confirm the morphologic suspect of APL and rapidly initiate tailored therapy, thus reducing the risk of potentially fatal hemorrhagic complications. The molecular analysis is currently based on RT-PCR or RQ-PCR assays, which can suffer from poor quality or insufficient amount of the extracted RNA, thus delaying the diagnosis.
Aims: Improvement of the molecular diagnosis of APL by introducing a novel ultra rapid and reliable screening test, robust for suboptimal RNA samples.
Methods: RT-Q-LAMP PML-RARa is an innovative, isothermal, non-PCR-based method for direct RNA amplification, extremely sensitive and specific. It consists of two fluorescent multiplex assays: one specific for the most frequent transcript isoforms (bcr1 and bcr3) and one for the infrequent bcr2 type. The reaction mix of both assays contains also additional primers to detect the endogenous GUSb housekeeping RNA as internal control. The reaction is performed in 40 minutes, with the amplification of positive samples requiring about 15 minutes, into the Liaison IAM instrument (DiaSorin) at constant temperature with a real-time monitoring of fluorescence. The data obtained are directly elaborated by the instrument that returns objective results and the proper discrimination of the transcript type.
Results: The triplex (bcr1-bcr3-GUSb) and duplex (bcr2-GUSb) RT-Q-LAMP assays have been tested on 33 PML-RARa positive and 20 PML-RARa negative clinical samples. In all cases RNA was extracted with the phenol-chloroform method and RT-PCR for PML-RARa was performed according to the Europe Against Cancer (EAC) protocol. All RNA samples presented heavy chemical contamination (ratio of 260/230 in the range of 0.29-0.99). In addition we tested 7 clinical samples that didn't produce an acceptable amplification result for the RT-PCR, due to strong RNA degradation (n=3), inhibition (n=2), and very low RNA amount availability (5ng/ul, n=2).The RT-Q-LAMP PML-RARa assays correctly amplified all the 53 samples with low 260/230 ratio, giving concordant results with those of conventional RT-PCR. Moreover, the 7 samples for which RT-PCR analysis was not possible, were all successfully amplified with RT-Q-LAMP in less than 30 minutes.
Summary and Conclusions: The fluorescent RT-Q-LAMP assays for PML-RARa fusion transcript detection is highly specific, sensitive and rapid. The rapidity and specificity of RT-Q-LAMP associated with the robustness for low concentrated and degraded samples, allow to reach a timely diagnosis in all cases, avoiding the need for analysis repetition or new sample extraction, as actually required by conventional RT-PCR. In addition, RT-Q-LAMP PML-RARa assays demonstrated to be not affected by the common PCR inhibitors, allowing the rapid amplification of samples presenting chemical contamination. These features increase the confidence in the results and make the assays suitable for even not highly specialized laboratories.

E938

THE ROLE OF AZACITIDINE IN THE TREATMENT OF ELDERLY PATIENTS WITH AML - RESULTS OF A RETROSPECTIVE MULTICENTER STUDY

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Background: Older patients with AML have significant comorbidities, poorer performance status.Overall results of intensive chemotherapy remain poor.Azacitidine (AZA) has significant activity in MDS.Use of AZA was associated with improved OS when compared to low dose cytarabine in patients with high risk MDS, including those with marrow blast counts ranging from 20 to 30%. In untreated or relapsed/refractory AML, few studies have also shown significant response rates of AZA.However, there is limited data showing efficacy of AZA in AML patients with >30% bone marrow (BM) blasts.
Aims: In this retrospective multicenter study, we aimed to investigate the efficacy and safety of AZA in elderly patients with AML (including patients with >30% BM blasts) defined according to WHO.
Methods: Between 2009-2014,≥60 year-old patients with AML from 16 centers

in Turkey were included.Eligibility criteria included ≥60 years-old AML patients treated with at least one dose of AZA.Demographic data, comorbidities, ECOG status, transfusion-dependency, cytogenetic risk status, blast ratios, treatment prior to AZA, adverse events, treatment responses were recorded.Assessment of response was performed after 4 cycles.
Results: 130 patients (72 men) receiving AZA were included into study.Median age was 73 (60-88 years).ECOG performance status (ECOG-PS) was ≥2 in 54.6%, there were comorbidities in 66.2% of cases (89.5% had <3 comorbidities).LDH was ≥225 IU/L in %75.4 and 40.8% had WBC count>10x109/L/mm3. 80 patients (61.5%) had >30% BM blasts.112 patients were transfusion-dependent. 50.8% had an intermediate karyotype.Patients received AZA for a median of 4 cycles (range 1-21). Initial ORR (including complete remission CR/CRi/PR) was 36.2%. Any hematologic improvement (HI) was documented in 37.7%.HI was also documented in 27.1% of patients who were unresponsive to treatment.Median overall OS was 18 months for responders, 12 months for non-responders (p=0.005).In unresponsive patient group, any HI has also improved OS compared to patients without any HI (median OS was 14 months versus 10 months, p=0.068).In univariate analysis of following parameters had significant effect on both treatment response and OS: ECOG-PS, number of AZA cycles and any HI. ECOG-PS≥2 was associated with a worse response to AZA (29.7% versus 53.2%, p=0.012) and with a worse OS (10 [95% CI 8.1-11.9] months versus 14.1 [95% CI 7.8-20.3] months,p=0.034).In patients receiving ≥5 courses of AZA versus <5 courses, response rate and OS were higher (61.9% versus 28%, p<0.001 and 14.1 [95% CI 8.5-19.6] months versus 9 [95% CI 4.0-14.0] months,p=0.011, respectively).Response rate and OS increased with any HI versus without any HI (59.6% versus 27.1%,p<0.001 and 18 [95% CI 10.0-26.0] months versus 10 [95% CI 5.1-14.9] months,p=0.002).OS was higher in patients with LDH<225 IU/L compared to patients with LDH≥225 IU/L (19 [95% CI 12.1-25.9] months and 11 [95% CI 8.6-13.4] months, respectively,p=0.018), but LDH level had no impact on treatment response (ORR is 38.5% versus 41.9% for LDH</≥225 IU/L, respectively,p=0.758).Transfusion-dependence prior to AZA was associated with a worse response to AZA (36.3% versus 66.7%,p=0.025) and with reduced OS, which was however not statistically significant (12.3 [95% CI 9.8-14.8] months versus 20 [95% CI 4.2-35.9] months,p=0.077).ECOG<2, increasing number of AZA cycles (≥5 courses) and any HI predicted better OS (Figure1). Age, AML type, BM percentage had no impact.Grade 3-4 neutropenia, thrombocytopenia anemia were documented in 34.6%,40.8%,39.2% of patients, respectively.Non-hematologic toxicities were mild;most common adverse events being mucositis, injection site pain, nausea.

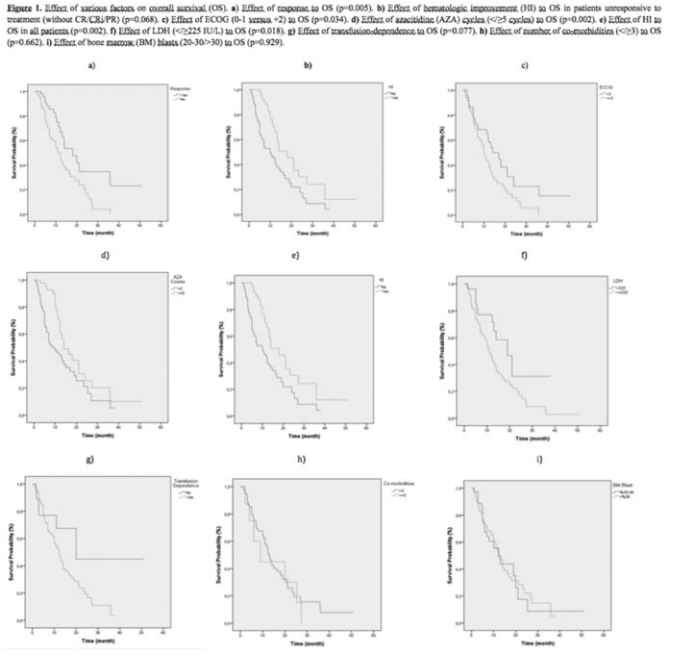

Figure 1.

Summary and Conclusions: In conclusion; AZA is effective and well tolerated in elderly, comorbid AML patients with fewer required erythrocyte and platelet transfusions, irrespective of BM blast count. And HI should be considered sufficient response to continue treatment and treatment should not be interrupted since OS and response to treatment increase with the increasing number of AZA cycles.

E939

CORE-BINDING FACTOR IN CHILDHOOD ACUTE MYELOID LEUKEMIA

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Background: Core-binding factor-acute myeloid leukemia (CBF-AML) (t[8;21] or inv[16]/t[16;16]) represents a favorable cytogenetic AML subgroup and have favorable prognosis
Aims: We reported the results of therapeutic and prognostic pediatric AML with rearrangement of CBF genes in a single center from Tunisia.
Methods: Our study is retrospective; it concerned patients under 20 years with AML with rearrangement of CBF genes de novo during the period between January 2006 and December 2013.The treatment protocol includes: a course of 7 +3 induction (Arac200mg/m2/day+DNR60mg/m2/day) followed in case of complete remission (CR) by 3 courses of consolidation: *1st course: Arac 6g/m^2/day (3d) +Mitoxantrone12mg/m2/day(3d), 2nd course: Arac200mg/m^2/day (7d) +DNR50mg/m^2/day VP16100mg/m2/day (5d) * 3rd course: Arac 6g/m2/day (3d) +L-asparaginase 6000UI/m2 in day 5. Allogenic bone marrow transplantation is indicated for patients who have a HLA-compatible family donor. Kaplan-Meier method is used to estimate overall survival (OS) and event-free survival (EFS).
Results: Of the 33 patients under 20 year with acute myeloid, 10 patients (33%) enrolled in our study with CBF genes. The sex ratio is 1,5. The mean age is 12 years [6 -18 years]. Distribution according to the FAB classification is as followed: 8 patients with AML2, 1 patient with AML4 and 1 patient with AML6. Cytogenetic abnormalities are found in 9 cases and 1 case have normal karyotype. Eight patients (80%) have t(8,21), one patient has inv 16 and one case has normal karyotype with positive CBF tanscript. Complete remission rate (RC) is 100%, relapse rate is 30 %. The bone marrow transplantation was performed in one patient in RC1. A second remission is achieved in 2 patients (67%). Overall survival, event-free survival and relapse-free survival at 5 years are respectively 89% and 65,5%.
Summary and Conclusions: The cytogenetic t(8;21) is predominant in CBF AML in our series (80%). Childhood CBF- AML are associated with a high CR rate and a good long-term prognosis in our series similar the literature.

E940

PROGNOSATIC RISK SCORE FOR HAEMORRHAGIC EARLY DEATH IN ACUTE PROMYELOCYTIC LEUKEMIA

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Background: Hemorrhagic early death (HED), w
ith reported rates between 5 and 26.52%, is a major impediment in the managing of acute promyelocytic leukemia (APL). Although previous studies identified several prognostic factors for HED such as poor performance status (PS), high white blood cell (WBC) count, high peripheral blast count, serum lactate dehydrogenase (LDH), low fibrinogen level, platelet count and prolonged prothrombin time (PT), a predictive model for HED has not yet been elaborated.
Aims: To identify factors predictive of HED and to develop prognostic scoring system.
Methods: We analyzed data on HED in 85 newly diagnosed PML-RARA positive APL patients (median age 45 years, range 18-78; 36/49 female/male ratio) managed in the Clinic of Hematology from 2004 to 2014 with all-trans retinoic acid combined with anthracyclines. Central nervous system (CNS), retinal, pulmonary or gastrointestinal haemorrhages were considered a severe bleeding event. HED was defined as death from hemorrhage from the first day of hospitalization up to the end of the induction treatment. The following parameters were evaluated as risk factors for HED: age, ECOG PS, patient's (time from the first symptoms to seeking medical care), medical (time from the first medical contact to ATRA initiation) and treatment delay (time from hospitalization to ATRA initiation), severe bleeding, WBC count, peripheral blast count, platelet count, fibrinogen level, PT, D dimer, ISTH DIC score, morphological disease type, CD15 and CD56 positivity, additional cytogenetic abnormalities, PML-RARA isoforms and *FLT3-ITD* mutation. Pearson chi test and Fisher test were used to analyze differences for categorical data. For multivariate analysis a binary logistic regression model was constructed for EHD adjusting for 6 variables. A backwards elimination procedure was used to exclude redundant or unnecessary variables. Integer weights for the risk score were derived from logistic regression model. Prognostic score was validated via 10-fold cross validation.
Results: HED occured in 12/85 (14.12%) patients. Predictors of HED in univariate analysis were: ECOG PS ≥2 (P=0.010), severe bleeding at diagnosis (P=0.023), WBC >20x10^9/L (P=0.015), fibrinogen level <2 g/L (P=0.05), PT <50% (P=0.005), and ISTH DIC score ≥7 (P=0.031) as the most statistically significant cutoff points. Independent risk factors in multivariate analysis were: severe bleeding at diagnosis (P=0.041), WBC >20x10^9/L (P=0.018) and ISTH DIC score ≥7 (P=0.024). A clinical prognostic scoring system was then developed: absence of severe bleeding at diagnosis, WBC <20x10^9/L, ISTH DIC score <7=0, WBC ≥20x10^9/L=1 point and ITSH DIC score ≥7=2 points. Accordingly patients were classified in three groups: low risk=0 points, intermediate=1-

2 points and high risk ≥3 points. The HED rate between these groups (low 2.4%, intermediate 26.1% and high 100%) was significantly different (p<0.001).
Summary and Conclusions: The prognostic model developed in this study allows for the identification of the patients at high risk for HED, needing more aggressive supportive measures. Further large population-based studies for testing new score systems and refinements therapeutic approaches for APL patients in high HED risk are needed.

E941

THERAPY-RELATED ACUTE MYELOID LEUKEMIA IN ADULTS: IS AGE YOUNGER THAN 40 CLINICALLY RELEVANT?

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Background: Therapy-related myeloid neoplasms (t-MN) occur as the complication of chemotherapy (CT) and/or radiation therapy (RT), and account for approximately 10 to 20% of all cases of myeloid neoplasm in adults. t-MN represent distinct clinical entities, confer a worse prognosis when compared to de novo counterparts and have a high prevalence of adverse-risk karyotypes. One of the most serious entities comprising t-MN is therapy-related acute myeloid leukemia (t-AML).
Aims: Most of the studies published in the past have focused on elder patients, so we aim to investigate the features of t-AML in patients younger than 40 years of age as compared to their older counterparts.
Methods: A retrospective review of cases encountered between 2003 and 2013 was performed following IRB approval. All cases of AML with a prior history of CT/RT were included in the study (n=70). The clinical data including age, sex, medical history and the time interval between the treatment for prior solid or hematologic malignancy and the development of t-AML were recorded. Cytogenetic, molecular and/or FISH data for t-AML were available for sixty-one patients. The cases were divided into two groups. Group 1 consisted of t-AML patients 40 years of age or younger and Group 2 consisted of patients older than 40. A survival analysis was performed.
Results: Table 1 shows the summary of characteristics for groups 1 and 2. Group 1 included 12 cases with a median age of 32 (20-40 years) and the M:F ratio of 0.5. In this group, the most frequent prior malignancy was hematologic cancers (n=5, 42%); Hodgkin lymphoma being the most common, followed by sarcomas majority of which are Ewing sarcomas (n=3, 25%). Cytogenetic findings were abnormal in 10 of 12 patients (83%), with MLL gene anomalies being the most common finding (n=5, 42%), followed by 7q deletion (n=4, 33%). Group 2 consisted of 58 cases with a median age of 61 (41-86 years) and the M:F ratio of 0.7. In this group, the most frequent prior solid and hematologic malignances were breast cancer (n=19, 33%) and follicular lymphoma (n=9, 16%), respectively. Cytogenetics and FISH (combined) revealed 7q deletion as the most frequent abnormality (n=16, 28%) followed by *MLL* gene abnormalities (n=9, 16%). NPM1 and FLT3 internal tandem duplications were found in 5% of cases. The median interval between the last CT/RT and diagnosis of t-AML for groups 1 and 2 were not significantly different; 47 and 36 months, respectively. There was no significant difference in cell counts or bone marrow blast counts. The OS in two groups were significantly different; 28 vs. 9 months in Groups 1 and 2, respectively (p <0.05) (Figure 1). There was no significant difference in OS between patients >60 and =<60 years old; 8 vs. 9 months, respectively (p=0.225).

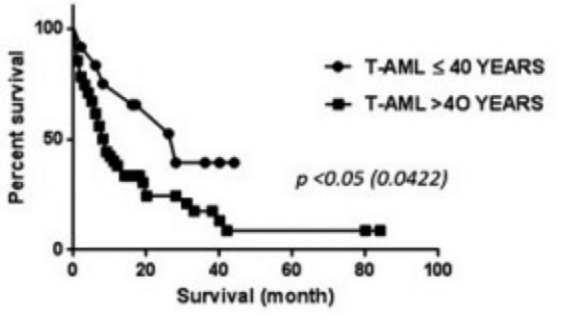

Figure 1. Survival analysis between Group 1 and Group 2.

Summary and Conclusions: The results of the current study show that age is an important prognostic factor regardless of the karyotypic abnormalities. In the two groups we analyzed, group 1 (=<40 years) showed more frequent association with poor prognostic karyotype when compared to group 2 (>40 years). There was no significant difference between two groups with regards to interval between prior treatment and t-AML, cell counts or blast count. t-AML being a dire complication in patients treated with cytotoxic therapy, prognostication is important to accurately predict the outcome in this group. Large scale studies are warranted to investigate other prognostic factors in addition to cytogenetic sub-classifications.

Tabella 1. Summary of patient char acter ist ics and outcomes.

	t-AML (Age =<40 years) (n= 12)	t-AML (Age >40 years) (n=58)
Age at diagnosis (median)	32 (20-40 years)	61 (41-86 years)
Prior radiation therapy	7 (58%)	27 (45%)
Prior chemotherapy	12 (100%)	53 (91%)
Prior malignancy		
Solid organ tumors	2 (17%)	33 (57%)
Hematologic malignancies	5 (42%)	22 (37%)
Germ cell tumors	1 (8%)	1 (2%)
Sarcomas	4 (33%)	0
Melanoma	0	1 (2%)
Other	0	1 (2%)
Interval between treatment and diagnosis of t-AML, median (months)	47	36
White blood cell count at diagnosis, median ($x10^3/\mu L$)	3.0	4.65
Absolute Neutrophil count at diagnosis, median ($x10^3/\mu L$)	0.15	0.85
Hemoglobin at diagnosis, median (g/dL)	7.9	8.6
Platelet count at diagnosis median ($x10^3/\mu L$)	44.5	43.5
Bone marrow blast count, median (%)	60	65
Molecular/karyotypic abnormalities at diagnosis (%)		
MLL	5 (42%)	9 (16%)
Del 7q	4 (33%)	16 (28%)
t(15;17)	0	2 (3%)
t(8;21)	0	2 (3%)
inv(16)	0	1 (2%)
NPM1	0	3 (5%)
FLT3-ITD		

E942

AZACYTIDINE THERAPY IN PREVIOUSLY UNTREATED ELDERLY AML PATIENTS: COMPARISON WITH CONVENTIONAL CHEMOTHERAPY IN A MATCHED-PAIRED ANALYSIS

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Background: The hypomethylating agent azacytidine (AZA) is increasingly used in acute myeloid leukemia patients (AML) with low marrow blast count or in elderly patients who are not eligible for intensive chemotherapy. We recently performed a retrospective analysis of AZA front line treatment in elderly AML patients and reported its efficacy and feasibility.
Aims: The aim of the present study was to compare the outcome of untreated elderly AML patients receiving AZA or conventional chemotherap by performing a matched-paired analysis.
Methods: We retrospectively analyzed the outcome of 42 elderly AML patients who received AZA as front line treatment in our Centre from June 2010 to December 2014. A control series of patients treated with conventional chemotherapy was selected through a matched-paired analysis. Matching variables were white blood cells count at diagnosis, age, karyotype, disease onset and sex. No significant differences were appreciable for those variables, whereas median age was significantly higher in AZA cohort than in FLAI-3 cohort (74 yy and 70 yy, respectively, p <0.05). Relevant comorbidities (diabetes mellitus, heart disease, hypertension, liver or lung diseases) were present in 70% of patients receiving AZA. AZA was delivered at the dosage of 75 mg/sqm s.c. with a 5+2 days schedule. Patients were given a median of 4 courses of treatment (range 1-27). Twenty-five patients (75%) received concomitant recombinant alpha erythropoietin therapy (40000 U once weekly). AZA therapy was continued until unacceptable toxicity or disease progression, regardless of response achieved. Conventional treatment consisted in a fludarabine-containing regimen with age-adjusted doses (FLAI-3 schedule: fludarabine 25 mg/mq, cytarabine 1000 mg/mq, idarubicin 5 mg/mq, days 1,2,3).
Results: Major infection during therapy were observed in 20/42 (47.6%) patients in AZA group and 15/42 (35.7%) in FLAI-3 group (p=0.356). Four and two patients died during induction in AZA and FLAI-3 group, respectively. Complete remission (CR) rates were significantly lower in AZA-treated patients (7/38, 18%), compared

to FLAI-3 (21/40, 53%) (p=0.002). Sex, disease onset, karyotype, WBC count and age did not significantly influence CR rates in neither of the two cohorts. Of note, none of the nine patients treated with AZA who presented with leukocytosis achieved CR, compared with 6/15 (40%) patients in the FLAI-3 cohort. However, this did not translate in a significant lower OS for patients with leukocytosis treated with AZA (2yy OS 50.4% and 24.2% for patients with WBC count lower or higher than 10.000/mmc, respectively, p=0,120). After a median follow-up of 25 months, 2-years overall survival (OS) was 36.2% in AZA group (median 14 months), significantly higher than FLAI-3 group (11.2%, median 9 months, p=0.048, Figure1). OS duration was significantly influenced only by achievement of CR in FLAI-3 cohort (p=0.000), whereas in AZA cohort was influenced only by younger age at diagnosis (p=0.048). Achievement of CR had only a borderline effect on OS duration in AZA cohort (p=0.050).

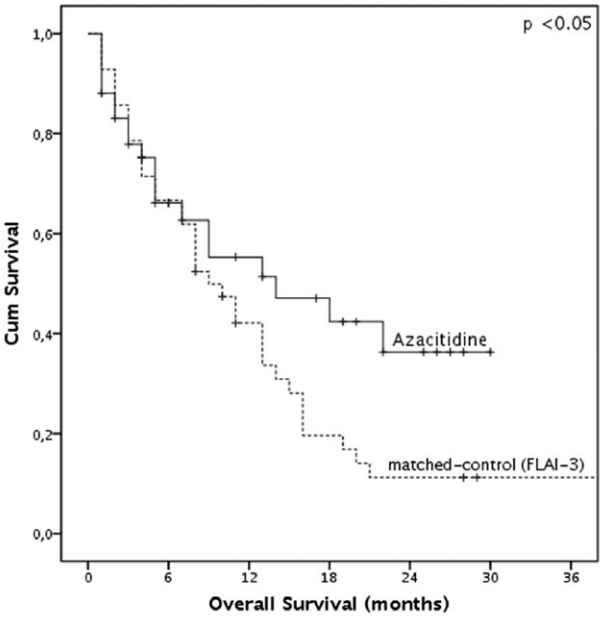

Figure 1. Overall survival according to treatment.

Summary and Conclusions: AZA was generally well tolerated, with acceptable toxicity profile, even in our cohort of very frail untreated AML patients. Even if CR rates were significantly lower when compared to a matched-paired cohort of conventionally-treated patients, OS rate were not inferior. Our data suggest that Azacytidine is a safe first-line therapy for frail AML patients, with comparable effectiveness with conventional intensive chemotherapy.

E943

COMBINATION OF CLOFARABINE AND CYTARABINE IN THE TREATMENT OF REFRACTORY OR RELAPSED ACUTE MYELOID LEUKEMIA

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Background: The use of combination chemotherapy is effective and curative in some patients with acute myeloid leukemia (AML). However, some of the newly diagnosed AML patients fail to achieve complete remission (CR) because of resistance to treatment and 40% of CR patients will relapse within 2 years. The prognosis of patients with primary refractory or relapsed AML is generally unfavourable, with salvage chemotherapy giving a remission rate of merely 10–40%. Clofarabine is a new generation deoxyadenosine analog that has shown some activity in relapsed or refractory AML. Because of the structural similarity between purine analogues, a putative synergism between clofarabine and cytarabine has been postulated. However data regarding efficacy and safety of clofarabine and cytarabine combination therapy in refractory and relapsed AML patients is limited.
Aims: To examine the efficacy and safety of sequential clofarabine and high dose cytarabine in adult patients with relapsed or refractory AML.
Methods: Data from 35 refractory or relapsed AML patients, at a median age of 39.4 years (range, 21-60 years), diagnosed between 2010 and 2014, were evaluated retrospectively. Clofarabine was administered as a 1-hour intravenous infusion at a dose of 40 mg/m^2 daily for 5 consecutive days on days 2 through 6 followed 4 hours later by cytarabine at a dose of 1 or 2 g/m^2 as a 2-hour intravenous infusion daily for 5 consecutive days on days 1 through 5. On day 1, only cytarabine was administered, and on day 6, only clofarabine was given. Patients who achieved CR received further consolidation chemotherapy with intermediate- (1.5 g/m2 every 12 h for six doses) or high-dose cytarabine (3 g/m2 every 12 h for six doses) or allogeneic hematopoietic stem cell transplantation (HSCT).
Results: All the patients had received at least one prior regimen. There were 29 primary refractory and 6 relapsed cases. Cytogenetic analysis was performed in 91.4% (32/35) of the patients. Twenty one patients (65.6%) had intermediate cytogenetic risk, whereas favorable and unfavorable cytogenetic findings were detected in 3 (9.4%) and 8 (25%), respectively. Internal tandem duplications of *FLT3* gene (FLT3-ITD) were found in 8 patients (22.9%). Eighteen patients (51.4%) achieved CR, whereas thirteen patients (37.1%) had resistant disease, and 4 (11.4%) died during induction. CR was achieved in 9 (60%) patients with diploid cytogenetics whereas CR was observed in 3 (100%) and 1 (12.5%) patient with favorable and unfavorable cytogenetic findings, respectively. Patients with FLT3-ITD achieved CR in 50% (4/8) of the cases. In patients with CR, 55.6% (10/18) were able to proceed to allogeneic HSCT. The 24 month overall survival (OS) for the whole patients' cohort was 28.8%. For CR patients, the 24 month OS and disease-free survival (DFS) were 33.8% and 40.8%, respectively.
Summary and Conclusions: Clofarabine in combination with cytarabine is effective in refractory and relapsed AML patients. In addition, it represents a useful remission induction strategy to serve as a bridge to transplantation in these patients.

E944

PROGNOSTIC IMPACT OF WT1 GENE EXPRESSION QUANTIFICATION DURING MINIMAL RESIDUAL DISEASE MONITORING OF ACUTE PROMYELOCYTIC LEUKEMIA

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Background: Acute promyelocytic leukemia (APL) is characterized by the highest Wilms tumor gene 1 (WT1) expression levels in comparison to other types of acute myeloid leukemia (AML). Several studies have reported the usefulness of WT1 expression assessment as a molecular marker of minimal residual disease (MRD) in AML patients. However, data on prognostic significance of WT1 gene expression during MRD monitoring of APL is lacking.
Aims: To quantify WR1 gene expression during MRD monitoring of APL patients
Methods: Data on WT1 expression in 22 *de novo PML-RARA* positive APL patients were analyzed prospectively (median age 42 years; female/male ratio 12/10; median WBC count 1.5 x10^9/L (range: 0.4-88), median follow-up of 27 months (range: 5-78)). The patients were managed with all-trans retinoic acid combined with anthracyclines. Fresh bone marrow (BM) samples were collected at diagnosis, after the first consolidation and after that every six months. WT1 expression levels were quantified by RQ-PCR method, using TaqMan chemistry. Abl served as a housekeeping gene. Relative quantification analysis was performed using comparative Ct method (2-ddCt), where ddCt=dCt(sample) - dCt(healthy(median)). A WT1 level of at least twice the maximum assessed in healthy controls was defined as WT1 overexpression (≥18.00).
Results: A total of 89 BM samples were collected during diagnosis and follow-up. Initially WT1 level was significantly higher in APL patients compared to healthy controls (mean 3765.99, range 13.64-13988.95vs. 1.95±1.18, range 0.06-8.77, p<0,001), with overexpression in 21/22 (95.45%) of patients. After first consolidation all patients reached molecular remission (MR). In 15/22 (68.18) patients MR was accompanied by WT1 levels ≥3 log reduction, in 4/22 (18.18%) by ≥2 log reduction and 1/21 (4.54%) <1 log reduction. Median WT1 gene expression after first consolidation was 24.34 (range: 0.95-72), with overexpression in 6/22 (27%) patients. Six relapses (5 molecular) was observed in 4/22 (18.18%) patients. The increase in WT1 expression was observed in all samples during relapse (>1.5 log in 2/6 relapses (30%), >1 log in 3/6 (50%) and <1 log in 1/6 (16.67%)) with mean level of 274.07 (range: 11.93- 1327.96). Increased WT1 expression was combined with PML-RARA positivity in 5/6 (83.3%) samples. In one case WT1 level increment was registered 6 months before PML-RARA positivity. Mean WT1 expression in patient with persistent MR was 21.17 (range: 3.34-59.76), with high level of variability and overexpression in 11/30 (36.7%) samples. However WT1 expression increment >1 log wasn't seen in patients with persistent MR. APL relapses were significantly associated with WT1 overexpression after first consolidation (3/4 (75%) *vs* 3/18 (16.67%), p=0,003), and >1 log WT1 expression increment during follow-up (5/6 (83.3%) *vs* 0, p=0,0001). No correlation was found with initial WT expression level (2471/7 *vs* 3994/51, p=0,516).
Summary and Conclusions: 1. WT1 decreased rapidly after induction but maintained residual levels in MR 2. WT1 expression was able to detect patients at high risk of relapse 3. Quantitative analysis of WT1 as post-remission control enables early detection of relapse. Further large prospective trials should eval-

uate whether this parameter should be included in risk stratification and MRD monitoring.

E945

CCAAT/ENHANCER BINDING PROTEIN ALPHA (CEBPA) NEW MUTATIONS IDENTIFIED BY THE HEMATOLOGY OF FLORENCE

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Background: CCAAT/enhancer binding protein alpha *(*CEBPA) is a transcription factor involved in lineage specification; it is crucial for the development of myeloid progenitors to differentiated neutrophils. Mutations in CEBPA gene are specific to AML and are not reported in other cancers. They are detected in 5-14% of cytogenetically normal AML patients (CN-AML). Typical alterations affect either the N-terminus with nonsense mutations leading to a dominant negative protein, and/or C-terminus resulting in decreased DNA binding of basic leucine zipper (bZIP) region. Several studies show that a single mutation in CEBPA (CEBPAsm) is prognostically neutral, whereas double mutations (CEBPAdm) have been associated with a relatively favourable outcome and therefore are emerging as a prognostic marker at the diagnosis. In the majority of CEBPAdm AML, the mutations are typically on different alleles and involve a combination of a N-terminal and a C-terminal mutation.
Aims: In order to obtain a better insight into the distribution of the various types of CEBPA mutations and evaluate their impact on clinical outcome, we screened for CEBPA a cohort of 230 de novo AML patients.
Methods: We analysed the entire coding region of CEBPA using three overlapping PCR primer pairs designed by Pabst *et al.* (2001) with minor modifications. PCR products were sequenced in both directions on ABI-3100 Genetic Analyzer (Applied Biosystems).
Results: By sequencing the entire CEBPA coding region, we identified a total of 33 mutations in 23 patients (10%) in our cohort of 230 patients de novo CN-AML. Within them, 10 patients had biallelic mutations (CEBPAdm) while 13 patients had single CEBPA mutation. Among these 33 mutations detected, 16 were already identified in previous studies, while the others (listed in table 1) had not been described before. The results can be summarized as follows: 8 of the 17 alterations described were frameshift mutations, 8 were insertions/duplications in-frame, 1 was a missense point mutation. According to literature data, insertions/deletions mutations (3n+1 bp) out-of-frame were concentrated in N-terminal region causing frameshift in the reading sequence and involving the formation of a new stop codon. All new mutations identified in the C-terminal region were in-frame and occurred between AA 301 and AA 318. We observed in four cases a "de novo" nucleotides insertion, while all the other patients showed long existing sequences duplication to 36 bp. Patient # 9 showed a nonsense nucleotide substitution in position 992 of the C-terminus, in which an thymine was replaced by a cytosine, corresponding to an Isoleucine Proline amino acid substitution (I with P), creating a novel stop codon.
Summary and Conclusions: In this analysis on a cohort of 230 de novo AML we identified 18 new CEBPA mutations not described before. Due to the relevant role of CEBPA in leukemogenesis, the identification of novel mutations aids to get more insight into the biology and prognosis of CEBPA-mutated AML subset. A longer follow up is necessary to evaluate the impact of CEPBAdm and particularly of these new mutation on the clinical outcome.

E946

CENTRAL NERVOUS SYSTEM INVOLVEMENT AT DIAGNOSIS IN PATIENTS WITH ACUTE MYELOID LEUKEMIA: RISK FACTORS, TREATMENT AND OUTCOME

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Background: Central nervous system (CNS) involvement at diagnosis of acute myeloid leukemia (AML) occurs in less then 5% of patients. It is rare but serious complication of AML with significant influence on poor outcome of these patients. Due to infrequency of CNS involvement (CNS+) in AML there is a paucity of literature on the optimal management of CNS disease.
Aims: The aims of this investigation were to estimate the incidence of CNS+at diagnosis AML, to determine risk factors for its occurrence and to define influence of CNS+on outcome of these patients: rate of complete remission (CR) and remission of CNS+, relaps-free survival (RFS) and overall survival (OS).
Methods: This single-center study involved 402 adult patients with nonpromyelocytic *de novo* AML during follow-up of 72 months. The following parameters were estimated as risk factors for CNS+at diagnosis of AML: age, WBC ($<30x10^9$/L *vs* $\geq 30x10^9$/L), serum lactate dehydrogenase (LDH) concentration >1.5x the upper limit of normal, expression of CD56 antigen on leukemic blasts (<20% *vs* ≥20%), AML subtype according to the French-American-British (FAB) Cooperative Group and cytogenetic risk group (assessed according to European LeukemiaNet recommendations). The presence of leukemic cells in cerebrospinal fluid (CSF) detected by cytomorphological and/or flow cytometric analysis of CSF is considered as CNS involvement. Patients were treated according with follow chemotherapy (CT): induction CT (daunorubicin 60 mg/m^2 three days and 7 days cytarabin 200 mg/m2) following three CT of consolidations (high dose of cytarabine, eg. 3 g/m2 every 12 h on day 1,3,5). In patients aged >60 years doses of induction CT were reduced for 50% and as consolidations CT were applied cytarabine in intermediate dose (1.5 g/m2 day 1 to 3). Patients aged >75 years treated with paliative therapy (Hydroxiurea or 6-mercaptopurine per os). Patients with CNS+treated with intrathecal (i.t.) CT: cytarabine 100 mg 2 x/week until clear of CSF. Patients who no achived remission of CNS+treated with radiotherapy of CNS with 18-24 Gy in total dose. Assesment of remission of CNS+was performed after 8 i.t. CT.
Results: The CNS+was recorded in 4.7% (19 pts) of AML patients at diagnosis. The mean age of CNS+patiens was 48 years (range 26-71 years) while the mean age of CNS negative (CNS-) patients was 58 years (range 19-85 yeras). Multivariate analysis identified CD56+as the most important risk factor for CNS+at diagnosis in AML patients: p=0.001, relative risk (RR)=18.241; 95% confidental interval (CI)=3.305-100.675. In group of CNS+patients, the CR rate was 68% (13 pts) while CNS remission rate was 16% (3 pts). RFS was significance different among CNS+and CNS- patients: 8 *vs* 20 months (p=0.001), as well as OS: 6 *vs* 18 months (p=0.002). Multivariate analysis indicated CNS+as the most significant independent predictor of poor RFS: p=0.002, RR=1.806, CI=1.241-2.628, and OS: p=0.008, RR=1.592, CI=1.130-2.243.
Summary and Conclusions: AML patients with CNS involvement at diagnosis have a poor prognosis and further studies need to improved and optimized their treatment.

E947

PHASE 1 STUDY: SAFETY AND TOLERABILITY OF INCREASING DOSES OF CB-839, AN ORALLY-ADMINISTERED SMALL MOLECULE INHIBITOR OF GLUTAMINASE, IN ACUTE LEUKEMIA

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Background: Glutaminase is a proposed tumor metabolism target implicated in the growth and survival of a variety of tumor types. The enzyme is essential to the utilization of glutamine by many tumor cells, converting incoming glutamine to glutamate for use in the TCA cycle and for production of glutathione and many other cellular constituents. Glutaminase is highly expressed in primary tumors, including acute myeloid leukemia (AML). CB-839 is a highly selective, reversible, allosteric inhibitor of glutaminase. Preclinial studies have demonstrated *in vitro* and *in vivo* anti-tumor activity across a broad array of solid and hematological tumor types and the compound was well tolerated in animals.
Aims: CX-839-003 is an ongoing Phase 1 study of escalating doses of CB-839 in acute leukemia patients to evaluate safety and tolerability, and to identify a recommended Phase 2 dose (R2PD) of the compound. Pharmacokinetics (PK) and pharmacodynamics (PD) of CB-839, as well as an exploration of biomarkers of tumor sensitivity, are being evaluated.
Methods: CB-839 was administered orally continuously in 21-day treatment cycles from 100 to 1000 mg three times daily (TID) to patients with relapsed and/or treatment-refractory acute leukemia. The plasma pharmacokinetics of CB-839 was determined after the first dose given on Days 1 and 15. At the RP2D, 11 patients will be enrolled in disease-specific treatment cohorts including AML; additional patients will be added if there are responses.
Results: 15 patients enrolled during dose escalation have received CB-839 with doses up to 1000 mg TID. The steady state plasma concentration of CB-839 on Day 15 was found to be above 250 nM continuously in most patients using doses of 600 mg TID and higher. In a companion solid tumor trial, CX-839-001, this plasma concentration was determined to result in >85% inhibition of glutaminase activity in platelets and 75% in solid tumors. Peripheral blood mononuclear cells (PBMCs) from three patients treated with 600, 800, and 1000 mg TID on the current trial were found to have between 10 and 58% leukemic blast counts and showed >94% inhibition of glutaminase activity.
CB-839 has been administered TID 1 hr before breakfast, at 3 pm, and prior to bedtime in all patients thus far. An increase in exposure was demonstrated with increasing dose. When CB-839 was administered at 600 mg BID with food in CX-839-001, the C_{max} was reached in 2-6 hr; trough drug levels exceeding 450 nM in all patients. This schedule of administration will be employed for all

patients going forward in this trial. There were no DLTs identified in this trial and treatment-related AEs (all grades) that occurred in >10% of patients were limited to increases in transaminases (4 patients) and bilirubin (2 patients). There were no Grade ≥3 AEs that were considered treatment-related in >10% of patients. Stable Disease (SD) for 4 – 10 cycles was observed in 5 (33%) of 15 efficacy-evaluable AML patients across all dose levels, with patients remaining on study for an average of 134 days (>6 cycles). One of these patients achieved a Complete Response in the bone marrow with incomplete recovery of peripheral counts (CRi) after 6 cycles of dosing; therefore, enrollment will continue with additional patients in the expansion cohort. All of these patients with SD or better were >65 years of age and not eligible for high dose therapy.
Summary and Conclusions: The glutaminase inhibitor CB-839 is well tolerated in AML patients using doses up to 1000 mg TID. One CRi was observed and 33% of patients enrolled during dose escalation showed stable disease that was prolonged for 4-10 cycles. This trial will continue to enroll at the expansion phase.

E948

PHARMACOKINETIC/PHARMACODYNAMIC (PK/PD) EVALUATION OF AG-221, A POTENT MUTANT IDH2 INHIBITOR, FROM A PHASE 1 TRIAL OF PATIENTS WITH IDH2-MUTATION POSITIVE HEMATOLOGIC MALIGNANCIES

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Background: The isocitrate dehydrogenase 1/2 (IDH1/2) enzymes catalyze the production of alpha-ketoglutarate (α-KG) from isocitrate. Mutant IDH1/2 enzymes are capable of novel enzymatic production of D-2-hydroxyglutarate (2-HG) from α-KG. 2-HG is an oncometabolite implicated in cancer initiation and progression that is found at highly elevated levels in several cancer types expressing mutant IDH, including acute myeloid leukemia (AML). AG-221 is a first-in-class, oral, selective, potent inhibitor of mutant IDH2. *In vitro* studies have shown that AG-221 reduces 2-HG levels by >90%, reverses histone and DNA hypermethylation, and induces cellular differentiation in leukemia cell models. In *in vivo* xenograft mouse models using U87MG and primary human AML cells, oral AG-221 also demonstrated robust 2-HG inhibition. These pharmacokinetic/pharmacodynamic (PK/PD) correlations between AG-221 exposure and 2-HG inhibition were used to project the efficacious dose in humans. Here we present updated PK/PD analyses from the ongoing, first-in-human, phase 1 trial of AG-221 in patients with IDH2 mutation-positive advanced hematologic malignancies (NCT01915498).
Aims: To model the human PK/PD profile of AG-221, to compare these results with *in vitro* as well as pre-clinical studies, and to assess the PK/PD profile of AG-221 across different IDH2 mutations.

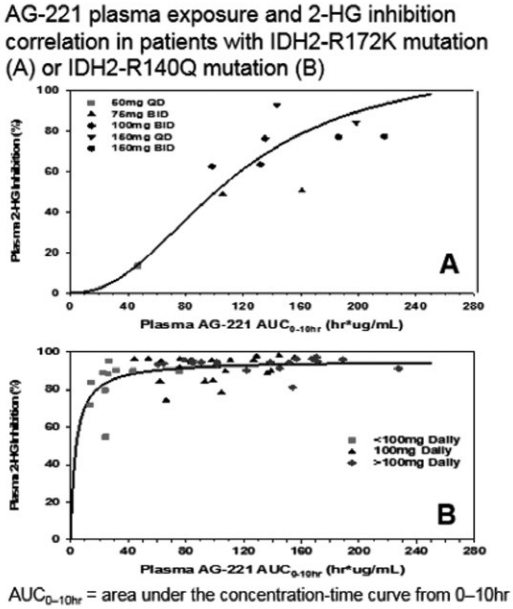

Figure 1.

Methods: In the phase 1, open-label, dose-escalation trial, patients who provided informed consent received oral AG-221 once (QD) or twice daily (BID) in continuous 28-day cycles. Dosing began at 30 mg BID and increased in subsequent cohorts. Blood and bone marrow samples were collected at multiple time points after a single dose at Day –3 and after multiple doses. AG-221 and 2-HG concentrations were measured using a qualified LC-MS/MS method for PK/PD analysis, which was performed with WinNonLin®. *In vitro* activity was assessed in cell lines carrying IDH2 mutations. Efficacy of oral AG-221 and PK/PD relationships were also evaluated in U87MG and primary human AML xenograft mouse models carrying the IDH2-R140Q mutation.
Results: Patients in this analysis received AG-221 at doses of 30, 50, 75, 100, and 150 mg BID and 50, 75, 100, 150, and 200 mg QD (total N=64). Patients bearing the two dominant IDH2 mutations, R140Q (88%) or R172K (12%), were enrolled. AG-221 demonstrated excellent dose-dependent plasma exposure with high accumulation after multiple doses and a half-life >40 hours. After multiple AG-221 doses, substantial, sustained reduction of 2-HG in plasma and bone marrow was observed. 2-HG inhibition in plasma was dose- and exposure- dependent, with up to 98% inhibition in IDH2-R140Q and 93% inhibition in -R172K mutation-positive patients. More than 30-fold different PD responses were modeled for each of the mutations (**Figure**), with an AG-221 free fraction 50% inhibitory concentration (IC_{50}) of 5 ng/mL for IDH2-R140Q (E_{max} model) and 160 ng/mL for IDH2-R172K (sigmoidal E_{max} model). This correlates with PK/PD analysis from *in vitro* studies, in which lower IC_{50} values were observed in IDH2-R140Q cells than in IDH2-R172K cells.
Summary and Conclusions: AG-221 demonstrates a favorable PK profile that supports oral QD dosing, based on the high plasma exposure and long half-life observed in this study. AG-221 inhibition of 2-HG production was more potent in IDH2-R140Q than in -R172K mutants, however, AG-221 reduces plasma 2-HG in patients with both IDH2-R140Q and -R172K mutations to within the normal range seen in healthy volunteers, in a dose- and exposure-dependent manner. The PK/PD findings translate well from *in vitro* to *in vivo* studies and from pre-clinical to human studies.

E949

REDUCED MEDICAL COSTS AND HOSPITAL DAYS WITH ORAL ARSENIC PLUS RETINOIC ACID COMPARED WITH INTRAVENOUS ARSENIC PLUS RETINOIC ACID AS THE FRONT-LINE TREATMENT OF ACUTE PROMYELOCYTIC LEUKEMIA

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Background: We recently demonstrated that oral arsenic (Realgar-*Indigo naturalis* formula,RIF) plus all-trans retinoic acid (ATRA) is not inferior to intravenous arsenic trioxide (ATO) plus ATRA as the front-line treatment of acute promyelocytic leukemia (APL). However, the cost-effectiveness of oral arsenic-based treatment has never been reported.
Aims: We aimed to compare the cost-effectiveness of between oral and intravenous arsenic.
Methods: we analyzed the results of 60 patients (30 patients in each group) involved in a randomized controlled trial at our center. The direct medical costs and hospital days for each patient were calculated.
Results: The median total medical costs were ¥83056 in the RIF group compared with ¥152063 in the ATO group (p<0.0001). This difference primarily resulted from the differential costs of induction therapy (p=0.016) and maintenance treatment (p<0.0001). The hospitalization length for the RIF group was significantly lower compared with the ATO group (24 *vs.* 31 days, p<0.0001) during induction therapy. During maintenance treatment, the estimated medical costs were ¥12897 for each patient in the RIF group treated at home compared with ¥71025 for each patient in the ATO group treated in an outpatient setting (p<0.0001).
Summary and Conclusions: We concluded that oral RIF plus ATRA significantly reduced the medical costs and length of hospital stay during induction and maintenance therapy compared with ATO plus ATRA in APL patients. Oral arsenic plus ATRA should be incorporated into the front-line treatment of patients with APL for medical and economic reasons.

E950

CLINICAL COURSE OF LEPTOMENINGEAL INVOLVEMENT OF ACUTE MYELOID LEUKEMIA: 14-YEARS SINGLE CENTER EXPERIENCE

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Background: The leptomeningeal involvement of acute myeloid leukemia is known to be rare in adult, and its prognostic importance is controversial.
Aims: We investigated risk, clinical course and outcome of acute myeloid leukemia patients with leptomeningeal involvement.
Methods: We examined medical records of the patients who were diagnosed with acute myeloid leukemia (AML) at Seoul National University Hospital from January 2000 to November 2013. Leptomeningeal involvement was defined as the presence of atypical or malignant hematopoietic cells in cerebrospinal

fluid. Molecular risk group was classify into three categories; good (t(8;21), t(15;17), inv(16) & NPM1), Poor (t(9;22), MLL rearrangement, MDS-related change, complex karyotype, FLT3-ITD) and Standard (normal karyotype and other abnormalities). Information about demographical data, treatments and outcomes, survival was also collected.
Results: Among 943 patients with acute myeloid leukemia, 155 patients (16.4%) have had lumbar puncture and cerebrospinal fluid examination. Leptomeningeal involvement of AML was confirmed in forty-three patients (4.6%). Most common symptom was headaches (15 patients, 34.9%). Median age was 51 years old and 27 patients (62.8%) were male. Patients in good risk group were fifteen (34.9%). Among these patients, t(15;17) was founded in 6 patients (40%). Fifteen were standard and eleven were high risk group. Twenty-three patients (53.5%) had involvements of extramedullary site except leptomeninges, and sixteen patients (37.2%) had chloromas in their brain parehchyma or spinal cord. Twelve patients (27.9%) discovered leptomeningeal involvement simultaneously with the initial diagnosis of leukemia. Among these patients, 3 (25.0%) belonged to good risk group. Thirty-one patients (72.1%) developed leptomeningeal involvement at the relapsed or refractory status, twelve (38.7%) of them were in good risk group. Thirty-six patients (83.7%) received intrathecal chemotherapy, then leukemic cells were eliminated from cerebrospinal fluid in thirty-one patients (86.1%). Whole-brain or spinal radiotherapy was performed in 13 patients (30.2%). The complete remission-rate of bone marrow after the first induction chemotherapy was 67.4%, and allogeneic hematopoietic stem cell transplantation was performed in 14 patients (35.5%). Median overall survival was 10.8 months. Radiotherapy and complete remission after the first induction chemotherapy were independent prognostic factors for overall survival in multivariate analysis.

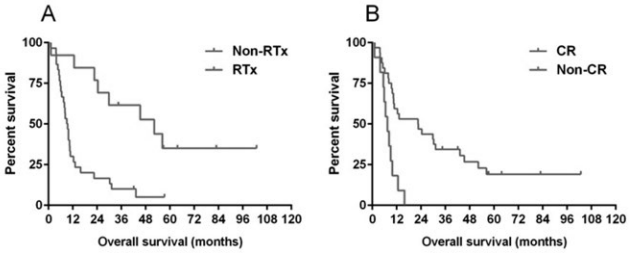

Figure 1. Overall survival. A; radiotherapy, B; Complete remission after 1st induction chemotherapy.

Summary and Conclusions: Leptomeningeal involvement in acute myeloid leukemia is rare condition, which are relatively common in relapsed or refractory status. Whole-brain radiotherapy and complete response to induction chemotherapy were thought to have contributed to improve their prognosis. Active treatment using intensive induction chemotherapy and leptomeninges-directed treatment including radiotherapy might be helpful improving their survival.

E951

A PHASE IB/II STUDY TO EVALUATE THE SAFETY AND EFFICACY OF VISMODEGIB IN RELAPSED/REFRACTORY ACUTE MYELOGENOUS LEUKEMIA

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Background: Vismodegib is a small molecule inhibitor of the hedgehog (Hh) signaling pathway currently approved for treatment of advanced basal cell carcinoma. Increased expression of Hh ligand leading to activation of the Hh pathway is thought to play a key role in the maintenance of the leukemic stem cell in myeloid leukemias. Specifically, the Hh ligand may contribute to leukemic stem cell self-renewal properties. Inhibiting the ability of leukemic stem cells to self-renew by targeting the Hh pathway could eradicate the malignant clone.
Aims: To assess the safety and efficacy of vismodegib in the treatment of relapsed/refractory acute myelogenous leukemia (AML) patients with a primary endpoint of overall response rate at week 8 of study treatment.
Methods: Patients (pts) with relapsed or refractory AML who gave informed consent were enrolled in a single arm treatment of vismodegib 150mg PO daily for 8 weeks or until progression evaluated by using the International Working Group criteria. Eligibility criteria included pts with documented relapsed/refractory AML (excluding APL), ≥18 years, ECOG ≤2, with adequate renal and hepatic function. Pts were stratified by the following factors: poor risk cytogenetics (based on NCCN guidelines), FLT3+mutation, or neither (patients who were not poor risk or FLT3+).
Results: 38 pts were enrolled (poor-risk karyotype n=15, FLT3+n=4, and neither n=19). Most pts had relapsed AML (60.5%), were white (89.5%) and male (55.3%) with a median age of 68.5 years. The ORR was 5.3% (1 CRi, 1 PR). Both of the responders were in the "neither" group with no identified cytogenetic abnormalities or other risk factors. The median duration of vismodegib therapy was 49 days, with a range of 5 – 225. 37 patients (97.4%) had at least one adverse event. The most common adverse events were pyrexia (39.5%), nausea (39.5%), dysgeusia (31.6%), epistaxis 26.3%), febrile neutropenia (23.7%), fatigue (23.7%), muscle spasms (23.7%), decreased appetite (23.7%), and peripheral edema (21.1%). Serious adverse events occurred in 26 patients (68.4%). The majority of serious adverse events were infections (53.8%). Most patients died due to disease progression on study, 27 of 33 pt deaths.
Summary and Conclusions: Single agent vismodegib treatment produced minimal clinical efficacy in pts with relapsed/refractory AML. However, this is the first documented single-agent response in a disease thought to be driven through the Hh ligand as opposed to a mutation in the pathway. The adverse event profile was most likely driven by the underlying AML. Serious adverse events were typically infections, which are common in relapsed/refractory AML.

E952

PHASE I/II STUDY OF DFP-10917 GIVEN BY CONTINUOUS INFUSION IN PATIENTS WITH RELAPSED OR REFRACTORY ACUTE LEUKEMIA: PHARMACOKINETIC, PHARMACODYNAMICS AND PHARMACOGENOMICS PROPERTIES

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Background: DFP-10917 is activated by deoxycytidine kinase (DCK) and inactivated by cytidine deaminase (CDA) similar to the deoxycytidine analogs, cytarabine and gemcitabine. Upon low dose administration DFP-10917 is converted to its nucleotide form and incorporated into tumor DNA causing DNA strand breaks and G2/M phase-arrest by cell-checkpoint regulators and ultimately tumor cell apoptosis.
Aims: To determine the maximum tolerated dose (MTD), the recommended phase II dose (RP2D) and dose-limiting toxicity (DLT) of DFP-10917 given by continuous infusion (CI) in patients with relapsed or refractory acute leukemia. To perform pharmacokinetic (PK), pharmacodynamic (PD) and pharmacogenomic (PGx) analyses.
Methods: In phase I, DFP-10917 was administered by 7-day CI followed by 21 days rest (stage 1) or 14-day CI followed by 14 days rest (stage 2) in pts with relapsed or refractory acute leukemia to determine the MTD. PK analysis was performed to measure DFP-10917 (CNDAC) and the primary metabolite at days 7 and 14. PD assessment utilized the comet assay to quantify DNA strand breaks in whole blood and bone marrow samples. PGx assessment was performed by DNA genotyping of CDA and DCK.
Results: In stage 1, twenty-seven pts received a 7-day CI of DFP-10917 at eight escalating doses ranging from 4 to 35 mg/m^2/day. At the 35 mg/m^2 dose level, one patient experienced a DLT of grade 3 diarrhea during cycle 1. The starting dose for stage 2 was calculated as two-thirds the cumulative 7-day DFP-10917 dose at the MTD of 30 mg/ m^2/day divided by 14-day resulting in a dose of 10 mg/m^2/day x 14 days. The 10 mg/m^2/day x 14-day CI dose resulted in DLTs of prolonged hypo-cellularity in 2 of 4 pts. At the 6 mg/m^2/day x 14-day dose level, 1 of 6 evaluable pts experienced a DLT of prolonged hypo-cellularity, and the MTD/RD was defined as 6 mg/m^2/day x 14-day CI. Initial efficacy assessments include leukemia responses observed in 7 of 10 AML pts (70%) receiving the 14-day DFP-10917 CI (3 bone marrow complete responses, 4 partial responses). PK data indicates dose-proportionality for steady state concentration (Css) of DFP-10917 and its primary metabolite. 2 of the 3 patients with prolonged hypo-cellularity DLTs demonstrated CNDAC Css of >10 ng/ml (0.0346 nM). The comet assay results indicate DNA strand breakage increased following the 7 and 14 day CI of DFP-10917; however, the 14-day CI was associated with a lower level of DNA strand breakage. DNA genotyping did not detect genetic mutations associated with CDA and DCK.
Summary and Conclusions: The MTD/RP2D of DFP-10917 in relapsed AML was established for 14-day CI at 6 mg/m^2/day, and the phase II study is ongoing. Prolonged myelosuppression was associated with CNDAC Css >10 ng/ml among 2 of 4 pts treated at 10 mg/m^2/day x 14 days of DFP-10917. PD data demonstrated DNA strand breakage at the doses tested. No genetic mutations associated with CDA or DCK were detected among leukemia patients treated in the Phase 1 study.

E953

EFFICACY AND TOXICITY OF FLUDARABINE BASED INDUCTION REGIMENS IN ACUTE MYELOID LEUKEMIA PATIENTS YOUNGER THAN 65 YRS

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Background: Fludarabine (Fluda)–Cytarabine combinations are commonly used as salvage chemotherapy in Acute Myeloid Leukemia (AML). The role and efficacy of Fluda based induction regimens in high risk AML younger than 65 yrs are still poorly understood.
Aims: To evaluate efficacy and toxicity of Fludarabine based induction regimens in patients with AML and younger than 65 yrs.
Methods: We retrospectively evaluated the efficacy and toxicity following induction chemotherapy with Fluda based regimens in 286 pts, with previously untreated Acute Myeloid Leukemia, followed over a 10 years period (2002-2012) at our Center. All patients were younger than 65 with a median age of 52 years. Diagnosis of AML was confirmed in all cases and Cytogenetic, Multidrug Resistance, FLT3 mutation analysis, were performed in all patients; 73% of these were poor-risk at diagnosis [high risk factors: BC >30x10^9/L, secondary AML, FLT3 ITD mutation, poor-risk karyotype]. The 286 Fluda based induction courses included: 235 FLAI courses (Fludarabine, Cytarabine, Idarubicine) and 51 FLAIE courses (FLAI course plus Etoposide). Patients were evaluated for response rate and treatment related adverse events. Toxicity was evaluated and graded according to National Cancer Institute criteria.
Results: After Induction with a Fluda based regimen 213/286 (74%) of patients achieved a Complete Remission (CR). Only a 3% of Death During Induction (DDI) was reported. Non hematologic toxicity was acceptable with 54% of pts presented FUO, 48 % bacteremia or sepsis and 20% grade II-III oral mucositis. As expected, all pts experienced grade IV hematological toxicity and (in CR pts) the median time to PMN (>1000/ml) and platelet (>50.000/ml) recovery was 24 (range 17 to 40) and 26 days (range 18-43), respectively. Supportive treatment consisted of a median of 13 RBC (range 5-26) and 7 platelet units (range 3-18). Median time to hospital discharge was 31 days (range 22-61). 1 yrs Overall Survival and DFS was 68% and 64%, respectively.
Summary and Conclusions: This data, from a large serie of cases, confirm that Fluda based induction regimens are effective and well tolerated in poor risk AML patients younger than 65 yrs with a high CR rate, favourable safety profile and very low DDI.

E954

OVERALL SURVIVAL (OS) AND CLINICAL OUTCOMES IN OLDER PATIENTS WITH ACUTE MYELOID LEUKEMIA (AML) TREATED WITH AZACITIDINE (AZA) OR LOW-DOSE CYTARABINE (LDAC) IN THE AZA-AML-001 STUDY

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Background: There is no standard treatment (Tx) for older patients (pts) with AML, who have especially poor OS due to adverse risk factors such as unfavorable karyotypes and poor performance status (PS). Older pts may be unfit for high-intensity induction chemotherapy (IC); such pts are often treated with LDAC or best supportive care (BSC) alone, which are associated with very poor outcome (Dombret, *Semin Oncol,* 2008). The international phase 3 AZA-AML-001 study compared AZA with conventional care regimens (CCR) in older pts with AML. Before randomization, investigators selected their preferred Tx option for each pt from 3 commonly used CCR for AML Tx: IC, LDAC, or BSC only. Pts were then randomized to receive AZA or CCR and received their preselected Tx.
Aims: To compare effects of AZA *vs* LDAC on OS and clinical outcomes in the subgroup of pts in AZA-AML-001 preselected to receive LDAC before randomization.
Methods: Pts aged ≥65 years with newly diagnosed *de novo* or secondary AML (>30% bone marrow [BM] blasts by local assessment), ECOG PS 0-2, WBC count ≤15x10^9/L, and intermediate- or poor-risk cytogenetics were enrolled. AZA dose was 75mg/m^2/day SC x7 days/28-day cycle and LDAC dose was 20mg SC BID x10 days/28-day cycle. OS and 1-year survival were estimated using Kaplan-Meier methods. OS was compared between Tx groups by log-rank test. An unstratified Cox proportional hazards model generated hazard ratios (HRs) and 95% CIs. Overall response rate (ORR) included complete remission (CR) +CR with incomplete blood count recovery (CRi) (IWG 2003). Proportions of pts with grade 3-4 infections and hematologic treatment-emergent adverse events (TEAEs), and TEAE incidence rates (IR) per 100 pt-years of Tx exposure, are reported.
Results: Of all pts in AZA-AML-001, most (312/488, 64%) were preselected to receive LDAC (AZA n=154, LDAC n=158). Median number of Tx cycles received was 7 (range 1–28) for AZA and 4 (1–25) for LDAC. At baseline in the AZA and LDAC groups, respectively, median ages were 76 (range 64–90) and 75 (65–88) years; 25% and 22% had ECOG PS of 2; centrally read median BM blasts were 70% (2–100) and 74% (4–100); and 29% and 34% of pts had poor-risk cytogenetics. Median OS in the AZA and LDAC groups was 11.2 *vs* 6.4 months (mon), respectively (HR=0.90 [95%CI 0.70, 1.16], p=0.43; Figure), indicating a 4.8-mon (95%CI 1.7, 7.9) longer median OS with AZA. The OS difference was not statistically significant due to convergence of the OS curves at ~20 mon. One-year survival rate was 48.5% with AZA *vs* 34.0% with LDAC, a difference of 14.5% (95%CI 3.5%, 25.5%). ORR was 27% with AZA and 26% with LDAC. Proportions [IRs] of AZA and LDAC pts, respectively, with grade 3-4 TEAEs were: anemia 19% *vs* 23% [26 *vs* 42]; neutropenia 25% *vs* 25% [33 *vs* 46]; febrile neutropenia 27% *vs* 30% [36 *vs* 56]; thrombocytopenia 25% *vs* 28% [34 *vs* 51]; and infections 49% *vs* 46% [66 *vs* 84].

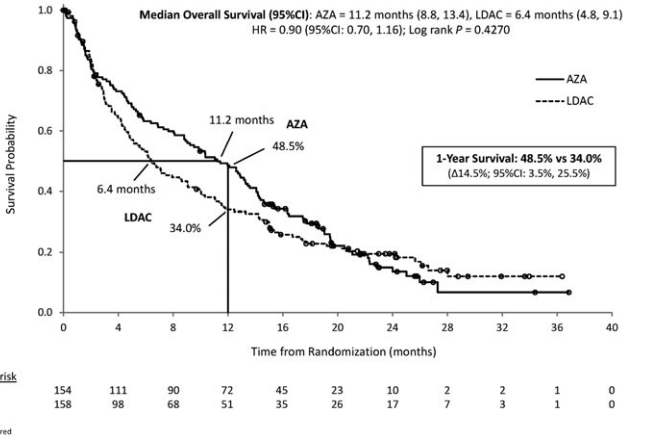

Figure 1. Overall survival in patients preselected to LDAC.

Summary and Conclusions: Analyzing outcomes within preselection groups allows assessment of Tx effects in pts with generally similar prognoses and clinical features, as preselection of the preferred CCR occurred before randomization. AZA was associated with a longer median OS of 11.2 mon *vs* 6.4 mon with LDAC, in pts with similar features at study entry. At 1 year, almost one-half of AZA-treated pts were alive, compared with approximately one-third of LDAC-treated pts. Quality of life outcomes during Tx with AZA or LDAC in this pt group are now being evaluated. AZA may offer potential advantages over LDAC as first-line low-intensity Tx in difficult-to-treat older pts with AML and high blast counts.

E955

RETROSPECTIVE ANALYSIS OF OUTCOMES BETWEEN PATIENTS WITH ACUTE MYELOID LEUKEMIA WHO ARE TREATED WITH HEPARIN AND RECOMBINANT HUMAN THROMBOMODULIN THERAPY

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Background: Recombinant thrombomodulin (rTM) is a promising anticoagulant and rTM therapy more significantly improves disseminated intravascular coagulation (DIC) and relieves bleeding complications in DIC patients, when compared with unfractionated heparin therapy. However, it is unknown as to whether or not the treatment with rTM affects acute myeloblastic leukemia (AML) patient's outcome.
Aims: we retrospectively analyzed 103 patients with AML (except adult acute promyelocytic leukemia), and compared outcomes between patients treated with low-molecular-weight heparin and rTM.
Methods: We retrospectively analyzed 103 patients with AML and compared the outcomes between the patients with low molecular weight heparin therapy and with rTM. The diagnostic criteria for DIC previously proposed by the Japanese Ministry of Health and Welfare. Comparisons between the qualitative variables were carried out using the χ^2 test. The survival probabilities were estimated by the Kaplan-Meier method, and differences in the survival distributions

were evaluated using the log-rank test.
Results: 47 developed DIC in association with chemotherapy or disease status. Fourteen patients were treated by rTM, and 33 patients were treated by low molecular weight heparin (LMWH). The overall survival was worse in the DIC group compared with the non- DIC group on the log-rank test (P=0.003). The overall survival was superior in the rTM group compared with LMWH group according to the log-rank test (P=0.016).

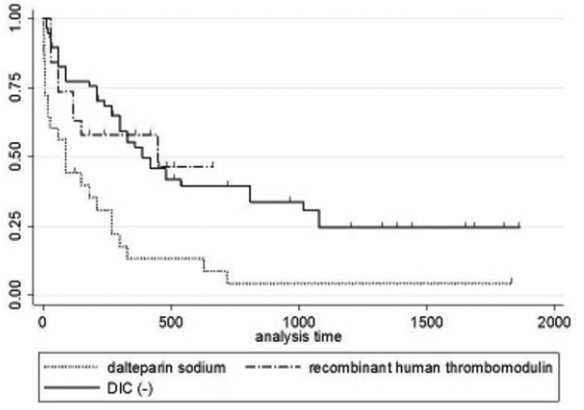

Figure 1.

Summary and Conclusions: Treatment with rTM is efficient compared with LMWH because of improvement of overall survival.

E956

POST REMISSION MONITORING OF MINIMAL RESIDUAL DISEASE BY WT1 AND LEUKEMIA ASSOCIATED IMMUNOPHENOTYPE PREDICTS RELAPSE IN ACUTE MYELOID LEUKEMIA (AML) PATIENTS

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Background: Although a complete remission (CR) can be achieved in 70-80% of newly diagnosed acute myeloid leukemia (AML) patients, relapses occur in up to the 50% of cases. Minimal residual disease (MRD) monitoring is a major issue for early detection of patients at high-risk of treatment failure and relapse.
Aims: to perform a comparative sequential monitoring of MRD by RT-qPCR on WT1 and by Multiparametric Flow Cytometry (MFC) on Leukemia Associated Immunophenotypes (LAIPs).
Methods: 104 newly diagnosed AML patients consecutively treated between 2010 and 2013 were monitored with RT-qPCR WT-1 from bone marrow (BM) and peripheral blood (PB) and with MFC on the LAIP on BM at baseline, after induction, after the first consolidation course and after the first intensification course.
Results: All the patients were studied at diagnosis for a LAIP and this was available in 80/104 (77%) cases. BM and PB-WT1 level at diagnosis was available in 92/104 (88%) and 70/104 (67%), respectively. Eighty-eight out of 104 patients (85%) achieved a complete remission (CR) after induction, 30/88 (34%) relapsed during follow up and 33/104 (32%) were addressed to allogeneic stem cell transplantation (allo-SCT). Univariate and multivariate analysis on relapse free survival (RFS) were conducted, censoring allotransplanted patients at the time of allo-SCT. By univariate analysis, after induction BM-WT ≥295x10^4/ABL (HR 7.8; p<0.0001) significantly identified patients at high risk of relapse. After 1st consolidation BM-WT1 ≥121x10^4/ABL (HR 5.2; p>0.0001), PB-WT1≥18x10^4/ABL (HR 7.9; p<0.0001), LAIP ≥0.2% (HR 3.3; p=0.002) and LAIP-decrease <85% (HR 3.6; p=0.001) were significantly correlated with adverse RFS. After 1st intensification cycle (before allo-SCT), BM-WT1 ≥150x10^4/ABL (HR 7.8; p<0.0001), PB-WT1 ≥16x10^4/ABL (HR 12.2; p<0.0001) ,and LAIP decrease <85% (HR 4.5; p=0.006) significantly affected the RFS. By multivariate analysis, after 1st consolidation BM-WT1 ≥121x10^4/ABL (HR 4.1; p=0.02) and LAIP decrease <85% (HR 3.0; p=0.0001) and after 1st intensification PB-WT1 ≥16x10^4/ABL (HR10.2; p=0.0001) were independently associated with averse outcome. Within the different ELN-risk categories, BM-WT1 ≥121x10^4/ABL after the 1st consolidation cycle significantly dissected the patients at high risk of relapse both in the ELN Int-1 [HR 14.4; 95% CI 1.7-118.6); p=0.01] and Int-2 risk category [HR 11.7; 95% CI 1.1-124.9); p=0.04]. Similarly, LAIP decrease <85% after the 1st consolidation cycle significantly identified the patients at high risk of relapse in the ELN Int-2 risk group only [HR 10.1; 95% CI 1.1-93.9); p=0.04]
Summary and Conclusions: Our data, although retrospectively collected, show that WT1 and LAIP MRD monitoring is useful to predict the relapse in AML patients and may help to guide the post-remission treatment strategy.

E957

TREATMENT WITH LOW-DOSE CYTARABINE (LD-ARAC) IN ELDERLY PATIENTS (AGE 70 YEARS OR OLDER) WITH ACUTE MYELOID LEUKEMIA (AML): A SINGLE INSTITUTION EXPERIENCE

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Background: The treatment outcome for patients with AML aged 70 years or older is unsatisfactory and has not improved significantly over the last two decades. Most of these patients do not receive intensive chemotherapy either because they decline or because they are not considered fit enough for such therapy. LD-AraC is still regarded as the standard of care in elderly patients with AML 'unfit' for intensive chemotherapy, against which novel drugs may be compared.
Aims: In this study, we compared the efficacy of LD-AraC with that of intensive chemotherapy (IC), best supportive care (BSC), or lower intensity therapy (LIT) based on hypomethylating agents in a single institution experience.
Methods: Three hundred and sixty six patients (aged 70 years or older) with newly diagnosed AML have been seen in our institution between 2000 and 2014. Among them, 60 patients (16%) received LD-AraC at 20 mg once or twice daily by subcutaneous injection for 10 consecutive days. Subsequent courses were administered after intervals of 4 to 6 weeks.
Results: They were compared to 85 patients treated by IC (anthracycline- and cytarabine-based chemotherapy), 34 patients treated by LIT (12 received decitabine and 22 azacitidine), and 43 patients receiving only BSC. The overall complete remission (CR) rate with LD-AraC was 7% (4/60 patients). The median number of courses to CR was 4 courses (3-9 courses). The CR rate was better in patients treated by IC (144/280 patients; 51%) and in patients treated by LIT (7/34 patients; 21%). Median OS of patients treated with LD-AraC was 9.6 months (95% CI, 5.8-13.5 months) with 3-year OS of 12%. Survival with LD-AraC was better than with BSC only (median OS: 9.6 months *vs* 3.4 months; *P*=0.001). Although not statistically significant, IC tended to be better than LD-AraC in terms of OS (median OS: 12.4 months *vs* 9.6 months; 3-year OS: 27% *vs* 12%; *P*=0.07). However, differences in favor of IC were only confirmed for patients aged less than 75 years (median: 12.7 months *vs* 9.2 months; 3-year OS: 28% *vs* 10%). In patients aged >or=75 years, median OS was better with LD-AraC (9.6 months *vs* 2.8 months). No significant differences were observed between LIT and LD-AraC (median OS: 16.1 months *vs* 9.6 months; 3-year OS: 22% *vs* 12%; *P*=0.1). There was no clear evidence that a beneficial effect of LD-AraC was restricted to any particular subtype of patients, except for cytogenetics (favorable and intermediate-risk *vs* unfavorable-risk) (median OS: 11.4 months *vs* 4.3 months; *P*=0.03). Of the patients who received LD-AraC, 24 patients were treated inside clinical trials, while 36 patients were not. Median OS was 13.2 months for the first ones *vs* 7.8 months for the others; 3-year OS: 18% *vs* 9%; *P*=0.21.
Summary and Conclusions: Overall, despite a trend in favor of IC and LIT over LD-AraC, no real significant advantage could be demonstrated, while LD-AraC showed a significant advantage comparatively to BSC. This tends to confirm that LD-AraC can still represent a baseline against which new promising agents may be compared either alone or in combination.

E958

CLINICAL SIGNIFICANCE OF THE DAY 5 PERIPHERAL BLASTS CLEARANCE RATE IN THE EVALUATION OF EARLY TREATMENT RESPONSE AND THE PROGNOSIS FOR AML PATIENTS

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Background: Acute myeloid leukemia (AML) is a group of clinically and genetically heterogeneous diseases. In spite of the landmark progress in treating acute promyelocytic leukemia (M3), current treatment of AML is still largely based on chemotherapy. The standard induction chemotherapy consists of anthracycline and cytarabine ("3+7" regimen) could achieve the complete remission (CR) rate about 75%, but due to the variability of individual genetic profile and drug sensitivity, the outcome is uncertain. Monitoring of the minimal residual disease (MRD) could reflect the treatment response in time, and then become the essential reference for AML patients to optimize chemotherapy strategy. In the past decades, multiparameter flow cytometry (MFC) method

has been used as a standard technique to track MRD in leukemia patients.
Aims: For AML patients, the MRD detection of bone marrow was usually performed after one course of induction chemotherapy. To optimize the chemotherapy strategies, it is essential to establish an earlier and easier method to monitor the early treatment response during induction period. Peripheral blood (PB) blasts clearance rate might serve as such a monitoring marker.
Methods: PB blasts were monitored using flow cytometry, the absolute counts were calculated before treatment (D0) and on the 3rd, 5th, 7th, 9th days (D3, 5, 7, 9) of induction chemotherapy. The cut-off value of Day 5 peripheral blasts clearance rate (D5-PBCR) was defined by a receiver operating characteristic (ROC) analysis. Prognostic effect was compared between the different patient groups according to D5-PBCR cut-off value.
Results: The D5 peripheral blasts clearance rate (D5-PBCR) cut-off value was determined as 99.55%. Prognostic analysis showed that patients with D5-PBCR≥99.55% were more likely to achieve CR (94.6% vs. 56.1%, *P*<0.001) and maintain the relapse-free status (80.56% vs. 57.14%, *P*=0.027). In survival analysis, both relapse-free survival (RFS) and overall survival (OS) were longer in the D5-PBCR≥99.55% patients group (2-year OS: 71.0% vs. 38.7%, *P*=0.011; 2-year RFS: 69.4% vs. 30.7%, *P*=0.026). Moreover, in cytogenetic-molecular intermediate-risk group, a subgroup with worse outcome could be further distinguished by D5-PBCR level (<99.55%) (OS: *P*=0.033, RFS: *P*=0.086).
Summary and Conclusions: In conclusion, our work established an effective early treatment response evaluation method by monitoring PB blasts using flow-cytometry. The D5-PBCR value (99.55%) could be a reliable reference to predict the treatment response and outcome in very early stage of chemotherapy, which may offer the opportunity of induction regimen modification, and help in optimizing the current cytogenetic-molecular prognostic risk stratification.

E959

PROPHYLACTIC USE OF SORAFENIB IN PATIENTS WITH FLT3-ITD-POSITIVE ACUTE MYELOID LEUKEMIA UNDERGOING ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Internal tandem duplication of FMS-like tyrosine kinase 3 (FLT3-ITD) mutations have been reported in 20%>30% of patients with acute myeloid leukemia (AML). FLT3-ITD-positive AML patients have an inferior survival, primarily due to lower complete remission (CR) rate and higher relapse rate. Although allogeneic hematopoietic stem cell transplantation (allo-HSCT) improves the outcomes of a proportion of FLT3-ITD-positive AML, a significant number will suffer disease recurrence after allo-HSCT. Sorafenib, an inhibitor of multiple kinases including FLT3, has shown promising activity in FLT3-ITD-positive AML. Recent studies have shown that sorafenib monotherapy or in combination with chemotherapy are effective in attaining CR, but they do not have significant improvement in relapse. Currently, prophylactic use of sorafenib after allo-HSCT has been rarely reported, and whether it can improve outcomes of FLT3-ITD-positive AML remains unclear.
Aims: To assess the feasibility and efficacy of prophylactic use of sorafenib in FLT3-ITD-positive AML after allo-HSCT for preventing relapse.
Methods: A total of 18 patients with FLT3-ITD-positive AML undergoing allo-HSCT from January 2012 to March 2014 at our single institute were enrolled in this retrospective study. The median age was 29 years (range 16-51 years), with 10 males and 8 females. Thirteen patients received related (9 sibling and 4 family donors), 5 unrelated donor transplants; 8 received HLA locus matched, 10 mismatched transplants. Seven patients were in first CR at the time of transplantation and all received standard conditioning. Eleven patients were not in CR (NR) at the time of transplantation and all received intensified conditioning. Sorafenib was used from day 30 to day 180 post-transplantation. The initial dose of sorafenib was 400 mg orally twice daily and was adjusted in case of suspected toxicity or resistance (dose range, 200-800 mg daily).
Results: Of the 18 patients, 7 patients received prophylactic sorafenib, including 3 in CR and 4 in NR pre-transplantation; 11 did not receive prophylactic sorafenib, including 4 in CR and 7 in NR pre-transplantation. The eleven patients in NR at the time of transplantation all achieved CR and had complete chimerism by day +30 post-transplantation. Of the 7 patients with prophylactic sorafenib, one developed grade II acute graft-versus-host disease (aGVHD) and 3 developed chronic GVHD (cGVHD, limited in 2 and extensive in 1). Of the 11 patients without prophylactic sorafenib, 3 patients developed aGVHD (grade II in 2 and III in 1) and 7 developed cGVHD (limited in 3 and extensive in 4), including 1 grade III aGVHD and 2 cGVHD after donor lymphocyte infusion (DLI). With a median follow up of 433 (range, 124-765) days post-transplantation, 7 patients with prophylactic sorafenib all had no recurrence and survived. Of the 11 patients without prophylactic sorafenib, 5 experienced leukemia relapse at a median time of 92 (range, 49 to 335) days post-transplantation, including hematologic in 4 and central nervous system (CNS) in 1. The patient with CNS relapse achieved CR after radiotherapy and DLI, and was still alive now. Of the 4 patients with hematologic relapse, one abandoned treatment and 3 received treatment, including sorafenib combined with chemotherapy and DLI. Two of the three patients achieved short CR after treatment and finally all of them died of relapse within half a year. With a median follow up of 343 (range, 135-863) days post-transplantation, 6 patients survived and 5 died in the 11 patients without prophylactic sorafenib. Causes of death included leukemia relapse (n=4) and cGVHD (n=1). The main side effect of sorafenib was rash, and none of patients experienced bone marrow suppression. Of the 10 patients with sorafenib treatment, 6 had hand-foot skin reaction and/or rash. Anti-allergy therapy was ineffective, and glucocorticoid was usually required. In 4 of the 6 patients, the skin rash covered gradually after reducing the dose of sorafenib and the use of glucocorticoid. Other two patients even required drug discontinuation, but sorafenib was tolerated after the reuse although the rash reappeared.
Summary and Conclusions: Early prophylactic use of sorafenib has an acceptable toxicity profile and could reduce relapse of FLT3-ITD-positive AML.

E960

COMPARISON OF DIFFERENT COMORBIDITY INDICES FOR PREDICTING OUTCOME IN PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background: Age is a dominant predictor of outcome in acute myeloid leukemia (AML). It is unclear to which extent comorbidities contribute to this effect. Unfortunately, data regarding the impact of pretreatment comorbidities on prognosis and outcome are limited.
Aims: The object of this study was to determine the effect of relevant comorbidities on the survival of patients with AML and compare three validated comorbidity indices in predicting outcome.
Methods: In a single-center retrospective study 194 AML patients aged older than 18 years treated between 1996 and 2012 were included. The Hematopoietic stem cell transplantation comorbidity index (HCT-CI), the Adult Comorbidity Evaluation-27 (ACE-27) score and the Cumulative Illness Rating Scale for Geriatrics (CIRS-G) at the time of initial AML diagnosis were evaluated to assess the degree of pretreatment comorbidity conditions. Data on demographics, cytogenetics, treatment and outcome were collected. Kaplan-Meier methods and Cox regression were used to assess survival.

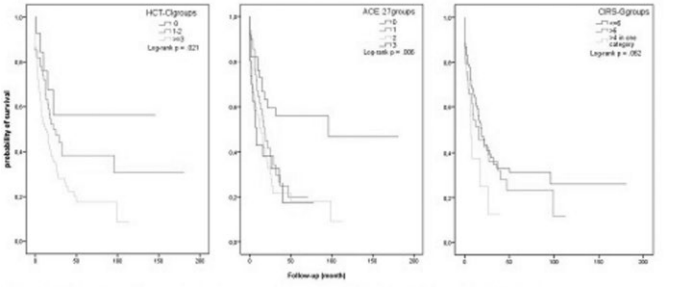

Figure 1. Survival curves according to HCT-CI, ACE-27 and CIRS-G.

Results: The study included 102 male and 92 female with median age at diagnosis of 60.9 years (range, 18-90). Median duration of follow-up was 9 month. One hundred twelve (57.7%) patients died, and 173 (89,2%)received intensive chemotherapy. Overall median survival was 17 month (range, 0-180). The most frequent comorbidities included cardiovascular disease (59.8%), infection (57.7%), hepatic disorders (45.9%) and prior malignancy (39.2%). In univariate analysis, presence of cardiovascular disease, severe hepatic disease and renal impairment were associated with inferior survival (26 *vs* 12 month, p=.005; 19 *vs* 4 month, p=.013; 17 *vs* 7 month, p=.016). Also, older age (≥60 years), poor cytogenetics and higher ECOG score (≥2) were significantly associated with reduced overall survival (25 *vs* 13 month, p=.003; 29 *vs* 12 month, p=.008; 21 *vs* 4 month, p=.000). For each index, the highest comorbidity burden was associated with poorer overall survival (Figure 1). However, in Cox-analysis the impact on overall survival was only significant for the ACE-27 score and the HCT-CI. Median survival by ACE-27 score was 96, 18, 14 and 8 month for patients with none, mild, moderate and severe comorbidities, respectively (p=.006). Median survival by HCT-CI was not reached, 22 and 12 month for patients in the lower- (0 points), intermediate- (1-2 points) and high- (≥3 points) risk groups, respectively (p=.021). Although the HCT-CI appeared to separate the risk groups better, in multivariate models only the ACE-27 score was significantly associated with overall survival. Adjusted hazard ratios were 1.9, 2.3 and 4.2 for mild, moderate and severe comorbidities, respectively, compared with no comorbidities (p=.004). Exploratory evaluations of the multivariate analysis suggested that higher ECOG (≥2), poor cytogenetics and presence of severe comorbidities according to ACE-27 had a significant impact on overall survival. Interestingly, higher age (i. e. ≥60 years) had no significant impact in multivariate analysis.
Summary and Conclusions: Comorbidities have a significant impact on survival of patients with AML. Patients with severe comorbidity had a greater than

50% decrease in survival, independent of age, ECOG and cytogenetics. A pre-treatment assessment of severity of comorbidities by comorbidity indices may help to identify patients with poor outcome. It appears that age at least partially impacts survival as a marker for comorbidities. Our data suggest that the ACE-27 score is a suitable index to predict survival in patients with AML.

E961

CLINICAL OUTCOMES OF ACUTE ERYTHROLEUKEMIA TREATED WITH AZACITIDINE: A RETROSPECTIVE MULTICENTER STUDY

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Background: Acute erythroleukemia (AML-M6) accounts for 4% of all AML and is often associated with high risk karyotypes and poor prognosis, with a median survival of 3-9 months from diagnosis. When treated with intensive chemotherapy (ICT), remissions last less than one year (Santos et al, Leukemia 2009). Azacitidine (AZA) prolongs overall survival (OS) in patients with MDS and AML who are not candidates for ICT (Fenaux et al, Lancet Oncology 2009) but there is limited data of its efficacy in AML-M6 (Almeida, Leuk Res Reports 2013).
Aims: We report the clinical outcomes of 88 AML-M6 patients treated with AZA.
Methods: Data were pooled from registries and/or clinical files from multiple nations. Survival was estimated using the Kaplan-Meyer method and log-rank test for group comparison.

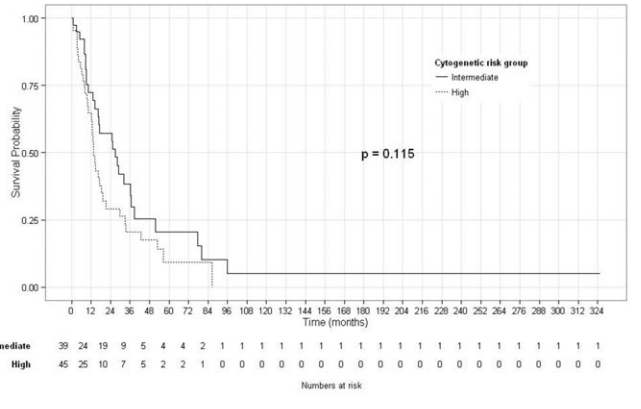

Figure 1.

Results: AZA was used as first line treatment in 41 patients (47%) and as ≥2nd line treatment in 51% (treatment line unknown in 2%).
Median age was 69 (range 28-88) years. MRC cytogenetic risk was intermediate in 44% and poor in 51%. The median time from diagnosis to treatment start was 21.9 days (1-552) and patients received a median number of 5 cycles (1-37). At the time of last follow-up 22 patients were still alive, of which 9 were still on AZA treatment; the main reasons for treatment discontinuation were disease progression (52%), infection/toxicity (12%), death (11%) and allogeneic transplantation (17%). At the time of writing, 66 patients had died from disease progression (48%), infectious complications (12%), and other (12%) or unknown causes (21%). Recorded grade 3-4 toxicities were predominantly hematological, and only 32 hospital admissions due to toxicities were reported. Overall marrow response was 34%, with 23% complete responses and 11% partial responses (assessed according to IWG criteria). Time to first and best response was 2.6 and 3.9 months, respectively. Progression free survival (PFS) in the whole AZA cohort was 5.1 months (range 3.4-9.2), and 9.4 months (range 4.3-14.5) in those treated with AZA first line. The median OS was 16.8 months (13.3-28.3) from diagnosis, 12.3 months (9.8-14.3) as of treatment start with AZA. Median OS from start of treatment for patients treated with AZA 1st line was 14.2 months (12.6-25.2) months, and 9.8 months (4.6 to 13.5) for those receiving AZA ≥2nd line. Response to AZA showed a significant impact on OS: 18.3 months in patients achieving CR/PR/HI, 16.2 months in patients with marrow SD and 9.1 months in patients with PD (p=0.008) Of importance, patients with poor-risk cytogenetics did not have an inferior survival when treated with AZA (Fig1). The median OS from start of treatment in a group of AML-M6 patients (n=34) treated with ICT alone was 7.6 months (5.4-20.3).
Summary and Conclusions: AZA demonstrates efficacy and shows promising response and survival results in our cohort of AML-M6 patients, not inferior to that seen with ICT. The median OS of 14.2 months for those treated with AZA 1st line compares favorably with data for WHO-AML patients treated with AZA 1st line in a randomized trial (10.4 months; Dombert H, EHA 2014, LB-2433) or from established patient registries (12.8 months; Pleyer L *et al.*, ASH 2014, #943). In addition, the observed median OS of 9.8 months in relapsed or refractory AML-M6 patients which generally confers a dismal prognosis (~3 months; Ferrara F, Haematologica 2004;998; Roboz G, JCO 2014;1919), is promising. Thus, AZA is a treatment option for 1st line treatment of AML-M6 patients ineligible for IC approaches, and also as salvage therapy for AML-M6 patients with relapsed/refractory AML.

E962

CLINICAL FEATURES AND OUTCOMES OF HYPOCELLULAR ACUTE MYELOID LEUKEMIA IN ADULTS: KOREAN AML REGISTRY DATA

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Background: The hypocellular variant of acute myeloid leukemia (AML) is defined as the presence of <20% of cellular bone marrow in a biopsy specimen at presentation.
Aims: We performed a retrospective analysis to determine the clinical features and survival outcomes of hypocellular AML in a Korean population.
Methods: We reviewed the medical records of all patients with AML who were diagnosed from 2006 to 2012 at 9 hospitals participating in the AML registry. Overall survival and event-free survival were calculated from the time of diagnosis until death or an event, respectively. An event was defined as relapse after remission, failure to respond or death from any cause.
Results: In total, 2110 patients were enrolled, and 102 (4.8%) were identified as having hypocellular AML. Patients with hypocellular AML were older than those with non-hypocellular AML (median age, 59 *vs. 49* years, respectively; p <0.001) and more frequently presented with leukopenia (mean white blood cell count, 5,810/μL *vs.* 40,549/μL, respectively; p <0.001). There were no differences in the presence of antecedent hematologic disorders (5.9% *vs.* 5.3%, respectively; p=0.809). No differences were seen between the hypocellular and non-hypocellular AML groups with respect to the complete remission rate (53.9% *vs*. 61.3%, p=0.139), early death rate (defined as any death before 8 weeks, 14.7% *vs.* 13.0%, p=0.629). Overall survival (OS) and event-free survival (EFS) were not different between two groups (median OS, 16 *vs.* 23 months, respectively; p=0.169; median EFS, 6 *vs.* 9 months, respectively; p=0.215).
Summary and Conclusions: Hypocellular AML is more frequently observed in older age, but the clinical outcomes of hypocellular AML do not differ from that of non-hypocellular AML.

E963

REDUCED-INTENSITY TRANSPLANTATION AS A PART OF STANDARD TREATMENT STRATEGY IN PATIENTS AGED 60 TO 70 YEARS WITH ACUTE MYELOID LEUKEMIA – SINGLE CENTRE EXPERIENCE

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Background: Outcome of patients (pts) over 60 years of age with acute myeloid leukemia (AML) treated only with intensive chemotherapy is poor. To improve treatment results reduced-intensity transplantation (RIT) was establish as a part of standard treatment strategy for pts aged 60 to70 years with AML (except of pts with prognostically favourable AML) in our centre from 2003.
Aims: With the aim to evaluate transplant feasibility and the role of RIT in the treatment of pts aged 60 to 70 years with AML we analysed outcome of such pts in our centre from 2003.
Methods: From 1/2003 to 11/2014 AML was diagnosed in 188 pts aged 60 to 70 years. 120 pts were intensively treated and 61 pts with median of age 63 years (range, 60-68 years) with AML in 1st CR (35 pts), in 2nd CR (5pts), with primarily resistant AML (9 pts) and with AML beyond CR (12 pts) underwent RIT (30% HLA identical related, 41% HLA matched unrelated, 26% HLA mismatched unrelated, 3% haploidentical related). Source of stem cells was in 85% peripheral blood and in 15% bone marrow with the median of infused CD 34+cells 4,8x10^6/kg (range, 1,7-14,9x10^6/kg). The conditioning regimen consisted of fludarabine (30mg/m2 for 4 days) and melphalan (140mg/m2 for 1 day), in 14 pts with ATG (ATG Fresenius 15mg/kg). CsA and methotrexate were used as GVHD prophylaxis except haploidentical RIT where posttransplant CPA and combination of CsA and MMF was used.
Results: The main reasons of impossibility to implement RIT in treatment of older pts with AML were death during remission induction treatment (39%), resistant or progressive AML (24%), non-availability of donor (16%), severe comorbidities (12%) and refusal of RIT (9%). However 51% of intensively treated pts with AML eventually underwent RIT. All pts fully engrafted and achieved complete remission (CR). 37 pts (60%) developed aGVHD (6 pts grade III-IV) and among 50 evaluable pts 22 (44%) of them developed chGVHD (9 limited, 13 extensive). With median follow-up 46 months (range, 4-123 months) 27 pts (44%) are alive (26 pts in CR). 14 pts (23%) relapsed and 13 of them died. 21 pts (34%) died due to NRM, 2 (3%) of them till day 100 after RIT and 11 (18%) of them till day 365 after RIT. The estimated probabilities of 3-years EFS and OS are 44% and 47%. 3-years OS for all intensively treated elderly pts was 38%.
Summary and Conclusions: Our data show that half of intensively treated pts aged 60 to 70 years with AML were able to undergo RIT and that RIT even in case of unrelated or HLA mismatched donor is associated with acceptable NRM and encouraging disease control of unfavourable AML (3-years OS 47%). The relatively high percentage of transplanted pts undoubtedly influenced the overall results of the group of intensively treated elderly patients with AML with a median survival of 15 months and estimated 3-year OS of 38%. RIT should be considered as standard treatment option for consolidation therapy in elderly patients with AML.

E964

HUMAN MYELOID INHIBITORY C-LECTIN (HMICL): A NOVEL ACUTE MYELOID LEUKEMIA MARKER

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Background: Acute myeloid leukemia (AML) is the most frequent acute leukemia affecting adults and its incidence increases with age. Leukemogenic mutations affect hemopoietic stem cells resulting in leukemic clones. It has been proven that leukemic stem cells reside in CD34+CD38- compartment of the bone marrow. Several studies were conducted to include different markers *e.g.* CD44, CD123, CD90 and CD133 for discriminating leukemic stem cell during treatment (minimal residual disease) or for targeted therapy. The human myeloid inhibitory C-type lectin-like receptor (hMICL) (also named CLL-1) was reported as possible leukemic stem cell marker and for targeted therapy.
Aims: The aim of this work is to determine the diagnostic impact and the applicability of the Human Myeloid Inhibitory C-Lectin (hMICL) in routine clinical flow cytometry (FCM) in the diagnosis of AML in Egyptian patients.
Methods: Bone marrow samples were drawn from 40 de novo AML and 20 de novo ALL patients. Mobilized peripheral blood was drawn from healthy donors after granulocyte colony-stimulating factor (G-CSF) stimulation. An informed consent in accordance with the Declaration of Helsinki was obtained. Bone marrow samples were stained with hMICL PE (clone: 687317, R&D systems, Minneapolis, USA) in combination with CD45 PE-PC5,CD34 ECD and CD38 FITC (Bechman Coulter,Maiami, USA) analysis done within 24 hours of sampling. Sample analysis was done by multicolor flow cytometry (Coulter Epics XL, Hialeah, USA). Gating strategy was applied using dim CD45/side scatter. Data analysis was done on Winlist 6 (Verity Software House, Topsham, ME).
Results: In AML cases, hMICL was expressed in all studied patients ranging from 20% to 95% (mean 60.3±19.9). This was found to be significantly higher compared to the healthy control subjects who ranged from 0.8% to 9.5% (mean 3.5±2.4) ($p<0.0001$) and to ALL patients who ranged from 0.9% to 7.2% (mean 3.3±1.9) ($p<0.0001$). In different FAB subtypes, expression of hMICL percentage was heterogeneous. The highest percentage was 95% in M1 and the lowest was 20% in M4. This heterogeneity of hMICL % expression was found to be significant ($p=0.02$). An interesting finding is that hMICL expression was also found in all CD 34- cases with percentage that reached 87% which pave the way for improved leukemia associated immunophenotype (LAIP) characterization and minimal residual disease (MRD) detection and quantification by multicolour FCM in the otherwise poorly described CD34- AML cases. hMICL expression has no statistical significance regarding age, gender, hemoglobin, platelet count, total leukocyte count, blast percentage, CD34, cytogenetic profile, and FLT3 ITD mutation. Using the receiver operating characteristic (ROC) curve, cut-off values for hMICL% and hMICL-MFI were identified which allow the most significant separation and differentiation between AML cases and normal subjects. The hMICL % cut-off value was 9.5% showing sensitivity of 95% and specificity of 100%. The hMICL MFI cut-off value was 2.3 showing sensitivity of 100%, specificity of 85%
Summary and Conclusions: hMICL is unique novel discriminator of AML reliable for diagnosis and MRD detection and it is considered a goal for targeted therapy.

E965

CLINICAL OUTCOMES OF AML PATIENTS TREATED WITH AZACITIDINE IN PORTUGAL: A RETROSPECTIVE MULTICENTER STUDY

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Background: Acute myeloid leukemia (AML) in elderly patients has a poor prognosis when treated with intensive chemotherapy (IC) and there is a need for new treatment options. Regardless of the blast count, elderly patients often display more indolent disease behaviour, resembling that seen in high risk myelodysplastic syndromes (MDS). Several studies have demonstrated the efficacy of Azacitidine (AZA), approved for use in MDS, in patients with AML unfit for IC.
Aims: We present a multi-centre retrospective analysis of efficacy of azacitidine in AML patients unfit for IC or relapsed/refractory to IC.
Methods: Retrospective data collection from clinical files was carried out following ethical committee approval. Survival was estimated using the Kaplan-Meyer method, post-hoc pairwise comparisons using log-rank test were performed and the p-values adjusted for multiple comparison using Hochberg method. Responses were assessed according to the IWG-MDS-2006 response criteria. [F1]Avaliação de resposta de acordo com que critérios.
Results: One-hundred and twenty three AML patients treated with AZA were identified. Median age at diagnosis was 69 years; 67% were men. At diagnosis, 75% of patients were anemic, 38% neutropenic and 46% thrombocytopenic. Secondary AML was diagnosed in 45% of patients. Karyotypes were normal in 40%, intermediate risk in 23% and poor risk in 21%. Patients started azacitidine a median of 1.5 months following diagnosis. It was first line therapy in 57% and second line following IC in 28%, unknown in 15%. Patients were treated until progression, toxicity or bone marrow transplant and received a median number of 5 cycles. Overall response rate was 27.6%, with 19.5% complete and 8.1% partial responders. Stable disease at time of assessment was seen in 17.9% and progression in 17.1%. Haematological improvement was achieved in 44.7% of patients. Of the whole population, 4 (3.3%) were not evaluable for survival. During the study period 86 deaths were reported. The main cause of death was disease progression (64%). The other main identified cause was infectious (12%). Median overall survival was 15.7 months. Response of any quality to AZA had a significant impact in overall survival. The median survival observed in patients achieving CR or PR was 19.2 months, in patients with SD was 24.6 months and in patients with PD was 6.4 months ($p=0.006$). Pairwise comparisons show a significant difference in overall survival between PD and CR+PR and between PD and SD but not between CR+PR and SD. The median survival was significantly higher in patients with haematological improvement (17 *vs* 13 months, respectively; log rank test: $p=0.048$).
The survival of the cohort treated with AZA was compared to that of a matched pair group treated with IC. The median time from diagnosis to start of IC in this comparator group was 0.13 months (range: 0-9.2 months). The median follow-up was 5.4 months (range: 0.1-43.4). There were 48 deaths and only 2 patients were still alive at the time of last follow-up. Median overall survival observed in this comparator cohort was 5.4 months. This was significantly inferior to that observed in the cohort treated with AZA ($p<0.0001$).
Summary and Conclusions: These results support the efficacy of AZA in AML in a similar fashion to that demonstrated in high risk MDS, increasing treatment options in these patients.

E966

THE DNMT3A R882 MUTATION WITH FLT3-ITD POSITIVITY IS AN EXTREMELY POOR PROGNOSTIC FACTOR IN PATIENTS WITH NORMAL KARYOTYPE AML AFTER ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION

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Background: The prognostic relevance of epigenetic modifying genes (*DNMT3A*, *TET2*, and *IDH1/2*) in patients with acute myeloid leukemia (AML) has been investigated extensively.
Aims: However, the prognostic implications of these mutations following allogeneic hematopoietic cell transplantation (HCT) have not been evaluated comprehensively in patients with normal karyotype (NK)-AML.
Methods: A total of 121 patients who received allogeneic HCT for NK-AML were evaluated for the *FLT3*-ITD, *NPM1. CEBPA, DNMT3A, TET2*, and *IDH1/2* mutations in diagnostic samples and analyzed for long-term outcome following allogeneic HCT.
Results: The prevalence rates for the mutations were: *FLT3*-ITD^{pos}(32.2%), *NPM1*^{mut} (43.8%), *CEBPA*^{mut} (double) (29.2%), *DNMT3A*^{mut} (32.2%), *DNMT3A* R882^{mut} (19.8%), *TET2*^{mut} (8.3%), and *IDH1/2*^{mut} (15.7%). The 5-year overall survival (OS) and event-free survival (EFS) rates were 57.9% and 56.2%, respectively, with a median follow-up duration of 69.2 months. A multivariate analysis revealed that *FLT3*-ITD^{pos} (hazard ratio, [HR], 2.122; p=0.009) and *DNMT3A R882*^{mut} (HR, 2.198; p=0.012) were unfavorable prognostic factors for OS. In addition, both mutations were significant risk factors for EFS and relapse risk. *DNMT3A* R882^{mut} had high coincidence with *FLT3*-ITD^{pos} (p=0.038). Additionally, patients with both mutations showed the worst OS, EFS, and higher relapse rates than those with the other mutations, which were confirmed in a propensity score matching analysis.
Summary and Conclusions: These results suggest that the *DNMT3A* R882^{mut}, particularly when accompanied by *FLT3*-ITD^{pos}, is a significant prognostic factor for inferior transplant survival outcome by increasing relapse risk, even afterallogeneic HCT. Thus, the association between *DNMT3A R882*^{mut}and *FLT3*-ITD^{pos} will clarify the prognosis in the patients with NK-AML after allogeneic HCT.

E967

LOW-DOSE CYTARABINE AFTER FAILURE OF 5-AZACITIDINE AS PALLIATIVE TREATMENT FOR ELDERLY PATIENTS WITH AML OR HIGH-RISK MDS INELIGIBLE FOR INTENSIVE CHEMOTHERAPY. A RETROSPECTIVE SINGLE CENTER ANALYSIS

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Background: The demethylating agents 5-azacitidine (5-AZA) or decitabine recently became a standard of care for first line treatment of elderly or unfit patients with high-risk myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML) who are not eligible for intensive induction chemotherapy or allogeneic stem cell transplantation. Although there is an improvement in overall survival with epigenetic therapies, almost all patients eventually develop disease progression, for which no standard therapeutic option is available.
Aims: The aim of our retrospective analysis was to evaluate the clinical benefit of low-dose cytarabine (ARA-C) treatment compared to best supportive care (without chemotherapy) as second-line palliative treatment options after failure of 5-AZA.
Methods: From 2009 to 2014 we treated 48 consecutive patients, 10 with newly diagnosed high-risk MDS and 38 with de novo AML, with 5-AZA (75mg/m2 s.c. d1-7, q-d29) as first-line therapy until progression. After failure of 5-AZA, 15/31 patients received a 2nd-line treatment with subcutaneous low-dose cytarabine (ARA-C, 2x 10 mg/m2/d, d1-10) and 16 patients received best supportive care only, without chemotherapy.
Results: After first-line 5-AZA 37/48 (76%) patients achieved a partial hematologic response (n=23) or stable disease (n=14). Median duration of response was 14 (2-52) months. After a median follow-up of 18 months, 31 patients had a disease progression, as defined by worsening of peripheral blood counts, increase of blasts or increase in transfusion frequency. Four of 15 patients, who were treated with ARA-C as second line therapy, achieved a partial hematologic response and 5/15 had stable disease with a median response duration of 4 months. The median overall survival from the time of progression on 5-AZA (OS-2) was 6 months, with a trend for longer survival in patients achieving at least a partial remission. There was no correlation between response and cytogenetic risk. In comparison to the ARA-C group, in 16 patients who received best supportive care only, the median OS-2 was 2.9 months and no objective responses were observed.
Summary and Conclusions: In conclusion, our analysis show that second-line treatment with ARA-C may add a survival benefit of approximately 3 months compared to best supportive care alone. The second overall survival (OS-2) of 6 months is within the range achieved with low dose ARA-C as primary treatment for this patient population. Our data indicate that ARA-C can be considered as a reasonable therapeutic option after failure of 1st-line treatment with demethylating agents like 5-AZA. This clinical observation is supported by recent pre-clinical data, showing that epigenetic modifications in AML cells can have a sensitizing effect for the subsequent administration of ARA-C.

LB2075

FAVORABLE OUTCOME OF PATIENTS WITH NORMAL KARYOTYPE ACUTE MYELOID LEUKEMIA HARBORING FLT3-ITD AND TREATED WITH CLADRIBINE ADDED TO DAUNORUBICIN AND CYTARABINE INDUCTION

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Background: *FLT3* internal tandem duplication (*FLT3*-ITD) is present in nearly 25% of patients with normal karyotype acute myeloid leukemia (NK-AML) and have been associated with poor prognosis. Because of inferior outcome of *FLT3*-ITD^{+} NK-AML patients when treated with standard chemotherapy regimens like daunorubicin (DNR)-cytarabine (AraC) 3+7 induction (DA), this group prompts the use of investigational therapy. Targeting the *FLT3* receptor signaling has recently become particularly attractive. However, so far clinical experience failed to demonstrate efficacy of such approach.
Aims: We retrospectively evaluated the effect of the addition of cladribine to induction regimen on patients outcome according to presence or absence of *FLT3*-ITD.
Methods: 227 patients with NK-AML either enrolled in clinical trials or treated with the same protocols outside prospective studies were included in the analysis. 103 patients were treated with daunorubicine-cytarabine-cladribine (DAC) while 124 patients received daunorubicine-cytarabine (DA).
Results: Among *FLT3*-ITD^{+} patients the use of DAC was associated with increased rate of complete remission rate (CR; 86% *vs* 61% in DA group; p=0.04) and improved overall survival (OS; 37% *vs* 14%, respectively; p=0.05). The effect on OS was most prominent when observations were censored at time of allogeneic hematopoietic stem cell transplantation. In multivariate analysis, DAC independently improved CR rate and predicted longer OS in the group of *FLT3*-ITD^{+} patients after adjusting for age and *NPM1* mutations (p=0.07 for CR rate; p=0.009 for OS). Such an effect could not be demonstrated for *FLT3*-ITD^{-} patients.
Summary and Conclusions: Thus, our results suggest that DAC regimen can abolish negative effect of *FLT3*-ITD on the prognosis of NK-AML patients.
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LB2085

ELEVATED PIM-2 GENE EXPRESSION IS ASSOCIATED WITH POOR SURVIVAL OF PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background: *PIM-2* gene belongs to the *PIM* family which encodes the serine/threonine kinases involved in cell survival and apoptosis. While *PIM-1* is described as an antiapoptotic protein in acute myeloid leukemia (AML), much less is known about the importance of *PIM-2*. The relations between *PIM-2* gene expression and AML outcome have not been fully defined
Aims: The aim of the presented study was to evaluate *PIM-2* gene as marker of AML malignancy. We also examined the role of *PIM-2* expression in apoptosis and proliferation of blastic cells isolated from patients with AML.
Methods: Ninety-one patients were enrolled in this study: aged 18-84, median age 50. Forty-eight patients reached complete remission (CR). For all patients, bone marrow samples were collected at the time of diagnosis, only samples with blastic cells for more than 80% of total cellularity were examined. In 20

patients bone marrow in CR phase was assessed. The control group consisted of 24 hematologically healthy bone marrow donors. External validation was performed on TCGA AML dataset. *PIM-2* expression levels assessed by RNAseq and represented as z-score were downloaded from cBioPortal. Expression of *PIM-2* gene was assessed by TaqMan RQ-PCRassay. Apoptosis was measured using FITC-Annexin V and propidium iodide staining. The BrdU staining was performed using FITC BrdU Flow Kit. Inhibition of *PIM-2* was performed with RNAi and apoptosis rate of HL-60/PAC-GFP and HL-60/PAC-GFP-shPIM-2 cells was analysed by standard methods. Expression of PIM-2, BAD, 4E-BP1 p-BAD, p-4E-BP1 proteins was assessed by Western blot. All statistical analyses were performed using GraphPad Prism 5 software.
Results: Median *PIM-2* gene expression in AML patients was significantly higher than in healthy control group. AML patients with high expression levels of *PIM-2* gene (above median) had significantly shorter leukemia free survival (LFS) than patients with low *PIM-2* expression (p=0.0082). External data from TCGA AML dataset was utilized to farther validate these observations. For patients with AML, Kaplan-Mayer analysis of overall survival confirmed that high *PIM-2* gene expression level is associated with poor prognosis (p=0.038). In addition, it was found that patients who achieved CR had significantly lower expression of the *PIM-2* gene than patients with no CR (p=0.0083). Importantly, elevated *PIM-2* gene expression assessed at presentation was restored to normal level in all patients in CR. Moreover we revealed overexpression of the PIM-2 kinase and its downstream effectors: native BAD and 4E-BP1 proteins as well as p-BAD (Ser112) and p-4E-BP1 (Ser65) in AML samples in comparison to controls. Negative, moderate correlation between *PIM-2* gene expression and the percentage of blastic cells in a total apoptosis was found (r^2=0.4332, p<0.0001). Cytometric analysis of HL60/PAC-GFP and HL60/PAC-GFP-shPIM2 cells revealed increase in the number of spontaneously apoptotic cells after inhibition of *PIM-2* gene.
Summary and Conclusions: In summary, we showed that elevated expression of the *PIM-2* gene in blastic cells is associated with poor prognosis of AML patients and their resistance to induction therapy, what can be linked with increase resistance of leukemic cells to apoptosis. We hypothesize that anti-apoptotic effect of the PIM-2 kinase is mediated, at least in part, by increased phosphorylation of BAD and 4E-BP1 proteins. Based on obtained data, we speculate that AML patients with elevated *PIM-2* expression more likely develop resistance to induction therapy, what may negatively affect their survival.
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Aggressive Non-Hodgkin lymphoma - Clinical

E968

RITUXIMAB MAINTENANCE VERSUS WW AFTER R-DHAP PLUS AUTOLOGOUS STEM CELL TRANSPLANTATION IN UNTREATED PATIENTS WITH MCL: INTERIM ANALYSIS OF THE LYMA TRIAL, A LYSA STUDY

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Background: The LyMa (ClinicalTrials.gov, NCT00921414) is a prospective randomised phase III trial conducted by the LYSA group. The study assessed the potential benefit of Rituximab maintenance after autologous stem cell transplantation (ASCT) in young previously untreated Mantle Cell Lymphoma (MCL) patients (<66y).
Aims: The primary endpoint was EFS at 4 years after randomization, EFS being defined as death of any cause, disease progression, severe allergic reaction or severe infection to Rituximab. PFS and OS were secondary objectives. Herein, we present the first planned interim analysis. Analysis was performed by intention to treat.
Methods: Patients were enrolled at times of diagnosis. All patients received 4 courses of R-DHAP followed by ASCT (4 additional courses of R-CHOP was given to patients who did not reach at least a PR after R-DHAP). The conditioning regimen of ASCT was Rituximab (500mg/m2) plus BEAM. Patients achieving a complete or partial response after ASCT were randomly assigned between 3 years of Rituximab maintenance therapy (375mg/m2, one injection every two months) versus wait and watch (WW) (1:1).
Results: From September 2008 to August 2012, 299 patients were included (one patient withdrawn his consent, data of one patient with incomplete data at time of the present analysis). Median age at registration was 57y (27-65) and 236 (78,9%) patients were male. MIPI score was low in 53,2% (n=159), intermediate in 27,4% (n=82) and high in 19,4% (n=58). In all, 257 (86%) patients proceeded to ASCT. The CR/CRu rates before and after ASCT were 81,4% and 92.7%, respectively. Last update was performed in November 2014. Median follow-up calculated from time of inclusion is 40.6 months. For all patients, median PFS and OS are not reached. The estimates 3y-PFS and -OS are 75% (95%CI ; 69.5-79.6) and 83.4% (95%CI ; 78.5-87.3), respectively. Last randomization was done in February 2013. Two hundred and thirty eight patients were randomised: 119 patients were assigned to rituximab maintenance and 120 to WW. The mFU (n=238) calculated from date of randomization is 34.3 months. The 3y-EFS is 88.1% (95%CI, 79.5-93.2) in the Rituximab maintenance arm versus 73.4% (95%CI, 62.6- 81.6) in the WW arm (p=0.0057). The 3y-OS does not differ between the two groups (85.5 in the WW arm *vs* 93.1% in the maintenance arm ; p=0.7175).
Summary and Conclusions: This planned interim analysis of the LyMa trial shows that a 3 years of rituximab maintenance after R-DHAP plus ASCT as first-line treatment for young patients with MCL significantly improves EFS and PFS.

E969

CLINICO-BIOLOGICAL CHARACTERISTICS AND OUTCOME OF PATIENTS WITH DIFFUSE LARGE B CELL LYMPHOMA (DLBCL) CARRYING HEPATITIS B VIRUS OR C VIRUS RECEIVING IMMUNOCHEMOTHERAPY

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Background: DLBCL patients carrying hepatitis B virus (HBV) or hepatitis C virus (HCV) are a management challenge in the clinical setting, particularly since rituximab-based treatments became the gold standard.
Aims: The aim of this study was to assess the HBV and HCV incidence and their clinical and prognostic impact in a series of DLBCL patients treated with immunochemotherapy.
Methods: 321 patients (161M/160F; median age 66) diagnosed with DLBCL in a single center between 2002 and 2013. Primary central nervous system lymphoma, primary mediastinal lymphoma and plasmablastic lymphoma were excluded, as well as transformed cases from other lymphomas, post-transplant lymphoproliferative disorders and HIV+patients. HCV+and HBV+were defined by the presence of IgG anti-HCV and HBV core-antibodies, respectively. Main clinico-biological characteristics and outcome were analyzed according to the viral status.

Results: 265 patients were virus negative, 32 HBV+(10%) and 29 HCV+(9%). 5 of the latter had HBV/HCV co-infection. Main initial features and outcome are detailed in the table. Elevated basal bilirubin correlated with higher liver toxicity during treatment (85% *vs* 40%, p<.001) and shorter overall survival (OS). After a median follow up of 49 months (range 2-146), median OS was not reached for patients without hepatitis, and was of 55, 38 and 14 months for HBV+, HCV+and HBV+/HCV+patients, respectively (p=.005). HCV+patients without liver cirrhosis showed an almost identical OS compared to hepatitis negative patients. When the analysis was restricted to patients receiving curative intention regimens, 5 year OS for hepatitis negative, HBV+, HCV+and HBV+/HCV+was 69%, 62%, 63% and 25%, respectively (p=NS).

Tabella 1. Summary of patient char acter ist ics and outcomes.

	No hepatitis (n=265)	HBV (n=32)	HCV (n= 29)	HBV/HCV (n=5)#
Gender (male) (%)	54	34*	35*	60
Age ≥60 years (%)	60	66	83*	80
Cell of origin $				
Germinal center (%)	45	57	38	0
Activated (%)	51	14	25	50
Undetermined (%)	4	29	38	50
Extranodal involvement (%)	70	63	59	40
Spleen (%)	14	29*	38**	60*
Liver (%)	8	13	24*	0
Ann-Arbor stage III-IV (%)	57	53	66	60
IPI risk high intermediate or high (%)	46	31	62*	40
Serum albumin <35 g/L (%)	18	31	44*	50
Serum LDH ≥450 UI/L (%)	49	47	72*	100
Serum B2m ≥2.3 mg/L (%)	53	73*	77*	100
Curative-intent treatment (%)	91	69**	76*	80
Liver toxicity grade ≥2 WHO	6	14	39**	75**
Complete remission (%)	73	61	55	40
5-year FPS (%)	53	39	37	20
5-year OS (%)	65	43*	42**	20

These patients are also included in HBV and HCV columns;*p<,05; **p<,005; $n= 64 patients

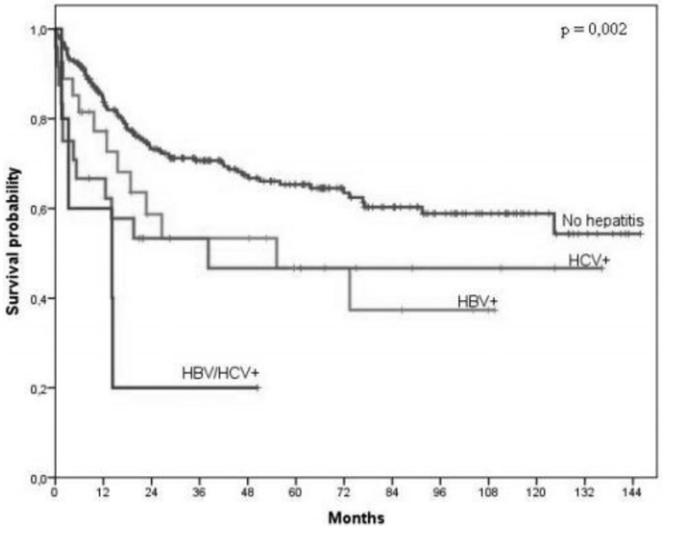

Figure 1.

Summary and Conclusions: The presence of HBV or HCV in DLBCL patients entails higher number of complications; however, liver impairment and not the hepatitis viral status is the key feature in the outcome of the patients.

E970

SIL INDEX IS A SIMPLE AND OBJECTIVE PROGNOSTIC INDICATOR IN DIFFUSE LARGE B-CELL LYMPHOMA

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Background: Rituximab (R) plus CHOP (R-CHOP) is the standard of care for patients with diffuse large B-cell lymphoma (DLBCL). The International Prognostic Index (IPI) and revised IPI were reported as prognostic indicators for DLBCL in 1993 and in 2007, respectively. Although they are widely accepted, the performance status (PS) factor is sometimes ambiguous or subjective. Therefore, we developed a new prognostic index, SIL, that includes only three objective prognostic factors: clinical stage (S), soluble interleukin-2 receptor level >2,500 U/mL (I), and elevated lactate dehydrogenase level (L) (Cancer Sci. 2012).

Aims: To compare the SIL index with other risk factors that comprise the IPI and to evaluate its utility in the risk stratification of patients with DLBCL.

Methods: Between 2003 and 2012, we registered and treated 781 consecutive patients with DLBCL, excluding those with mediastinal large B-cell lymphoma, intravascular large B-cell lymphoma, and primary effusion lymphoma. All the included patients were scheduled to undergo primary therapy with six cycles of full-dose R-CHOP. Patients in whom the initial therapy dose was reduced by >20% were excluded. Finally, 572 of 781 patients were retrospectively analyzed. Patients with partial remission (PR) after the initial four cycles underwent eight R-CHOP cycles in total, whereas those who did not achieve PR after the initial four R-CHOP cycles or those who exhibited disease progression at any given time received salvage therapy. If deemed necessary by the attending physician, additional local irradiation was performed in patients with PR or complete remission.

Results: The median age at diagnosis was 63 years (range, 18-89 years). The median number of therapy cycles was 6 (range, 1-8), and 90% of the patients received ≥6 cycles. Sixty-one patients (11%) received radiation therapy as primary treatment, which was often used to treat sites of residual masses at the end of chemotherapy. The median observation time for survivors was 55 months (range, 1-131 months). For the 572 patients, the 5-year progression-free survival (PFS) and 5-year overall survival (OS) rates were 70% and 81%, respectively. According to the SIL index, 367 patients (64.2%) and 205 patients (35.8%) were classified as having standard (SIL index: 0 or 1) and high (SIL index: 2 or 3) risks, respectively. Five-year PFS rates in the standard and high risk groups were 79% and 53%, respectively (*P* <0.0001). Five-year OS rates in the standard risk and high risk groups were 90% and 66%, respectively (*P* <0.0001) (Figure). Cox regression analysis of the SIL index, age (>60 years), PS (2-4), extranodal involvement sites (>1), and sex showed that the SIL index (*P* <0.0001; hazard ratio [HR]: 2.38) and PS (*P*=0.005; HR: 1.73) were independent risk factors for PFS. Similarly, the SIL index (*P* <0.0001; HR: 2.62) and PS (*P*=0.006; HR: 1.89) were independent risk factors for OS. When the patients were divided into two groups by age (≤60 years and >60 years), the SIL index was a good prognostic indicator for PFS and OS in both groups. When they were divided by the number of extranodal involvement site (0-1 and >1), and sex, the SIL index was still a good prognostic indicator for PFS and OS in both groups. Lastly, when they were divided by PS (0-1 and 2-4), the SIL index was effective in the good PS group. In the poor PS group however, the SIL index showed a statistically significant difference in the OS, but not in the PFS.

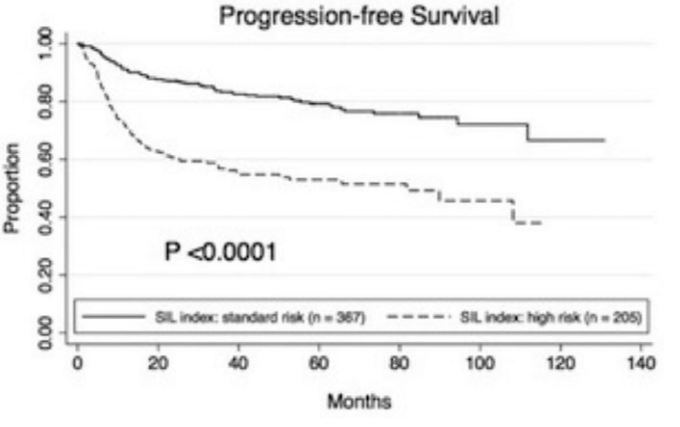

Figure 1.

Summary and Conclusions: The SIL index is a simple and objective prognostic indicator for DLBCL patients treated with R-CHOP.

E971

CLINICAL FEATURES AND OUTCOMES IN PATIENTS WITH PRIMARY TESTICULAR LYMPHOMA

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Background: Primary testicular lymphoma (PTL) is a rare type of extranodal non-Hodgkin lymphoma. Because of its rarity, clinical features, and optimal treatments remain uncertain.
Aims: To assess clinical features, outcomes and prognostic factors in patients with PTL.
Methods: In total, 62 patients were diagnosed with PTL at 8 institutions of the Yokohama City University Hematology Group from 1998–2014. Of 62 patients, 57 (92%) were diffuse large B-cell lymphoma (DLBCL). Our retrospective analysis included these 57 patients with PTL-DLBCL. We defined stage IE as mono or bilateral involvement of the testis, and stage IIE as the stage IE lesion with concomitant regional lymph node involvement. Stage III or IV, although undistinguishable from a nodal lymphoma in advanced stage, was defined as testicular involvement with distant lymph nodes and/ or extra-nodal sites involvements.
Results: The median age at diagnosis was 68 years (range, 42–85 years). The clinical stage of PTL was stage I, II, III, and IV in 25, 15, 7, and 10 patients respectively. The median observation period in the surviving patients was 53 months (range, 7–124 months). Fifty-five patients underwent orchiectomy. Fifty-five patients received systemic chemotherapy; 46 with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) therapy, 7 with CHOP therapy, and 2 with other regimens. Of the remaining 2 patients, 1 had orchiectomy and radiation, and 1 had best supportive care (BSC). Thirty-two patients received contralateral testicular irradiation in addition to systemic chemotherapy. Prophylactic intrathecal methotrexate (IT) or high-dose intravenous methotrexate (HdMTX) for central nervous system (CNS) relapse was administered in 40 patients; IT for 32, and HdMTX for 8. Among the 56 patients who underwent treatments other than BSC, 48 (86%) achieved complete remission, and 3 (5%) partial response. The 4-year progression-free survival (PFS) and overall survival (OS) rates of all patients were 57% and 59%, respectively. Twenty-one patients had disease progression after treatment for PTL. Extranodal recurrence was reported in 18. CNS was the most common site of extranodal relapse observed in 6 patients, although 5 of them had prophylactic IT. In total, 21 patients died: 13 of lymphoma, 2 of infection during chemotherapy, 1 of pneumonia in remission, 2 of secondary malignancy, and 2 of cardiopulmonary arrest from unknown cause. Absence of B symptoms, normal levels of serum lactate dehydrogenase, good performance status (PS), and addition of contralateral testicular irradiation to chemotherapy were associated with better PFS and OS at univariate analysis. Patients that received chemotherapy plus contralateral testicular irradiation had a tendency to be in earlier stages (P=0.03), and in better PS (P=0.008) than those who received chemotherapy alone. However, even when the analysis was limited to the patients with stage I and II, the benefit of radiation therapy added to chemotherapy remained statistically significant for PFS (P=0.015), and OS (P=0.002). The impact of additional radiation therapy diminished in advanced stages. In the multivariate analysis, any prognostic factors were significant for OS, whereas only additional contralateral testicular irradiation was associated with better PFS (HR 0.29; 95%CI 11-83; P=0.02).

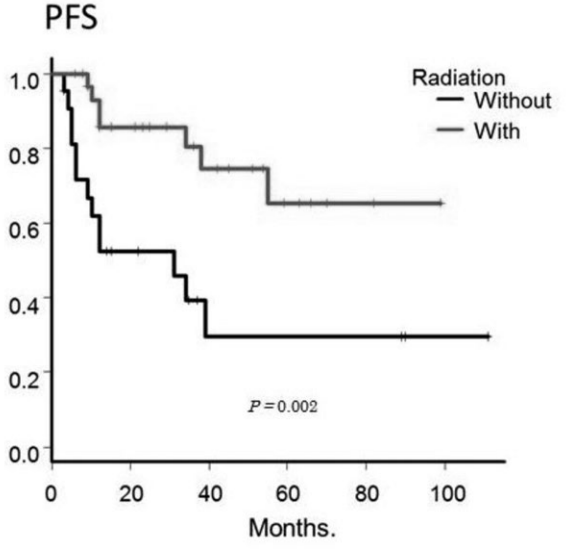

Figure 1. PFS according to contralateral testicular irradiation.

Summary and Conclusions: PTL-DLBLC had poor outcomes, and a high frequency of extranodal relapse, especially in CNS. Our study showed that chemotherapy with contralateral testicular irradiation may improve the outcomes.

E972

STATIN ADMINISTRATION AND THE RISK FOR NON-HODGKIN LYMPHOMA: A NATIONWIDE POPULATION-BASED CASE-CONTROL STUDY

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Background: 3-Hydroxy-3-methylglutary–coenzyme A reductase (HMG-CoA) inhibitors, also known as statins, are common cholesterol-lowering medications that are widely used for the primary and secondary prevention of cardiovascular disease and stroke. In addition to their cholesterol-lowering effects, several *in vitro* and *in vivo* studies have suggested that statins may have anticancer properties via various mechanisms.
Aims: The purpose of this study was to investigate whether statin administration was associated with a decreased risk of non-Hodgkin lymphoma (NHL).
Methods: This nationwide population-based case–control study was conducted retrospectively using the National Health Insurance Research Database (NHIRD) of Taiwan. The NHL group consisted of the patients with a first-time diagnosis of NHL between 2005 and 2008. The cases of the control group were pair-matched to the NHL group by sex, year of birth and index date. The usage of statins was analyzed retrospectively in both groups since January 1, 1996 to the index day. The cumulative defined daily dose (cDDD) was estimated to evaluate the statin exposure. Usage of other lipid-lowering agents, aspirin, non-steroidal anti-inflammatory drugs, cyclooxygenase-2 inhibitors and angiotensin-converting enzyme inhibitors was also identified. Adjusted odds ratios (ORs) and 95 % CIs (95 % confidence intervals) were estimated using multiple logistic regression.
Results: This study enrolled 1715 cases with NHL and 17150 controls for the analysis. Compared with the group without the statin administration, the group with any previous statin usage had an adjusted OR of 0.54 (95% CI=0.46-0.64). In addition, the chemo-preventive effect of the statins was exerted in a dose-response manner. The adjusted ORs were 0.69 (95% CI= 0.51-0.94), 0.56 (95% CI=0.42-0.76), 0.55 (95% CI=0.42-0.71), and 0.37 (95% CI=0.26-0.54) for statin administration of fewer than 28, 28 to 90, 91 to 365, and more than 365 cumulative defined daily doses (cDDDs), respectively, relative to the group without the statin administration.
Summary and Conclusions: The results of this study suggest that the administration of statins may reduce the risk of NHL.Further studies, especially prospective randomized trials, to confirm our findings are warranted.

E973

HIGHLY SUSPECTED LYMPHOMA ASSOCIATED HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS WITHOUT PATHOLOGICAL EVIDENCE: PET-CT FEATURES AND TREATMENT OUTCOME

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Background: Hemophagocytic lymphohistiocytosis (HLH) is a life threatening syndrome of hyper-inflammation, caused by uncontrolled activation and proliferation of lymphocytes and antigen-presenting cells. Up to now, few researches have described the characteristic findings of PET/CT in patients with HLH, especially for those with out known pathological evidences. Further studies about identifying the causative agents and finding efficacious regimens to lymphoma associated HLH patients are seldom reported.
Aims: The aims of the present study were to: (1) investigate the characteristics of PET-CT image in patients with sHLH; (2) find out the differences of PET-CT characteristics between lymphoma associated HLH (LAHLH) and non-lymphoma associated HLH (non-LAHLH); (3) discuss the diagnostic performance of PET-CT for the detection of underlying lymphoma, especially when pathological evidences were unavailable; (4) compare the efficacy of different regimens for sHLH without pathological basis.
Methods: A total of 44 adult patients first diagnosed with HLH who had undergone F-18 FDG PET-CT scanning before clinical treatment were enrolled in this retrospective analysis. Their clinical manifestations, laboratory findings, PET-CT characteristics, and received therapies or survival data were collected and compared. PET-CT parameters were comparatively analyzed between LAHLH patients and non-LAHLH patients. Moreover, the efficacies of initial therapies to highly suspected lymphoma were compared by OS analysis.
Results: All of the patients had at least 3 organs involved, including 37 cases (82.2%) showing splenomegaly, 35 cases (77.8%) with bone lesions, 34 cases (75.6%) lymphadenopathy, 35 cases (77.8%) inflammatory changes in the lung including pneumonia and/or atelectasis, 29 cases (64.4%) serous effusions, 26 cases (57.8%) pleura thickening and/or pleura adhesion, 17 cases (37.8%) cholecystitis or cholelithiasis, 16 cases (35.6%) sinusitis, 15 cases (33.3%) heart lesions, 13 cases (28.9%) brain tissue or cerebrovascular lesions, 10 cases (22.2%) hepatomegaly, 8 cases (17.8%) pericardium thickening. The maximum SUV levels of the spleen (SUV_{Sp}), bone marrow (SUV_{BM}), lymph node (SUV_{LN}), SUV_{max}, $SUV_{LN/Li}$ and $SUV_{max/Li}$ in LAHLH group were signif-

icantly higher than those in non-LAHLH group (p= 0.002, p=0.042, p=0.001, p<0.001, p=0.041, p=0.022, respectively). However the level of SUV_{Li}, $SUV_{Sp/Li}$, $SUV_{BM/Li}$ in two groups revealed no significant difference. HLH patients with an absolute SUV_{max} value >5.5, absolute SUV_{LN} value >2.6, and an absolute SUV_{sp} value >4.8 were more like to be LAHLH (p<0.001, sensitivity=92.9% and specificity=82.4%, AUC=0.933; p=0.001, sensitivity=85.7% and specificity=70.6%, AUC=0.851; p=0.001, sensitivity=71.4% and specificity=100%, AUC=0.836). The incidence of multiple lymphadenopathys with increased FDG uptake in LAHLH patients was significantly higher than those in non-LAHLH group (p=0.004); the incidence of multiple bone lesions in LAHLH patients was significantly higher than those in non-LAHLH group (p=0.016). Furthermore, OS analysis revealed that highly suspected LAHLH patients treated with lymphoma-chemotherapy had better prognosis (365 days) than those treated with non-lymphoma therapy (35 days) (p=0.003).

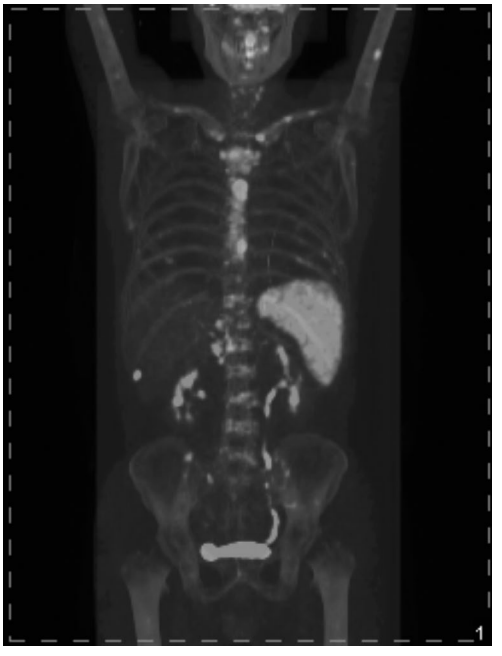

Figure 1.

Summary and Conclusions: PET-CT may play an important role in confirming the diagnosis of LAHLH without pathologic evidence. Patient in LAHLH group had remarkablely higher levels of SUV_{Sp}, SUV_{BM}, SUV_{LD}, SUV_{max}, $SUV_{LD/Li}$, and $SUV_{max/Li}$ than those in non-LAHLH group. In addition, sHLH patients who presented in PET-CT images with multiple lymphadenopathys and/or multiple bone lesions accompanied by increased FDG uptake were more like to be LAHLH. For the initial treatment of these highly suspected LAHLH, lymphoma chemotherapy, rather than HLH-2004 protocol or immunosuppressive/cytokine-regulating therapy, may have a relatively favorable effect and better clinical outcomes.

E974

HIGH THROUGHPUT TCR SEQUENCING PROVIDES ADDED VALUE IN THE DIAGNOSIS OF CUTANEOUS T-CELL LYMPHOMA

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Background: Diagnosis of early-stage CTCL can be challenging because skin lesions contain a mixture of both diverse benign and clonal malignant T cells. High throughput sequencing (HTS) of the TCR gamma (TCRG) and TCR beta (TCRB) CDR3 regions provide markers of the total repertoire of T cell clones in a specimen, the breadth of repertoire diversity, and an exact quantitation of individual T cell clones.
Aims: Two independent sample cohorts from two different institutions have been analyzed by HTS in an attempt to validate this approach and discriminate benign from malignant skin disease.
Methods: The two independent studies together involve the analysis of >100 patients with CTCL and approximately 100 patients with benign skin disease. Skin biopsies were studied from patients with histopathologically and clinically suspicious or diagnostic CTCL (over 80% Stage I or Stage II), treated CTCL patients, patients with benign inflammatory diseases, and normal skin. Genomic DNA was extracted from FFPE or OCT sections of skin biopsy specimens of patients. T cell receptor gamma (TCRG) and beta (TCRB) chain sequences were then independently amplified using multiplex PCR with optimized primer sets. Following HTS, a bioinformatics pipeline clusters the sequences into distinct clonotypes to determine overall frequencies and to identify diagnostic clones. V, (D), and J genes are also identified for each clonotype.
Results: The assays defined the dominant clonal sequences in the majority of malignant cases and distinguished likely gamma/delta from alpha/beta T cell lymphoma. Using the fraction of the top two TCRG sequences divided by two as a fraction of the total nucleated cell population defined a cut off of approximately 1/1000 above which the assay approached 100% specificity for malignant disease and below which the assay approached 100% specificity for non-malignant or treated malignant disease. TCR PCR was negative in a significant fraction of lesions suspicious of CTCL—most of these were among those determined to be clonal by HTS. There was also a significant false positive rate for TCR PCR.
Summary and Conclusions: HTS analyses of DNA extracted from skin biopsies of patients with skin disorders can provide important quantitative data of T cell number, clonality, repertoire, and frequency and in so doing provide a useful discriminator of benign versus malignant disease, extent of disease, and disease response.

E975

THE CLINICAL IMPLICATION OF THE ACTIVATION OF NF-KB SIGNALING PATHWAY IN DIFFUSE LARGE B CELL LYMPHOMA: PRE- AND POST-RITUXIMAB ERA

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Background: The nuclear factor-κB (NF-κB) comprises a family of transcription factors that control genes implicated in the activation, proliferation and resistance to apoptosis in normal B lymphocyte and malignant lymphoma cells, especially diffuse large B cell lymphoma (DLBCL). Gene expression studies on DLBCL lines have discovered that several genes which are targets of NF-κB are expressed on a subgroup of DLBCL (activated B-cell subtype). However, the clinical implication of activated NF-κB was not fullly elucidated, especially in the Rituximab era.
Aims: The aim of this study is through antibodies against the members of NF-κB family to delineate the activation of NF-κB, and to evaluate the clinical implication of this signaling pathway.

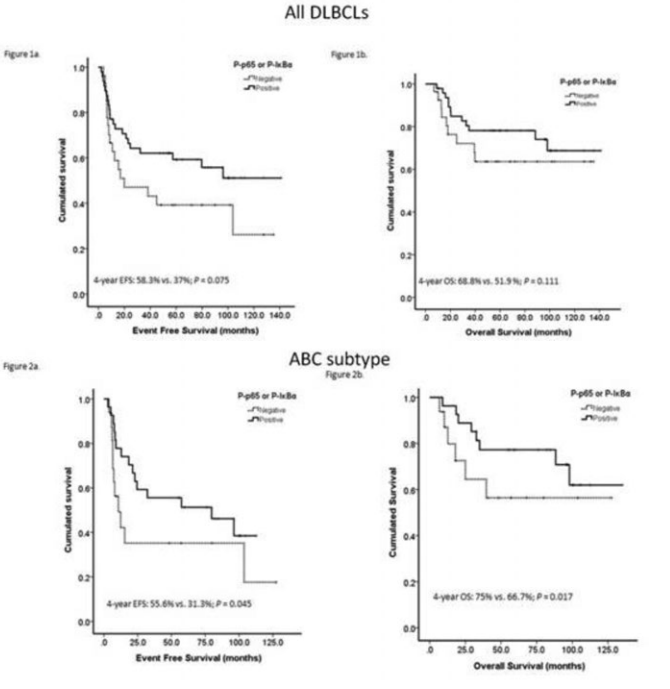

Figure 1.

Methods: Seventy-six DLBCLs diagnosed between 2002~2009, treated with antracycline containing regimen with (n=40; 53%) or without Rituximab (n=36; 47%) were evaluated with antibodies against phosphorylated p65(P-p65), phosphorylated IκBα (P-IκBα), p52, BCL-2, BCL-6, CD10, MUM-1 and c-MYC. IκB phosphorylation and degradation results in liberation of NFκB dimers (p65/p50) and its activation. Besides, there is evidence that phosphorylation of p65 on

serine 536 leads to NFκB activation, independently of IκB degradation. Therefore, activation of NFκB was defined as presence of positive P-p65 or P-IκBα. The impact of activated NF-κB was analyzed comparing clinical features and outcome including 4-year event free survival (4-year EFS) and 4-year overall survival (4-year OS) of DLBCL patients. We also evaluate the association between activated NF-κB and DLBCL subtypes, clinical features or other IHC stain markers (includes BCL-2, BCL-6 and C-MYC).

Results: Seventy-six DLBCLs were enrolled, and 48 cases had activated NF-κB. In the group with activated NF-κB, one case cannot be subdivided into GCB or ABC subtype. Twenty (20/47; 43%) belonged to GCB subtype and twenty-seven (27/47; 57%) belonged to non-GCB subtype (*P*=0.743). There were no difference betweent NFκB activation (+) and NFκB activation (-) in Ann Arbor stage, international prognosis index (IPI ≥3 vs. <3), and age (≥60 vs. <60). Treatments (with or without Rituximab) given to patients with NFκB activation (+) or NFκB activation (-) also had no difference.

With a median follow-up of 58 months, the group of activated NF-κB had trend toward better 4-year EFS(58.3 vs. 37%, P=0.075). 4-year OS was also better in the group with activation of NFκB but with no significance (68.8% vs. 51.9%, P=0.111). Because the gene expression studies on DLBCL cell lines suggested NFκB is preferentially activated in ABC-like DLBCL. Subgroup analysis revealed that in the no-GCB group, patients with activated NFκB had better 4-year EFS (55.6% vs. 31.3%; P=0.045) and 4-year OS (75% vs. 66.7%; P=0.017). We further analyzed if addition of Rituximab to chemotherapy could affect the clinical outcome according to the activation status of NFκB. We found that patients with activated NFκB who also received treatment with Rituximab containing regimen had the best 4-year EFS and 4-year OS (P=0.036 and P=0.058, respectively). Similarly, in non-GCB subgroup, patients with activated NFκB who received treatment with Rituximab containing regimen also had the best 4-year EFS (P=0.024). In multivariate analysis, activated NFκB was correlated with trend for better 4-year EFS (HR 0.521, P=0.056) and trend for better 4-year OS (HR 0.479, P=0.062).

Summary and Conclusions: NFκB activation through classical pathway can be detected through immunohistochemical stain of P-p65 or P-IκBα. Activation of NFκB is associated with good prognosis. Besides, patients with activated NFκB also had better outcome to Rituximab containing regimen. Subgroup analysis revealed that in non-GCB DLBCL, patients with activated NFκB also had better prognosis which is not observed in GCB DLBCL. NFκB activation is not confined to non-GCB subgroup and is not correlated with other IHC markers (BCL-6, BCL-2 or c-MYC).

E976

HISTOLOGICAL DISTRIBUTION, TREATMENT AND PROGNOSIS OF PERIPHERAL T-CELL LYMPHOMAS: AN ANALYSIS OF 505 PATIENTS IN TAIWAN

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Background: Peripheral T-cell lymphomas (PTCL) are a heterogeneous group of lymphoid malignancies. The distribution of pathological subtypes differs in different geographic areas and the prognostic factors, optimal therapy and roles of stem cell transplantation (SCT) remain to be defined.

Aims: We aimed to determine the clinical characteristics of PTCL in Taiwan.

Methods: We examined histological subtypes, patient characteristics, treatment, and outcome among 505 consecutively newly diagnosed adult PTCL patients from 1998 to 2014.

Results: PTCLs represented 14.3% (505/3539) of all non-Hodgkin lymphomas (NHL). PTCL subtypes included NK/T-cell lymphoma (NKTCL) (21.0%), angioimmunoblastic T-cell lymphoma (AITL) (20.2%), PTCL, not otherwise specified (PTCL-NOS) (17.0%), anaplastic large cell lymphoma (ALCL) (15.6%), adult T-cell leukemia/lymphoma (ATLL) (overall 7.3%, acute type 4.5%), others (3.0%), and cutaneous T-cell lymphomas (CTCL) (15.9%) including mycosis fungoides (7.3%), primary cutaneous ALCL (4.6%), subcutaneous panniculitis-like T-cell lymphoma (3.2%), and rare subtypes of CTCL (0.8%). Median age was 56 (range 20-90) years, 53% had stage III/IV disease, 53% had international prognostic index (IPI) ≥2, and 38% had prognostic index for T-cell lymphoma (PIT) ≥2. 391 patients received chemotherapy: CHOP (77%), COP (23%), proceeding to high-dose chemotherapy (ESHAP, VPBBM, MINE, ICE or EPOCH regimen) (22%) and SCT (6%). The overall response rate was 61%, complete response (CR) rate was 51%, and 37% had primary refractory disease. With a median follow-up of 63.7 months, 3-year progression free survival (PFS) and overall survival (OS) rates were 38% and 45% for all patients. The 3-year OS rate for each subtype was 77% (CTCL), 63% (ALK-positive ALCL), 52% (NKTCL), 39% (AITL), 39% (ALK-negative ALCL), 28% (PTCL-NOS), 18% (ATLL), and 9% (others). Both IPI (HR 3.9, *P*<0.001) and PIT (HR 3.8, *P*<0.001) had significant impact on OS. In multivariate analysis, age ≥60 (HR 1.7, *P*<0.001), ECOG performance status ≥2 (HR 1.9, *P*<0.001), extranodal sites ≥2 (HR 1.6, *P*=0.006), bone marrow involvement (HR 1.5, *P*<0.018), elevated LDH (HR 1.4, *P*=0.017), and hypoalbuminemia (HR 1.5, *P*=0.02) were independent poor pre-treatment prognostic factors for OS. OS benefit was observed in patients who received CHOP-like compared to COP regimen (HR 0.6, *P*=0.004), and in patients who achieved CR (HR 0.2, *P*<0.001) or received SCT (HR 0.5, *P*=0.052).

Summary and Conclusions: The overall frequency of PTCL among NHL and the frequencies of NKTCL and ATLL in our cohort were higher compared to western countries. The distribution of PTCL subtypes are mostly in line with other Asian series except for a lower frequency of ATLL and higher frequencies of ALCL and CTCL compared to Japanese series. IPI and PIT predicted OS. Hypoalbuminemia was a poor prognostic factor for OS. Patients who received CHOP-like regimen or achieved CR survived longer. SCT provided a trend of improved OS in PTCL patients.

E977

THE INFLUENCE OF TREATMENT FACILITY VOLUME ON SURVIVAL OF DIFFUSE LARGE B-CELL LYMPHOMA

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Background: Diffuse large B-cell lymphoma (DLBCL), the most common form of non-Hodgkin lymphoma, is a relatively uncommon cancer with annual incidence of ~21,000 cases in the US.

Aims: The goal of this study was to determine the extent to which the number of DLBCL patients treated annually in a facility (facility volume) affects overall survival (OS).

Methods: We included DLBCL patients identified from the US National Cancer Data Base (NCDB) from 1998 to 2011. NCDB, a joint program of the American College of Surgeons and the American Cancer Society, is a nationwide oncology outcomes database for more than 1,500 cancer programs in the US and Puerto Rico capturing about 70% of all newly diagnosed cases of cancer in the US. We classified treatment facilities by quartiles based on facility volume (mean DLBCL patients/year): Quartile 1 (Q1; ≤2), Quartile 2 (Q2; 3-6), Quartile 3 (Q3; 7-11) and Quartile 4 (Q4; >11).

Results: 116,615 DLBCL patients were cared for in 1,547 facilities. The distribution of patients according to facility volume was Q1 (2.0%), Q2 (14.3%), Q3 (23.0%) and Q4 (60.7%) and according to facility type was academic (30.3%), comprehensive community (11.0%), community (54.1%) and other (4.6%). The unadjusted median OS by facility volume was: Q1: 27.5 months, Q2: 38.5 months, Q3: 49.1 months and Q4: 61.3 months. After multivariable analysis adjusting for demographic (sex, age, race, ethnicity), socioeconomic (education, income, primary payor), geographic (area of residence), disease-specific (NHL subtype, stage) and facility-specific (type and location) factors, we show that facility volume remains an independent predictor of OS. Compared to patients treated at Q4 facilities, patients treated at lower quartile facilities had a worse OS (Q3HR: 1.03 [95% CI, 1.06-1.13]; Q2HR: 1.09 [1.06-1.13]; Q1HR: 1.21 [1.314-1.29]). We performed sensitivity analyses removing primary payor and facility type (collinearity with age and facility volume, respectively) as well as adjusting for Charlson-Deyo co-morbidity score (available for patients diagnosed after 2003) in secondary models and found similar results.

Summary and Conclusions: Patients who receive treatment at higher volume facilities for DLBCL are likely to have longer OS than those who are treated at facilities with a lower volume.

E978

CLINICAL SIGNIFICANCE OF CO-EXPRESSION OF MYC AND BCL2 PROTEIN IN AGGRESSIVE B-CELL LYMPHOMAS TREATED WITH A SECOND LINE IMMUNOCHEMOTHERAPY

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Background: Recent studies have shown that concurrent expression of MYC and BCL2 protein evaluated by immunohistochemistry (IHC) in *de novo* diffuse large B-cell lymphoma (DLBCL) is associated with worse survival when standard R-CHOP is applied. However, little is known about the clinical significance of concurrent expression of MYC/BCL2 protein in aggressive B-cell lymphomas which failed to benefit from conventional, upfront immunochemotherapies.

Aims: We show the clinical impact of co-expression of MYC and BCL2 protein among patients with relapsed or refractory aggressive B-cell lymphomas, who were uniformly treated with a second line immunochemotherapy.

Methods: This is a retrospective analysis of patients with relapsed and refractory aggressive B-cell lymphomas, who were consecutively treated with the R-IVAD regimen consisting rituximab (375 mg/m^2, day 1), ifosfamide (1500 mg/m^2 on day 3-7), etoposide (150 mg/m^2, day 3-5), cytarabine (100 mg/m^2, day 3-5) and dexamethasone (40 mg/body, day 3-5) followed by consolidative high-dose chemotherapies at Nihon University Itabashi Hospital from 2001 to 2009. MYC

and BCL2 protein were analyzed by IHC assay using formalin-fixed paraffin-embedded tissue specimens for all available cases. Cut-off values of positivity for MYC and BCL2 protein were set as 40% and 50% of stained tumor cell, respectively. Lymphomas showing concurrent positivity for MYC and BCL2 protein were defined as "Double expresser lymphoma (DEL)".
Results: A total number of 27 patients with a median 61 years (range 38-79) of age were analyzed. Nineteen patients were relapsed and the other eight patients were refractory to upfront immunochemotherapies. Histological subtypes consisted of 20 cases of DLBCL and seven cases of grade 3/transformed follicular lymphoma. Totally, 12 cases (44%) were categorized into DEL. Clinical characteristics such as risk factors consisting IPI or relapsed/refractory status were generally balanced, whereas a histological form of DLBCL was more common in the DEL group as compared with the non-DEL group (100% *vs* 53%, *P*=0.008). The overall response (OR) and the complete response (CR) rate to R-IVAD for all patients was 70% and 56%, respectively. The DEL group was less likely to respond to R-IVAD, as compared with the non-DEL group (OR: 50% *vs* 87%, *P*=0.087, CR: 33% *vs* 73%, *P*=0.057). The numbers of patients proceeding to consolidative therapies in the DEL group and the non-DEL group was 4 (33%) and 9 (60%) (*P*=0.252), respectively. Within a median 47 months (range 2-152) of observation period, the 2-year EFS and the OS rate for all patients was 33% and 59%, respectively (Figure a, b). As for the status of MYC/BCL2 protein, both the 2-year EFS and the OS rate were significantly lower in the DEL group than in the non-DHL group (Figure c, d). Multivariate analysis revealed that the negative impact of MYC/BCL2 expression on OS was still significant (HR 3.05, 95%CI 1.05-9.65, *P*=0.040), after it was adjusted for the other prognostic factors (*i.e.* high/high-intermediate risk for IPI, and refractory disease).

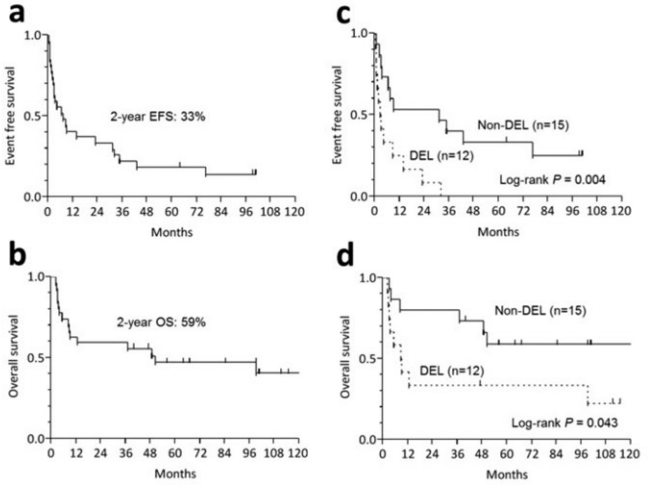

Figure 1.

Summary and Conclusions: This analysis confirmed that dual expression of MYC/BCL2 protein in IHC among relapsed/refractory aggressive B-cell lymphomas was common especially in DLBCL, and strongly associated with worse clinical outcome. Our findings suggest that MYC/BCL2 related high-risk aggressive B-cell lymphomas need highly discreet strategies in the context of second line setting as well as upfront therapies. The standardization of diagnostic procedures for DEL should therefore be established.

E979

OUTCOMES OF SPLENECTOMY IN NON-HODGKIN'S LYMPHOMAS COMPLICATED BY IMMUNE CYTOPENIAS

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Background: Autoimmune hemolytic anemia (AIHA) and immune thrombocytopenia (ITP) complicate the clinical course of Non-Hodgkin's lymphomas (NHL) in 15-25% of patients. In cases of ineffective steroid- and chemotherapy and in the presence of massive splenomegaly, such patients undergo splenectomy (SE).
Aims: To retrospectively analyze the efficacy of splenectomy in patients with NHL associated with AIHA and/or ITP.
Methods: Ninety seven patients with NHL underwent SE during 1986-2014. In 49 out of them (50.5%), immune cytopenias were diagnosed. Warm-type AIHA was observed in 7 (7.2%) patients: mantle cell lymphoma (MCL) in one, splenic marginal zone lymphoma (SMZL) in 2, diffuse large B-cell lymphoma (DLBCL) in 4. ITP was found in 28 (28.9%) patients: SMZL in 8, DLBCL in 13, diffuse large T-cell lymphoma (DLTCL) in one, hepatosplenic T-cell lymphoma (HCTCL) in one, and in 5 patients NHL were classified according to the Working Formulation (WF). NHL with AIHA and ITP was diagnosed in 14 (14.4%) patients: MCL in 2, follicular lymphoma (FL) in one, SMZL in 6, while one of them had splenic lymphoma with villous lymphocytes (SLVL), DLBCL in 4, and DLTCL in one. Splenomegaly was revealed in all patients, some of them developed a giant-size spleen weighing 6-8 kg. Patients were followed up to 1-270 months after splenectomy.
Results: SE has been effective in 25 (89%) out of 28 patients suffering from NHL with ITP: a large tumor mass was removed, the platelet count was normalized, and signs of hemorrhagic syndrome disappeared. In 3 patients (DLBCL – 1, DCTCL – 1, and HCTCL – 1), splenectomy proved ineffective. They died within 4 months following surgery due to a thrombocytopenia relapse and disease progression. Mean post-splenectomy survival of patients with NHL with ITP reached 67.3 months. SE performed in 14 patients with NHL associated with AIHA and ITP proved effective in 9 (64%) patients: abdominal discomfort was relieved, hemolysis was reverted, and the platelet count was normalized. One patient died immediately after operation due to acute adrenal insufficiency, and four patients died within 1-6 months following SE (DLBCL – 1, DLTCL – 1, and SMZL with SLVL – 1 patient). Mean survival after SE reached 50.5 months. The worst long-term outcomes of SE were observed in patients with NHL and AIHA: 6 patients survived 2-22 months after operation, whereas only one has been living for 270 months following SE. Mean survival after SE is 46.2 months.
Summary and Conclusions: SE remains to be an effective treatment option in NHL associated with splenomegaly and immune cytopenias. The worst long-term outcomes of SE are observed in NHL with AIHA. SE proved to be ineffective in DLTCL and HCTCL.

E980

LONG-TERM RESULTS OF TREATMENT OF DIFFUSE LARGE CELL LYMPHOMA ASSOCIATED WITH HEPATITIS C (DLBCL+C)

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Background: Materials. 94 patients with diffuse large cell lymphoma and markers of hepatitis C (DLBCL +C) and 72 patients with diffuse large cell lymphoma without markers of hepatitis C (DLBCL - C) were included in the study. All the patients were under the supervision of an oncohematologist and therapist from 2003 to 2013.
Aims: The age of patients with DLBCL +C ranged from 21 to 76 years (mediana 47). In control group age ranged from 23 to 81 years (mediana 55) (p=0.02). The ratio of men and women in the two groups was equal - in DLBCL +C -1:1.3 in the group DLBCL-C-1:1.7. Stage I and II were in 11% of patients in group DLBCL +C, and 48% of patients in the control group; III and stage IV were in 89% of patients in the DLBCL +C, 52% in the control group (p=0.00002). Extranodal lesions were 72% of patients in the DLBCL +C, 46% of patients with DLBCL - C (p=0.006).
Methods: In the group of patients with GCB DLBCL+C was 55% non-GCB was 45%; in the control group GCB DLBCL-C was 36%, non-GCB was 64% of patients (p=0.001).
Results: In the group of patients with DLBCL+C antibodies to hepatitis C were positive in all 94 patients. Positive PCR HCV was 78% (74 patients). Median viral load at the start of chemotherapy was 2x104 copies / mL. 15 patients with viral load was high - more than 1 x106 copies / mL. All patients received chemotherapy CHOP / R-CHOP. After treatment in patients with DLBCL+C and DLBCL-C complete remission was achieved in 60% and 63% respectively. Disease free survival in patients with DLBCL+C was 25 months in the control group - 61 months. The median overall survival in DLBCL+C group was 40 months in the control group it was 71 months (p=0.0003). Median progression free survival in patients with GCB DLBCL+C was 40 months in the patients with GCB DLBCL-C - 60 months (p=0.003). Median overall survival in the group of patients with GCB DLBCL+C was 45 months in the control group to 70 months (p=0.002). Median progression-free survival in patients with non-GCB DLBCL+C was 10 months in the control group non-GCB DLBCL-C -60 months (p=0.00001). Overall survival in the group of patients with non-GCB DLBCL+C was 18 months in the patients with non-GCB DLBCL-C overall survival was 70 months (p=0.00001).
Summary and Conclusions: DLBCL with markers of viral hepatitis C is a separate group of lymphomas with characteristic clinical, morphological features. This allows us to separate of a hepatitis C-associated DLBCL. Despite the lack of differences in the effectiveness of the therapy, long-term results of therapy was significantly worse in patients DLBCL+C.

E981

INTERIM 18F-FGD PET/CT MAY NOT PREDICT THE OUTCOME IN PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA PATIENTS TREATED WITH INTENSIVE METHOTREXATE AND CYTARABINE

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Background: ^{18}F-fluoro-2-dexoy-*D*-glucose-positron emission tomography (PET)/computed tomography (CT) is a useful imaging technique for monitoring treatment response in cases of malignant lymphoma.
Aims: We investigated the value of interim brain PET/CT (I-PET/CT) for monitoring the response to intensive methotrexate chemotherapy in primary central nervous system lymphoma (PCNSL) patients with diffuse large B cell lymphoma (DLBCL).
Methods: Of 94 PCNSL patients treated with intensive methotrexate and cytarabine chemotherapy between September 2006 and December 2012, 66 PCNSL patients with DLBCL underwent brain PET/CT at diagnosis, during therapy (I-PET/CT), and at the end of therapy (final [F]-PET/CT) and were included in this study.
Results: The patient cohort consisted of 66 individuals (43 men and 23 women), with a median age of 59 years (range, 17–75 years). During chemotherapy, 36 patients (54.5%) showed a negative metabolism in the scan of I-PET/CT, and 47 patients (71.2%) showed a negative F-PET/CT at the end of therapy. The baseline characteristics were similar between I-PET/CT-negative (n=36) and I-PET/CT-positive patients (n=30). After a median follow-up of 27.5 months, there was no difference in the progression-free survival (PFS; P=0.701) or overall survival (OS; P=0.620) between the I-PET/CT-negative and I-PET/CT-positive groups. However, PFS in the F-PET/CT-negative group was significantly longer than in the F-PET/CT-positive group (P <0.001) without significant difference in OS (P=0.892)
Summary and Conclusions: Our findings suggest that I-PET/CT might not predict the survival outcome of PCNSL patients with DLBCL treated with intensive methotrexate and cytarabine chemotherapy. Prospective trials are required to fully evaluate the role of I-PET/CT.

E982

CLINICAL RELEVANCE OF BONE MARROW HEMOPHAGOCYTOSIS IN THE DIAGNOSIS OF HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS IN ADULTS: A SINGLE CENTER, RETROSPECTIVE ANALYSIS

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Background: Hemophagocytic lymphohistiocytosis (HLH) is an immune-mediated life-threatening condition diagnosed by the diagnostic criteria for HLH (HLH-2004). However, the clinical relevance of HLH-2004 criteria is still not clear in adult patients because it was mainly based on clinical experience in pediatric patients. Thus, we analyzed bone marrow hemophagocytosis in adult patients presenting suspicious hemophagocytic syndrome.
Aims: The primary objective was to analyze underlying causes, clinical features and outcomes of adult patients with bone marrow hemophagocytosis, and the secondary objective was to explore the feasibility and clinical usefulness of HLH-2004 criteria in adult patients.
Methods: We reviewed the electronic data base of medical records at the Samsung Medical Center, and selected 264 patients who had evidence of hemophagocytic histiocytosis in bone marrow aspiration and biopsy between January 2000 and June 2014.
Results: All patients had bone marrow hemophagocytosis, and their median age was 53 years (range, 18-83). Malignant disorders were predominant underlying causes of adult HLH (n=170, 64%), especially aggressive T/NK-cell (n=82) and B-cell non-Hodgkin lymphomas (n=45). Among non-malignancy associated HLH (n=94, 36%), infectious disease (n=48) and idiopathic HLH (n=17) were more common than autoimmune disease. There was no primary/familial HLH in these patients. Fever (68%) and splenomegaly (55%) were most common clinical manifestations, and more than 80% of patients had increased level of serum ferritin and lactate dehydrogenase (89% and 84%, respectively) whereas the frequency of hypofibrinogenemia, hypertriglyceridemia, and bicytopenia were less common findings (<40%). Thus, only 45% of patients who had bone marrow hemophagocytosis could be diagnosed with HLH based on the current diagnostic criteria (≥five positive criteria). However, the remaining patients also had clinically significant hemophagocytic syndrome. Malignancy was more common cause of HLH than non-malignant disorders in patients who fulfilled the diagnostic criteria (63% vs. 37%). After a median follow-up of 30 months, the median overall survival (OS) was significantly worse in patients with malignancy (9.0 months, 95% CI: 5.6-12.5) than patients without malignancy (71.8 months, 95% CI: 56.5-87.1, p <0.001). The presence of bi-cytopenia, splenomegaly, and hypertriglyceridemia was not associated with poor OS (p >0.05). However, the presence of fever, elevation of ferritin, and hypofibrinogenemia was significantly associated with poor OS in malignancy-associated hemophagocytosis. Especially, patients with very high ferritin level (>10,000 ng/mL) showed extremely worse OS than patients who did not.
Summary and Conclusions: Bone marrow hemophagocytosis might be a crucial finding in the diagnosis of HLH in adult patients regardless of other diagnostic clues. Considering high incidence of malignancy, especially lymphoma as predisposing disorders for HLH in adults, immediate evaluation and management should be done in adult patients with bone marrow hemophagocytosis.

E983

TOTAL BODY SURFACE AREA (TBSA) AS A NEW PROGNOSTIC VARIABLE IN MYCOSIS FUNGOIDE AND SÉZARY SYNDROME

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Background: Mycosis fungoides and Sézary syndrome (MF/SS) are the most common forms of primary cutaneous T cell lymphomas. There is no standard chemotherapy regimen for advanced stages of disease. The only curative option is allogeneic stem cell transplantation but it is limited to eligible patients. Currently, it is still difficult to establish prognosis taking into account clinical and biological features at diagnosis.
Aims: Our aim is to analyze the applicability of cutaneous lymphoma international prognostic index (CLIPi) in our population with MF/SS and also evaluate the total body-surface area (TBSA) at diagnosis and the type of skin lesions.
Methods: A retrospective analysis identified in our center 134 patients diagnosed with MF/SS between 1976 and 2013. (see table 1)

Tabella 1. Characteristics of MF/SS patients (n=134).

Table 1. Characteristics of MF/SS patients (n=134)		
Gender	Male: 59.7% (n=80)	Female: 40.3% (n=54)
Age	Median: 61 years (range: 9-90 years)	
Histological subtype of MF/SS	Classic: 79.1%	(n=106)
	Folliculotropic Follicular: 6.7%	(n=9)
	Pagetoid: 0.7%	(n=1)
	Granulomatous: 0.7%	(n=1)
	Sézary: 12.7%	(n=17)
ISCL/EORTC Stage	Ia - IIb: 82.8%	(n=111)
	IIIa - IVb: 17.2%	(n=23)
TBSA score	< 40% score: 38%	(n=51)
	≥40% score: 62%	(n=83)
B symptoms	No: 64.4%	Yes: 33.6%
Median time of follow up	96 months (range: 0 – 450 months)	

Results: The overall survival (OS) at median time of follow up (96 months) was 75.6% (CI 95%, 62.0-98.5%). In the univariate analysis, OS was analyzed by age (≤60 vs. >60 years), advanced disease (<IIb *vs* ≥IIb) according to ISCL/EORTC, presence of erythroderma, type of skin lesions and TBSA (<40% *vs* ≥40%) at diagnosis; all showed differences in OS (p<0.01) (see figure 1). In the multivariate analysis there was a significant increased relative risk of survival (p<0.05) in those patients younger than 60 years, with TBSA less than 40% and early stage (<IIb).

Tabella 2. Independent prognostic factors for survival.

Table 2. Independent prognostic factors for survival			
Prognostic factors	Hazard ratio	95% confidence interval	P-value
Age ≤ 60 years	5.481	2.572 – 11.679	<0.0001
TBSA < 40%	2.971	1.372 – 6.433	<0.006
Stage < IIb	4.862	1.653 – 14.301	<0.004
Lesions with patch	1.281	0.540 – 3.040	0.573
Erythroderma	0.647	0.215 – 1.947	0.439

CLIPi was calculated for both early and advanced disease. There were differences in probabilities of survival in early and advanced disease, but patients with CLIPi 0 and 1 in both groups were poorly discriminated.

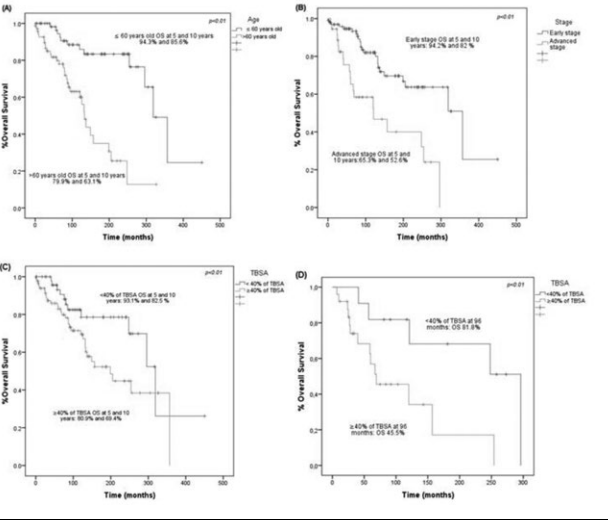

Figure 1. Overall Survival at 5 and 10 years: (A) OS by Age. (B) OS by Stage. (C) OS by TBSA. (D) OS in advanced.

Summary and Conclusions: In conclusion, the CLIPi is a reliable tool to identify poor prognostic patients, but those with initially early stages were not fully discriminated. The addition of the TBSA data is a simple, ready-to-use method in the daily clinical practice that could improve the prognostic classification of those patients with early-stage disease. This combined score, CLIPi plus TBSA, may be used to identify early-stage patients that may benefit from new systemic therapies.

E984

DIRECT ANTIGLOBULIN TEST POSITIVITY IS A PROGNOSTIC MARKER IN DIFFUSE LARGE B-CELL LYMPHOMA PATIENTS

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Background: The revised International Prognostic Index (r-IPI) is conventionally used for prognostication of DLBCL patients but has limited ability to stratify patients with a probability of survival less than 50%. There is thus a need to identify readily available biomarkers to ifurther classify prognostic groups within this category.
Aims: We investigated the prevalence of, and prognostic value of subclinical autoimmunity directed against red blood cells [demonstrated by a positive direct antiglobulin test (DAT)] in DLBCL
Methods: Eligibility criteria for inclusion in the study were: patients aged >18 years, histological diagnosis of DLBCL at the Canberra Hospital from 2004-14, DAT performed at the time of diagnosis, and availability of relevant clinical and laboratory data. In addition to descriptive statistics and Kaplan-Meier curves, Cox proportional hazard regression (SPSSv22.0) was used to calculate the prognostic effect of DAT positivity on progression free survival (PFS) at 5 years and overall survival at 3 years (OS) controlling for R-IPI, gender, and anaemia.
Results: Of the 96 cases that fulfilled the eligibility criteria for inclusion in the study, 77 were DAT negative (80.2%) and 19 DAT positive (19.8%). DAT positivity resulted in a poorer 5-year PFS (HR=2.3, p= 0.046) and 3-year OS (HR=2.605, p=0.035). This effect was accentuated in the 'poor prognosis' subset of patients identified by an r-IPI score of 3 or higher (OR=5.630, p=0.004).
Summary and Conclusions: The findings of this study demonstrate that the prevalence of DAT positivity is much higher in DLBCL than the background reported rate of 1:10,000. Further, it is a strong negative prognostic marker in DLBCL and may be particularly useful in identifying patients with a very poor prognosis. It is likely that the impact of subclinical autoimmunity on survival is mediated by effects on the tumour microenvironment and further studies are required to understand this epiphenomenon.

E985

FEATURES OF EBV VIREMIA AND OUTCOMES IN PATIENTS RECEIVED ALLOGENEIC STEM CELL TRANSPLANTATION: A CHINESE MULTICENTER SURVEY

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Background: EBV reactivation and associated diseases have been well recognized as one of the life threatening complications after SCT, which leads to the establishment of prophylactic and preemptive treatment for EBV viremia. However since then, few epidemic data was shown in SCT recipients, especially in China.
Aims: An observational data base study was conducted in patients with hematological disorders who received allo-SCT from Jun 2011 through Jun 2014.
Methods: EBV-negative status was confirmed before transplant for both donors and recipients. EBV-DNA was screened weekly until Day 100 post SCT by qPCR, and then every 2-4 weeks until 1 year. Additional tests were carried out when clinically indicated. All patients received ganciclovir during conditioning regimen, and then switched to acyclovir or valaciclovir after stem cell infusion until 1 year post SCT. Ganciclovir and/or foscarnet were used when DNAemia developed, as well as rituximab for high risk patients such as high viral load or persistent DNAemia.
Results: This study recruited 892 evaluable cases, including 91 cases of benign diseases and 801 cases of malignancies. EBV-DNA was detected in 165 cases with a median duration of 55 days (16-618days) post SCT, and the long-term cumulative incidence of DNAemia was 21.3±1.5%. Totally 7 patients developed probable or proven PTLD. Log-rank test showed EBV-DNAemia had impact on neither 2 year-OS (67.2±6.3% *vs* 66.2±1.9%, P=0.341) nor 2 year-NRM (19.4±4.0% *vs* 25.1±1.8%, P=0.272). Cox model analysis revealed that haploidentical donor (RR 1.973 [95%CI 1.383-2.814], P<0.001) and the use of ATG/ALG (RR 4.831 [95%CI 2.469-9.524], P<0.001) were independent risk factors (Figure 1). While focusing on leukemia patients, RIC regimen was identified as an additional risk factor (RR 3.378 [95%CI 1.058-10.870]), with a weaker association (P=0.040).
Summary and Conclusions: Reported EBV viremia incidence post SCT ranged from 0.6 to 26%. A relatively higher incidence in this study was probably due to higher proportion of haploidentical donor SCT. Fortunately, OS and NRM were comparable between EBV-positive and EBV-negative patients, and PTLD was rarely observed, which implied the efficacy of preemptive treatment. Previously described risk factors of EBV reactivation include unrelated or HLA-mismatched donor, use of T cell depletion *in vivo* and onset of GVHD. Our results were partially in accordance with those literatures. Acute GVHD had not been identified as an independent risk factor, and a probable reason is that physicians paid more attention to virus infection during the treatment of GVHD. For haploidentical donor SCT recipients, the EBV prophylactic strategy needs to be further explored.

E986

A MODIFIED-INTERNATIONAL PROGNOSTIC INDEX INCLUDING PRETREATMENT HEMOGLOBIN LEVEL FOR EARLY STAGE EXTRANODAL NK/T CELL LYMPHOMA

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Background: Anemia is a common finding in cancer patients, even before patients have received radiotherapy or chemotherapy and even when there is no bone marrow infiltration. Recent studies have found that pretreatment anemia status is correlated with shorter survival in patients with several hematological malignancies.
Aims: We aimed to define the relationship between pretreatment hemoglobin level and survival outcomes in patients of extranodal NK/T cell lymphoma, nasal type (ENKTL).
Methods: We retrospectively investigated the prognostic role of pretreatment hemoglobin level in 352 patients with stage I/II ENKTL.
Results: Of 352 patients, 67% had stage I disease, and 79.8% had international prognostic index (IPI) scores between 0 to 1. There were no significant correlations between baseline hemoglobin level and age, ECOG performance status score, lactate dehydrogenase (LDH) level, and Ann-Arbor stage. The complete response rate in patients with anemia was 64.7%, slightly lower than that in patients without anemia (75.6%, P=0.076). The overall response rate in both groups were similar (84.1% and 88.7%, P>0.05). At a median follow-up time of 83.8 months, the 5-year and 10-year PFS rate were 48.0% and 40.0%, respectively, and the 5-year and 10-year OS rate were 63.0% and 60.0%, respectively. Patients with pretreatment hemoglobin level<120g/L had significantly inferior PFS and OS than those with hemoglobin level≥120g/L (P<0.05). In a multivariate Cox regression model, age, ECOG performance status, LDH level, Ann-Arbor stage, and pretreatment hemoglobin level were all independent prognostic factors for PFS and OS (P<0.05). Using these five parameters, a modified-IPI model (mIPI) was constructed and three prognostic groups were classified: group 1, no adverse factors; group 2, one or two factor; group 3, three or more factors. This mIPI could categorize three groups with significantly different PFS and OS (both P<0.0001). Using the cohort of patients who received asparaginase-based therapy as a validation set, this mIPI retained its prognostic value.
Summary and Conclusions: This large-cohort retrospective study confirmed the independent prognostic role of pretreatment hemoglobin level in early stage ENKTL, and a newly modified IPI including pretreatment hemoglobin level could be used to further optimize treatments for patients with stage I/II ENKTL, even in the era of asparaginase.

E987

CARBOPLATIN, ARACTYIN PLUS DEXAMETHASONE WITH OR WITHOUT RITUXIMAB (DHAC+/-R) IS A FEASIBLE AND EFFECTIVE TREATMENT FOR LYMPHOMA PATIENTS

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Background: Salt platins (cisplatin, oxaliplatin, carboplatin) are used in relapsed/refractory (R/R) lymphomas. The so-called DHAP regimen with or without Rituximab (R) combines dexamethasone, cisplatin plus high-dose cytarabin (AraC-HD). However, cisplatin-induced nephrotoxicity is a major concern: 10-20% of tubular complication sometimes leading to persistent reduced glomerular filtration that jeopardizes autologous stem cell transplantation (ASCT). Oxaliplatin may replaces cisplatin but it is associated with neuropathy toxicity. Carboplatin is widely used in solid tumours and combined with ifosfamide (ICE regimen) in lymphomas. However, the carboplatin plus AraC-HD and dexamethasone (DHAC+/-R) regimen has never been evaluated.

Aims: Taken into account the potential benefice of carboplatin, we investigated this regimen. Herein, we report the toxicity and efficacy of DHAC+/-R in lymphomas patients.
Methods: From September 2008 to September 2014, all R/R lymphoma patients treated in our institution received DHAC+/R instead of DHAP+/-R. Only patients with Mantle Cell Lymphoma (MCL) or contra-indication to anthracycline was allowed to receive upfront DHAC+/-R. Rituximab (375mg/m^2) was administrated only in CD20+lymphoma entities. DHAC consisted of: Carboplatin (targeted AUC =5, Calvert's formula [Dose (mg)= AUC x (GFR +25)]), AraC-HD 2g/m^2 two times a day (day 2) and IV or oral dexamethasone from day 1 to 4. Interval between courses was 21 days. Number of cycles was adapted to patients and therapeutic program including or not ASCT.
Results: 182 patients received DHAC+/-R. Diagnoses were as followed: Diffuse Large B Cell Lymphoma (DLBCL) in 97 cases (53%), Follicular Lymphoma (FL) in 24 cases (13%), Hodgkin Disease (HD) in 22 cases (12%), MCL in 22 cases (12%), Peripheral T Cell Lymphoma (PTCL) in 6 cases (3%) and 7 cases presented with low grade B lymphomas and 3 patients had plasmablastic lymphomas. Twenty-two patients received upfront DHAC+/-R while 54 were in relapse (n=54, 30%), 61 in partial response (PR) (34%), 19 in stable disease/refractory (SD) (11%) and 23 patients had progressive disease (PD) (13%). The median number of treatments line before DHAC+/-R was 1 (range, 1-4). At time of first cycle, median age was 53 years (range, 18-75). Before start of DHAC+/-R, ASCT was planned for 133 patients (73%). 48% of patients received DHAC+/-R with overnight stay and 38% in day care hospital. For all patients, a median of 3 cycles of DHAC+/-R was administrated. Median dosing of carboplatin at cycle 1, 2, 3, 4, 5 and 6 were (mg/m²) 350, 350, 350, 350, 310, 315, respectively. Median dosing of AraC-HD was 4g/m² through all cycles. 78% of patients with advanced disease completed the scheduled number of cycles. Major cause of arrest of DHAC+/-R was PD or SD (32 out of 41). In term of toxicity, Grade >=3 haematological toxicities were reported mainly thrombopenia (n=53) and anaemia (n=41). Transfusion support was used in 106 patients (58%), with a median of 2 red blood cell units (2-14) and 2 platelets units (1-27) during the treatment period. 11 sepsis were reported. One patient died of septic shock at day 8 of first cycle of DHAC. No grade >= 3 renal (4 cases of grade 1/2), and only one grade 3 neurological toxicities were reported. In term of response, Percentage of response after DHAC was as followed: complete remission (CR) in 27%, PR in 42%, SD in 11% and 20% PD. 60% of the patients at diagnosis reached CR, while 72% of patients with relapsed lymphoma reached PR. For the entire cohort, the median follow-up was 24m (range, 2-71), median OS was not reached (estimated 2-year OS was 74%), and median PFS was 46m. According to lymphoma entities, median PFS were 3.6m for PTCL, 21m for DLBCL, NR for FL, NR for MCL and 51m for HD. When focusing on DLBCL with insufficient response before ASCT, the 2y OS was 85% and the 2y PFS was 64% (41-77). Among the 133 patients scheduled for ASCT, 96 were in response after DHAC+/-R and they all underwent ASCT.
Summary and Conclusions: Herein, we show that DHAC+/-R is a safe chemotherapy regimen associated with good clinical response in all lymphoma entities and does not jeopardize ASCT when PR is reached.

E988

C-MYC AND BCL2 TRANSLOCATION FREQUENCY IN DIFFUSE LARGE B CELL LYMPHOMAS

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Background: Diffuse large B-cell lymphomas (DLBCLs) are a biologically heterogeneous group with varied clinical courses, histology, molecular and cytogenetic characteristics.
Aims: DLBCLs have been associated with multiple cytogenetic abnormalities including *MYC* with *BCL6* or *BCL2* rearrangement. Three antibody panel of immunohistochemical stains can be used to subclassify DLBCL into prognostically significant germinal center B-cell like (GCB) DLBCL and activated B-cell like (ABC) DLBCL subtypes. Both CD 10 and BCL6 are considered as germinal center markers while MUM 1 is expressed in activated B-cells and plasma cells.The aim of this study was to investigate the frequency and prognostic impact of BCL2 and MYC rearrangements.
Methods: In the present study, we evaluated the expression patterns of CD 10, BCL6 and MUM 1 by immunohistochemistry on contructed 121 cases (62; GCB, 59 ABC), (60; female, 61; male) of DLBCL in tissue microarray. MYC, BCL2 rearrangements were investigated by interphase fluorescence *in situ* hybridization (FISH) on tissue microarrays in 97 DLBCLs.
Results: Mean age of patients was 54.8 year. There were 60 women (49,58%) and 61 men (50,41%) patients. Tumors were located at lymph nodes in 59 patients (48,76%). Mean of survival time was 65,74 months. FISH was successfully performed in 97 cases. No significant differences were observed with regard to age (<60 *vs* -60years), sex, primary site of presentation (nodal *vs* extranodal), stage (I-II *vs* III-IV) among GCB and ABC subgroups. MYC rearrangements were observed in 11 (11,3%) of 97 DLBCL patients. There was no association with other clinical features, including age, sex, nodal/extranodal disease. MYC rearrangement was associated with significantly worse overall survival (p=.00). MYC rearrangement was more common in cases with GCB phenotype (9 cases GCB; 2 cases ABC). BCL2 rearrangements were observed in 14 (14,4%) of 97 DLBCL patients. Six of them were extranodal lymphoma, eight cases were nodal lymphoma. BCL2 rearrangements were more common in nodal than in extranodal presentations. There was no association with other clinical features, including age, sex. BCL2 rearrangement had prognostic impact on outcome (p= .00). No significant differences were observed with regard to age (<60 *vs* -60years), sex, primary site of presentation (nodal *vs* extranodal), stage (I-II *vs* III-IV) among GCB and ABC subgroups.No significant differences were observed with regard to BCL2 rearrengement among GCB and ABC phenotype. MYC and BCL2 rearrangements were observed in 3 (3,09%) of 97 cases. There was no significant difference between GCB and ABC phenotype cases with regard to MYC and BCL2 rearrangements. Three cases were 53 (female), 53, 63 years years old, respectively. Two of them were GCB type of DLBCL. The patients were died in 24.,18. and 35. months after diagnosis. Double-hit lymphoma patients included one female and two males with a median age of 56 years (range, 53-63). Two cases had primary nodal and one case primary extranodal presentations. The majority of patients had stage IV disease.
Summary and Conclusions: Cases in which clinical, morphological and immunohistochemical diagnosis are not adequate to discriminate DLBCL-BL, cytogenetic studies are beneficial. In this way, not only the patient will be able to reach the correct diagnosis but also get the effective treatment. We concluded that C-MYC and BCL2 may contribute to aggressive transformation, and more mechanism-based therapy should be explored. Analysis of MYC gene rearrangement along with BCL2 is critical in identifying high-risk patients with poor prognosis.

E989

R-DA-EPOCH VS R-CHOP IN PATIENTS WITH PRIMARY MEDIASTINAL LARGE B-CELL LYMPHOMA: INTERIM RESULTS OF RANDOMIZED (PROSPECTIVE) MULTICENTER STUDY

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Background: The standard frontline therapy of primary mediastinal large B-cell lymphoma (PMBL) does not establish. Different anthracycline–based regimens are applied. Therefore, the effort to determine the most effective and well-tolerated treatment of such category of patients is reasonable.
Aims: To compare the treatment efficacy and toxicity of R-da-EPOCH and R-CHOP regimens in patients with PMBL.
Methods: 57 patients with newly diagnosed PMBL from five Ukrainian centers were included into the study. Median age was 30 years (range 17-45), 39 females (68.4%) and 18 males (31.6%). 70.2 % of patient had early stage (I-II) of disease, 80.7 % of patients had greatest size of the tumor mass in mediastinum more than 10 cm. The tumor pleurisy, pericarditis were revealed in 40.3% and 42.1%, respectively. Patients were randomized in two groups to receive R-da-EPOCH (32 patients) or R-CHOP (25 patients). Primary and post treatment assessment of disease included PET-CT or CT of the whole body. The treatment efficacy in both groups was evaluated according to Cheson criteria 1999, 2007 and Deauville criteria. All patients received 6 cycles of R-chemo±mediastinal radiation therapy (30-36 Gy).
Results: The ORR was 100% in the group of R-da-EPOCH and 78% in group of R-CHOP, p<0.01. CR rate (including CR unconfirmed) was 75% and 48%, respectively, p<0.05. Primary refractory disease was diagnosed in 3 pts (12%) of R-CHOP group. 1 patient from R-da-EPOCH-group had early relapse up to 6 months after treatment. All 57 patients had manifestations of treatment toxicity. 54 (94.7%) patients completed full treatment plan. There was no significant difference in toxicity rate in both groups. Treatment was complicated by anemia grade 3-4 in one case in R-da-EPOCH group *vs* two cases in R-CHOP group. FN was detected in 4 pts (12.4%) *vs* 3 pts (12%), respectively. Thrombocytopenia grade 3-4 was not observed. All cases of non-hematological toxicity were grade 1-2.
Summary and Conclusions: The treatment of patients with PMBL with both regimens was quite efficient, but ORR and CR rate was significantly higher in R-da-EPOCH group. The treatment toxicity rate was comparable in both groups. The study should be continued to evaluate and analyze long-term results.

E990

A PHASE IIA STUDY OF SINGLE-AGENT MOR208, AN FC-OPTIMIZED ANTI-CD19 ANTIBODY, IN PATIENTS WITH RELAPSED OR REFRACTORY B-CELL NON-HODGKIN'S LYMPHOMA

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Background: A number of second-generation monoclonal antibodies (mAbs) that target the antigens CD20 and CD19 have been evaluated across a range of non-Hodgkin's lymphoma (NHL) subtypes combined with chemotherapy, as a single agent, or as maintenance therapy. Although these agents are usually well tolerated and have demonstrated clinical activity in patients (pts) with NHL, there remains a high unmet medical need for new therapies for pts with relapsed or refractory (R-R) B-cell NHL. MOR208 is an Fc-engineered, humanized mAb that targets the B-cell-specific antigen CD19 and which possesses significantly enhanced antibody-dependent cell-mediated toxicity, a key mechanism for tumor cell killing.
Aims: To evaluate the preliminary efficacy and safety of MOR208 in adult pts with relapsed or refractory NHL.
Methods: This is a non-randomized, open-label, multicenter, two-stage, phase IIa study of MOR208 in pts with R-R NHL previously treated with rituximab who were not candidates for high-dose chemotherapy with stem cell support. Adult pts with diffuse large B-cell lymphoma (DLBCL), mantle cell lymphoma (MCL), follicular lymphoma (FL), or other indolent NHL (iNHL), were treated with single-agent MOR208, at an intravenous dose of 12 mg/kg, weekly, over two 28-day cycles. Pts with at least stable disease according to the 2007 International Response Criteria were to continue treatment with MOR208 for another cycle. Pts achieving a complete or partial response (CR or PR) could then receive maintenance MOR208 every two or four weeks, depending on the investigator's decision, until progression. Overall response rate (ORR=CR +PR) was the primary endpoint.
Results: By 17 November 2014, all pts (N=89) had been enrolled (DLBCL, n=35; FL, n=31; MCL, n=12; iNHL, n=11); median age was 67 (range 35–90) years. Of these pts, 88% had stage III–IV disease and the median number of prior therapies was 2 (range 1–4). The mean number of cycles completed was 2.2 (0–3). The investigator-assessed responses across all NHL subtypes are shown in Table 1; the highest ORR was recorded in the DLBCL cohort (26%). Preliminary median duration of response was 7.7 months in the DLBCL group and 2.6 months in the FL group. Grade ≥3 non-hematologic treatment-emergent adverse events (TEAEs) were recorded in 30 pts (34%); disease progression, reported in 10 pts (11%), was most common. Grade ≥3 hematologic TEAEs were recorded in 8 pts (9%); neutropenia, reported in 5 pts (6%), was most common. Infusion-related reactions, reported in 8 pts (9%), were all grade 1–2 except for one case of dyspnea, which was grade 4. There were no treatment-related deaths.

Tabella 1. Investigator-assessed responses in HHL subtypes (safety population).

	NHL cohort				
Response, n	DLBCL N = 35	FL N = 31	MCL N = 12	iNHL N = 11	Overall N = 89
CR	2	1	0	1	4
PR	7	6	0	3	16
SD	5	14	6	3	28
PD	11	4	5	3	23
NE	10	6	1	1	18
ORR, n (%)	**9 (26)**	**7 (23)**	**0 (0)**	**4 (36)**	**20 (22)**

CR, complete response; DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma; MCL, mantle cell lymphoma; NE, not evaluable; iNHL, indolent non-Hodgkin's lymphoma; ORR, overall response rate; PD, progressive disease; PR, partial response; SD, stable disease.

Summary and Conclusions: MOR208 demonstrated encouraging single-agent efficacy with CRs observed in pts with R-R DLBCL, FL, and iNHL. MOR208 is well tolerated without significant infusional toxicity. Protocols are being developed to investigate MOR208 in combination with other agents.

E991

PROPOSED PROGNOSTIC MODEL FOR PERIPHERAL T-CELL LYMPHOMA: PSU-PROGNOSTIC INDEX FOR T-CELL LYMPHOMA (PSU-PIT) SCORE

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Background: Peripheral T-cell lymphoma (PTCL) is an uncommon lymphoma entity which is composed of various histological subtypes and unfavorable outcomes. Compared with Western countries, the prevalence of PTCL is higher in the Asian population and accounts for 15-20%.
Aims: This objective of the study was to identify prognostic factors and to propose a new prognostic model for PTCL patients.
Methods: The clinical characteristics, histopathology, treatment outcomes, and survival of 182 newly diagnosed PTCL patients from 2001 to 2014 in Songklanagarind Hospital were retrospectively reviewed. The clinical and outcome data were analyzed to define the factors associated with the response rate and survival outcomes.
Results: The histological subtypes of our patients included PTCL, NOS (33.5%), AITL (17.6%), nasal NKTCL (15.4%), CTCL (12.6%), SPTCL (12.1%), ALCL (4.4%), and others (4.4%). Overall clinical characteristics of the patients included a median age of 53 years (range 15-89 years), 70.3% male, 87.4% ECOG score 0-1, 80.2% extranodal lesions with 28.1% extranodal lesions >1, 48.4% Ann Arbor stage III-IV, 50% had B symptoms, 63.7% had high LDH level, 23.5% had bone marrow (BM) involvement, and 10.4% had thrombocytopenia (platelet count $<150x10^9$/l). Eighty-five percent of the patients were treated with chemotherapy and CHOP regimen was used in 77.4%. Of the 148 patients evaluated, the overall response rate was 69.6% with 47.3% complete remission (CR). With the median follow-up time of 18 months, the median overall survival (OS) was 23.9 months and the 3-year OS was 43.8%. From multivariate analyses, 6 factors were significantly associated with poor OS including the ECOG score 2-4 (p=0.000), extranodal >1 (p=0.013), Ann Arbor stage III-IV (p=0.005), B symptoms (p=0.003), BM involvement (p=0.003), and platelet count $<150x10^9$/l (p=0.001). A new prognostic (PSU-PIT) model was constructed by combination of these prognostic variables: score 0-1 as low-risk group, score 2-3 as intermediate-risk group, and score >3 as high-risk group. The PSU-PIT score was able to differentiate 3 risk-groups of PTCL patients with significantly different response rates (p=0.016) and survival outcomes (p=0.000) as the table.

Tabella 1.

PSU-PIT risk group	N (%)	CR rate (%)	Median OS (mo)	3-year OS (%)
Low	101 (55.5)	50	47	54.6
Intermediate	47 (25.8)	34	13	38.5
High	34 (18.7)	12	9	13.8
p value		0.016	0.000	0.000

Summary and Conclusions: A new prognostic model for PTCL was proposed. The PSU-PIT score efficiently predicted the CR rate and 3-year OS among the three risk groups of our PTCL patients.

E992

RESULTS OF TREATMENT OF LYMPHOBLASTIC LYMPHOMA AT THE CHILDREN CANCER HOSPITAL EGYPT- A SINGLE CENTER EXPERIENCE

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Background: Lymphoblastic lymphoma (LL) and acute lymphoblastic leukemia (ALL) and are neoplasms of immature B or T-cell precursors. They are considered as a unique biological entity in the 2008 World Health Organization Classification of Hematologic Neoplasm. Both entities are arbitrarily separated by a cut-off point of 20-25% of blast cells in the bone marrow. Treatment of LL has evolved over time from conventional high-grade NHL schedules to ALL- derived protocols.
Aims: The aim of this work is to estimate the EFS, OS, and common chemotherapy toxicities of LL patients treated at the Children Cancer Hospital Egypt during 5.5 years period.
Methods: A Retrospective review of patients charts diagnosed and treated as LL during the period between July 2007 till end of December 2012 was done. Patients were treated according to St. Jude Children Research Hospital ALL Total Therapy XV protocol, standard risk arm.
Results: This study included 77 patients. T- cell LL patients were 87%, while B-cell were 13%. The median age at diagnosis was 9 years (range 1–17 years). The majority were males (70.1%). Stage III was the most common at presentation (74%). Two patients were excluded from analysis as they died before receiving chemotherapy. Complete remission post induction chemotherapy was seen in 25.3% of the patients, partial remission in 72%. Progressive disease was the event in 6.6% of the patients, while 5.3% suffered from a disease recurrence. The most common chemotherapy toxicities were cerebral venous thrombosis (20%), followed by bone infarcts ((10.6%), and avascular necrosis of head of femur (9.3%). One patient developed secondary AML after 3 years of FU. Disease recurrence was local in 4% and systemic in 5% of the patients. By the end of the study, 84% of the patients were alive, 16% were dead. Mean duration of follow up was 48.6 months (range 1-89 months). The 4 years overall survival was 82.7% while event free survival was 82.2%.
Summary and Conclusions: Disease progression and chemotherapy related toxicity are the main causes of death in pediatric LL patients. While cerebral vascular thrombosis and steroids induced musculoskletal complications are the major chemotherapeutic adverse events.

E993

VALIDATION OF THE NCCN IPI IN 70 YEARS AND ABOVE AGGRESSIVE PATIENTS TREATED WITH R-COMP

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Background: The new NCCN IPI has been suggested as a better outcome predictor than the classic IPI in patients with DLBCL in the Rituximab era (blood 2014). The MDA-LNH-2011-01 study analyzed the effectiveness and toxic profile of R-COMP in patients 70 and above with diagnosis of DLBCL and grade 3 Follicular Lymphoma, G3FL (EHA 2013).
Aims: The aim of this study is to evaluate the new NCCN IPI in 70 yr old and above aggressive lymphoma patients treated with R-COMP (Rituximab 375 mg/m2/day1, Ciclofosfamide 750 mg/m2/day1, Vincristine 2 mg/day1, Liposomal non pegilated Doxorubicin 50 mg/m2/day1 and Prednisone 60 mg/m2/days 1-5).
Methods: We performed the analysis of the ORR, CRR (as *Revised-Response-Criteria-for-Malignant-Lymphoma v2007)*, OS and PFS (Kaplan meier) using the new NCCN IPI as well as the classic IPI in the patients included in the MDA-LNH-2011-01 study. This study was approved by the Spanish Medicines Agency (AEMPS).
Results: The MDA-LNH-2011-01 study included 100 patients from 15 Spanish sites, data to perform the new NCCN IPI were available in 84 pt. Mean age 77.3 (70-91), age >75yr 52pt (62%), M/F 46/38pt, DLBCL/G3FL 74/10pt, Ann Arbor III/IV 54pt (64%), extranodal involvement 52pt (62%), ECOG≥2 38pt (45%), LDH 1-3 49pt (58%), LDH>3 8pt (9.5%). NCCN IPI: int-low 14pt, int-high 36pt, high 34pt. Classic IPI: low 10pt, int-low 23pt, int-high 19pt, high 32pt. Effectiveness: CRR 55pt (65.5%), mean OS 24.6 months, mean PFS 22.7 months. Toxicity: Febrile neutropenia (FN) 29pt (34%). In the univariate analysis ECOG≥2 predicts OS and PFS (p<0.01 and p 0.02) and extranodal involvement predicts PFS (p0.02). The NCCN IPI index results in statistical significant differences for OS (p0.01), classic IPI don't (p0.07). Both index predict OS (p<0.01) and have correlation with FN incidence, if we make two groups, low and int-low versus int-high and high. We didn't observed correlation between other clinical baseline characteristics, age >80 yr, creatinine >1.3 mg/dL, Hb <10g/dL and OS.
Summary and Conclusions: In our experience NCCN IPI is useful to predict OS in 70 and above aggressive lymphoma patients treated with R-COMP. Making two groups (low and int-low versus int-high and high) both index are useful to predict OS and FN.

E994

PROSPECTIVE PHASE II TRIAL OF LENALIDOMIDE IN ASSOCIATION WITH CHOP IN ELDERLY PATIENTS WITH ANGIOIMMUNOBLASTIC T CELL LYMPHOMA (AITL): INTERIM ANALYSIS OF A LYSA STUDY

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Background: Angioimmunoblastic T-cell lymphoma (AITL) is one of the most frequent subtypes of T-cell lymphoma encountered in Western countries. Its optimal first-line treatment remains ill-defined with only 30% of patients experiencing long-term disease free survival when treated with anthracycline-based regimens. This disorder derives from the "follicular helper T cells" (TFH) subset developing within a unique malignant microenvironment. Owing to the importance of this microenvironment and given the promising activity of lenalinomide in a relapsed/refractory setting in AITL, we postulated that AITL patients might benefit from a treatment with lenalinomide combined with a classical CHOP regimen.
Aims: This multicenter, open label, phase 2 trial (NCT01553786) investigates the combination of lenalidomide with CHOP in previously untreated elderly patients with AITL.
Methods: Patients older than 59 years were treated with 8 cycles of lenalidomide +CHOP 21 (lenalidomide 25 mg/day (d), d1 to 14 - cyclophosphamide 750 mg/m², d1- doxorubicin 50 mg/m², d1- vincristine 1.4 mg/m², d1-prednisone 40 mg/m², d1 to 5, with pegfilgrastim at day 6) and received intrathecal methotrexate as central nervous system prophylaxis with the first 4 cycles. A thrombosis prophylaxis was mandatory. A dose adjustment of lenalidomide was planned according to related toxicities. A PET was performed at diagnosis and at the end of treatment. Histologic samples and PET were centrally reviewed. The primary objective was to evaluate the complete response (CR) rate based on a visual interpretation (Deauville five points scale) of PET according to the Lugano 2014 Classification. Secondary endpoints were safety, progression free survival and overall survival. Based on a Simon two-stage design comparing a CR rate of 60% with treatment to an unacceptable CR rate of 45% (CHOP alone), 17 or more CR out of 37 evaluable patients were required to declare the treatment worthy of further testing.
Results: Between November 2011 and July 2014, 19 LYSA centers enrolled 38 patients, of whom 37 were evaluable. Median age at diagnosis was 68 (59-79), 47 % were male, 68% had a performance status of 0 to 1, 97% an Ann Arbor stage ≥III, 84% IPI≥3 and 82% a PIT score≥2. The mean number of cycles delivered was 5.9. A total of 21 patients received the 8 planned cycles. A metabolic CR was obtained in 17 patients (46% [IC95%:29.5-63.1]) and 3 patients achieved a partial response, providing an overall response rate of 54%, 12 patients progressed and 5 could not be evaluated (one death of a septic shock after cycle 1, one withdrawal of consent, 2 patients with a major protocol violation retrieved after one cycle and one patient who refused to continue lenalidomide after one cycle). Toxicity was in the range of CHOP regimen with 70% and 30% grade 4 neutropenia and thrombocytopenia, respectively. Four episodes of thrombosis occurred during treatment. No second cancer was reported.
Summary and Conclusions: This pre-planned interim analysis shows that a combination of 25 mg of lenalidomide for 14 days with CHOP cycles gives acceptable activity and toxicity in AITL elderly patients. The study will continue as planned for a total of 70 evaluable patients.

E995

SUVMAX ON 18F-FLUORODEOXYGLUCOSE POSITRON EMISSION TOMOGRAPHY SCAN IS ASSOCIATED WITH CLINICOPATHOLOGICAL FACTORS AND PROGNOSIS IN NEWLY DIAGNOSED PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA

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Background: Although 18F-fluorodeoxyglucose positron emission tomography (PET) imaging for diffuse large B cell lymphoma (DLBCL) have been widely utilized to evaluate the staging and response assessment after treatment with high sensitivity, the relevance of the maximum standard uptake value (SUVmax) on pretreatment PET scan to disease outcome has been poorly studied.
Aims: The aim of this study was to investigate relationships between SUVmax on pretreatment PET scan and clinicopathological factors, and the prognostic value of baseline SUVmax in patients with newly diagnosed DLBCL.
Methods: In the present study, 140 patients with newly diagnosed DLBCL who had undergone pretreatment PET scan from 2004 to 2014 in single institute using an identical protocol were reviewed and analyzed. Correlations between SUVmax at the predominant lesion on PET scan and patient clinical features, immunohistochemical parameters of glucose transporter-1 (Glut-1), Glut-3 and Ki-67 reactivity were evaluated. SUVmax were evaluated for their association with progression-free survival (PFS) and overall survival (OS).
Results: The median SUVmax was 12.2 (1.7–42.7). The median follow-up time was 30 months (range, 4-124months). There was significant association between high SUVmax and patient characteristics, including Ann-Arbor stage, B symptoms, number of extranodal involvement, serum lactate dehydrogenase level, and Prognostics Index (R-IPI). High SUVmax was significantly associated with high expression of Glut-3 (immunoreactive score ≥6 *vs* <6: 16.7±10.1 *vs* 9.7±5.8 *P*=0.008) and Ki-67 (%expression ≥50% *vs* <50%: 15.1±10.0 *vs* 9.4±4.9 *P*=0.001), but not with Glut-1 (*P*=0.668). The 3-year PFS rates in patients with low SUVmax (SUVmax≤9) and those with high SUVmax (SUVmax>9) were 92.2 and 63.6%, respectively (*P*=0.000). The 3-year OS rates were 95.5 and 78.3%, respectively (*P*=0.003). This outcome advantage was demonstrable in patients with very good prognosis group and poor prognosis group according to the R-IPI categories (PFS, *P*=0.044 and *P*=0.011, respectively). Multivariate analysis revealed that high SUVmax is a significant poor prognostic factor for PFS (hazard ratio (HR) 4.784 [1.429–16.022], *P*=0.011), independent of R-IPI. The combination of SUVmax and R-IPI risk group provided a powerful discriminator of outcome.

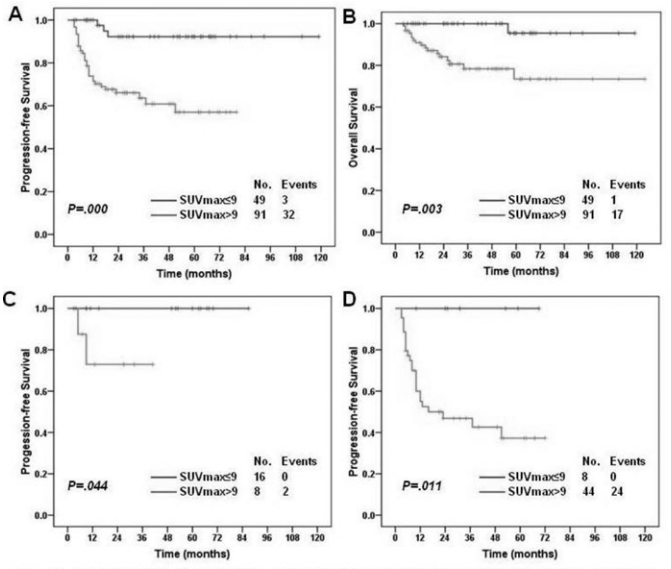

Fig. PFS (A) and OS (B) of all patients according to SUVmax using cut off value of 9;PFS of of patients in the "very good" prognosis group by R-IPI according to SUVmax (C), and of patients in the "poor" prognosis group according to SUVmax (D).

Figure 1.

Summary and Conclusions: In conclusion, for newly diagnosed patients with DLBCL, there was significant relationship between SUVmax on pretreatment PET and clinicopathological factors including R-IPI, Glut-3 and Ki-67. SUVmax can provide prognostic information in DLBCL beyond that which can already be obtained by the R-IPI. Therefore, the baseline SUVmax is an important prognostic tool of disease progression in DLBCL.

E996

PROGNOSTIC VALUE OF INTERIM POSITRON EMISSION TOMOGRAPHY-COMPUTED TOMOGRAPHY IN 377 PATIENTS WITH NON-HODGKIN LYMPHOMA: A SINGLE INSTITUTIONAL ANALYSIS OVER THE 8 YEARS

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Background: Positron Emission Tomography combined with computerized tomography (PET-CT) is increasingly used in non-Hodgkin lymphoma (NHL), and its availability of a staging also improves the accuracy of subsequent response assessment. Recently, interim PET-CT (iPET) that is often used to assess the early response and to guide the subsequent treatment, although its role is still controversial other than in Hodgkin disease.
Aims: The present study was performed to evaluate the prognostic value and clinical significance of iPET with regard to NHL in assessment of early response, and the usefulness for guiding subsequent treatment.
Methods: Between June 2006 and December 2014, 377 patients with NHL who was had iPET after two to four courses of treatment and end of treatment at Kameda Medical Center, Kamogawa-shi, Japan were retrospectively analysed. All of the patients with CD20-positive B-cell lymphoma received rituximab combined treatment. iPET was performed just before the next cycles of treatment to avoid the post chemotherapeutic change due to previous chemotherapy. A negative interim scan was defined as FDG uptake less than or equal to liver uptake at any site of FDG-positive disease identified in the baseline study. A positive scan was defined as any FDG uptake greater than liver background activity with a corresponding structural abnormality on CT scan. Overall survival (OS) and progression free survival (PFS) differences between curves were calculated by two-sided log-rank test. OS and PFS were compared between the patients with negative and positive results on iPET.
Results: The study population consisted of 219 males and 158 females with a median age of 63 years. The most frequent lymphoma diagnoses were diffuse large cell lymphoma (DLBCL; n=225), followed by indolent lymphoma (n=83) and T-cell lymphoma (n=69). Percentage of patients whose treatment was modified by iPET results were 13% in patients with DLBCL, 39% with T-cell lymphoma, and 5% of indolent lymphoma. iPET and at the end of treatment PET-CT status correlated with conventional response criteria. With a median follow up of 34 months, 2-year progression free survival rate was 81.2% for iPET negative versus 45.7% for iPET positive (P<0.001). Percentage of patients who became iPET negative was 70% (157/225), 71% (59/83), and 48% (33/69) in patients with DLBCL, indolent lymphoma and T-cell lymphoma, respectively. iPET status was associated with high serum LDH and soluble interleukin 2 receptor by Fisher's exact test (p<0.01 and P<0.001, respectively). On the whole, the patients who achieved the iPET negative showed significantly longer OS and PFS compared to those with positive (P<0.001). In the analysis of lymphoma subtypes, patients with DLBCL and T-cell lymphoma who had iPET negative showed significant longer PFS and OS (P<0.001), however, iPET did not have a predictive values in patients with indolent lymphoma (P=0.7 for OS and P=0.22 for PFS). We also analyzed maximal standardized uptake value reduction (ΔSUVmax) at baseline and iPET. Using the receiver operating characteristic (ROC) curve, ΔSUVmax between baseline and iPET of >85% was predictable for better PFS and OS (sensitivity 55.2%, specificity 85.7%, area under curve 0.719, 95% confidence interval=0.667-0.771).

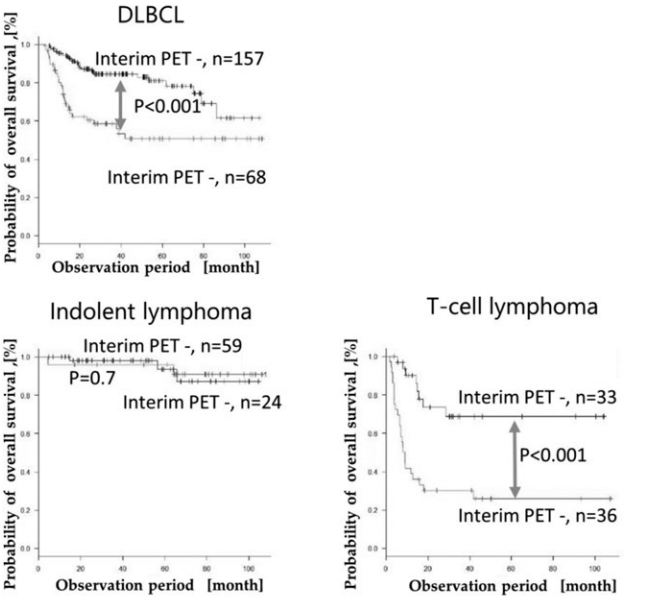

Figure 1. Impact of interim PET-CT negative on OS.

Summary and Conclusions: iPET effectively predict outcomes of patients with DLBCL and T-cell lymphoma, but it appeared to have a limited predictive value in patients with indolent lymphomas. ROC analysis revealed theΔSUVmax between baseline and iPET>85% is also predictable for better survival in both PFS and OS.

E997

LONG-TERM OUTCOME OF ADULTS WITH FIRST-RELAPSED OR REFRACTORY SYSTEMIC ALCL: A LYSA STUDY

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Background: Long-term outcomes of adults with first-relapsed or refractory (R/R) systemic anaplastic large-cell lymphoma (ALCL) are not definitively established and should be evaluated.
Aims: To evaluate long-term outcomes of adults with first R/R systemic ALCL.
Methods: We previously reported the long-term outcomes of adult patients treated at first diagnosis of systemic ALCL in three LYSA prospective clinical trials (Sibon D et al, J Clin Oncol 2012). All patients had confirmed systemic ALCL after immunohistopathologic review and defined ALK expression status. Here we report the long-term outcomes of these patients with R/R systemic ALCL.
Results: Among the 138 (64 ALK+and 74 ALK-) adult patients treated at first diagnosis in clinical trials, 40 (14 ALK+and 26 ALK-) had R/R systemic ALCL after the frontline chemotherapy and their long-term outcomes were analyzed. Median follow-up of R/R patients was 12.5 years (ALK+13.6 years; ALK- 12.1 years). The median age at first relapse/progression was 35 (19-76) years for ALK+patients and 61 (34-82) years for ALK- patients. All patients relapsed/progressed after polychemotherapy with an anthracycline-based regimen. Five (4 ALK+and 1 ALK-) patients relapsed/progressed after planned high-dose therapy/autologous stem-cell transplantation as first-line treatment consolidation. Median time from initial inclusion in clinical trials to relapse/progression after primary therapy was 6 months (46 days – 2.8 years) for ALK+patients and 11

months (32 days – 5.6 years) for ALK- patients. Median progression-free survival (PFS) after the first relapse/progression (second PFS) was 5 months for ALK+patients and 4 months for ALK- patients. Median overall survival (OS) after the first relapse/progression was 11.9 months for ALK+patients and 7.7 months for ALK- patients, and 10-year OS rates were 14% for ALK+patients and 15% for ALK- patients, without significant difference (p=0.99; figure). ALCL was the main cause of death. Impact of treatments will be presented.

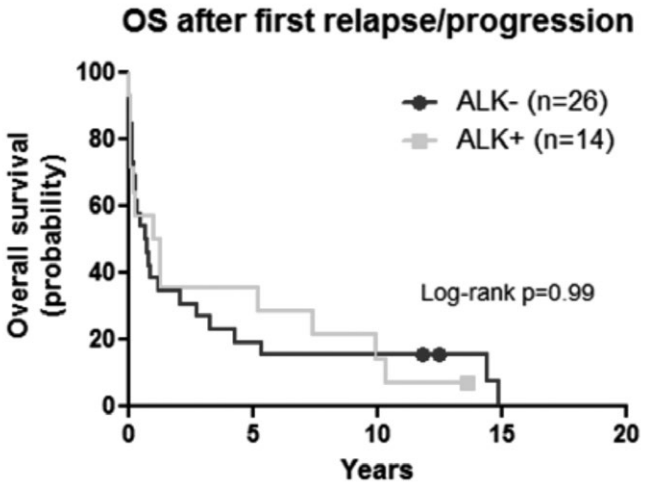

Figure 1.

Summary and Conclusions: Most patients with first R/R ALCL have poor outcomes with short survival, without significant difference between ALK+and ALK- patients. These results could be used as reference in the evaluation of new drugs for R/R ALCL.

E998

HIGH-THROUGHPUT SEQUENCING OF BOTH IMMUNOGLOBULIN LIGHT AND HEAVY CHAIN GENE REARRANGEMENTS IMPROVES DETECTION OF NEOPLASTIC CLONES IN MATURE B-CELL LYMPHOMAS

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Background: The detection of recurrent/persistent disease in mature B-cell lymphomas is important for individualized patient care. Amplification and high-throughput sequencing (HTS) of CDR3 sequences to describe the repertoire of B-cell and T-cell receptors is emerging as a low-invasive and sensitive method to identify and monitor minimal residual disease (MRD), and we have previously applied this technology to MRD monitoring in precursor T- and B-lineage acute lymphoblastic leukemias (Sci. Transl. Med. 2012; 4: 134ra63, Clin. Cancer Res. 2014; epub). However applying this technology to mature B-cell lymphomas present a unique challenge; some germinal-center derived mature B-cell lymphomas may have somatic hypermutation (SHM) of the *IGH* locus that may limit primer binding and amplification of these rearrangements. Prior work suggests that analysis of multiple gene rearrangements, IGH, IGK, and IGL, may increase the likelihood of identifying a tractable clone (Biol Blood Marrow Transplant. 2014; S1083-8791(14)00257-2).
Aims: Here we evaluate the potential of sequencing immunoglobulin heavy and light chains (*IGH* and *IGK/IGL*) in mature B-cell lymphomas by testing if we could identify tractable B-cell clones using HTS of the IGH and IGK/IGL repertoire.
Methods: First we defined tractable clones by defining the normal proportion of reactive clones in lymphoid tissues and peripheral blood by measuring the relative abundance of B-cell clones in healthy controls. We amplified the *IGH* and *IGK/IGL* repertoire of 20 control samples using an amplification bias-controled multiplex PCR assay and HTS sequencing. We defined clones as abnormal and representing a neoplastic population in patient samples, if the clones occurred at a proportion of >5 standard deviations above mean frequency of the most abundant rearranged IGH, IGK/IGL in control samples. Next we used the same assay to amplify the IGH and IGK/IGL repertoire of a cohort of 60 mature B-cell lymphomas assembled from residual material. This set included cased from: 20 diffuse large B-cell lymphoma (DLBCL), 10 mantle cell lymphoma (MCL), 20 follicular lymphoma (FL), and 10 chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL). As described, we then used the distribution of abundant clonal IGH and IGK/IGL CDR3 sequences in healthy controls to define probable neoplastic clones.
Results: We detected a clonal IgH gene rearrangement suitable for disease tracking in all MCL and CLL/SLL samples. By contrast, for many follicular lymphoma samples (11 of 20) and for some DLBCL samples (3 of 20), a clonal *IGH* gene rearrangement was not detected, most likely due to SHM. Additional high-throughput sequencing analysis of *IGK* or *IGL*, however, allowed a tractable clone to be detected in nearly all samples (18 of 20 for follicular lymphoma and 19 of 20 for DLBCL). Taken together, in this cohort, ninety-five percent of samples (57 of 60 cases) had at least one tractable clonal, immunoglobulin gene rearrangement.
Summary and Conclusions: We conclude that high-throughput sequencing of *IGH* is suitable for the majority of mature B-cell lymphomas. Lymphomas that may undergo somatic hypermutation, such as diffuse large B-cell lymphoma and follicular lymphomas, benefited most from additional immunoglobulin light chain gene sequencing. As high-throughput sequencing of antigen receptors can describe the breadth of the B-cell repertoire and simultaneously quantify the proportion of individual clones, this technology could contribute to the clinical monitoring of recurrent/persistent disease in these patients.

E999

Abstract withdrawn

E1000

AN INHERITED MUTATION OF THE NOD2 GENE IS ASSOCIATED WITH POOR SURVIVAL OF PATIENTS WITH DIFFUSE LARGE B CELL LYMPHOMA

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Background: The *NOD2/CARD15* gene is involved in the regulation of immune function through activation of the transcription nuclear factor – kappaB (NF-kappaB). A single truncating mutation of *NOD2/CARD15* gene, 3020insC is present in the Polish population with a frequency of approximately 7%. This allele has been shown to be associated with chronic inflammatory disease such as Crohn's disease and also with increased risk of colorectal cancer. The mutation has also been found to confer an increased risk of gastric MALT lymphoma, but not of other subtypes of lymphoma.
Aims: In the current study we analysed whether or not this mutation is associated with the risk of diffuse large B cell lymphoma (DLBCL), disease progression and survival among patients with DLBCL.
Methods: We genotyped 141 patients diagnosed with DLBCL between the November 2000 and the August 2009 for the 3020insC allele using PCR-RFLP. We compared the frequency of this allele in DLBCL patients to that reported previously in the general population of Poland using Fisher exact test. The patients were followed from date of diagnosis (November 2000 to August 2009) to the date of death (for deceased patients) or the date of last follow up (for alive patients). The mean follow up time was 4.4 years (range 0.04 – 12.9 years). For clinical evaluation we used a retrospective analysis of: International Prognostic Index (IPI) and its modifications, first full blood count (FBC) parameters at diagnosis (absolute lymphocyte count (ALC), presence of anemia or thrombocytopenia), progression incidence. Progression free survival rates, overall survival and disease specific survival were compared between carriers of the allele and non-carriers.
Results: The 3020insC allele was present in 11 of 141 (7.8%) patients with DLBCL, compared to 140 of 1910 (7.3%) population controls and the difference was not significant (OR=1.1, 95%CI=0.6 - 2.0, p=0.9). In the univariate Cox proportional hazards model carriers of the NOD2 mutation were found to have 2.7-fold increased risk of DLBCL progression (95%CI=1.1 - 6.5, p=0.02) and 3.1-fold increased risk of death because of the lymphoma (95%CI=1.1 - 9.4, p=0.03), but association with death of any cause was not significant (HR=2.0, 95%CI=0.8 - 4.6, p=0.12). The analysis of survival curves showed that the presence of 3020insC was associated with both a shorter progression-free survival (log-rank p=0.04) and disease-specific survival (log-rank p=0.045), but not with overall survival (log-rank p=0.15). In multivariate Cox regression analysis where age of onset, sex, aaIPI and thrombocytopenia at the time of diagnosis were included as covariates, the 3020insC mutation was associated with increased risk of DLBCL-related death (HR=7.7, 95%CI=1.8 - 32.9, p=0.006).
Summary and Conclusions: Our study suggests that an inherited mutation of the *NOD2* gene is an independent factor associated with poor disease-specific survival in patients with diffuse large B cell lymphoma, however, further studies are needed in this regard.

E1001

MYC/BCL2 DOUBLE EXPRESSION INCREASES A RISK OF RELAPSE OR PROGRESSION IN DIFFUSE LARGE B-CELL LYMPHOMA PATIENTS TREATED WITH INTENSIVE CHEMOTHERAPY M-NHL-BFM-90 PLUS RITUXIMAB

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Background: Diffuse large B-cell lymphoma (DLBCL) represents a highly aggressive type of lymphoma. A potential role of MYC and BCL2 synergetic interaction continues to be widely discussed in context of tumor chemoresistance. Previously it was reported that MYC/BCL2 double expressor (DE) DLBCL had a poor prognosis in patients, treated with CHOP plus rituximab (R) and in pts with non-GCB DCBCL who underwent R-(DA)-EPOCH chemotherapy.
Aims: To investigate a prognostic significance of MYC/BCL2 double expression in DLBCL patients treated with intensive chemotherapy m-NHL-BFM-90+R.
Methods: 62 DLBCL patients (35 males and 27 females) which underwent modified protocol of intensive chemotherapy – m-NHL-BFM-90+R in National Research Center for Hematology (Moscow) between 2004 and 2013 years were included in current study. Hans algorithm was used to determine GCB or non-GCB immunohistochemical subtypes of DLBCL. Tumor samples were stained with antibody to BCL2 (clone 124, Dako) and MYC (clone Y69, Epitomics). We used a previously reported cut off for MYC expression ≥40% and BCL2 ≥50% (N. Johnson *et al.*, 2012). G-banding data were available in 19 patients. 1 patient had a translocation t(8;14)(q24;q32). In all other cases FISH didn't reveal *c-MYC* or *BCL2* rearrangements with DNA probes Vysis LSI MYC Dual color, Break Apart Rearrangement Probe, Vysis LSI BCL2 Dual color, Break Apart Rearrangement Probe. We used Kaplan-Meyer and Cox regression analysis to estimate treatment results (SAS 9.3).
Results: By clinical characteristics patients corresponded to a high-risk group according IPI (3-5) in 48 (78%) cases. According to MYC and BCL2 expression status patients were divided into 4 groups: MYC+/BCL2- -9 (14,5%), MYC+/BCL2+- 15 (24%), MYC-/BCL2+- 21 (34%), MYC-/BCL2- 17 (27,5%) patients. All patients had comparable clinical characteristics, except of age. Median age of DE MYC/BCL2 patients were statistically higher - 61 (25-73) years old than in others - 47 (15-73) years old, (P<0,05). MYC/BCL2 double expression was revealed more frequently in non-GCB (29%) than in GCB subtype (17%), (P=0,18). 45 (78%) of patients achieved a complete remission, 4 from them had second remission. Relapses and progression of DLBCL developed in 9 (15%) and 7 (11%) patients, respectively. 5 (8%) patients died because of other reasons. There wasn't significant differences in 4-year OS depending on MYC and BCL2 expression: MYC+/BCL2- - 100%, MYC-/BCL2 – 78%, MYC+/BCL2+- 57%, MYC-/BCL2+- 53% (P=0,09). Although DE DLBCL patients had a statistically higher risk of relapse or disease progression within 4 years: MYC+/BCL2- - 14%, MYC-/BCL2 – 14%, MYC+/BCL2+- 65%, MYC-/BCL2+- 24% (P=0,02) (Picture 1). If compare DE patients with all others statistical significance increased (65% *vs* 15%, P=0,003). GCB/non-GCB subtype increases a probability of DLBCL relapse or progression within 4 years (17% *vs* 39, P=0, 07). In multivariate analysis MYC/BCL2 double expression had an independent prognostic power (HR 4,717, P=0,0024) from IPI, GCB/non-GCB subtype.

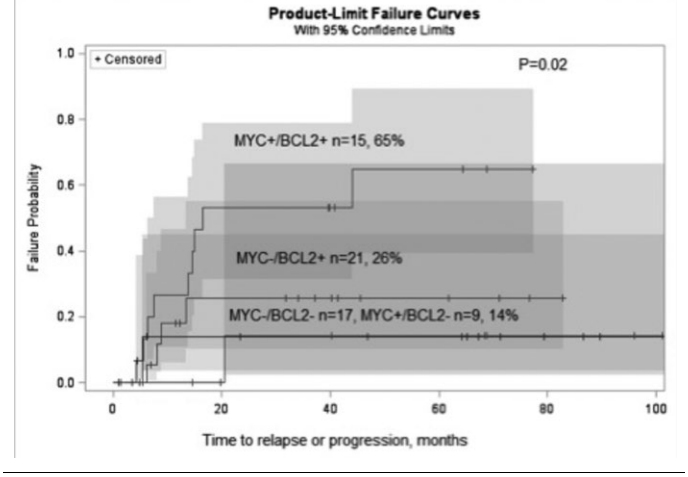

Figure 1.

Summary and Conclusions: MYC/BCL2 double expression is an independent risk factor of DLBCL relapse or progression in patients treated with intensive chemotherapy m-NHL-BFM-90+R, independently of IPI, GCB/non-GCB subtype.

E1002

CLINICAL FEATURES AND OUTCOMES OF PATIENTS WITH EXTRANODAL NK/T-CELL LYMPHOMA, NASAL TYPE: A PROSPECTIVE MULTICENTER STUDY FROM THE THAI LYMPHOMA REGISTRY

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Background: Extranodal NK/T-cell lymphoma (ENKTL), nasal type is a rare subtype of lymphoma with a strong geographic predilection for Asian and South American populations. At diagnosis, most of ENKTL, nasal type patients present with destructive lesions in the nasal cavity and paranasal sinuses.
Aims: This study aims to describe the clinical features and treatment outcomes of Thai patients with ENKTL, nasal type.
Methods: A clinical review of patients with ENKTL, nasal type according to WHO 2008 classification obtained from The Thai Lymphoma Registry between 2002 to 2014 was performed.
Results: Among 4,019 cases of lymphoma, there were 81 cases (2%) of ENKTL, nasal type. The median age was 45 years old (range, 21-91). The male: female ratio was 2.5: 1. At diagnosis, approximately half of the patients had stage I/II disease (55.6%), International Prognostic Index (IPI) score of ≤1 (51.9%), B symptoms (54.0%), and elevated serum LDH (54.0%). A majority of the patients (92.6%) presented with good ECOG performance status. The patients were treated with chemoradiotherapy in 34.6%, chemotherapy alone in 35.8%, and radiotherapy alone in 11.1%. Fifteen patients (18.5 %) did not receive any treatment. The most common first-line induction chemotherapy regimen was CHOP (92.9%). Of the 66 evaluable patients, the overall complete remission (CR) rate and overall partial remission (PR) rate was 39.4% and 6.1%, respectively. The CR rate was achieved in 71.4%, 27.6%, and 22.2% of the cases treated with chemoradiotherapy, chemotherapy alone, and radiotherapy alone, respectively. With the median follow-up time of 9.7 months (range, 0.5-316.2), the median overall survival (OS) and progression-free survival (PFS) for the whole group was 10.8 months (95% CI: 0.2-21.5) and 8.2 months (95% CI: 3.0-13.5), respectively. The median OS of the patients treated with chemoradiotherapy was significantly higher than those treated with chemotherapy alone (5.8 years [95% CI: 1.2-10.4] vs. 0.4 year [95% CI: 0.1-0.7]; *P*=0.002) and radiotherapy alone (5.8 years [95% CI: 1.2-10.4] vs. 0.3 year [95% CI: 0.1-0.5]; *P*=0.021). The median PFS of the patients treated with chemoradiotherapy was significantly higher than those treated with chemotherapy alone (17.1 months [95% CI: 8.7-25.5] vs. 4.1 months [95% CI: 2.2-5.9]; *P*=0.014) and radiotherapy alone (17.1 months [95% CI: 8.7-25.5] vs. 3.1 months [95% CI: 1.6-4.6]; *P*=0.024). Furthermore, the IPI score was of prognostic significance to predict the median OS (IPI score=0 [6.4 years] vs. IPI score ≥1 [0.4 year]; *P*=0.016) and 5-year OS (IPI score=0 [56.9%, 95% CI: 30.8-76.3] vs. IPI score ≥1 [25.2%, 95% CI: 14.5-37.5]; *P*=0.012) in this cohort.
Summary and Conclusions: ENKTL, nasal type is rare among Thai population. At presentation, approximately half of the patients had early stage diseases, B symptoms, elevated serum LDH, and low IPI score. Chemoradiotherapy was the most effective treatment. In this cohort, the IPI score significantly predicted the OS.

E1003

CD30 EXPRESSION IN DE NOVO DIFFUSE LARGE B-CELL LYMPHOMA: CLINICAL FEATURES AND OUTCOME

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Background: Diffuse large B-cell lymphoma (DLBCL) is the most common and one of the most heterogeneous lymphomas. Therefore, it is critical to further stratify cases of DLBCL into biologically similar and clinically meaningful subgroups, which will not only guide prognostic assessment and facilitate therapeutic decisions, but also stimulate further research to understand the pathogenesis and develop potential novel treatments. One promising candidate is brentuximab vedotin, an antibody-drug conjugate targeting CD30-expressing cells. Previous observational studies have suggested that CD30 may be expressed in 10 to 20% of DLBCLs.
Aims: The aim of this study was to determine the prevalence of CD30 expression in DLBCL by immunohistochemistry, and to explore possible relationships with outcome.
Methods: We retrospectively identified cases of DLBCL diagnosed between January 2010 and January 2013 at our Institution. The following large B cell lymphoma subtypes were excluded from this analysis: post-transplant lymphoproliferative disorders with a DLBCL morphology, Primary Mediastinal large cell lymphoma and unclassifiable lymphomas with intermediate features between either DLBCL and Burkitt's lymphoma or DLBCL and Hodgkin's lymphoma. Immunohistochemistry was performed as part of the routine workup

and CD30 was considered positive when ≥30% of neoplastic cells stained positive. A total of 82 cases of de novo DLBCL treated with R-CHOP were included in the training set for further analysis There were 45 men and 37 women with a median age of 57 years (range, 16-84); 35 patients (43%) presented with B symptoms, and 49 (60%) had advanced Ann Arbor stages. Most of the patients had a good performance status (Eastern Cooperative Oncology Group score 0- 1, 87%), elevated serum lactate dehydrogenase level (61%), and low or low-intermediate International Prognostic Index (IPI) risk (IPI score 0-2, 63%). Involvement of multiple extranodal sites (>=2) was seen in 22% of cases, and bulky disease in 32% of cases.
Results: The median follow-up time was 47 months. Among the 82 cases in the training set, CD30 was positive in 24 cases (29%). No difference in response rate was observed between CD30 positive and CD30 negative patients. Patients with CD30+DLBCL showed a significantly superior OS and PFS compared with those with CD30–. The 3-year OS was 79% in patients with CD30+vs 59% in CD30– (P <0.05); 3-year PFS was 73% in patients with CD30+vs 57% in CD30– (P<0.05).
Summary and Conclusions: CD30 is expressed in approximately 29% of all DLBCL and defines a novel subgroup of diffuse large B-cell lymphoma with a more favorable prognosis. The advent of brentuximab vedotin and its well-established effectiveness in other types of relapsed lymphomas opens the possibility of its application in this subset of patients.

E1004

PHASE II STUDY OF SMILE CHEMOTHERAPY FOR RELAPSED/ REFRACTORY PERIPHERAL T-CELL LYMPHOMA

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Background: We previously reported that SMILE (Steroid, Methotrexate, Ifosfamide, L-asparaginase and Etoposide) regimen is effective for newly-diagnosed stage IV, relapsed or refractory extranodal NK/T-cell lymphoma, nasal type (J Clin Oncol 2011; 29: 4410-6). Because of the many similarities in extranodal NK/T-cell lymphoma, nasal type and peripheral T-cell lymphoma (PTCL), the SMILE regimen was applied for PTCL.
Aims: The aim of this study is to examine the efficacy and safety of SMILE regimen for relapsed/refractory PTCL.
Methods: The phase II study of SMILE for PTCL was carried out according to the Simon's two-stage design. Patients with relapsed or refractory PTCL after first-line chemotherapy, aged 15-69 years old, and with a performance status of 0-2 were eligible. Eight subtypes of PTCL were subjected, comprising PTCL, not otherwise specified (PTCL-NOS), angioimmunoblastic T-cell lymphoma (AITL), ALK-positive and -negative anaplastic large cell lymphoma (ALCL), hepatosplenic T-cell lymphoma, enteropathy-associate T-cell lymphoma (EATL), primary cutaneous gamma-delta T-cell lymphoma (PCGDTL), and primary cutaneous CD8+aggressive epidermotropic cytotoxic T-cell lymphoma. The primary endpoint was overall response rate (ORR) after 2 cycles of SMILE chemotherapy.
Results: From November 2009 to February 2014, a total of 42 patients were enrolled. The median age was 56 years (range, 28 to 69 years), and the male:female ratio was 33:9. The diagnosis was PTCL-NOS in 19, AITL in 14, ALK-positive ALCL in 1, ALK-negative ALCL in 4, EATL in 3, and PCGDTL in 1. Twenty-six were relapsed PTCL, and 16 were refractory to initial treatment with anthracycline-containing regimen. Complete and partial response was achieved in 6 and 13 patients, respectively, with an ORR of 48% (90% confidence interval, 34-62%). The response was stable disease in 6, progressive disease in 15, but was not evaluable in 2. The ORR was 64% (90% confidence interval, 46-80%) for relapsed PTCLs, but was 27% (90% confidence interval, 10-51%) for relapsed patients, and the difference was statistically significant (P=0.048). One patient died of sepsis and another patient died of disease in the treatment period.
Summary and Conclusions: These results indicate that SMILE regimen is effective for relapsed or refractory PTCL. The efficacy was lower than that for extranodal NK/T-cell lymphoma, but the present T-SMILE study included more relapsed patients. Further follow-up defined in the protocol is required to determine the duration of response and long-term efficacy.

E1005

GASTROINTESTINAL TRACT POST-TRANSPLANT LYMPHOPROLIFERATIVE DISORDER (PTLD) AFTER KIDNEY TRANSPLANT: A 17-YEAR BRAZILIAN SINGLE-CENTRE EXPERIENCE

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Background: Post-Transplant Lymphoproliferative Disorder (PTLD) is a well recognized complication following both solid organ and allogenic hematopoietic stem cell transplantation. This condition is characterized by the development of lymphoid or plasma cell neoplasms in the setting of post-transplant immunossupression. Epstein-bar virus is considered the most important causative factor, with EBV positivity observed within up 90% of tumor lymphocytes. Gastrointestinal (GI) involvement of PTLD is a relatively common life-threatening condition, usually associated with aggressive presentation and high mortality rates.
Aims: To analyze the cases of PTLD with Gastrointestinal involvement dignosed between 1998 and 2014 on our center.
Methods: From 1998 to 2014, a total of 11,284 kidney transplants were performed at Hospital do Rim e Hipertensão/UNIFESP (Federal University of São Paulo). We retrospectively analyzed cases of PTLD with GI tract involvement diagnosed over the past 17 years. Only confirmed cases of PTLD with available clinical and epidemiological data were included.
Results: PTLD was reported in 94 recipients. GI tract involvement was present in 39% of the cases (37 patients). Two patients were excluded from the analysis due to incomplete data. The median age at diagnoses was 41 (range 4 to 64). The M:F ratio was 1,92:1. The median time from transplant to PTLD was 57 months. Twelve (32%) of our cases received induction therapy either with antithymocyte globuline (ATG) (50%) or Basiliximab (50%) at the time of transplant. The immunossupressive regimen the patients were receiving at the time of diagnosis of PTLD were: Prednisone (100%), azathioprine (88%), tacrolimus (62%), cyclosporine (37%), mycophenolate mofetil (8%) and 1 patient received FTY720. Regarding EBV positivity, 28 patients (76%) were EBV positive, 4 patients (11%) were EBV negative and 5 patients (13%) were not tested or had inconclusive results. Monomorphic PTLD was present in 32 patients (86%), the majority (90%) was diagnosed as diffuse large B-cell non-Hodgkin lymphoma, Polymorphic PTLD corresponded to only 4 cases (11%) and 1 patient could not be classified. Eight (22%) patients had multiple gastrointestinal tract involvement, 11 (31%) patients had only small intestine involvement, followed by 9 (24%) patients with stomach involvement, 8 (22%) with hepatic involvement and 1 patient had esophageal involvement. Seventeen (46%) had concomitant non GI tract extranodal involvement (lung/pleura, CNS, bone marrow, bone, skin and ovaries). After diagnosis, all patients had their immunossupression regimens reduced or suspended. Twenty-eight (76%) patients received anti-CD20 based therapy with 21 (75%) patients receiving R-CHOP, 5 patients (13.5%) did not received chemo/immune therapy, eleven patients (30%) required surgery (gastrectomy or enterectomy) and in 4 (11%) cases surgery was the only treatment. Nineteen (51%) of our patients achieved complete remission (CR). 17 patients (46%) died, most of them (10 patients) from infectious complications, 3 patients due to GI perforation, 2 patients from pulmonary embolism and 2 from undetermined causes. At total, 6 (16%) cases of GI perforation were observed.
Summary and Conclusions: In our population, GI tract was the most common PTLD involved site. EBV was positive in the majority of cases. Although 19 patients achieved CR, a high number of deaths were observed, mostly due to infection. This unique condition should be considered in every transplant recipient presenting with abdominal symptoms.

E1006

THE ROLE OF UPFRONT AUTOLOGOUS STEM CELL TRANSPLANTATION FOR HIGH-RISK PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA

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Background: Primary central nervous system lymphoma (PCNSL) is an aggressive extranodal non-Hodgkin lymphoma. The patients with International Extranodal Lymphoma Study Group (IELSG) score of more than one or those who did not achieve complete remission (CR) after two courses of high-dose methotrexate (HD-MTX)-based chemotherapy (non-CR1) have been shown inferior treatment outcomes. Upfront autologous stem cell transplantation (ASCT) as consolidation after HD-MTX-based chemotherapy has shown better efficacy compared to whole brain radiotherapy consolidation.
Aims: To investigate the role of upfront ASCT for high-risk PCNSL patients
Methods: We retrospectively analyzed a total of 66 newly diagnosed high-risk PCNSL patients between February 2003 and December 2014 in Severance Hospital. Patients who were younger than 65 years of age with IELSG score ≥2 (n=56) and/or non-CR1 (n=37) were included in this study. All the patients (n=66) were HIV negative, received HD-MTX-based chemotherapy as initial treatment and achieved at least partial response (PR) after initial treatment.
Results: The median age at diagnosis was 52 (range 21-64) years and 43 (65.2%) patients were male. Median follow-up for surviving patients was 22 (range 2-123) months. The upfront ASCT group included more patients aged 50 or less (68.4% vs. 31.9%, *P*=0.007). Nineteen (28.8%) patients have received upfront ASCT, and all of them received busulfan-based conditioning regimens including busulfan plus thiotepa (n=18). The disease status at the

time of upfront ASCT was CR in 17 (89.5%) patients and PR in 2 (10.5%) patients. Transplant-related mortality occurred in 1 (5.3%) patient after upfront ASCT. In non-upfront ASCT group (n=47), 28 (59.6%) patients achieved CR after completion of therapy and 16 (34.0%) patients received whole-brain radiotherapy as consolidation. Twelve patients in the non-upfront ASCT group underwent ASCT after relapse. Non-upfront ASCT was associated with shorter overall survival (OS) and progression-free survival (PFS) in multivariate analysis (hazard ratio (HR) 8.24; 95% CI 1.31-51.99; *P*=0.025, and HR 4.26; 95% CI 1.49-12.14; *P*=0.007, respectively). The 2-year OS and 2-year PFS of all patients were 65.9% and 39.9%, respectively. The upfront ASCT group showed superior OS and PFS compared to the non-upfront ASCT group (*P*=0.022 and *P*=0.005, respectively). Among the 56 patients with IELSG score ≥2, upfront ASCT was performed in 17 (30.4%) patients and showed survival benefit for OS and PFS (*P*=0.036 and *P*=0.005, respectively). OS and PFS between the upfront and the non-upfront ASCT group also showed similar results in patients with 37 non-CR1 patients (*P*=0.009 and *P*=0.021, respectively).

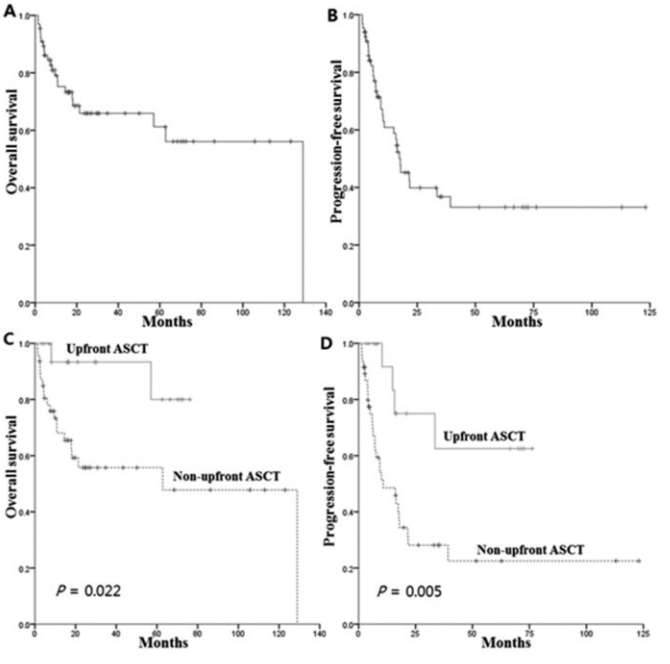

Figure 1.

Summary and Conclusions: Upfront ASCT as consolidation after HD-MTX-based induction chemotherapy in high-risk PCNSL showed better survival results with acceptable toxicities. Upfront ASCT overcomes a negative impact of IELSG score ≥2 and/or non-CR1 in PCNSL.

E1007

FAVORABLE OUTCOME IN PRIMARY MEDIASTINAL LARGE B-CELL LYMPHOMA IN A SINGLE CENTRE: THE PADUA EXPERIENCE

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Background: Primary mediastinal large B-cell lymphoma (PMBCL) is a distinct clinical, pathological and molecular subtype of diffuse large B-cell lymphoma. The optimal management of PMBCL is still unknown, however several retrospective analyses showed an improvement of outcome with dose-intensive chemotherapy regimens and radiotherapy on residual mass.
Aims: In this study we evaluate activity, feasibility and safety of the third generation regimen R-VACOP-B and mediastinal radiotherapy (RT) in the treatment of PMBCL.
Methods: We retrospectively evaluated data from 34 previously untreated patients with PMBCL between September 2002 and January 2014, referred to the Hematology and Clinical Immunology Unit of Padua University Hospital. Patients were treated with R-VACOP-B+RT according to Todeschini G. et al Br J Cancer 2004. Progression free survival (PFS) and Overall survival (OS) were calculated as date of initial presentation to progression (event) or death for any cause (event), respectively, or last known follow-up (censored). Survival analyses were performed by Kaplan-Meier method and Log-rank test to compare survivals. p values <0.05 were considered significant.
Results: Among recruited patients 50% of them were female, the median age at diagnosis was 33 years, 79% had a mediastinal-limited disease, 88% had IPI 0 or 1 and ECOG 0 or 1, and the median follow-up was 69 months. All patients completed the planned 8 cycles R-VACOP-B followed by RT. Treatment was well tolerated; neutropenia G3-4 occurred in 38% of patients, anemia G3-4 in 3% but no piastrinopenia G3-4 was observed. 38% of subject underwent a major infective event. Extrahematological toxicity mainly consisted in transient neuropaty (21%) and catheter related thrombosis (12%). Focusing on late cardiac toxicity, a decrease in left ventricular ejection fraction (EF) was observed in 18% of the cohort but an EF<40% was reported only by 1 patients. Considering pulmonary toxicity 50% of subjects had a slight to moderate reduction of CO diffusion, but none of them developed a life threatening interstitial pneumonia. Finally, hypothiroidism and mammarian nodule was observed in 6% of patients. Interim PET-CT was performed in 23 patients (68%) and for 18 (78%) of them it was negative. Interestingly, all of these 18 subjects achieved a complete remission (CR) at the end of therapy. 14 patients (41%) had partial response at interim analysis, 10 of them covered to CR after the eighth cycle, the remaining 4 developed a relapse-refractory (R/R) disease. The median PFS was not reached during the follow-up and the 5-year PFS and OS were 82% and 100% respectively. 6 patients (18%) had a R/R disease and all progressed within the first 15 months. All these 6 patients were successfully saved by R-DHAP followed by autologous stem cell transplantation. 1 (3%) patient required allogenic bone marrow transplant due to acquired bone marrow failure. All 34 subjects were still alive after 10 years of follow-up. Furthermore, we observed that IPI and staging at diagnosis significantly correlated with PFS (IPI 0-1 *vs* >1, 86% *vs* 50%, p=0.0385 and Stage II *vs* III-IV, 89% *vs* 50%, p=0.0234).
Summary and Conclusions: In this single center retrospective study, R-VACOP-B+RT induced a very-high remission rate with an acceptable toxicity profile. IPI and ECOG correlated with PFS and all patients were still alive after a follow-up of more than 10 years. We provide evidence that R-VACOP-B is a safe and effective radio-chemo-immunotherapeutic protocol for the treatment of patients with PMBCL. In the next future, we will compare R-VACOP-B+RT *vs* R-DAEPOCH.

E1008

HUMAN T-LYMPHOTROPIC VIRUS TYPE I TAX SPECIFIC CYTOTOXIC T-LYMPHOCYTE ANALYSIS OF AGGRESSIVE TYPES OF ADULT T-CELL LEUKEMIA/LYMPHOMA PATIENTS WITH LONG-TERM SURVIVAL AND COMPLETE REMISSION

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Background: Adult T-cell leukemia/lymphoma (ATLL) is a peripheral T-cell malignancy caused by human T-lymphotropic virus type I (HTLV-I). Most ATLL cells are CD4 and CCR4 positive, and CD8 negative. ATLL is classified into 4 clinical subtypes: smoldering, chronic, acute, and lymphoma. Acute and lymphoma type ATLLs, which are aggressive types, have a very poor prognosis.
Aims: We report here the extremely interesting results of aggressive ATLL patients with long-term survival and complete remission (CR) after activation of cellular immunity against ATLL cells.

Tabella 1. A summary of HTLV-I tax specific CTL analysis and HTLV-I provirus load in the peripheral blood.

Patient No.	HLA		CTL (%)	HTLV-I provirus (copies/1000 cells)	Mainly used TCR VB (%)
1	A02:01	2012	0.11	35.4	VB3 (71.0)
		2014	0.13	61.9	
2	A02:01	2012	0.78	24.4	VB2 (4.1)
		2014	1.56	14.7	VB8 (6.0)
					VB17 (5.4)
					VB22 (6.8)
3	A02:01	2012	1.06	7.1	VB1 (12.53)
		2014	1.31	13.3	VB3 (7.95)
	A24:02	2012	0.03		
		2014	0.03		
4	A24:02	2012	2.07	26.4	VB17 (12.2)
		2014	3.64	27.9	VB20 (13.5)

Methods: We retrospectively evaluated 46 patients with aggressive ATLLs diagnosed at our hospital between January 2001 and August 2011. Of these, 7 patients had long-term survival greater than 3 years with complete remission (CR) after intensive chemotherapies. Four of these 7 patients had HLA-A02:01

or HLA-A24:02, and were investigated using cytotoxic T-lymphocyte (CTL) analysis. T-cell receptor (TCR) V beta (VB) repertoire and the HTLV-I provirus load in peripheral blood were also analyzed.
Results: The Table summarizes the results. HTLV-I specific CTL analysis and HTLV-I provirus load in the peripheral blood were performed twice, once in 2012 and once in 2014. HTLV-I tax specific CTLs were detected in all 4 patients. Although all patients maintained CR, HTLV-I proviruses were detected in the peripheral blood in all patients. This phenomenon was observed both in 2012 and in 2014. TCR VB repertoire was analyzed using flow cytometry in January 2015. Clonal dominance was present in all patients. In particular, HTLV-I tax specific CTL expressing VB3 was found in a high percentage (71%) in Patient No. 1.
Summary and Conclusions: The findings from this study suggest that HTLV-I specific CTLs can be induced with clonal dominance in patients with aggressive types of ATLL. In patients having long survival with CR, these CTLs can contribute to treatment and may play a roll inhibiting the relapse of ATLL. The development of efficacious methods to induce HTLV-I specific CTLs in individual ATLL patients may leads to improved outcomes for aggressive types of ATLL.

E1009

PROPHYLACTIC USE OF ACETAZOLAMIDE REDUCES WEIGHT GAIN AND AMELIORATES RENAL COMPLICATIONS ASSOCIATED WITH HIGH DOSE METHOTREXATE

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Background: High dose methotrexate (HDMTx) is an effective drug in the treatment of lymphoid malignancies such as CNS lymphoma, especially combined with high dose cytarabine or radiotherapy. Adequate MTx levels in the CSF require rapid infusion, with 2 - 4 hours deemed the optimal duration. HDMTx toxicities include mucositis, hepatotoxicity, renal impairment and myelosuppression. HDMTx-induced acute kidney injury (HDMTx-AKI) is a rare complication; it can range from asymptomatic serum creatinine (Cr) rise to life-threatening AKI. HDMTx-AKI can increase the risk of other MTx related toxicities, and cause a reduction of the optimal MTx dose administered. HDMTx-AKI is attributed to tubular obstruction due to precipitation of MTx and its metabolite (crystal nephropathy), which may be exacerbated by acidic urinary pH and hypovolemia. Acetazolamide (AZL) is a carbonic anhydrase inhibitor in the renal proximal convoluted tubule. It causes increased bicarbonate excretion and therefore urine alkalinization. Shamash et al found AZL 500 mg QID effectively alkalinized urine in patients treated with HDMTx, with satisfactory plasma MTx elimination. Prophylactic intravenous fluids are also essential in preventing HDMTx-AKI, and hence fluid overload is a common complication of HDMTx therapy.
Aims: We conducted a dual centre, retrospective cohort study examining AZL's renoprotection effect in patients receiving HDMTx for haematological malignancies. Parameters examined included AKI incidence, days of elimination and urinary pH. Since 2010, the use of prophylactic AZL was recommended for patients undergoing HDMTx therapy at our institutions.

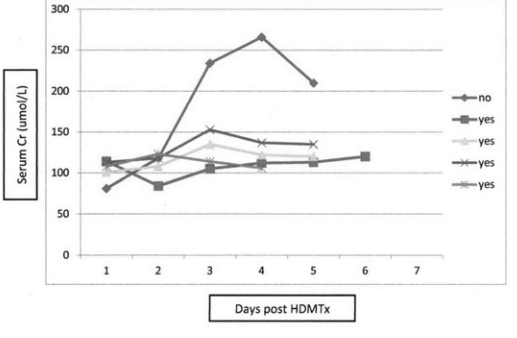

Fig 1. A graph of a patient's serial serum creatinine (Cr) in 4 cycles of HDMTx is shown, depicting much higher serum Cr without acetazolamide (blue line) versus serum Cr levels with acetazolamide in subsequent cycles.

X axis - days post HDMTx
Y axis - serum Cr levels (umol/L)

Figure 1.

Methods: Data of patients who had HDMTx from 2007 to 2012 was analysed. In total, 92 patients were given HDMTx, resulting in 256 cycles. The AZL group (250mg q8h) and non-AZL group were comparable in the majority of variables that could have impacted on the incidence of HDMTx-AKI. The primary endpoint was AKI (Cr>100umol/L) or prolonged MTx elimination (>5 days).
Results: 1. AZL showed a strong trend in preventing either AKI (Cr>100umol/L) or prolonged MTx elimination (>5 days). a) AKI or MTx elimination>5 days occurred in 24/164 (14.6%) of the non AZL group, versus 4/68 (5.9%) of the AZL group, p=0.063. There is a strong trend that AZL use reduced renal complications. A graph of a patient's serial Cr in 4 cycles of HDMTx is shown (Fig 1), depicting much higher Cr without AZL versus Cr levels with AZL. b) HDMTx induced AKI was correlated with increased days of elimination (mean 6.5 days for AKI versus 4.0 days for non AKI, p=0.001). c) AZL benefit on HDMTx-AKI or prolonged MTx elimination was more obvious in males, who were more prone to these effects. In the non AZL group, 4/64 (6.3%) females had either AKI or prolonged MTx elimination, versus 20/100 (20%) males, p=0.015. AZL abolished this gender disadvantage, with 2/29 (6.9%) females versus 2/39 (5.1%) males, p=0.759. 2. AZL use resulted in significantly less weight gain and less acidic urinary pH (pH<7) and was tolerated well a) weight gain - median weight gain of 3.0kg (range 0 - 13, n=151) in the non AZL group, compared to 1.0kg (0 - 6, n=85) in the AZL group, Mann-Whitney p<0.001. b) urine pH <7.0 - 40/166 (24.1%) in the non AZL group had at least one episode of urinary pH<7, versus 10/86 (11.6%) in the AZL group, Chi-square p=0.029. c) AZL was well tolerated, with mild hypokalaemia being the only common complication (48.5% of AZL group). This was preventable by prophylactic oral potassium use.
Summary and Conclusions: This retrospective, dual centred cohort study examined AZL's renoprotection effect during HDMTx. AZL showed a strong trend in preventing either AKI or prolonged MTx elimination, especially in males, who appeared to be at higher risk. A larger patient sample is required to confirm the significance of this finding. AZL use also resulted in significantly less weight gain and less acidic urinary pH (pH<7) during HDMTx; these factors could influence patient management and length of hospital stay. In summary, this study demonstrated that AZL could be used as an adjunct renoprotective treatment in HDMTx regimens.

E1010

CD30 EXPRESSION IN DLBCL, INCIDENCE AND PROGNOSIS VALUE: A SINGLE-INSTITUTION EXPERIENCE

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Background: DLBCL is the most common subtype of NHL, with R-CHOP, the standard of treatment, we obtain CR and 5 years PFS in more than 50% of patients. It has been identified biological markers with impact on the outcome of these patients. CD30 is a cell-surface marker expressed by several types of NHL, including a subset of DLBCL (±15%). A better outcome has been suggested for CD30 positive patients.
Aims: The aim of this study is to analyze the incidence of CD30 in DLBCL and to evaluate the prognostic impact and the possible correlation with clinical and biological characteristics.
Methods: We carried out a single institution study with DLBCL samples from patients diagnosed in the period 1994-2011 and treated with chemotherapy or R-chemotherapy. DLBCL diagnosis was reconfirmed. Tissue fixation and processing were performed using standard methods. Tissue microarrays (TMAs) that contained two representative 2-mm cores from each tumor were prepared. Internal control cores were present in each TMA. Immunohistochemical stainings were performed using fully automated protocols. Sections were subjected to staining protocols with the antibody CD 30(Dako). CD30 expression was evaluated by an anatomopathologist and a hematologist and was considered positive 20% and above. CD30 expresion was also evaluated using computerized image analysis with Image Pro Plus 6.0. A mean number of 1000 cells were evaluated per case. Both readings were compared and scoring discrepancies (differences >10%) were resolved using a multi-head microscope. Correlation with clinical and biological characteristics were analyzed with chi-square test and OS and PFS with kaplan Meier (SPSS19). This study has been approved by local ethic committee.
Results: Samples from 140 DLBCL patients (95/45pt R-chemo/chemo) were analyzed, median age 70 (23-76), male/female 73/67pt, Ann Arbor stage III or IV: 73pt (52%), high LDH 72pt (51%), high β2-microglobullin 62pt (44%) IPI low 53 pt, int-low 26pt (19%), int-high 31pt (23%), high 28pt (20%) IPI ≥3: 59pt (42.1%). All patients ORR 79pt (58%), CRR 53pt (39%). We observed CD30 overexpressed (>20%) in 14pt (10%). In univariate analysis patients with CD30 overexpression had better CRR, 91% versus 68% (p0.08). CD30 also appears to predict a better outcome, OS p 0.1 and PFS p0.1. We didn't observed correlation between CD30 overexpression and clinical baseline characteristics.
Summary and Conclusions: In our experience the incidence of CD30 expression seems lower than the previous reported (10% versus 15%), these CD30 positive patients have a higher CRR, better OS and better PFS.

E1011

PROPOSAL OF NEW PROGNOSTIC INDEX FOR PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA IN THE RITUXIMAB ERA

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Background: The International Prognostic Index (IPI) has been useful prognostic tool to predict prognosis of aggressive non-Hodgkin lymphoma in the last 20 years. Since the advent of R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone) chemotherapy for diffuse large B-cell lymphoma (DLBCL), its utility has been challenged and other prognostic index including revised IPI and National Comprehensive Cancer Network (NCCN)-IPI were proposed, which are not popularly used yet.
Aims: We aimed to develop new prognostic model for DLBCL in rituximab era.
Methods: Between March 2004 and June 2012, patients with DLBCL treated with R-CHOP were identified in the database of the Asan Medical Center (AMC) Lymphoma Registry. Primary end point was to devise a new prognostic index for DLBCL. Secondary end point was to validate the NCCN-IPI in our cohort. We tested new prognostic index model in the training set of AMC cohort consisted of randomly selected 80% of the sample (503 patients). The remaining 20% (118 patients) was used as an internal validation set.
Results: The AMC cohort consisted of 621 patients. Median follow-up duration was 43.3 months (6.2-122.5 months). Median age was 57 years (range, 16-85 years). Median β-2 microglobulin (β-2 MG) was 2.10 mg/L (range, 1.0-66.0 mg/L). The univariate analysis of baseline characteristics revealed that age (≤60 vs. >60 years), LDH (within normal vs. increased), ECOG performance (0 or 1 vs. ≥2), advanced stage (Ann Arbor stage I/II vs. III/IV), extra-nodal involvement (≤1 vs. >1), B symptoms (no vs. yes), and β-2 MG (≤2.5 vs. >2.5) could predict overall survival (OS), whereas bulky disease and gender did not (p value 0.140, 0.621, respectively). In the multivariate analysis, age, LDH, ECOG performance status, and β-2 MG were significantly associated with OS (p value 0.001, <0.001, 0.004, and 0.019, respectively), while stage, extra-nodal involvement, and B symptom did not (p value 0.057, 0.233, and 0.577, respectively). We developed a new prognostic model with these 4 significant factors in the multivariate analysis. One point is assigned for each of the risk factors without refined categorization. Four risk groups were composed as followings: low (0 point), low-intermediate (1 point), high-intermediate (2-3 points), and high (4 points). The new prognostic model showed better discriminative power compared with classic IPI (Figure 1A). Five-year OS of low- and high-risk subgroup in new scoring model and classic IPI model in AMC cohort were 95% and 32% versus 89% and 45%, respectively. Our model was validated in an internal validation set (Figure 1B). NCCN-IPI also could stratify four risk groups (Figure 1 A and B).

Figure 1. IPI versus NCCN IPI versus new prognostic index model

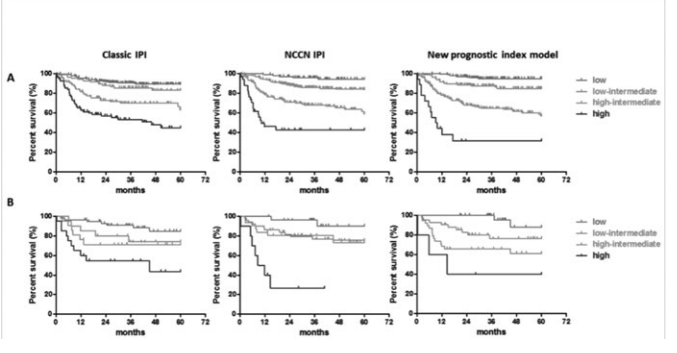

Figure 1.

Summary and Conclusions: We propose a new prognostic index model for DLBCL in rituximab era with age, LDH, ECOG performance and β-2 MG, which has good discriminative power and convenient to apply. It warrants further validation using an independent cohort.

E1012

AN INVESTIGATION OF RADIATION THERAPY FOR DLBCL AFTER CHEMOTHERAPY FOR ACHIEVING COMPLETE REMISSION: A SINGLE INSTITUTION EXPERIENCE

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Background: Radiation therapy (RT) for localized- refractory or -relapsed diffuse large B cell lymphoma (DLBCL) after chemotherapy is a sort of standard therapy. Some cases can achieve complete remission (CR) after RT for local residual disease, but others cannot. If we can distinguish more radioactive DLBCL from radio-resistant DLBCL in advance, more appropriate care could be possible.
Aims: To clarify which cohorts of patients (pts.) are more radiosensitive and which cohorts aren't.
Methods: We analyzed DLBCL receiving RT on the purpose of achieving remission after chemotherapy at Cancer Institute Hospital from October 2005 to January 2014. All pts.' histopathology samples were reviewed by expert hematopathologists, according to the WHO classification. All 3-dementional conformal RT were planned by an expert lymphoma radiation oncologist.
Results: A total of 180 pts. were included in this study. Baseline pts.' characteristics were as follows; a median age of 65.2 years (range, 17.3-88.1 years), 105 male and 75 female. We classified them into four categories; 87 pts. (48.3%) received RT as an adjuvant therapy after CR by R-CHOP therapy, 43 pts. (23.9%) received RT after partial metabolic response (PMR) at positron emission tomography-computed tomography (PET-CT)-based response of the Lugano classification by R-CHOP therapy, 38 pts. (21.1%) received RT for no metabolic response (NMR) at the PET-CT-based response by R-CHOP therapy, and 12 pts. (6.7%) received for progressive metabolic disease (PMD) after previous chemotherapy. The median dose of RT for CR settings was 30.6Gy (range, 16.2-45.0Gy), for PMR setting was 40.0Gy (range, 30.0-54.0Gy), and for NMR/PMD were 40.0Gy (range, 20.0Gy-50.4Gy). Disease progression rates within RT field were 0% of CR group, 2.3% (5 pts.) of PMR group, 43.2% (13 pts.) of NMR group, and 16.7% (2pts.) of PMD group. Disease progression outside of the radiation fields were 5.8% (5 pts.) of CR group, 11.6% (5 pts.) of PMR group, 50% (19 pts.) of NMR group, and 83.3% (10pts.) of PMD group. There was no treatment related mortality during RT. At a median follow-up time of 48.2 months (range, 6.5-78.8), the 2 year progression free survival (PFS) after RT and overall survival (OS) rates of all the pts. are 75.9%, 83.0%, respectively. In CR group, PFS and OS were 95.1%, 96.4%, in PMR group, 89.9%, 90.4%, in NMR group, 37.3%, 47.5%, and in PMD group, 16.7%, 72.7%, respectively.
Summary and Conclusions: RT as an adjuvant therapy resulted in a good prognosis as previously reported in the literature. RT for PMR after chemotherapy also resulted in a good prognosis. However, RT for NMR and PMD after previous chemotherapy showed poor prognosis and also revealed high rates of disease progression outside the RT field, suggesting the limitation of localized treatment for DLBCL. Because RT causes few severe adverse events, it is tolerable even for elderly people and pts. with long history of chemotherapy. Though such an advantage, it might be desirable for DLBCL of NMR and PMD after chemotherapy to treat with a systematic therapy if their health conditions allow for achieving remission.

E1013

UNDERWEIGHT AND LOW BODY SURFACE AREAS FOR CHEMOTHERAPY DOSING ARE ASSOCIATED WITH INFERIOR SURVIVAL IN MALE PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA: A STUDY OF 658 R-CHOP TREATED PATIENTS

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Background: Chemotherapeutic drugs are usually dosed according to patient weight or body surface area (BSA). Therefore, anthropometrics may influence survival in patients with diffuse large B-cell lymphoma (DLBCL).
Aims: To examine the impact of body mass index (BMI) and BSA on overall survival (OS) in DLBCL patients.
Methods: This retrospective study included adult patients with newly diagnosed DLBCL seen at the hematology centers of Aalborg (2003-2010), Holstebro (2006-2012), Odense (2006-2012), and Copenhagen (Rigshospitalet, 2010-2012). All patients had been treated with R-CHOP/CHOP-like regimens. Patient lists were obtained from the Danish Lymphoma registry (LYFO). All medical records were reviewed for information about weight and height at diagnosis, disease-related weight loss (≤5kg, >5kg), and BSA values used for chemotherapy dosing. Patients were grouped according to the WHO defined BMI categories for underweight (<18.5), normal weight (≥18.5, <25), overweight (≥25, <30) and obesity (≥30). The 3-year overall survival (OS) fractions were estimated using the Kaplan-Meier method. Cox regression analyses were used to assess the prognostic impact of BMI and BSA.
Results: A total of 658 DLBCL patients were included in the present study. The median age was 67 years (range 16-95 years) and the male:female ratio was 1.3. Patients with full international prognostic index (IPI) scores (n=615) were categorized as low-risk (27%), low-intermediate (26%), intermediate-high (27%) and high-risk (20%). By the time of first chemotherapy, 4% of the patients were underweight, 46% were normal weight, 34% were overweight, and 16% were obese. BSA values used for chemotherapy dosing were <1.8 m^2, 1.8-2.0 m^2, and >2,0 m^2 in 36%, 31%, and 33% of the patients, respectively. The used BSA value was not reported in five patients. The Table shows the prognostic impact of BMI and BSA on OS. There were no significant differences in the

BMI-specific 3-year OS estimates with the exception of inferior OS for underweight patients vs. overweight patients. In the sex-stratified analyses, underweight was associated with a higher risk of death in male patients, but not in female patients. BSA values between >1.8 m^2were associated with a better OS for male patients, but only BSA values ≥2.0 m^2 retained significance in multivariate analysis.

Tabella 1.

	3-year OS (95% CI)			Crude HR (95% CI)		Adjusted HR (95% CI)*	
	All	Men	Women	Men	Women	Men	Women
Normal weight	70% (64-75)	67% (59-75)	72% (63-79)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
Underweight	57% (37-73)	33% (5-68)	63% (40-80)	3.85 (1.39-10.70)	1.50 (0.70-3.23)	5.67 (1.96-16.39)	1.86 (0.80-4.28)
Overweight	74% (67-79)	74% (66-81)	73% (61-82)	0.85 (0.55-1.31)	0.99 (0.58-1.70)	0.80 (0.49-1.28)	1.33 (0.73-2.43)
Obesity	69% (59-77)	67% (53-78)	72% (55-83)	1.02 (0.59-1.76)	1.02 (0.53-1.97)	1.29 (0.71-2.34)	1.15 (0.54-2.45)
BSA<1.8 m^2	66% (59-72)	52% (36-66)	70% (62-76)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
BSA ≥1.8, <2.0 m^2	73% (66-79)	72% (63-79)	76% (64-84)	0.50 (0.29-0.86)	0.76 (0.44-1.29)	0.60 (0.33-1.08)	1.05 (0.58-1.90)
BSA ≥2.0 m^2	72% (65-78)	72% (65-78)	66% (34-85)	0.46 (0.28-0.77)	0.83 (0.33-2.09)	0.55 (0.32-0.96)	0.77 (0.24-2.50)

*Adjustment for IPI risk groups and weight loss>5 kg. HR=hazard ratio

Summary and Conclusions: Consistent with previous reports, being underweight at the time of diagnosis was associated with an inferior survival in male DLBCL patients. Lower BSA values were also associated with a worse outcome in male patients, which highlights the ongoing discussion of a possible inadequate dosing of immunochemotherapy in these patients.

E1014

18-F FDG PET/CT VERSUS BONE MARROW BIOPSY FOR ASSESSMENT BONE MARROW INVOLVEMENT IN PATIENTS WITH NON-HODGKIN LYMPHOMA

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Background: Bone marrow involvement is an important parameter that affects the stage, prognosis and management of lymphoma patients. Bone marrow involvement in patients with non-Hodgkin lymphoma (NHL) shows extensive stage of the disease. The bone marrow biopsy (BMB) is the method of choice for the detection of bone marrow infiltration, despite its technical limitations. BMB may demonstrate high false negative results. In last years 18-F FDG PET/CT imaging has become a very useful technique for diagnosis, staging and therapy control in NHL patients.
Aims: To evaluate the value of 18-F FDG PET/CT and its concordance with BMB in determining the bone marrow involvement in patients with NHL.
Methods: Data from 70 patients (39 men and 31 women), aged 21- 69 years, with histologically verified non-Hodgkin lymphoma were analyzed retrospectively. All the patients were investigated by 18-F FDG PET/CT, according to the accepted protocol. Iliac crest BMB was performed according to standard procedure. The 18-F FDG PET/CT results were compared with the BMB results in order to evaluate the bone marrow involvement.
Results: Both 18-F FDG PET/CT and BMB were negative in 52 (74%) patients. In 18 patients (26%) 18-F FDG PET/CT was positive for bone marrow infiltration. The focal and diffuse bone marrow involvement was detected in 13 and 5 patients respectively. Of the eighteen 18-F FDG PET/CT positive patients, both methods gave concordant data in 5 (28%) patients. Thirteen patients of the 18-F FDG PET/CT positive patients showed a negative BMB. These patients had multifocal 18-F FDG uptake but excluding the iliac crest biopsy site.
Summary and Conclusions: 18-F FDG PET/CT has a complementary role with BMB for the detection of bone marrow involvement in patients with non-Hodgkin lymphoma, especially in the focal bone marrow infiltration. 18-F FDG PET/CT positive results may change the treatment in patients with negative BMB.

E1015

INTRAPLEURAL RITUXIMAB FOR THE TREATMENT OF MALIGNANT PLEURAL EFFUSION DUE TO CD20-POSITIVE LYMPHOMA

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Background: Pleural effusion due to lymphoma can be massive and resistant to chemotherapy treatment. When systemic therapy fails, local therapy has to be considered. Rituximab was proven effective for the treatment of CD20-positive B cell lymphoma when given intravenously. The intrapleural route of administration was not explored. The intrapleural route might allow achievement of a high enough concentration of the antibody for lymphoma cell killing in the pleural cavity.
Aims: We assess the use of intrapleural rituximab in controlling malignant pleural effusion due to CD20-positive lymphoma.
Methods: Patients with malignant pleural effusion due to lymphoma were identified. Immunophenotyping study of the pleural fluid was performed to confirm CD20 positivity. They were given systemic chemotherapy and pleural tapping. Those with persistent pleural effusion were recruited and given intrapleural rituximab. Rituximab (50-100mg in 50ml saline as a bolus) was given intrapleurally and the chest tube was clamped for two hours and then released.
Results: Two patients with refractory pleural effusion were given intrapleural rituximab. The first patient was a 61-year-old man with stage IV mantle cell lymphoma. There was bone marrow and pleural involvement by lymphoma with massive left pleural effusion. Immunophenotyping study of the pleural fluid showed malignant B cell (CD20+) involvement. He was given two cycles of standard R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine and prednisolone) chemotherapy three weeks apart but the pleural effusion persisted. At the third cycle of chemotherapy, rituximab (100mg in 50ml saline as a bolus) was added and given intrapleurally. Repeat chest X-ray on day two showed resolving pleural effusion. There was no untoward side effect. After the fourth cycle of R-CHOP, the patient had lymphoma progression with central nervous system involvement. Further salvage chemotherapy including high dose methotrexate and cytarabine was given. The lymphoma did not respond to salvage chemotherapy. Despite this, there was no recurrence of the pleural effusion. The second patient was a 44-year-old man suffering from marginal zone B-cell lymphoma. He presented with extensive lymphadenopathy and left massive pleural effusion. Immunophenotyping study of the pleural fluid also confirmed malignant B cell (CD20+) involvement. Two cycles of R-CHOP chemotherapy at standard dosing was given but there was persistent massive effusion that required repeated chest drainage. Rituximab (50mg in 50ml saline as a bolus) was then administered intrapleurally together with the first day of intravenous chemotherapy and rituximab at the third cycle of chemotherapy. Two more doses of rituximab (100mg in 50ml saline) were given intrapleurally three weeks apart in subsequent cycles. The patient tolerated the intrapleural rituximab and the effusion subsided gradually.
Summary and Conclusions: Intrapleural rituximab has an adjuvant role in the control of malignant pleural effusion due to CD20-positive lymphoma. Our patients demonstrated a good response to rituximab given through this route, with resolution of the effusion. This route of administration was well tolerated. It is worthwhile exploring further this novel route of administration.

Bleeding disorders (congenital and acquired)

E1016

LOCALLY ADMINISTERED HAEMOSTATIC DRUG TREATMENT OF DIFFUSE LIFE-THREATENING PULMONARY BLEEDS, REFINING TECHNICAL APPROACH

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Background: Fulminant diffuse, critically severe pulmonary bleedings, particularly diffuse alveolar haemorrhage (DAH) might be treated with iv. recombinant Factor VIIa (rFVIIa) and quickly stopped by intratracheal application of suitable sized haemostatic compounds, including rFVIIa (Orphan Drug Status in DAH: Europe, UK and FDA).
Aims: DAH is well known, sometimes critically severe complication of bone marrow (or organ) transplantation and might also be the consequence of systemic pulmonary vasculitis. There is limited, anecdotic experience on intrapulmonary rFVIIa administration, which had been summarised by Hesley, 2012 (Biologics 6:37). Intratracheal rFVIIa might powerfully stop DAH bleeding, without unwanted systemic coagulatory effects. However, the optimal dose, applied volume, way of application is far from being elucidated. Limited data are available on systemic or intrapulmonary tranexamic acid in DAH. Our aim was first of all stop life-threatening DAH bleeds, and try to improve our skills in intrapulmonary haemostatic drug delivery (timing, combinations and sequence).
Methods: Three fulminant DAH cases will be presented on the poster. All three cases received intrapulmonary rFVIIa and or tranexamic acid first iv, but due to refractoriness locally, by bronchoscopy or inhalation or both. The dose of intrapulmonary rFVIIa was 50 ug/kg and tranexamic acid was applied as 500 mg in 5 ml. Bronchoscopic administration was followed, if needed by a second inhalatory administration one day later. The control of bleeding was assessed by bronchoscopic lavage, and measuring respiratory parameters, along with haemodynamics and haemostasis monitoring.
Results: Pulmonary bleeding was stopped or significantly decreased in all of our cases, within a few minutes. The most complex and relapsing case was a young male patient, in whom DAH developed following autologous bone marrow transplantation therapy of AL amyloidosis. His fulminant DAH needed complex systemic therapy including repeated iv 40 ug/kg rFVIIa and tranexamic acid, which measures could not stop the progression of DAH. Following that intrapulmonary rFVIIa administration (with quick cessation of bleeding), but later, when restrictive heart failure became even more serious it turned to be refractory and the patient died. In the other two patients (case 2: liver failure with DAH, case 3: DAH following acute leukaemia chemotherapy) intrapulmonary rFVIIa +tranexamic acid proved to be effective quite promptly, achieving durable responses. We used the recommended rFVIIa dosing schedule (50ug/kg), however, we found critical over flood phenomenon if we applied the originally suggested 50 ml volume –divided into the two lungs, so at the end we reduced the volume to 5-10 ml/lung. We did not observe any systemic haemostatic changes or thrombotic events following our intrapulmonary treatments.
Summary and Conclusions: Careful intrapulmonary administration of rFVIIa or probably tranexamic acid is safe and effective way to stop otherwise refractory, massive pulmonary haemorrhage and DAH. This form of treatment is safe, feasible, but needs well trained and instrumented intensive care unit, and should be available and applicable in transplant and larger oncohaematological centres. However, the optimal technique, mode of application, dosage seems to be uncertain, *e.g.* the volume recommended by the literature seemed to be critically oversized. It is not clear weather intrapulmonary administration could be the first approach instead of iv route or not. It would be important to collect and summarise anecdotic experiences, until then each treating unit should develop an own protocol and improve skills.

E1017

THE USE OF PROTHROMBIN COMPLEX CONCENTRATE FOR INDICATIONS OTHER THAN VITAMIN-K ANTAGONIST REVERSAL: AN ALTERNATIVE AND EFFECTIVE MEANS OF CORRECTING ACQUIRED COAGULOPATHIES

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Background: In accordance with a number of national guidelines, prothrombin complex concentrate (PCC) is widely used for the emergency reversal of the anticoagulant effect induced by vitamin-K antagonists. The licenced use also includes the treatment and prophylaxis of bleeding in all situations where there may be an acquired deficiency of coagulation factors. Despite this, fresh frozen plasma (FFP) is preferentially used in these scenarios, partly because of clinician familiarity, but also because of lack of data supporting PCC use in this context.
Aims: To determine the frequency of PCC utilisation at Central Manchester NHS Foundation Trust (CMFT) for indications other than the emergency reversal of vitamin K antagonists; the indications for this use and it's efficacy in terms of correction of routine coagulation parameters.
Methods: Data was collected retrospectively for two 13-month periods, January 1st 2012 – January 31st 2013 and January 1st 2014 – January 31st 2015. Using electronic records held by the CMFT transfusion laboratory, all patients that had been issued PCC during these periods were identified. These records were cross-referenced with electronic request forms and discharge documentation to determine which patients had been anticoagulated with vitamin K antagonists at the time of PCC issue and excluded. Besides laboratory results and demographic information, the following data was collected: Patient sex; coagulopathy aetiology; Liver function test and albumin results; PCC indication; dose issued; pre and post-PCC administration PT and APTT.
Results: A total of 41 patients were identified to have received PCC for indications other than vitamin-K antagonist reversal. This included 20 males and 21 females. The average PCC dose issued was 2463 iu (equivalent to 35iu/kg for a 70kg patient). 18 patients (44.0%) had evidence of significant liver impairment (at least grade A, according to Childs-Pugh classification). All patients had baseline coagulation parameters available; 32 patients (78%) had a repeat measurement within 24 hours of receiving PCC. Of those that had a repeat measurement, the PT and APTT corrected to within 0.5 seconds of the reference range in 34.4% and 4.0% of patients, respectively. With the less stringent target of correction to within 1.5x reference range, the PT and APTT corrected in 75.0% and 71.9%, respectively.
Summary and Conclusions: In comparison to FFP, there are a number of advantages to using PCC to correct coagulation abnormalities. It can be administered rapidly and, relative to FFP, incurs a low volume fluid load. These are issues particular pertinent in the management of critically ill and decompensated patients. Our data suggests that, at therapeutic doses, PCC is effective, correcting routine coagulation parameters to with 1.5x of reference range in the majority of patients. In combination with its clinical advantages, we suggest this data supports wider advocacy of PCC use in acquired coagulopathies.

E1018

VON WILLEBRAND FACTOR DEFICIENCY CORRECTED BY LUNG TRANSPLANTATION.

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Background: In experimental models with von Willebrand disease (vWD) pigs, plasma von Willebrand factor (vWF) was significantly increased after lung transplantation because lung endothelial cells strongly express vWF. However these findings have not been confirmed in human beings.
Aims: To confirm that bilateral lung transplantation could correct a mild von Willebrand defect.
Methods: A 26-year-old man, with mild bleeding episodes (epistaxis) and family history (mother with menorrhagia), was diagnosed in November 2012 with type 1 vWD (FvW:Ag 39 IU/dL; FvW:RCo 44 IU/dL; FVIII 99%). The patient was referred to our institution for lung transplantation as a result of cystic fibrosis. The vWF deficiency was confirmed by low levels of vWF and a normal multimeric plasma vWF pattern. Bilateral lung transplantation was performed in April 2013. The mechanical ventilation time was 48 hours and PaO2/FiO2 was 358. Ischemic times were 315 minutes (right lung) and 435 minutes (left lung). *Ex vivo* lung perfusion was performed with Organ Care System (preservation time 120 minutes). FVIII/vWF concentrate was administered both during and after surgery, without any relevant bleeding events. The patient developed primary lung graft dysfunction grade 2 although he was extubated on the second postoperative day with good oxygenation. However he developed progressive lung infiltrates with respiratory failure and extracorporeal membrane oxygenation (ECMO) was administered for 10 days. At hospital discharge in June 2013 he was taking immunosuppression with oral tacrolimus, prednisone and mycophenolate mofetil, which has continued until the present day (+22 months).

Tabella 1.

	APTT (s) (29.2-40.0)	FVIII (%) (60-150)	FvW:Ag (IU/dL) (50-160)	FvW:RCo (IU/dL) (55-140)	PFA 100 COL/EPI (s) (94-193)
At diagnosis		99	39	44	
Month -5	46.6	85	48		158
Day 0 (surgery)	43.3	69	74	Not detected	
Day +2	40.9	169	203	157	
Month +1	38.7	227	206	151	
Month +5	36.9	122	101	89	125
Month +11	40.9	97	111	85	167
Month +14	40.0	95	85	73	174
Month + 22	41.0	123	96	84	103

Results: Plasma vWF level increased during the postoperative days, presumably due to endothelial injuries and the infusion of vWF concentrate. Laboratory

tests post-lung transplantation demonstrated sustained normalization of all parameters.
Summary and Conclusions: In the present case, bilateral lung transplantation resulted in a sustained increase of plasma vWF levels produced by normal lung endothelial cells that corrected the defect. To our knowledge, this is the first case of von Willebrand deficiency corrected through lung transplantation.

E1019

PREDICTING BLEEDING RISK IN PATIENTS ON VITAMIN K ANTAGONISTS TREATMENT.
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Background: The patients on vitamin k antagonists, anticoagulant treatment (VKA) have a high risk of bleeding which is difficult to evaluate and to predict.
Aims: Assess the bleeding risk in patients on VKA using the ISTH/SSC scoring system. And evaluated the efficiency of the scores: OBRI, HAS-BLED, HEMORR2HAGES and ATRIA in predicting the risk of bleeding.
Methods: We have studied and evaluated the bleeding history of 57 patients on VKA treatment. For each patients we calculate ISTH/SSC, OBRI, HAS-BLED, HEMORR2HAGES and ATRIA score; this score was correlated with biological and clinical data.
Results: 52% of the patients have a low bleeding risk (ISTH average score after VKA treatment: 1.27), 39% of the patients have an intermediate bleeding risk (ISTH average score after VKA treatment: 5.59), and 9% of the patients have a high bleeding risk (ISTH average score after VKA treatment: 9.8). 79% of the patients have a labile INR with a bleeding incidence estimated at 48.1% per year, the incidence become 20% when the INR is not labile, whereas the Major bleeding incidence is estimated at 2.5% per year. The scores: OBRI, HAS-BLED, HEMORR2HAGES and ATRIA; have a low sensibility and specificity (sensibility <41% and specificity between 20% and 50%).
Summary and Conclusions: Good correlation was found between the high score results (ISTH/SSC) and bleeding risk in the patients with Atrial fibrillation on VKA treatment (INR [2-3]). The scores: OBRI, HAS-BLED, HEMORR2HAGES and ATRIA have a limited ability to predict the bleeding risk.

E1020

THE P2Y12 H1 HAPLOTYPE IS ASSOCIATED WITH IMPAIRED ADP INDUCED AGGREGATION IN TWO PATIENTS WITH PLATELET-TYPE BLEEDING DIATHESIS.
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Background: At least two platelet receptors are required for full platelet activation and aggregation in response to ADP: P2Y1 that mediates a rapidly reversible wave of aggregation and P2Y12 that is responsible for slowly progressive and sustained platelet aggregation. Markedly reduced and reversible aggregation in response to (4μM) ADP (≈10%) was observed in two patients free of any antiplatelet treatment with a bleeding diathesis suggesting an underlying molecular defect in P2Y12 receptor.
Aims: To investigate the above patients for molecular defects in the P2Y12 gene.
Methods: DNA & RNA extracted from patients' whole blood were subjected to PCR and RT-PCR respectively, followed by sequencing and real-time PCR.
Results: Both subjects had normal coagulation tests (fibrinogen, prothrombin time and activated partial thromboplastin time), vonWillebrand factor antigen and ristocetin cofactor activity but prolonged bleeding time (≥15min). Patient KG had low platelet count (≈120x10^3/μL). Sequencing analysis of P2Y12 gene revealed patient KG to be heterozygous for a non coding G-to-T transversion (G52T) in exon 2 and an intronic T-to-C transition (i-T744-C). Therefore patient KG carries both H1/H2 P2Y12 haplotypes. Patient DT exhibited no polymorphic variation in these sites or at positions i-ins801A and i-C139T, indicating that the patient carries two H1 alleles (H1/H1). Platelet P2Y12 mRNA, quantified by real-time PCR in both patients, was expressed similarly to healthy blood donors' platelets exhibiting normal aggregation in response to ADP. Sequencing analysis of the P2Y12 cDNA revealed no sequence variations.
Summary and Conclusions: In conclusion, no loss of function mutation or transcriptional defect in the P2Y12 gene has been found in our patients. However, it has been published that the H2 and H1 haplotypes of P2Y12 gene are associated with higher and lower maximal aggregation respectively in response to ADP in healthy subjects. Therefore we can speculate that the presence of the H1 P2Y12 haplotype could be associated with a poor ADP response leading to bleeding diathesis in these subjects.

E1021

PREGNANCY MANAGEMENT IN VON WILLEBRAND'S DISEASE
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Background: Pregnancy and childbirth are considered as the situations predisposing to a high risk of bleeding in women with von Willebrand disease (VWD). A clinical vigilance and a treatment if necessary minimize the number of complications during pregnancy and postpartum.
Aims: Establishment of algorithms for a management of pregnancy, delivery and postpartum in VWD is crucial to pregnancy outcomes. We have been optimizing strategies for the management of pregnant patients with VWD.
Methods: Since 2010 we have analyzed 24 pregnancies in 22 women with VWD (previously diagnosed VWD-20, determined during pregnancy-2). VWD Type 1 established in 16 patients, type 2 in 5 patients, type 3 in 1 case. All of them underwent monitoring and a preventive treatment when necessary. The laboratory assessment included complete blood count, activated partial thromboplastin time, prothrombin time, fibrinogen, von Willebrand panel (FVIII, VWF:Ag, VWF:RCo), platelet aggregation studies and thromboelastography. In addition, we examined the newborns to detect VWD.
The preventive treatment administered if analyses revealed hypocoagulation signs or a clotting factor levels reduction. We used DDAVP during pregnancy in type 1 on demand, a clotting factor concentrate (CFC) containing VWF until the levels were above 40-50 IU/dl and a tranexamic acid.
Results: No bleeding events registered during pregnancy. Most women with VWD type 1 developed an increase of clotting factor levels as a physiological response to pregnancy. Only 4 (16,7%) cases required a regular CFC treatment throughout pregnancy, including the patient with VWD type 3 received CFC all observation time. Therapy on demand was performed in 9 (37,5%) cases. The CFC preventive treatment during labour was performed in 8 (33,3%) cases, a postpartum therapy - in 17 (70,8%) cases. Pregnancies resulted in a birth of the full-term newborns in all 24 cases. No spontaneous miscarriage or neonatal mortality has occurred. Caesarean sections were performed in 3 (12,5%) of births. We detected VWD in 9 (37,5%) newborns. Despite the preventive treatment, we observed one intraoperative hemorrhage in the case of placenta previa and non-severe secondary postpartum hemorrhage (PPH) in 3 cases. Close monitoring was conducted over 4-6 weeks postpartum for preventing delay PPH.
Summary and Conclusions: The risk of hemorrhagic complications during pregnancy and postpartum in VWD may be minimized by applying the management algorithm including a preventive treatment. For women with low factor levels the preventive treatment with CFC is necessary. Women with VWD need pregnancy planning, clinical vigilance and observation by multidisciplinary team during pregnancy, puerperium and postpartum.

E1022

EFFICACY AND SAFETY PROFILE OF RIVAROXABAN FOLLOWING HIP AND KNEE ARTHROPLASTY
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Background: The incidence of venous thromboembolism (VTE) after hip and knee arthroplasty has been quoted as 1.1% and 0.53% respectively despite thromboprophylaxis. Furthermore, the incidence of myocardial injury after non-cardiac surgery is 8.0%[1]. There is a burden of thromboembolic events in orthopaedic patients during the perioperative period. Rivaroxaban is recommended as thromboprophylaxis following total hip and knee replacement surgery.
Aims: To investigate the efficacy and safety profile of rivaroxaban in patients undergoing hip and knee arthroplasties.
Methods: Patients who underwent total hip or knee replacement surgery between April 2013 and August 2014 were included in the retrospective study, carried out in accordance with the UK Good Clinical Practice. Data regarding patient characteristics and postoperative morbidity were collected using medical records. Patients were followed up with telephone surveys to investigate any bleeding events/adverse effects experienced. Primary efficacy was defined as composite of pulmonary embolism (PE), deep vein thrombosis (DVT), myocardial infarction (MI) and stroke. Safety profile was defined as major or minor bleeding. Data analysis was performed using IBM SPSS statistical software.
Results: 445 patients were included in the study. Preliminary results are presented for a sub-cohort of 99 patients. PE occurred in three (3%) patients, of which one patient died, and is higher compared to other studies[1]. No incidences of DVT, MI or stroke were reported. One major bleeding event and seven minor bleeding events occurred in five patients. The major bleeding event followed a lobectomy and required blood transfusion. Minor bleeding events included epistaxis, wound, gastrointestinal and skin bleeds. There was a statistically significant association between diabetes and PE (p=0.02), and ischaemic heart disease and bleeding events (p=0.04) according to Fisher's exact test.
Summary and Conclusions: Prophylactic management of VTE should be individualised according to patients' risk factors for thrombosis and bleeding. A small percentage of patients experienced bleeding events and strategies for reversal of rivaroxaban remain limited as there is no specific antidote currently available. Future work includes long-term follow up of orthopaedic patients initiated on rivaroxaban and preventative strategies of VTE events.

References
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E1023

FACTOR V DEFICIENCY IN WEST OF ALGERIA: SINGLE CENTER EXPERIENCE

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Background: Factor V deficiency is one of the rare inherited bleeding disorders the frequency in world is 1/ 10^6. But in algeria the high consanguinity rates have increased the number of patients with Rare Bleeding Disorders (RBDs)
Aims: -It was established a clinical and biological classification of patients with rare deficiency disorders. It was explored the relationship between coagulation factor activity level and bleeding severity for this patients.
Methods: A retrospective study conduct from 2008 to 2014. It was concerned 63 patients with a mean age 29 years (range1 year to 73 years) and Sex ratio ♂/♀=1.17. We use assigned categories of clinical bleeding severity of The European Network of Rare Bleeding Disorders (EN-RBD): **1. Asymptomatic**: No documented bleeding episodes. **2. Grade I:** bleeding Bleeding that occurred after trauma or drug ingestion (antiplatelet or anticoagulant therapy). **3. Grade II**: bleeding Spontaneous minor bleeding:bruising, ecchymosis, minor wounds, oral cavity bleeding, epistaxis and menorrhagia. **4. Grade III**: bleeding Spontaneous major bleeding: hematomas*, hemarthrosis, central nervous system,gastrointestinal and umbilical cord bleeding.
Results: 15 patients (23.8%) of RBDs were diagnosed with Factor V deficiency, the coagulation factor activity levels that were necessary for patients to remain asymptomatic were: (39.58±13.56) %. Grade 1: (27.50±21) %. Grade 2: 12 %. Clinical bleeding showed that the most common symptoms in patients with FV deficiency were oral cavity bleeding followed by hemarthrosis.
Summary and Conclusions: There was no association between coagulation factor activity level and clinical bleeding severity for FV. R^2=0.143. Results of the literature review suggest that the severity of symptoms is variable and correlates poorly with laboratory phenotype. Platelets provide a concentrated supply of FV: although most FV is present in plasma, approximately 20–25% of the circulating FV is found within platelet a-granules. After a granule release upon platelet activation, FV can presumably bind immediately to surface receptors optimizing prothrombinase complex activity. This probably protects some patients with FV deficiency from severe life-threatening bleeding, and may explain the weak association between laboratory phenotype and clinical severity.

E1024

COMPOUND FACTOR V AND FACTOR VIII DEFICIENCY IN ALGERIA

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Background: The frequency of the compound Factor V and factor VIII deficiency is 1/1000000. The gene is located on chromosome 18. Level factor are variable between 5% and 25%.
Aims: To assess the relationship between factor level and bleeding severity.
Methods: Retrospective study conducted for 7 years (2008 to 2014) in the west of Algeria, 63 patients has been tested for diagnosis of rare bleeding disorder. We use the Assigned categories of clinical bleeding severity of European Network of Rare Bleeding Disorders (EN-RBD): 1. Asymptomatiqc (no documented bleeding episodes). 2. Grade I bleeding (bleeding that occurred after trauma or drug ingestion). 3. Grade II bleeding (spontaneous minor bleeding; bruising, ecchymosis, minor wounds, oral cavity bleeding, epistaxis and menorrhagia). 4. Grade III bleeding (spontaneous major bleeding ; hematomas, hemarthrosis, central nervous system, gastrointestinal and umbilical cord bleeding).
Results: 16 patients (25.4%) diagnosed with compound Factor V and factor VIII deficiency, 11 patients have factor level <20 % classified with severe form and 05 patients had factor level betwene 20 and 40 % classified with moderate form. According the proposition of europen network of rare bleeding disorder. Regarding the clinical severity 3 are asymptomatic (factor level between 25 and 30%), 9 patients classified on grade 1 (factor level between 10 and 25%) and 4 on grade 2 (factor level between 5 and 10%).
Summary and Conclusions: A good corelation was found between factor level (factor V and VIII) and the clinical severety (R^2=0.83). Factor level **<**10% is associated with severe bleeding, and a level factor > 25 % are asymptomatic.

E1025

Abstract withdrawn

E1026

IN VITRO FACTOR VIII ACTIVITY AFTER RECONSTITUTION WITH DILUENT FLUID FOR CONTINUOUS INFUSION

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Background: Major surgery for hemophilia patients is very critical situation for one's life. For the successful result one's blood coagulation factor level during and after surgery is important. Continuous infusion method has merit of saving product as much as 30%. Because the drug activity will be decreased gradually after dilution even *in vitro* the latter portion of infusion will have decreased activity also.
Aims: We evaluate *in vitro* factor VIII activity after reconstitution with diluent fluid for continuous infusion.
Methods: We checked the *in vitro* activities on 0, 8, 16 and 24 hours after dilution with one commercial plasma derived FVIII product. And three commercial FVIII products (2 recombinant FVIII and 1 plasma derived concentrates) were used for *in vitro* FVIII:C activities at 0, 2, 4, 6, and 8 hours after reconstitution in both the exposure to indoor light group and the light shield group.
Results: *In vitro* activity was gradually decreased to 91±5.3%, 80±6.9%, and 56±6.7% at 8 hr, 16 hr and 24 hr on room temperature and on room light exposure respectively. Better results of 94±4.8%, 89±6.5% and 73±6.4% on light shield were observed (p<0.001). In the three drugs, *in vitro* FVIII:C was decreased from 0 to 8 hours after reconstitution (p <0.001). The decline of FVIII:C followed linear model (p<0.001). *in vitro* FVIII:C was 95.3±1.91% and 90.6±2.53% at 4 and 8 hours after reconstitution, respectively, in exposure to room light group. On light shield group, FVIII:C was 95.4±1.12% and 90.9±1.67% at 4 and 8 hours, respectively (p=0.849).
Summary and Conclusions: We aware the longer *in vitro* dilution state the lower factor activity. So, we have to adjust the required amount according to the frequency of changing bottle for the best bleeding control during and after surgery. Reconstitution of lyophilized powder with diluent every 4 hours is desirable to maintain effective blood factor level.

E1027

FREQUENCY AND CHARACTERISTICS OF FACTOR VIII INHIBITORS IN TUNISIAN HEMOPHILIA A: EXPERIENCE OF A SINGLE CENTER

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Background: Development of an inhibitory antibody to factor VIII is a rare but serious complication of hemophilia treatment of patients with hemophilia A.
Aims: The aim of this study is to determine the prevalence of inhibitors in hemophiliacs patients and to analyse their characteristics in our center.
Methods: From May 2008 to May 2013, 75 patients with severe hemophilia A admitted in department of hematology in Sfax from Tunisia were evaluated. Those who had abnormal mixing study, antibody against FVIII were measured. High titer inhibitor was defined as having a titer of >5 BU/ mL at any time. Data were collected and analyzed. Our patients received treatment on-demand with plasma-derived or recombinant FVIII and or cryoprecipitate.
Results: Of the 74 with hemophilia A enrolled in our study, 50 patients (68%) were with severe form, 16 patients (21%) were with moderate form and 8 patients (11%) mild form. From this group, sixteen (21%) were developed inhibitors. Frequency of inhibitors in severe, moderate and mild form were respectively 26%, 19% and 0%. The mean age of patients at inhibitor development was 15 years (3 -31years). Seven patients (44%) were high responders while 9 patients (56%) were low responders. All low inhibitors have disappeared spontaneously despite continued treatment with FVIII. One patient died by severe bleeding and one patient developed compressive spinal hematoma and keeps a flaccid paraplegia.
Summary and Conclusions: In our series the incidence of inhibitors in Haemophilia A (21%) is similar to the literature (20 à 30 %). Inhibitors are more frequently encountered in persons with severe hemophilia compared to those with moderate or mild hemophilia. The median age of inhibitor development in our series is higher than in developed countries (three years or less). Development of an inhibitory remains a major problem in the management of hemophilia threatening the vital and functional prognosis in our patient population

E1028

12 NOVEL MUTATIONS IDENTIFIED IN PATIENTS OF CONGENITAL AFIBRINOGENEMIA IN PAKISTAN.

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Background: Consanguinity is a hot issue in multiple cultures around the globe. Pakistan is included in the list where interfamilial wedlock is highly preferential and enriched source of inherited bleeding disorders.
Aims: This study focuses to detect the mutation in fibrinogen gene (*(FGA, FGB &FGG)* by DNA sequencing in Pakistani patients.
Methods: This descriptive and cross sectional study was conducted in Karachi and Lahore and fully complied with the Declaration of Helsinki. Patients with fibrinogen deficiency (tested by Fibrinogen functional assay from Laboratoire Stago, Asnieres, France) were screened for mutations in the Fibrinogen gene alpha (FGA), beta (FGB) and gamma (FGG) genes by direct sequencing.
Results: Total 17 mutations have been identified in 13 patients. 10 mutations were novel and identified in *FGA*, 4 in *FGB* and three in *FGG*. *FGA* gene mutation is seemingly the most commonly occurring mutation in our population with 8 novel mutations followed by 2 novel mutations identified in *FGB* and two novel mutations in *FGG*. Results were matched with wild type of *FGA,FGB* & *FGG* using HGMD*(http://www.hgmd.cf.ac.uk/ac/index.php)*.
Summary and Conclusions: Congenital afibrinogenemia is a rapid growing problem in countries such as Pakistan where consanguinity is frequently practiced. In this study amongst 8 novel mutations in *FGA* gene, five are identified as Missense mutation in *FGA* in our population which has not been reported yet in literature. This study transpires the fact that mutations in *FGA* frequently exist in local population followed by *FGB and FGG* mutations.

E1029

EFFICACY AND SAFETY OF PROPHYLAXIS IN PREVIOUSLY UNTREATED PATIENTS WITH SEVERE HEMOPHILIA A IN IRAN

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Background: Hemophilia is an inherited bleeding disorder that is associated with wide range of bleeding episodes. Primary prophylaxis is the best method of treatment to save the health and quality of life especially in children. Recently the low dose tailoring method of primary prophylaxis has been introduced for Previous Untreated Patients (PUP).
Aims: To evaluate the efficacy and safety of low dose tailoring method of primary prophylaxis in PUPs with severe hemophilia A.
Methods: In this pre-post interventional study, 16 patients with severe hemophilia A (activity <1%) were included. All patients were attending at the two main comprehensive care centers in Iran. Primary continuous modified prophylaxis was used for patients with age less than 3 years old, after first and before the second episode of obvious clinical bleeding in large joints or large soft tissue hematoma or major bleedings. All the hemophilic subjects in our study were previously untreated patients and randomly selected into two groups receiving recombinant factor 8 concentrate (Kogenate Bayer) and plasma derived (Emoclot Kedrion). Prophylaxis was started by 25-30 IU/kg once per week and increased to twice or three per week if necessary according to defined bleeding events for at least 50 exposure days (ED) or one year, each of occurred later. None of the patients had positive titer of inhibitor at the beginning of study. Descriptive data were presented as median and interquartile range (IQR) and also summarized at table 1.
Results:: Median and IQR for age were 3 and 2.7 years respectively. Median and IQR for age at diagnosis and age of starting prophylaxis were: 6 (5.7) and 9.5 (12) months respectively. Median and IQR for Annual Bleeding Rate (ABR) of patients after prophylaxis were 1 and 2.75 episodes per year respectively. Inhibitor was present in 5 patients (31%) after prophylaxis (patients number 2, 3 and 5: inhibitor titer =4; number 12: inhibitor titer =400; and number 13: inhibitor titer =32).

Table 1. Clinical characteristics of previously untreated patients with severe hemophilia A

	Age (Year)	Age at diagnosis (Month)	Age of starting Prophylaxis (Month)	Bleeding episode/year after prophylaxis	Bleeding type after prophylaxis	Dose of prophylaxis IU/kg	Dose of prophylaxis number/week
Patient N 1	1	At birth	6	0	No	25	1
Patient N 2	2	4	4	2	hematoma	25	2
Patient N 3	3	7	8	1	hematoma	25	1
Patient N 4	5	8	9	1	hematoma	25	1
Patient N 5	3	6	6	0	No	25	1
Patient N 6	5	2	30	0	No	25	1
Patient N 7	6	9	10	3	hemarthrosis	30	1
Patient N 8	1	7	7	3	Hematoma, ICH*	25	2
Patient N 9	7	6	35	1	hematoma	25	1
Patient N 10	4	6	6	0	No	25	1
Patient N 11	3	9	18	1*	hemarthrosis, gum bleeding	25	1
Patient N 12	1	5	7	1	hematoma	25	1
Patient N 13	2	2	17	0	No	25	1
Patient N 14	3	1	16	1	hematoma	25	1
Patient N 15	3	9	18	2	Hematoma, hemarthrosis	25	1
Patient N 16	3	At birth	10	3	Hematoma, tongue bleeding	25	1

Summary and Conclusions: Our study showed modified prophylaxis once per week can be cost- benefit in comparison with other known protocols and leads reduced morbidity in patients with severe hemophilia A comparing with On Demand treatment. On the other hand, incidence of inhibitor was 31% in our study which is comparable with previously published data (5-39%).

E1030

DISTRIBUTION OF THE ALLELES HLA II CLASS IN UKRAINIAN HEMOPHILIA A PATIENTS, DEPENDING ON THE INHIBITORS

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Background: The development of neutralizing antibodies against factor VIII and IX is the major complication in haemophilia care today. The antibodies neutralize the biological activity of FVIII and FIX and render replacement therapies ineffective. Antibodies against infused FVIII affect 20–30% of patients with severe HA and usually occur in childhood within the first 20-50 exposure days (ED) of therapy Antibodies are generated as a result of a cascade of tightly regulated interactions between different cells of the innate and the adaptive immune system located in distinct compartments. Candidates for immunogenetic investigation are genes involved in the immune response such as human leucocyte antigen (HLA) alleles. The HLA class II molecules are of particular interest because they present extracellular antigens (*e.g.* exogenous FVIII) to the patient's immune system. Previous immunogenetic studies in severe HA have shown the implication of HLA alleles in inhibitors formation.
Aims: The aim of our study was to investigate a possible link between the alleles HLA II class and the inhibitor and its type in Ukrainian haemophilia A patients (pts)
Methods: The study included 47 haemophilia A pts, aged 3 to 65 years (13 children and 34 adults) who were divided into 3 groups: I - HA pts without inhibitors (n=13); II –HA permanent inhibitor pts (n=20); III – HA transient inhibitor pts (n=14). All non-inhibitor pts had more than 150 ED; Informed consent was obtained from pts, children's parents or adolescents. To measure the FVIII activity and establish haemophilia diagnosis, the one-stage clotting assay was performed. To detect inhibitors against FVIII, the Bethesda method was applied. Genomic DNA was extracted from peripheral blood leukocytes by salting. Genotyping DRB1, DQA1 and DQB1 performed using PCR. Investigated 16 gene allele HLA-DRB1, 10 alleles of the gene HLA-DQA1 gene alleles and 19 HLA-DQB1.
Results: Statistical analysis of the results allowed to distinguish alleles associated with inhibitor in patients with haemophilia A in Ukraine. To significantly increase set for alleles DRB1*15:01 (χ^2=4,61, p <0,0), DQB1*06:02 (χ^2=4,61, p <0,05), and is likely to significantly reduce DQB1*0302 (χ^2=6,14, p <0,05). This means that the alleles DRB1*15:01 and DQB1 *06:02 can be seen as alleles - aggressors, and allele DQB1*03:02 rather as protectors allele. The calculation showed that carriers of allelic groups DRB1*15:01 and DQB1*06:02 and increases the risk of inhibitor in patients with haemophilia 10 times each (OR=10,7; CI 95%: 0,58-198,91) as opposed to carrier DQB1*03:02, which reduces the risk of inhibitor 5 times (OR=5,5; CI 95%: 1,29-23,17).
Summary and Conclusions: The majority of associations of immune response genes to the production of inhibitors in Ukrainian HA pts are related to HLA class II alleles HLA- DRB1*15:01, DQB1*06:02 and DQB1*03:02 haplotype. Further research must continue in a large group to determine the possible characteristic of certain groups alleles and investigate their role in the development of the FVIII inhibitor.

Bone marrow failure syndromes incl. PNH - Biology

E1031

THE RATIO OF LEUKOCYTE AND RED BLOOD CELL CLONES IN PATIENTS WITH AA/PNH AND THEIR CORRELATION WITH LDH LEVEL

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Background: It is known that patients with AA in 70% and more can have PNH-clone of various size: from minor clones to clones of considerable size with the development of severe hemolysis. There are cases of discrepancy of granulocyte and monocyte PNH-clone sizes in the same patients. In addition relationship of leukocyte PNH-clone size with a level of LDH as indicators of hemolysis is understood not enough.
Aims: Aim was to examine the ratio of granulocytic and monocytic PNH-clones of patients with aplastic anemia associated with the PNH (AA/PNH) and assess the relationship between the level of LDH and PNH-clone size among the different types of blood cells.
Methods: We heve examined 33 patients with AA/PNH, including 23 patients in dynamics (total 78 analyses) with the definition of the size of PNH-clone among white blood cells and red blood cells (RBC) using highly sensitive method of 4-colour flow cytometry. The PNH-clone size, defined among the granulocytes, was equal to or higher than 10% in 12 patients (Group 1) and was less than 10% at 21 (Group 2).
Results: In most of the examined patients (19 of 33) the sizes of granulocyte and monocyte PNH-clones did not significantly differ among themselves in the same person. 13 patients have shown a higher percentage of monocyte PNH-clone, including more than 10% difference in 5 patients: 4 in Group 1 and 1 – in Group 2. The predominance of granulocytic PNH-clone was observed in 1 patient with a clone size within 20-30%. In a survey of the dynamics in the absolute majority of patients trends persisted. In the whole group of patients there were observed highly reliable correlation values of granulocyte and monocyte PNH-clone size with LDH level ($r=0.62$ and $r=0.74$, respectively, $p<0.01$), mostly due to the patients of Group 1. In Group 2 there persisted a correlation between monocyte PNH-clone size and LDH level ($r=0.61$, $p<0.01$) and there was no correlation between the granulocyte clone size and LDH level ($r=0.07$). Worthy of note is the fact that in one third of this group of patients the size of the monocyte PNH-clone was significantly higher than that among the granulocytes. Indicators of pathological clone size among RBC and ratio of type II and III RBC could be variable in the same patient, depending on the presence and intensity of hemolysis. But examination outside the periods of active hemolysis in patients with clinical manifestation of PNH-clone identified general trends. There was reliable correlation between the level of LDH and total RBC PNH-clone ($r=0.51$, $p<0.01$) in the whole group of patients, mainly due to RBC type III ($r=0.51$, $p<0.01$), whereas the relationship between the level of LDH and RBC type II was much weaker ($r=0.24$). The relationship between the size of granulocyte and total RBC PNH-clone was stronger in patients of Group 1 compared with that of Group 2 ($r=0.53$ and 0.46, respectively). On the contrary, the relationship between the sizes of RBC clones type II and III was more pronounced in patients of Group 2, compared to one in Group 1 ($r=0.36$ and 0.24, respectively).
Summary and Conclusions: 1. In examined AA/PNH patients we have revealed variability of the ratios PNH-clone sizes among granulocytes, monocytes and different types of RBC. Noted more frequent prevalence of monocyte PNH-clone compared to granulocyte one in patients with PNH-clone <10%.
2. In cases with the amount of granulocytic PNH-clone >10% there was a clear correlation between the level of LDH (the primary intravascular hemolysis indicator) with the value of the granulocyte and monocyte PNH-clone and RBC type III (with an absolute deficiency of GPI-linked proteins). In cases of small PNH clone (<10%) LDH correlated only with monocyte and RBC clones.

E1032

VITAMIN D LEVELS OF CHILDREN WITH FANCONI ANEMIA

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Background: Fanconi anemia (FA) is a congenital syndrome associated with chromosomal fragility and aplastic anemia. Conflicting data exist regarding whether low bone mineral density is associated with FA. Factors that contribute to development of peak bone mineral during critical periods of childhood and adolescence include adequate calcium and vitamin D intake, general nutritionaladequacy, exercise, and normal hormonal and pubertal progression, along with genetic factors. Severe vitamin D deficiency may cause myelofibrosis in some children.
Aims: The aim of this study was to determine the frequency of vitamin D deficiency and insufficiency in children with FA.
Methods: None of the patients had previously received vitamin D supplementation. No patient had clinical or radiologic findings of rickets or seropositivity of hepatitis C virus or HIV or had been made bone marrow transplantation. Age, gender, serum level of ferritin, hemoglobin F, peripheric blood count, ALT, AST, calcium, phosphor, alkaline phosphatase, 25-OH vitamin D, and parathormon level were recorded. 25-OH vitamin D levels have measured by HPLC. 25-OH vitamin D level below 10 ng/ml is accepted as severe vitamin D deficiency and between 10 and 19.99 accepted as vitamin D deficiency, 20 and 29.99 was vitamin D insufficiency.
Results: Seventeen children with FA were included in the study. The median age of the patients was 13 (range, 4–20) years. The median vitamin D level was 11.73 ng/ml (range, 4.23–35.00). Vitamin D levels were below 10 ng/ml (severe vitamin D deficiency) in 4 cases (23%), between 10 and 19.99 ng/ml (vitamin D deficiency) in 10 cases (59%), between 20 and 29.99 ng/ml (vitamin D insufficiency) in 2 cases (12%), and above 30 ng/ml (normal vitamin D level) in 1 cases (6%).
Summary and Conclusions: A majority of our patients with Fanconi anemia (94%) had low vitamin D levels. Vitamin D levels will be different in different geographic regions even in the same country. Our study is from the north region of Turkey. New studies are therefore required from different regions. All centers should determine the frequencies themselves and develop a prophylaxis policy based on this data. We advise routine checking of vitamin D levels twice a year and vitamin D supplementation to maintain its level between 30 and 100ng/ml.

Bone marrow failure syndromes incl. PNH - Clinical

E1033

A PROPOSAL OF CLASSIFICATION FOR PAROXYSMAL NOCTURNAL HEMOGLOBINURIA.

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Background: Paroxysmal nocturnal hemoglobinuria (PNH) is a hematopoietic stem cell disorder characterized by hemolysis, thrombosis and a frequent association with bone marrow failure syndromes. The size of the GPI deficient clone (PNH clone) and the intensity of the hemolysis and pancytopenia vary widely among patients and during the evolution of the disease. There is a general agreement that the current PNH classification does not cover the spectrum of these clinical manifestations and should be updated, especially in the eculizumab era.
Aims: We propose a new PNH classification based on the clinical characteristics of the patients and introducing the concept of "evolving PNH" in order to allow a better identification of the differences in prognostic and treatment of each subgroup.
Methods: We propose to define four main PNH groups. (a) **Predominantly hemolytic with or without mild cytopenias**, defined by: LDH>3 x ULN, reticulocytes >150 x10^9/L, neutrophils >1.5 x10^9/L, platelets count>100 x10^9/L. (b) **Predominantly hemolytic with significant cytopenias**, defined by: LDH>3 x ULN, reticulocytes >150 x10^9/L, neutrophils <1.5 x10^9/L, platelets <100 x10^9/L. (c) **Predominantly hypoplastic with mild hemolysis**, defined by LDH high but<3 x ULN, reticulocytes high but <150 x10^9/L, and patients may have hypoplastic bone marrow with significant cytopenias (as defined above). (d) **Subclinical**, characterized by a PNH clone with no evidence of hemolysis, in general in the context of a severe aplastic anemia (2 of the following 3 values: neutrophils count<0.5 x10^9/L, platelets count<20 x10^9/L, reticulocytes<80 x10^9/L and bone marrow cellularity<25%) or a normal hemopoiesis.
Results: Thirty two patients (median age 35, range 6-71, years; males 63%) diagnosed with PNH at 7 institutions between July 1979 and August 2013 have been studied. At diagnosis, 12 (37.5%) patients were predominantly hemolytic, 7 (22%) predominantly hemolytic with significant cytopenias, 4 (12.5%) predominantly hypoplastic with mild hemolysis, and 9 (28%) subclinical PNH. Of the 32 patients, 17 (53%) experienced an evolution of the disease along the time, with clinical changes that modified their initial classification. Thus, 9 (28%) patients presenting at diagnosis with severe aplastic anemia or with significant cytopenias normalized the peripheral neutrohil and platelet values and evolved into a more hemolytic disease, while 2 (6%) patients showed the opposite, a progressive trend to an aplasia with decreasing hemolysis. Two (6%) patients developed a myelodysplasia with 7q deletion, 2 (6%) patients evolved to a subclinical PNH with normal neutrophil and platelet values and without hemolysis, and 2 (6%) patients achieved a complete response after receiving a bone marrow transplantation.
Summary and Conclusions: The proposed PNH classification was able to classify all 32 PNH patients in four groups. It constitutes a dynamic classification, showing that more than 50% of the patients change of category along the time. Furthermore, this classification could be used to better define treatment indications, according the predominance of hemolysis or aplasia, among different hospitals and groups interested in PNH.

E1034

RABBIT ANTITHYMOCYTE GLOBULIN AT >3,5 MG/KG/DAY/X 5 DAYS FOR TREATMENT OF ACQUIRED APLASTIC ANEMIA

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Background: Aplastic anemia (AA) is a life-threatening hematological condition that can be cured with prompt and correct management, including combination of the rabbit ATG Thymoglobuline (TG) plus cyclosporine A (CsA). However, optimal dose of TG is still unknown.
Aims: To analyze outcome of a large series of patients treated with TG at >3,5 mg/kg/day/5 days plus long-term CsA (>1 year) (TG-3,5).
Methods: Sixty-three AA patients (36 male, 27 female) treated with TG-3,5 were included in this retrospective analysis, across 22 centers. Median age was 45 yo (2-78). Infection prophylaxis and dose of steroids used to prevent serum sickness (SS) were administered according to each local center's policy. Response (complete [CR] plus partial [PR]) was assessed at days +90, +180 and at 1 year.
Results: Early (≤4 months) mortality rate was 15,9% (10 patients from 6 centers, 7 of them due to infections). Late mortality (>4 months) was 0%. If we excluded the 12 alive patients with follow-up <1 year, the overall response was 76,5% (39 out of 51). Five patients with no response at day +90 reached response at day +180 or 1 year (late responders).
Summary and Conclusions: Our series of AA patients treated with TG at >3,5 mg/kg/day/5 days plus long-term CsA showed: 1) Infectious early mortality in around 1 out of 10 patients, which highlight the need to focus on infection prophylaxis and limit the total dose of steroids employed after the 5 days of ATG administration in patients who do not develop SS, as steroids may significantly increase infectious complications. 2) 1-year overall response (CR plus PR) superior to 75%. 3) An increase in response rate over time in a cohort of patients (late responders). 4) Prospective randomized studies, as the ongoing Asia-Pacific study, are warranted to find out the ideal dose of TG to treat AA patients.

E1035

A STUDY OF THE MARKERS OF THROMBIN GENERATION AND INFLAMMATION IN PAROXYSMAL NOCTURNAL HEMOGLOBINURIA IN A SUBSET OF PATIENTS SEEN AT A TERTIARY CARE CENTRE IN NORTH INDIA

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Background: Data from studies on PNH patients from western countries and few Asian countries suggest more hemolytic and thrombotic manifestations in Caucasians compared to Asians. There is limited data reported on PNH from the Indian subcontinent.
Aims: Study of clinical features and outcomes of PNH in Indian population and to measure the markers of thrombin generation and inflammation in PNH patients presenting to our centre.
Methods: We studied disease characteristics, with emphasis on thromboembolic complications in 18 patients with PNH who were registered in our department between January 2004 and January 2014. Patients were sampled for markers of hemostatic activation; thrombin-antithrombin complex (TAT), D-Dimers, soluble P-Selectin (Sp-Selectin) and inflammatory cytokine Interleukin-6 (IL-6). The samples were also collected for these assays from 35 age and sex matched healthy control individuals in order to determine the cut off values in Indian population. Plasma TAT, Sp-Selectin and IL-6 levels were measured using commercial enzyme-linked immunoassays (Abcam TAT ELISA, Abcam Inc, MA, USA; Human sP-selectin/CD62P ELISA & Human IL-6 Quantikine HS ELISA, R & D Systems Inc, Minneapolis, MN, USA respectively). D Dimer was measured with automated STA LIATEST immunoturbidimetric D-dimer assay performed on the STA-Compact analyzer (Diagnostica Stago, France).

Table 1.

Patient characteristics	Present study	Hillmen P et al	International PNH registry Schrezenmeier et al	French data de Latour et al	Chinese data Ge et al
Number of patients	18	80	1610	460	280
Median age (years)	30	42	42	34.2	32
Females	28%	N/A	53.2%	54.3%	42.5%
Disease duration (median) prior to diagnosis	4 years	N/A	4.6 years	N/A	N/A
Fatigue	100%	N/A	80%	N/A	89.3%
Abdominal pain	33%	N/A	43%	18.2%	1.8%
Hemoglobinuria	72%	84%	62%	N/A	26.1%
Thrombosis	17%	39%	15.5%	7.2%	3.6%
Infection	5%	5%	N/A	15.2%	37.1%
Erectile dysfunction (% Male patients)	62%	N/A	38%	N/A	N/A
Renal dysfunction	22%	N/A	13.9%	N/A	N/A
Classical PNH	95%	71%*	52%	26%	18%
PNH/AA	5%	29%*	43.5%	52%	31%
Anemia alone	61%	N/A	N/A	22.8%	16.2%
Anemia & thrombocytopenia	11%	N/A	N/A	25.3%	25.3%
Anemia & neutropenia	None	N/A	N/A	4%	3.6%
Pancytopenia	17%	N/A	N/A	39.1%	49.5%
Median Lactate Dehydrogenase X ULN	11	N/A	1.96	N/A	N/A
Haemoglobin (Median)	58 g/L	N/A	106 g/L	N/A	63 g/L
Median Absolute neutrophil count	2 x 10^9/L	N/A	1.7 x 10^9/L	N/A	0.75 x 10^9/L
Platelet count (Median)	125 x 10^9/L	N/A	131 x 10^9/L	N/A	21x10^9/L
Median Granulocyte clone	85%	N/A	68.1%	N/A	N/A
Classical PNH	85%	N/A	83%	46%	39.1%
PNH/AA	52%	N/A	35%	20%	23.5%

*: Diagnosis of AA preceded the diagnosis of PNH in 29% of the patients in this study

Results: Thromboembolism was present in three (17%) PNH patients in our study (two cerebral venous thrombosis and one portal vein thrombosis). All the patients with thromboembolism had PNH clone size more than 85% at presentation. The majority of the patients in our study (95%) fit criteria for classical PNH. Bone marrow examination findings were correlated with clinical presentation in 13 patients. Five (28%) patients had peripheral blood cytopenias fulfilling the IAAAG criteria for aplastic anemia. However only one patient with PNH/AA had decreased bone marrow cellularity and normal ME ratio. Peripheral blood cytopenias with increased BM cellularity were present in 4 (22%) of patients. One of the two patients with anemia and thrombocytopenia had massive splenomegaly with hypersplenism secondary to portal vein thrombosis. Two patients with pancytopenia had myeloid hypoplasia in addition to erythroid hyperplasia in BM. The optimal cut-off values for TAT (2.9 ng/ml) & sP-Selectin (58.41 ng/ml) were determined in our study population using Youden's index. IL-6 levels were not significantly elvated in PNH patients as all the patients were on prednisolone therapy at the time of study. We found significant elevation of markers of thrombin generation (TAT and D-Dimer) and endothelial and platelet activation (sP-Selectin) in patients with PNH in our study. A positive correlation of TAT levels with sP-selectin and D-Dimer levels was observed in our study suggesting a contributory role for platelet and endothelial activation in activation of thrombosis in PNH. There was no correlation of hemolysis (LDH) with markers of thrombin generation (D-Dimer, TAT) or platelet and endothelial activation (sP-Selectin) in our study.
Summary and Conclusions: The prevalence of thromboembolism in PNH reported in our study is similar to that reported from other Indian studies and is comparable to the rates of thromboembolism reported in the western PNH patients. We found significantly elevated markers of thrombin generation and endothelial and platelet activation in PNH patients in our study. Further, there was a lack of correlation between serum LDH and markers of thrombin generation suggesting that thrombin generation in patients with PNH occurs may be through pathways independent of hemolysis.

E1036

ELTROMBOPAG IS BENEFICIAL IN THE TREATMENT OF CHILDREN WITH ACQUIRED APLASTIC ANEMIA

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Background: Acquired Aplastic Anemia (AA) in children is a rare disorder characterized by peripheral blood pancytopenia and hypocellular bone marrow, caused by immune-mediated processes. The first line treatment is allogeneic bone marrow transplantation from an HLA-matched sibling donor. Patients not eligible for bone marrow transplantation receive immunosuppressive treatment (IST), with the most commonly used schema consisting of antithymocyte globulin, cyclosporine, and methylprednisolone with the optional addition of Granulocyte colony-stimulating factor (G-CSF). Eltrombopag is an oral thrombopoietin mimetic, licensed for chronic immune thrombocytopenic purpura in adult patients. Treatment of adult patients with AA with eltrombopag has been shown to be beneficial. The use of eltrombopag in pediatric patients with AA has not been reported.
Aims: To evaluate the hematologic response following eltrombopag use in children with AA.
Methods: We report the use of eltrombopag in 4 children diagnosed with AA treated in our unit during the past two years. The patient received the medication as off-label compassionate use and after having received approval from regulatory authorities and Ethics committee. The patients were 3 males and 1 female, with their age being 6, 10, 15 and 16 years old, respectively. Possible identifiable cause for AA was the use of non-steroid anti-inflammatory drugs in one patient, while two patients presented with elevated transaminases, suggestive of infectious or auto-immune etiology. All patients fulfilled criteria for severe AA (absolute neutrophil count <500, platelet count (PLT) <20.000 /μL). As they had no HLA-matched sibling donor, they received IST. Eltrombopag was started at a dose of 100 mg daily per os, as they continued to have suboptimal platelet response, within 2 months after IST in the 3 patients, while the 4th patient started at 15 months after IST for persistent suboptimal response and parental hesitation. Treatment was continued until they reach optimal platelet count levels.
Results: Hematological response to the treatment with eltrombopag was observed within the first weeks of treatment. All of the patients increased their PLT levels at a percentage of >50%, and became platelet independent with PLT counts>20.000/μL within the first month of treatment. Continuation of therapy was related to continuous platelet improvement (figure 1). Neutrophil count and hemoglobin levels paralleled platelet response. Duration of treatment was at least for 6 months. One patient ended treatment after 6 months and is currently off Eltrombopag for 8 months with stable PLT counts>100.000/μl. The other 3 patients continue to be on therapy, in 6, 7 and 8 months respectively. Discontinuation of eltrombopag in one patient resulted in drop in the platelet count. When the drug was restarted, the platelet count rose again. This observation verifies the direct effect of eltrombopag. No adverse events related to eltrombopag use were observed.

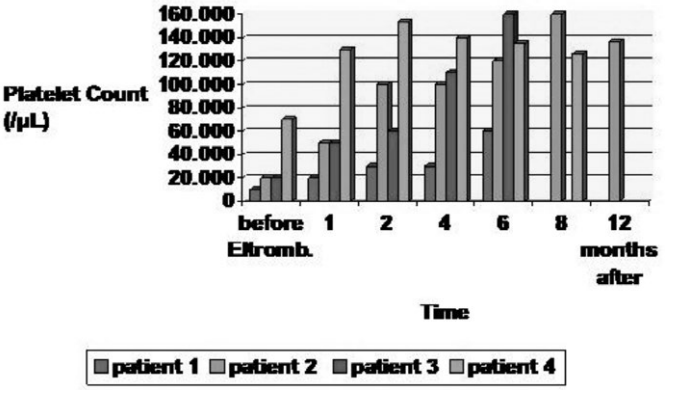

Figure 1.

Summary and Conclusions: Eltrombopag use was clearly associated with an increase in platelet count and possibly with a multilineage clinical response. Addition of Eltrombopag should be considered in pediatric patients with AA.

Chronic lymphocytic leukemia and related disorders - Biology

E1037

CHARACTERIZATION OF THE DNA-DAMAGE RESPONSE DOWNSTREAM OF B-CELL RECEPTOR SIGNALING IN CLL

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Background: Despite the considerable efforts which have been undertaken to develop new treatment strategies, the classical chemotherapy is still indispensable for the treatment of B-cell chronic lymphocytic leukemia (B-CLL). Chemotherapeutic agents damage the genome of neoplastic cells which triggers the DNA damage response (DDR) to either halt the cell cycle and repair the damage or, if the extent of damage is beyond repair capacity, to send the cell into apoptosis. However resistance against chemotherapy sooner or later occurs and leads to a relapse of the disease. In order to develop new strategies to overcome this resistance, a better understanding of the pro-survival signaling cascades in CLL and their link to the DDR machinery is necessary. One important pro-survival pathway in CLL is the PI3K/AKT pathway, which is activated through B-cell receptor (BCR) signaling. Since the literature states several links between AKT and the DDR, we hypothesize that AKT signaling downstream of BCR activation interacts with the DDR also in B-CLL.
Aims: In the presented study we aim to clarify the importance of BCR signaling and subsequent AKT activation for the resistance of CLL cells to classical chemotherapy and try to identify molecular targets involved in DDR signaling which respond to BCR activation.
Methods: We either stimulate the BCR by cross linking with soluble anti-IgM antibodies or inhibit the receptor signal by blocking BTK (Ibrutinib) or PI3K (Cal-101) in primary CLL cells to analyze the impact of BCR signaling on cellular resistance towards genotoxic stress. We measure cellular survival by flow-cytometry and activation of DDR signaling by immunofluorescence staining of γ-H2AX as well as immunoblotting of p53, Chk2 and DNAPK.
Results: We observed no induction of DDR signaling by BCR stimulation alone, but a clear activation of the investigated signaling molecules in response to DNA damage induced by bendamustine. Stimulation with anti-IgM antibodies clearly protects CLL cells from cell death induced by bendamustine and this protective effect is abandoned by inhibition of BCR signaling with Ibrutinib or Cal-101. However, the investigated DDR molecules show a very diverse activation pattern when genotoxic stress is combined with BCR stimulation. This diversity became even more pronounced when also Cal-101 was added.
Summary and Conclusions: In CLL the BCR generates a distinct pro-survival signal which protects the cells from apoptosis caused by genotoxic stress, however the activation of DDR signaling in response to BCR activation is not that clear-cut. Since CLL is a very heterogeneous disease, our results potentially reflect the existence of different CLL subgroups. We are currently subdividing our patient samples according to different criteria, *e.g.* mutational status of the immunoglobulin heavy chain, cytosolic calcium signaling in response to BCR stimulation, deletion of chromosomes 11q or 17p or clinical response to treatment in order to identify a correlation to the DDR patterns observed.

E1038

CD69 EXPRESSION POTENTIALLY PREDICTS RESPONSE TO BENDAMUSTINE IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Bendamustine, a drug that combines the properties of an alkylator and a nucleoside analogue, is currently being used for the treatment of chronic lymphocytic leukemia (CLL). Clinical responses are very heterogeneous and no specific markers to predict drug sensitivity have been reported.
Aims: The purpose of this study was to identify suitable biomarkers useful to predict bendamustine response in CLL patients.
Methods: The *in vitro* response to bendamustine 25 μM at 24 hours was determined in peripheral blood mononuclear cells from 80 CLL cases by Annexin V/PI labeling and flow cytometry. A gene expression profile was done in 38 CLL samples in order to identify the differentially expressed genes (DEGs) between the bendamustine-sensitive and the -resistant samples. Most relevant genes were validated by qPCR in a larger cohort of 77 CLL cases and the correlation of gene expression with bendamustine cytotoxicity was analyzed. The protein levels of the genes showing the strongest correlation with bendamustine response were then validated by flow cytometry. The effect of the microenvironment was also analyzed by co-culture with stromal cell lines.
Results: We found 416 DEGs between bendamustine-sensitive and -resistant cases. The biological functions of these genes included cell-to-cell signaling and interaction, cellular development, cellular growth and proliferation and cell death and survival. According to the fold change, we selected 66 genes for validation by qPCR, including 21 that showed a significant correlation with the *in vitro* response to bendamustine. The combination of *CD69 (*CD69*)* and *ITGAM* (CD11b) genes was the best predictor mRNA signature of response. When we interrogated the predictive value of the cell surface protein expression of these two molecules, we showed that the single expression of the activation marker CD69 was the most reliable predictor of bendamustine sensitivity in CLL cells. Importantly, a multivariate analysis revealed that the prognostic value of CD69 was independent from other biological and clinical CLL features, including Binet stage, mutational *IGHV* status, previous treatment, deletions on 17p and 11q and expression of ZAP-70, CD38 and CD49d. We also confirmed that the chemoresistance mediated by the microenvironment was accompanied by an upregulation of CD69 protein levels when CLL cells were cocultured with the stroma. Likewise, tissue-derived CLL cells expressed higher CD69 levels than their respective peripheral blood cell counterparts and, concordantly, were less sensitive to bendamustine.
Summary and Conclusions: We have identified CD69 as a maker of bendamustine resistance in CLL patients. Thus, CD69 quantification by flow cytometry may be useful to stratify patients who may receive bendamustine alone or in combination regimens. Acknowledgements: This work has been funded by Mundipharma Research.

E1039

SERUM CHEMOKINES AND CYTOKINES IN CLL PATIENTS TREATED WITH DUVELISIB, AN ORAL DUAL INHIBITOR OF PI3K-DELTA,GAMMA

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Background: PI3K-δ and PI3K-γ play complementary roles in malignant B-cells and the tumor microenvironment. Inhibition of PI3K-δ blocks cytokine-mediated CLL proliferation, while inhibition of PI3K-γ blocks M2 macrophage polarization and T-cell migration *in vitro*. Duvelisib, a dual oral inhibitor of PI3K-δ,γ, has shown clinical activity in a phase 1 study in patients with hematologic malignancies (Study IPI-145-02), including patients with relapsed/refractory (R/R) CLL (O'Brien, ASH 2014).
Aims: Examine changes in serum chemokines and cytokines from patients with R/R CLL following treatment with duvelisib.
Methods: Serum from 52 patients with R/R CLL and 30 healthy subjects was analyzed for 72 analytes (cytokines, chemokines, and matrix metalloproteinases) using Luminex xMAP^{O} technology at baseline, Cycle 1 Day 8 (C1D8), C2D1, and C3D1. Median change from baseline was analyzed for statistical significance using a paired t-test with Bonferroni correction for multiple hypotheses; threshold for reduction was ≤50% of baseline and for increase was ≥150% of baseline. Comparison between CLL and healthy subjects utilized a 2-sample t-test with Bonferroni correction.
Results: Following treatment with duvelisib, the median serum levels of 12 analytes decreased to ≤50% of baseline ($p < 0.0002$) by C1D8. These included CCL1, CCL3, CCL4, CCL17, CCL22, CXCL10, CXCL13, IL-6, IL-10, IL-12p40, MMP-9 & TNFα. All of these were significantly elevated ($p < 0.0042$) at baseline in CLL patients compared to healthy subjects and reduced towards normal range following duvelisib treatment. In addition, MMP1 was the only analyte for which median serum levels increased above the threshold of ≥150% of baseline ($p < 0.0002$). Together, these 13 analytes were further explored for potential associations with clinical efficacy. TNFα was significantly elevated ($p < 0.0013$) at C2D1 & C3D1 in patients who did not exhibit a nodal response.
Summary and Conclusions: Most of the analytes reduced following duvelisib treatment are known to be involved in the communication between CLL cells and the tumor microenvironment. Furthermore, one of these analytes (TNFα) exhibited elevated levels at C2D1 and C3D1 in patients who did not achieve a nodal response. Together, these data indicate that modulation of the tumor microenvironment via PI3K-δ,γ inhibition may be an important mechanism of action in supporting clinical activity of duvelisib in patients with CLL.

E1040

TREG CELLS IN CLL: DEFECTIVE FUNCTION AND HIGH CD39 EXPRESSION

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Background: Chronic lymphocytic leukemia (CLL) is a lymphoid malignancy which is derived from monoclonal B-lymphocytes. Tumor reactive T cells were determined in patients with CLL especially in early stages and it was suggested that immunosuppressive mechanisms allow CLL cells to escape from immunosurveillance. Regulatory T cells (Treg cells) are defined as a subgroup of T cells which express the markers CD4, CD25 and FOXP3. It was shown that Treg cells can prevent autoimmune and inflammatory diseases and inhibit antitumor responses. The numbers of Treg cells increase in other malignancies and CLL. Possible mechanisms about Treg cells' effects in immunoregulatory activity include cell-cell contact dependent suppression, secretion of anti-inflammatory cytokines like interleukin 10, transforming growth factor beta, and accumulation of pericellular adenosine. CD39 is another marker of Treg cells which has ectoenzyme- ectonucleoside triphosphate diphosphohydrolase activity for producing adenosine monophosphate (AMP). After production of AMP, another ectoenzyme (CD73) hydrolyses this substrate for accumulating pericellular adenosine which contributes to Treg cells' suppressor activity and by binding to its receptors on cytotoxic T cells (CTL) they can also suppress anti tumor activity of CTLs.
Aims: In our study we have measured Treg cells in the peripheral blood of 39 CLL patients and 21 healthy controls (HCs) by using flow cytometry. We have also evaluated the anti-proliferative effect of Treg cells on effector CD4 positive T cells with co-culture.
Methods: Peripheral blood CD4+CD25+and CD4+CD25-T cells from patients and HCs isolations were performed. Cells were co-cultured triplicate in anti-CD3 coated 96 well plates at a ratio of 1:1 ($5x10^3$cells: $5x10^3$cells) for 5 days and medium supplemented with soluble CD28 at 5µl/ml concentration. Cross mixing experiments were performed by co-culturing patients' and HCs' Treg cells with the autologous and the converse CD4+CD25- cells either HCs or patients. On the first day and the fifth day of culture WST-1 was performed in order to measure proliferation. The measurements were performed in triplicate. The results were evaluated according to the unpaired T-test by using SPSS statistical program. The difference were indicated as statistically significant if $p<0.05$ and highly significant if $p<0.01$.The proliferation index was calculated according to the formula mentioned as $[(\text{Cell \#})_{last}-(\text{Cell\#})_{initial}]/ [(\text{Cell\#})_{initial}]$
Results: As demonstrated in the table the percentages of CD4+CD25+and CD4+CD25+FOXP3+cells were not different between patients and HCs. However the expressions of CD39 on CD4+CD25+cells are significantly higher in patients than HCs. In addition, the cell percentage of CD39+cells in the population of lymphocytes was found approximately equal to those of CD4+CD25+cell population. The effect of Treg cells on effector T cells are shown in figures We evaluated the proliferation index for CLL patients and HCs. In this context, first, we compared proliferation index of 1:1($CD4^+CD25^{high}$: $CD4^+CD25$-) autologous cell co-culture versus 0:1 ($CD4^+CD25^{high}$: $CD4^+CD25$-) cell culture in patients and HCs separately. We demonstrated that patient $CD4^+CD25^{high}$ Treg cells could not inhibit proliferation of autologous $CD4^+CD25$- cells. In contrast, $CD4^+CD25^{high}$Treg cells from HCs supressed the proliferation of their own $CD4^+CD25$- significantly (p=0,018). Second, we performed a cross-mixing experiment in which patient and control regulatory $CD4^+CD25^{high}$cells were co-cultured with the converse $CD4^+CD25$- cells from either controls or patients. We compared proliferation index of A) Patient $CD4^+CD25^{high}$:HCs $CD4^+CD25$- (1:1) versus HCs $CD4^+CD25$- (0:1) B)HCs $CD4^+CD25^{high}$:Patient $CD4^+CD25$- (1:1) versus patient $CD4^+CD25$- (0:1). We found that while patients Treg cells have no inhibitor effect on HCs' $CD4^+CD25$- cells, in contrast HCs' Treg cells could inhibit the proliferation of patient $CD4^+CD25$- cells however the difference was not significant.

	CD4+CD25+ (min-max) (%)	CD4+CD25+ FOXP3+ (min-max) (%)	CD4+CD25+CD39+ (min-max) (%)	CD39+ (min-max) (%)
Patients	0,74 (0,07-8,889)	5,71 (1,07-50,48)	20,62(0,10-77,33)	21,46 (0,20-80,60)
Healthy Control	1,37(0,10-18,21)	7,91(0,30-52,33)	6,34(0,19-12,62)	3,68(0,09-13,51)
P value	0,114	0.963	<0,01	<0,01

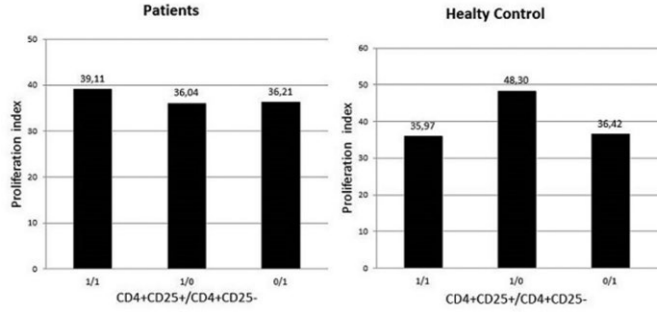

Figure 1.

Summary and Conclusions: CD4+CD25+cells are sensitive to toxicity induced by ATP, high expression of CD39 on these cells can reduce this sensitivity and this mechanism can provide survival advantage for Treg cells. In addition, it can be possible that high expression of CD39 on Treg cells in CLL can serve more AMP for CD73 which can result pericellular accumulation of adenosine. It is probable that by this mechanism, inhibition of anti tumor activity of CTL can be resulted as enhanced surveillance of malign CLL cells. On the other hand we demonstrated defective function of Treg cells in co cultures in CLL. An increased incidence of autoimmunity is a known entity in CLL. The question is whether this defective cell-cell contact dependent suppression can be responsible from this increased incidence.

E1041

ELIMINATION OF MUTATED P53 PROTEIN AND INDUCTION OF P53 DOWNSTREAM GENES IN CHRONIC LYMPHOCYTIC LEUKEMIA CELLS USING PRIMA-1MET

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Background: In chronic lymphocytic leukemia (CLL), *TP53* mutations represent the most adverse predictive and prognostic factor. As we have reported, particularly poor prognosis is associated with missense mutations located in DNA-binding motifs of the p53 protein. These alterations frequently lead to accumulation of mutated p53 protein with presumably oncogenic properties. Thus, the affected patients could potentially benefit from innovative therapy targeted directly to the mutated p53 protein. PRIMA-1^{MET}(Tocris Bioscience) is a small molecule currently tested in clinical trials, which has been declared to be able to change mutated p53 to wild-type (wt) conformation and rescue its activity.
Aims: To test a possibility of subtle molecular manipulation with accumulated mutated p53 in CLL cells using small molecule PRIMA-1^{MET}.
Methods: CLL patients' peripheral blood mononuclear cells (leukemic cells' proportion over 85%) used for reactivation study were chosen from the cohort characterized for *TP53* status by FASAY and direct sequencing. The samples were analyzed for p53 protein level by western blot (WB). A metabolic activity (viability) after PRIMA-1^{MET} treatment was determined by WST-1 assay. Impact of 48 h PRIMA-1^{MET} exposure on p53 protein level was analyzed by WB. The p53 downstream genes' induction was assessed by qRT-PCR.
Results: Firstly, the impact of PRIMA-1^{MET} on CLL cells' viability was assessed to determine appropriate concentration range for the reactivation. We observed clear concentration-dependent curve of viability in all studied samples (n=5) between 0.5 and 4µM. Therefore, 2µM and 4µM were further applied for the testing of reactivation at molecular level. Five out of eleven mutated samples with high baseline p53 level showed clearly diminished or even absent p53 protein after 4µM PRIMA-1^{MET} treatment and four other samples manifested partially reduced level. The effects at 2µM were also apparent, but less pronounced. The best response was observed for mutations p.Y205H, p.R248W, and p.C275R, in which the p53 protein was completely eliminated even at 2µM concentration. The p53 downstream genes' expression was heterogeneous among the samples, with the induction of *CDKN1A* (*p21*) and *GADD45* being much more prominent compared to *PUMA*. The expression induction reached up to 11800% (*p21*) and 4500% (*GADD45*) in comparison with untreated control set at 100%. The *BAX* and *MDM2* genes' expression was not increased after treatment. This indicates that observed elimination of mutated p53 is not probably caused through the induction of the p53 basic negative regulator MDM2. Concerning the specificity of observed effects with respect to *TP53* status, we noted the following: (i) in line with other studies, we noted similar impact of PRIMA-1^{MET} on both p53-wt and p53-mut samples' viability, and (ii) certain induction of p53 target genes was also evident in p53-wt samples. In addition, we also analyzed 10 samples with *TP53* mutation, but without p53 protein stabilization, for induction of the downstream genes. Again, the effects of PRIMA-1^{MET} were apparent, especially in case of *p21* and *GADD45*.
Summary and Conclusions: We have been able to demonstrate that PRIMA-1^{MET} can significantly diminish or even completely eliminate p53 protein accumulated due to the high-risk mutations in *TP53* gene. It still remains to be elucidated, what is the specificity of the p53 pathway activation with respect to *TP53* status. Supported by grants NT/13519-4/2012 and MUNI/A/1180/2014.

E1042

MICROVESICLES IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Normal and malignant cells secrete extracellular microvesicles (MV), usually defined as shedding membranous vesicles (50-1,000 nm diameter) that are produced by budding from the plasma membrane. It has been demonstrated that elevated levels of circulating MV in chronic lymphocytic leukemia (CLL) are able to activate bone marrow stromal cells and, in turn, to enhance production of VEGF, a pro-survival factor for CLL B-cells
Aims: We analyzed serum from untreated CLL patients searching for the number of circulating MV in order to test their possible prognostic role when evaluated at diagnosis.
Methods: Sera from 101 CLL patients (mean age 70 years, range 41 – 89; 60 M, 41 F; 52 patients with A, 36 with B, and 13 with C Binet clinical stage, respectively) were collected at diagnosis. Briefly, 1 ml of serum was processed with serial centrifugations using Optima XE ultracentrifuge (Beckman Coulter): 2,000 x g for 15 min; 10,000 x g for 30 min; 100,000 x g for 70 min. Resulting pellets were washed with pre-filtered (0.22 µm) phosphate buffer saline (PBS) and centrifuged at 100,000 x g for 70 min. Pellets were suspended in 200 µl of pre-filtered PBS. All steps of MV isolation were performed at 4°C. MV enriched pellets were analyzed by a FACSCalibur (Becton Dickinson) cytometer using Cell Quest software. Standard micro beads (0.3 – 0.9 – 3 µm) were used to define the size limit for MV and their size assessment. Pre-filtered PBS was also used to set the lower limit of MV gate. TruCOUNT beads (BD) were also added immediately prior to analyze samples by flow cytometry to determine the number of MV/µl. To identify and characterize MV, allophycocyanin-conjugated anti-CD19 monoclonal antibody and isotype-matched controls were used.
Results: A higher mean number of MV was found in CLL patients with respect to 28 control healthy subjects (991.8±768.4/µl *vs* 270±325/µl; p <0.001). Of interest, in CLL patients about 15% of MV were found to be CD19+. A higher number of MV was found in patients with advanced clinical stage (Binet A+B 950/µl±770 *vs* C 1,234/µl±661, p 0.02), while a trend was observed with surface expression of CD38, (p 0.06). No correlation was found according to CD49d and ZAP-70 expression, IgVH mutational status and cytogenetics abnormalities detected by FISH. A correlation was also found with respect to the number of circulating B-lymphocytes, with either total and CD19+MV (r=0.23 and r=0.25, respectively, p<0.05). Overall, patients who required therapy showed a more elevated number of MV at diagnosis (1,333/µl±941) with respect to those who did not (764/µl±565) (p 0.003). The time to treatment was longer in patients with lower number of both total (p 0.05) and CD19+MV (p<0.05). Finally, overall survival was shorter in patients with higher levels of total MV (p 0.001), while the number of CD19+MV was not significant.
Summary and Conclusions: Our study suggests a possible prognostic relevance of the circulating MV number detected at diagnosis in CLL patients. Interestingly, it has been previously reported that: i) the majority of circulating MV is platelet-derived (about 80%), while only a minority of them directly belongs to endothelium (about 10%) or leukocytes (about 10%); ii) a phenotypic shift from predominantly platelet/megakaryocyte-derived CD61+MV occurs in early stages ,while more "leukemic" CD19+B-cell derived MV are seen in advanced clinical stages; iii) *in vitro* stimulated CLL cells generate MV which preferentially express CD52, but not CD19 antigen. In light of these observations, we are currently evaluating the complete phenotype of the CD19-negative MV.

E1043

CHANGES IN LYMPHOCYTE SUBSETS, ANGIOGENIC FACTORS AND PLASMA CYTOKINES IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA DURING TREATMENT WITH OFATUMUMAB AND LENALIDOMIDE

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Background: Chronic lymphocytic leukemia (CLL) is characterized by a complex immune dysregulation. In CLL, lenalidomide exerts both a direct antitumor effect and a pleiotropic activity on the immune system. The addition of an anti-CD20 monoclonal antibody to lenalidomide is associated with improved clinical responses in patients with CLL.
Aims: The aim of this study was to analyze the *in vivo* effects of the combination of ofatumumab and lenalidomide on circulating T and NK cell numbers, and on plasma levels of angiogenic factors, chemokines and cytokines.
Methods: Thirty-two patients with relapsed or refractory CLL were treated in a phase II clinical study with ofatumumab and lenalidomide. Peripheral blood mononuclear cells (PBMC) and plasma samples were collected at baseline and after 3, 6, 9, and 12 months of therapy. Lymphocyte subsets were defined by the expression of CD3, CD4, CD5, CD8, CD16, CD19, CD20, CD25, CD56, CD57, and Forkhead box protein P3 (FoxP3) by flow cytometry. PBMC from 34 age-matched healthy individuals were used as controls. A multiplex bead-based immunoassay was used to quantify plasma concentration of bFGF, VEGF, Trombospondin-1, IFN-γ, CCL2, CCL3, CCL4, IFNα2, and IL-12p70. Clinical responses to therapy were assessed according to the 2008 International Workshop on CLL criteria.
Results: Treatment with ofatumumab and lenalidomide decreased the number of circulating T and NK cells. The greatest decrease was observed after 3 months of therapy as compared to baseline (median $CD3^+$T cells 950/µl vs. 1830/µl, $CD4^+$T cells 460/µl vs. 790/µl, $CD8^+$T cells 460/µl vs. 700/µl, Tregs 50/µl vs. 80/µl, $CD16^+CD56^+$NK cells 120/µl vs. 160/µl, and $CD57^+CD56^+$NK cells 32/µl vs. 70/µl; p≤0.02 for all comparisons). We hypothesized that T cell number could impact the response to therapy, and therefore compared T and NK cell populations in patients who achieved a complete response (CR) versus those seen in non-responders (NR). We found that the baseline number of $CD4^+$T cells and $CD57^+CD56^+$NK cells was higher in CR patients as compared to NR patients (median absolute number 1595/µl vs. 715/µl, p=0.04, and 358/µl vs. 70/µl, p=0.02). We also compared baseline T and NK cell populations of CR patients to healthy donors, and found that the former had significantly higher numbers of $CD16^+CD56^+$and $CD57^+CD56^+$NK cells (median 356/µl vs. 80/µl, p=0.03, and 358/µl vs. 29/µl, p=0.0008). Interestingly, baseline numbers of $CD16^+CD56^+$and $CD57^+CD56^+$NK cells were comparable between NR patients and healthy individuals. Plasma levels of bFGF, VEGF and IFN-γ were also significantly decreased at month 3 compared to baseline in all analyzed patients (p≤0.02 for all comparisons). No modulation of Trombospondin-1 levels was detected. Consistently with our previous report in patients treated with lenalidomide as monotherapy, we observed significantly lower levels of circulating CCL2, CCL3, CCL4, IFNα, and IL-12p70 during treatment in patients who achieved CR, compared to NR (p <0.05 for all comparisons).
Summary and Conclusions: Patients with CLL achieving CR to treatment with ofatumumab and lenalidomide have higher baseline numbers of CD4+T cells and CD57+CD56+NK cells, suggesting that these cells play a key role in response to therapy. The *in vivo* effect of this combination in modulating the interactions between the microenvironment and the CLL clone is supported by the induced changes in plasma angiogenic factors and cytokine levels.

E1044

CD49D EXPRESSION IN TRISOMY 12 CLL HAS LITTLE PROGNOSTIC POWER FOR SHORTER OVERALL SURVIVAL

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Background: Trisomy 12 CLL cells have increased expression of many integrins, including CD49d, compared with non trisomy 12 CLL. CD49d is one of the most powerful prognostic markers in CLL and CD49d positive cases have an average 23% reduction in OS at 10 years. The high fraction of CD49d positive cases would predict an high risk disease, in contrast with the intermediate risk category assigned to the generic case bearing trisomy 12.
Aims: To verifiy if the high fraction of CD49d positive cases in trisomy 12 CLL is still significantly correlated to shorter overall survival.
Methods: We performed a multi-center pooled analysis on 2157 untreated CLL cases all evaluated by FISH for the common cytogenetic aberrations. Cases were classified according to the hierarchical model of Dohner in the following subgroups: 41% del13q (n=892), 28% negative (n=599), 14% trisomy 12 (n=303), 17% high risk (n=363, del17p or del11q). The following prognostic variables were evaluated: IGHV mutational status, CD49d, CD38 and ZAP-70 expression. The endpoints overall survival (OS) and treatment free survival (TFS) were correlated with prognostic variables by Kaplan-Meyer plots and Cox regression analysis.
Results: The rate of death across cytogenetic subgroups was as expected: 10.5% in del13q cases, 14.3% in negative cases, 15.8 in trisomy 12, 26% in high risk cases. By comparing low risk cytogenetic subgroups (del13q, negative and trisomy 12) we found in trisomy 12 a remarkable higher fraction of cases expressing CD49d, CD38, ZAP-70 and UM IGHV. This was most evident for CD49d (78% of cases CD49d positive), one of the most powerful prognostic markers in this setting. We compared the hazard ratio (HR) for death in trisomy 12 and negative cytogenetics subgroups, which shared a similar rate of death and median follow-up (3.5 year trisomy 12, 4.7 years negative). By univariate analysis all the variables had a significant HR, however, in trisomy 12 cases the HR magnitudo and its statistical significance were lower for CD49d, CD38 and ZAP-70, with a barely significant p-value for CD49d (p=0.0489). By contrast, UM IGHV had a slight higher HR with the same level of statistical confidence. By Kaplan-Meyer analysis, the 10-years OS rate of the few CD49d negative cases (84%) was superimposable to that of del13q/negative subgroups

(82% and 76%). However, in multivariate analysis only UM IGHV remained a significant predictor for shorter OS.
Summary and Conclusions: Our data suggest that in trisomy 12 CLL IGHV mutational status may still be used for prognostic assessment, on the contrary CD49d, CD38 and ZAP-70 expression lose their usual prognostic power. These features, toghether with other characteristics, support the idea that CLL bearing trisomy 12 constitute a CLL subgroup with a different biology from all other CLLs.

E1045

MESENCHYMAL STROMAL CELLS IMPROVE SURVIVAL OF B-CLL CELLS: ROLE OF CELL-CELL CONTACT AND SOLUBLE FACTORS IN THE MICROENVIRONMENT

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Background: The malignant behavior of chronic lymphocytic leukemia (CLL) cells can be ascribed to intrinsic features involving an abnormal signal transduction mediated by B Cell Receptors (BCR) and to factors originating from the surrounding microenvironment in which CLL B cells engage complex interactions with stromal cells. Mesenchymal Stromal Cells (MSCs) represent the dominant population in CLL-marrow stroma and are involved in supporting leukemic B cell survival and conferring drug-resistance. During last years, several molecules have been developed to inhibit a variety of kinases in the BCR signaling that are essential not only for activation of multiple survival pathways, but also for migration and adhesion of B cells in the microenvironment.
Aims: The aim of this work was to evaluate how MSCs influence CLL B cell behavior and whether Bafetinib, CAL-101 and Ibrutinib kinase inhibitors could affect the MSC-CLL B cell cross-talk.
Methods: MSCs isolated from the BM of 50 CLL patients were expanded *ex vivo* and characterized through flow cytometry analysis and differentiation cultures (adipocytes and osteocytes). Freshly isolated CLL peripheral blood B cells were co-cultured with MSCs. For experiments using kinase inhibitors, B cells were treated with 5μM Bafetinib, 5μM CAL-101 and 5μM Ibrutinib. Apoptosis was measured by Annexin V test and western blotting analysis. Chemokines and cytokines released in culture supernatants were collected for the human Bio-Plex™ 27 plex Cytokine Assay.
Results: In presence of MSCs, we observed an extended survival of leukemic B cells (60%±17.3 with MSCs *vs* 14%±11.7 with *medium* alone). Through a transwell system to avoid lymphocyte-MSC direct contact, CLL B cell survival in presence of MSCs was minimally reduced (60%±17.3 with MSCs *vs* 52.4%±23.5 with MSCs in transwell system), suggesting that the anti-apoptotic effect is mainly ascribed to soluble factors produced by MSCs. In order to identify soluble factors responsible for the pro-survival effect, MSCs secretion profile was evaluated before and after CLL B cells exposure. We observed a stronger increase of IL-8, IL-15, CCL11 and CXCL10 production under co-culture conditions, suggesting their potential involvement in leukemic B cell survival. Moreover, we found that MSCs were not able to rescue CLL B cells from apoptosis induced by kinase inhibitors. In fact, CLL B cell viability co-cultured with MSCs was: 84.7%±4.9% without inhibitors, 57.2%±14.9% with Bafetinib ($p<0.01$), 49%±16.5% with CAL-101 ($p<0.01$), and 38.2%±10.8% with Ibrutinib ($p<0.001$).
Summary and Conclusions: We demonstrate that MSC-CLL B cell co-culture represents a reproducible *in vitro* system mimicking the *in vivo* bone marrow conditions, pointing out that the heterogeneity of the disease is reflected also by CLL B cell ability to respond to MSCs signals. Therefore, to identify patients who may benefit from treatments targeting the cross-talk with supportive cells, it is mandatory to test the effect of new therapeutic agents in the context of the microenvironmental influence. For this reason, in our co-culture system we tested the effect of Bafetinib, CAL-101 and Ibrutinib kinase inhibitors involved in BCR pathway, finding that all the molecules were able to reduce CLL B cell viability despite the MSCs presence. As a result of the ability of kinase inhibitors to inactivate BCR signaling pathway, leukemic B cells lose not only their ability to proliferate and survive but also their ability to home in, invade, and persist in the lymphoid tissue, a necessary condition to pursue the eradication of the disease.

E1046

QUANTITATIVE AND QUALITATIVE ANALYSIS OF REGULATORY T CELLS (TREGS) IN B CELL CHRONIC LYMPHOCYTIC LEUKEMIA (B-CLL)

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Background: T regulatory cells are immunosuppressive cells considered to play an important role in cancer biology and autoimmunity by suppressing host immune response and autoreactive lymphocytes respectively. Accumulated data indicate a significant role of T cell dysfunction in the pathogenesis of CLL.
Aims: The scope of this study is the analysis of numerical and functional abnormalities of Tregs in B-CLL with the view to elucidate their role in the pathogenesis of the disease.
Methods: Treg cells derived from 28 untreated B-CLL patients with a median age of 62 and 17 healthy donors were analyzed by Flow cytometry. Peripheral blood was obtained from 15 patients with B-CLL. Mononuclear cells were isolated using Ficoll-Paque gradient centrifugation. $CD4^+CD25^+$(Treg cells), $CD4^+CD25^-$ (T effector cells, Teff), $CD5^+CD19^+$(B-CLL) and $CD5^-CD19^+$(Normal B, NB) cells were separated using magnetic antibody cell sorting. To test the functionality of the assayed Tregs, the isolated cell populations were cultured in a 96-well plate (Tregs, Teff, B-cll, NB cells, B-cll: Tregs in 1:20 ratio, B-cll: Teff in 1:20 ratio, NB cells: Tregs in 1:20 ratio, NB cells: Teff in 1:20 ratio) and their proliferative capacity was measured using the BrdU assay. To further analyze the functional role of Tregs, peripheral blood was obtained from 12 patients with B-CLL and 12 healthy donors. Mononuclear cells were isolated using Ficoll-Paque gradient centrifugation. $CD4^+CD25^+CD127^{dim/-}$ (Treg cells), $CD5^+CD19^+$(B-CLL) and $CD5^-CD19^+$(Normal B, NB) cells were separated using magnetic antibody cell sorting and were co-cultured in a 96-well plate in a 1:10 ratio. The apoptosis of B cells was determined by the Annexin V/PI method.
Results: FACS analysis of the Treg cells resulted at the following observations: The Treg absolute cell number (cells/μL), estimated either as the number of $CD4^+CD25^+CD127^-$ cells or as the number of $CD4^+CD25^+FoxP3^+$cells, was significantly higher in patients' samples than in controls ($CD127^-$ $p=0.047$, $FoxP3^+p= 0.036$). Annexin V expression in Treg cells from BCLL patients was significantly lower compared to controls ($p=0.027$). The functional analysis of Treg cells through BrdU assay and Annexin V/PI method indicated that CLL Tregs were able to suppress the proliferation of Teff cells ($p=0.002$) and that Teff cells were in turn able to significantly suppress the proliferation of B-CLL cells ($p=0.05$). After the co-culturing of NB cells with Treg CLL cells, the apoptosis of NB cells was significantly increased compared to NB cells (16.10 *vs* 6.22, $p<0,005$). Likewise, when B-CLL cells were co-cultured with Treg CLL cells, their apoptosis was significantly increased compared to control (24.06 *vs* 12.66, $p<0,01$). Interestingly, no significant alterations were observed after culturing NB or B-CLL cells with Tregs from healthy donors.
Summary and Conclusions: In CLL patients, Treg cells are significantly higher and present with lower apoptotic levels compared to healthy donors. The functional analysis indicates that Teff cells suppress the proliferation of B-CLL cells whereas they are suppressed by Tregs, indicating that increased Tregs observed in CLL contribute indirectly to the proliferation of the CLL clone. Moreover, CLL Tregs, unlike Tregs from healthy donors, enhance the apoptosis of both NB and B-CLL cells, suggesting the existence of distinct functional differences between CLL Tregs and their normal counterparts, which need to be further elucidated.

E1047

FCGAMMARIIB HOMMOAGREGATION CAN INDUCE A PROSURVIVAL STATE IN CHRONIC LYMPHOCYTIC LEUKAEMIA CELLS

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Background: FcγRIIb is a low-affinity receptor that regulates B-cell activation by inhibiting signalling from the B-cell receptor (BCR) in murine and normal B cells. This inhibitory capacity depends on the colligation of FcγRIIb with BCR and it is mediated by phosphorylation of SHIP and inhibition of the AKT pathway. Besides this BCR-dependent inhibitory effect, in murine B-cells FcγRIIb has been also implicated in delivering pro-apoptotic signals upon homoaggregation. However, the effects of FcγRIIb hommoagregation have not been extensively investigated in human normal-B cells and CLL cells.
Aims: To evaluate the effects of FcγRIIb signalling on viability, activation, and proliferation of human CD19+normal B-cells and CLL cells. Also, to investigate whether the SHIP/AKT pathway is involved in FcγRIIb hommoagregation signalling in these cells.
Methods: B-cells were isolated from cryopreserved PBMCs from 31 patients with CLL (14 *IGHV* mutated, M-CLL, and 17 *IGHV* unmutated, U-CLL) and 10 healthy donors, cultured in RPMI 10% FBS in the presence or absence of the novel and specific anti-human FcγRIIb mAb from Macrogenics. At 48 hours, cell viability was determined as the percentage of AnnexinV/TO-PRO®-3 double negative cells by flow cytometry, and cell activation was determined as the percentage of CD69+cells. Cell proliferation was determined at 72 hours by

using the Click-iT® EdU Alexa Fluor® 488 Imaging Kit. For signaling analyses, reactions were stopped after 5 minutes of exposure, and total protein extracts were performed to analyze the expression of phosphorylated SHIP and AKT by western blot.
Results: In human CD19+normal-B cells and CLL cells, the hommoagregation of FcγRIIb did not induce changes in B-cell viability nor in activation as compared to basal levels. However, the FcγRIIb hommoagregation induced an increase in proliferation at 72h in CLL cells from 15 out of 31 CLL samples and in CD19+normal B-cells from 2 of 10 healthy donor samples (48.4% *vs* 20%, p=0.152). Within CLL subgroups, 9 of 14 U-CLL and 6 of 17 M-CLL cases responded with an increase in proliferation after FcγRIIb hommoagregation (64.3% *vs* 35.3%, p=0.156). At the molecular level, we found that in 17 of 20 patients with CLL analyzed by western blot, CLL cells did respond to FcγRIIb hommoaggregation with an increase in AKT phosphorylation, while in CD19+normal-B cells, only 1 of 10 healthy donor samples did respond to FcγRIIb crosslink with a slight increase of AKT phosphorylation (p<0.01). No significant differences were observed between U-CLL and M-CLL cases, both groups showing an increase AKT phosphorylation in 90% and 80% of cases, respectively. SHIP protein did not appear to be involved in FcγRIIb hommoaggregation signalling in CD19+normal-B cells and in CLL cells.
Summary and Conclusions: In contrast to what has been described in murine B cells, the homaggregation of FcγRIIb in human normal B-cells and CLL cells did not trigger B-cell apoptosis. Moreover, in CLL cells FcγRIIb hommoagregation induced AKT-phosphorylation activating signals, an effect that was not observed in CD19+normal-B cells. Further experiments are needed to investigate whether FcγRIIb stimulation may enhance CLL cells survival and thereby contributing to the progression of the disease.

E1048

CHRONIC LYMPHOCYTIC LEUKEMIA CELLS ARE HIGHLY SUSCEPTIBLE TO DIRECT INHIBITION OF CHECKPOINT KINASE 1

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Background: Chronic lymphocytic leukemia (CLL) is incurable disease with as yet no optimal treatment options, particularly for patients with mutations in *TP53* or *ATM* genes. Checkpoint kinase 1 (Chk1) is involved in molecular pathways preserving genome integrity in all cell cycle checkpoints (G1/S, intra-S, G2/M) and also in non-dividing cells. SCH900776 (Schering-Plough) is highly specific Chk1 inhibitor currently tested in clinical trials for selected solid tumors and leukemia. We have prepared its potentially more metabolically stable analog OH209EN1, which possesses highly unusual N-trifluoromethyl pyrazole moiety. OH209EN1 phenocopies short interfering RNA-mediated Chk1 ablation and greatly sensitizes tumor cells to nucleoside analogs.
Aims: (1) to assess the impact of direct Chk1 inhibition on CLL cells´ viability using our innovative inhibitor, and (2) to correlate observed effects with the presence of *TP53* and *ATM* defects.
Methods: *TP53* and *ATM* mutations were identified by next-generation sequencing and Sanger sequencing. Deletions 17p and 11q were detected by I-FISH. Peripheral blood mononuclear cells (CLL cells´ proportion over 85%) were treated in 96-well plates using 500 000 cells/well. Cell lines were tested the same way using 50 000 cells/well. The final viability after 72h inhibitor exposure was assessed using metabolic WST-1 assay. Apoptosis (Annexin-V/PI) was measured by flow-cytometry in full blood samples in lepirudin treated with OH209EN1 for 24 h at 37ºC, 5% CO_2.
Results: Firstly we determined appropriate concentration range of OH209EN1 for viability testing using cell lines with known genetic status and healthy cells. The LC50 values after 72h treatment were following: 80 nM for GRANTA-519 (MCL, ATM mutation), 250 nM for MEC-1 (CLL/PL, p53 mutation), and 300 nM for NALM-6 (B-cell precursor leukemia, ATM/p53-wt). Negligible effect on three healthy PBMNC cultures and three non-cancerous fibroblast cell lines was noted up to 400 nM of the inhibitor. Further, Chk1 autophosphorylation on Ser-296 (Chk1 activation marker) was readily eliminated (on western blot) using 200 nM OH209EN1 in the cell line with high baseline autophosphorylation level (NALM-6). According to these results, we used concentrations 100, 200, 300 and 400 nM of OH209EN1 for the testing in CLL cells. Fifty-four CLL cultures were used and a clear concentration-dependent decrease of viability was noted in the very most of them. At the highest concentration 400 nM, the final median viability in individual genetic categories was following (listed according to sensitivity): 33% in samples with ATM inactivation (mutation and 11q-; n=15), 44% in samples with sole 11q- (n=10), 46% in samples with p53 inactivation (mutation with/without 17p-; n=13), and 62% in samples having no *ATM* or *TP53* abnormality (n=16). Interestingly, the inhibitor SCH900776 tested in parallel in 44 cultures showed substantially weaker effects with final median viability 80%. The concentration-dependent (OH209EN1, 100-400 nM) induction of apoptosis was clearly noted in two full blood samples from CLL patients, while no apparent effect was observed in one tested healthy control blood.
Summary and Conclusions: Although CLL cells from peripheral blood are non-dividing and exhibit rather low Chk1 expression, they manifest surprisingly high intrinsic sensitivity to specific Chk1 inhibitor. This good response concerning also *ATM* and *TP53* mutated samples seems to be in contrast to non-cancerous cells and thus provides potential clinical utility. Supported by grants NT13519-4 and MUNI/A/1180/2014.

E1049

Abstract withdrawn

E1050

REGULATION OF CD38 BY IRF4 IN CHRONIC LYMPHOCYTIC LEUKAEMIA

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Background: Chronic lymphocytic leukaemia (CLL) typically follows a very heterogeneous course in different patients. A number of prognostic markers have been determined in CLL, of which CD38 surface expression is one of the most widely used, with positivity indicating a poorer outcome. Interferon regulatory factor 4 (IRF4) is a transcription factor with vital roles in B cell differentiation. A single nucleotide polymorphism (defined by rs872071) in the 3'UTR region of the *IRF4* gene has been identified in genome wide association studies as defining a strong common risk allele for development of CLL (OR=2.72 [1.93-3.82 95% C.I.]). Interestingly, it is also significantly associated with CD38 positivity (p=0.004).
Aims: Given that the promoter and first intronic region of *CD38* both carry perfect consensus binding sites for IRF4, we hypothesised that IRF4 plays a role in the regulation of CD38 expression.
Methods: Chromatin immunoprecipitation (ChIP) was used to investigate binding of IRF4 to *CD38* in a panel of immortalised B cell lines (TK6, MEC-1, SU-DHL-6) and primary CLL samples. Primary cells were cultured on a CD40 ligand-expressing fibroblast layer in order to mimic the tumour microenvironment, prior to ChIP. Short interfering RNA (siRNA) was used in B cell lines to generate cells with transient knockdown of IRF4 protein. B cell lines were also transduced with short hairpin RNA (shRNA) and then cloned on sloppy agar to generate cell clones with constitutive IRF4 knockdown. Protein knockdown was confirmed by Western blotting. CD38 expression in knock down and wild type cells was determined by flow cytometry, and the effects on cell proliferation and sensitivity to cytotoxic agents were investigated by growth inhibition assay.
Results: ChIP demonstrated binding of IRF4 at *CD38* in two out of three cell lines (SU-DHL-6 and MEC-1). In both cases, binding at *CD38* intron 1 was consistently greater than the binding at the *CD38* promoter, and this reached significance in SU-DHL-6 (p=0.02). In contrast, binding at the *CD38* intron 1 was negligible in TK6 cells. IRF4 knockdown slowed proliferation in TK6 cells (p=0.02) and sensitized them to fludarabine-induced cytotoxicity. These effects were not observed in the other cell lines. In addition, despite evidence of IRF4 knockdown confirmed by western blotting, there was no effect on surface CD38 expression in any of the cell lines. Co-culture of primary cells on a fibroblast monolayer was associated with upregulation of IRF4 protein expression. ChIP in these primary cells did not show consistent evidence of binding at the *CD38* promoter or first intron. In separate experiments, IRF4 upregulation in primary cells cultured on the fibroblast layer was not associated with any change in CD38 expression.
Summary and Conclusions: These data suggest that IRF4 binds to *CD38*, specifically at the first intron. IRF4 is known to bind its target genes as a heterodimer with Ets family transcription factors, and the cell-line specificity of the IRF4-*CD38* binding demonstrated here, suggests that the expression of IRF4 binding partners in cell lines may be relevant. Despite evidence of IRF4-*CD38* interaction however, we were unable to demonstrate any impact of altered IRF4 expression (knockdown or upregulation) on surface CD38 expression. While CD38 remains a potential therapeutic target in CLL, these data suggest that IRF4 plays a relatively minor role in the functional regulation of surface CD38 in CLL.

E1051

13Q DELETION IS PREDOMINANT CYTOGENETIC ABERRATION NEWLY AQUIRED DURING CHRONIC LYMPHOCYTIC LEUKEMIA COURSE IRRESPECTIVE OF DISEASE ACTIVITY AND TREATMENT STATUS

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Background: In Chronic lymphocytic leukemia (CLL) four recurrent cytogenetic aberrations with different prognostic impact are routinely tested: deletions 11q, 13q, 17p and trisomy 12. Their prognostic impact is generally described using Dohner's hierarchy: del17p >del 11q >tris 12 >normal >del 13q. The aberrations are usually tested once at the diagnosis but their spectra can change during disease evolution.
Aims: Repeated analysis of the four recurrent CLL cytogenetic aberrations was aimed to monitor the changes in aberration distribution in the different disease stages.
Methods: Four common cytogenetic aberrations (deletions 11q, 13q, 17p, and trisomy 12) were detected using interphase FISH analysis.
Results: Based on the disease course and therapy administration, CLL patients were stratified into two groups: (A) patients with indolent disease – 16 % (52/335), no treatment required, median follow-up 75 months; (B) patients with progressive disease requiring therapy intervention either after an inactive phase (56 %; 186/335; median time to first treatment 35 months; median follow-up 74 months), or soon after diagnosis (28 %; 97/335; median time to first treatment 14 months, median follow-up 54 months). In total, newly acquired cytogenetic aberration was detected in 33 % (109) from 335 repeatedly tested patients. In the indolent group A, 23 % (12/52) of patients gained a new aberration during follow-up. In almost all cases (92 %; 11/12) the newly acquired aberration was deletion 13q. In the progressive group B, patients were tested repeatedly before the first therapy and during relapse where possible. 11 % (30/283) of patients gained a new aberration already before therapy - 20 % (6/30) at inactive phase and 80 % (24/30) at active phase of CLL. Del 13q comprised 83% (5/6) of aberrations gained at inactive phase. The distribution of aberrations gained at active phase of CLL was following: del 13q in 63 % (15/24), del 17p in 17 % (4/24), del 11q in 13 % (3/24), and tris 12 in 7 % (2/24) of cases. 71 % (200/283) of CLL patients were tested again in progression after therapy. New chromosomal aberration was detected in 34 % (67/200) of cases: del 13q in 61 % (41/67), del 17p in 28 % (19/67), del 11q in 16 % (11/67), and tris 12 in 1 % (1/67).
Summary and Conclusions: In our study we showed that 33 % of repeatedly tested patients acquired additional cytogenetic aberrations during the CLL course. The most frequently acquired aberration was 13q deletion (66 %). Regarding the disease activity, deletion 13q comprised 92 % newly acquired aberrations among patients with indolent CLL without clinical manifestation of the disease progression. Surprisingly, 13q deletion comprised 60 % of all aberrations acquired before therapy and 61 % of all aberrations acquired after therapy also in the group with progressive disease. Our data suggests that the proportion of the deletion 13q among newly acquired aberrations appears independent on the disease activity and therapy intervention. In contrary, 17p deletion acquisition increases with disease activity and foregoing therapy, 13 % before vs. 28 % cases after treatment in progressive group. Supported by: *IGA MZCR NT13519, IGA MZCR NT13493, CZ.1.05/1.1.00/02.0068, CZ.1.07/2.3.00/30.0009, NGS-PTL/2012-2015/no.306242, MSMT-CR (2013-2015, no.7E13008)*

E1052

NOVEL NO-ASA DERIVATIVES ARE NEW PROMISING AGENTS IN THE THERAPY OF CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) AND SOLID CANCERS

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Background: CLL is characterized by an accumulation of B cells in the peripheral blood, bone marrow and lymphatic tissues. Patients harbouring inferior prognostic markers as the 17p deletion or the *TP53* mutation show high resistance to standard therapy in CLL. It is well known that NO-ASA (Nitric-oxide donating acetylsalicylic acid) is a potential drug for the treatment of diverse cancers by inducing apoptosis *in vitro* & *in vivo*.
Aims: Our main objectives were to determine whether NO-ASA is a specific therapeutic substance in high risk CLL and further solid cancers. Further we intend to illuminate the mechanism of its cytotoxic effect.
Methods: A group of 22 new developed NO-ASA derivatives were tested for their efficacy to induce apoptosis on primary CLL cells and PBMCs of healthy donors via Annexin V/7AAD assay. The anti-tumor effect analysis of the most promising derivatives (B9, B12, B13) was enlarged to the analysis of diverse cancer cell lines (SW480, MelJuso, HCC-44, SH2, COLO-407). In addition the *in vivo* effect of B1 (para-NO-ASA), B9, B12 and B13 were analyzed via JVM3-xenograft mouse model. To elucidate the biological mechanism of the derivatives we performed a kinase array, Western Blots and immunofluorescence analyses with a fluorescence coupled B9 (B20).
Results: The effective dose (and therefore the toxicity) in most of the NO-ASA derivatives were significant lower in CLL cells compared to healthy PBMCs (*e.g.* EC_{50} CLL: B9 1.3 µM, B12 1.05 µM, B13 1.08 µM; EC_{50} PBMCs: B9 93.4 µM, B12 55.4 µM, B13 77.2 µM). We detected a strong reduction of cell viability in all tested cell lines and a cytotoxic effect even on *TP53* mutated and pretreated CLL with the highest effects by B9 and B12. Derivative B9 and even B12 demonstrated a strong anti-tumor efficacy in the xenograft mouse with a maximal tumor inhibition rate of 65-70%. Cell imaging assumes that B20 might be localized and acts in structures near the endoplasmatic reticulum. The human phosphor kinase array and Western Blot analyses showed preliminary results of the impact of the NO-ASA derivatives on the cell cycle checkpoint signaling and the MAPKinase signaling pathway. After 3 h NO-ASA treatment (B9, B12) on primary CLL cells the phosphorylation of the kinases p38 alpha, MKK3/ MKK6, Chk2 and CREB were induced.
Summary and Conclusions: We showed that our new developed NO-ASA derivatives (especially B9 and B12) are novel potential therapeutic drugs in the treatment of CLL patients including these with bad prognosis. For the first time we identified the three NO-ASA derivatives B9, B12 and B13 as potential drugs for diverse solid cancers.

E1053

A RETROSPECTIVE CASE CONTROL STUDY ASSOCIATING CHRONIC LYMPHOCYTIC LEUKEMIA AND ITS SPECIFIC ABERRATIONS WITH CERTAIN RISK FACTORS

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Background: The role of environmental or occupational genotoxic exposure in the development of Chronic Lymphocytic Leukemia (CLL) is limited and inconclusive. In addition, no investigation has been conducted to elucidate the putative role of lifestyle and occupational factors in the formation of CLL specific chromosomal aberrations.
Aims: Regarding the above, the present study examines through detailed questionnaires the potential environmental and occupational chemical exposure as well as the lifestyle and cancer family history of cases and healthy controls to assess the contribution of specific risk factors to CLL incidence. Moreover, these postulated risk factors are evaluated for the first time in respect to CLL specific chromosomal abnormalities.
Methods: A total of 138 unselected patients diagnosed with CLL and 141 unrelated healthy men and women, age and sex matched to CLL cases, were enrolled in this study. Face-to-face interviews were conducted on the study participants using a standardized and structured questionnaire to obtain a lifetime demographic, occupational and medical history as well as other risk factor information. In addition, cytogenetic analysis was performed on unstimulated and stimulated bone marrow cells of all CLL patients at the time of diagnosis so as to associate CLL chromosomal abnormalities with specific risk factors. Study results were analysed using univariate and multivariate statistical analysis to control the overall influence of risk factors.
Results: CLL was positively associated with certain lifestyle and medical/clinical risk factors such as smoking (P=0.005), family history of CLL or other malignancy (P<0.0001), episodes of pneumonia (≥1) within 5 years before CLL diagnosis (P=0.016) and exposure to medical radiation (P=0.003). Similarly positive associations were revealed between CLL and occupational/environmental chemical exposures including exposure to petroleum (P<0.0001), metals (P=0.024), pesticides/chemical fertilizers (P<0.0001) and detergents (P<0.0001). It was also observed that if someone is exposed to more than two risk factors in lifetime, he/she is at ~1.5 fold greater risk of developing CLL compared to those who have experienced only one toxic exposure (OR=1.42, 95% CI=1.17-1.73, P<0.0001). Analyses by specific risk factors and CLL chromosome abnormalities showed that del(11q) and del(13q) were found more frequent in patients having been exposed to pesticides (P=0.050) and rubber (P=0.044) respectively while patients who had a father suffering from CLL or other malignancies tended to carry more often del(11q), del(13q) and +12.
Summary and Conclusions: Our study provides evidence for the involvement of genetic predisposition and exposure to specific occupational and lifestyle risk factors in the onset of CLL. Moreover, it was indicated that the more different risk factors one has been exposed to, the greater is the risk of developing CLL. Notably, the association of certain risk factors with CLL specific chromosomal aberrations for the first time revealed positive correlations between deletions of 11q and 13q and exposure to pesticides and rubber respectively. The above results reinforce the idea that the interaction between specific exposures and genetic predisposition may be a possible cause for the development of CLL and its specific chromosomal abnormalities.

E1054

HIGH RESOLUTION MELT (HRM) CURVE ANALYSIS OF P53 MUTATIONS IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) PATIENTS

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Background: Disruption of p53 by deletion, mutation or both occurs in at least 10% of patients with chronic lymphocytic leukaemia (CLL) prior to first-line treatment and is associated with both chemo-refractoriness and a short median survival of 3-5 years. Del (17p) detected by FISH analysis identifies up to 80% of p53 disrupted patients, but the remainder can only be confirmed by Sanger sequencing, which is not feasible in a diagnostic molecular laboratory. p53 disrupted patients follow a specific treatment pathway with alemtuzumab and allogeneic transplantation if <65 years, but more recently the novel, effective agents ibrutinib and idelalisib have been licensed. The European research initiative on CLL (ERIC) recommended screening all patients for evidence of p53 disruption prior to therapy.
Aims: To develop a pre-sequencing screening assay for p53 mutations, using a high resolution melt (HRM) analysis to detect sequence alterations in exons 5-8 which harbour ≥95% of p53 mutations in CLL prior to confirmatory sequencing.
Methods: Consensus p53 PCR programme was employed to amplify exons 5-8 and optimise HRM analysis. The HRM predictive value and sensitivity were evaluated using the cell lines (K-562 and JURKAT) and DNA from known del(17p) patients of whom 80% will have a mutated second allele. We performed HRM screening on 45 CLL patient blood samples (12 treatment–naïve and 33 relapsed / refractory) and lymph node (LN) DNA from 4 patients with Richter's transformation (RT). Samples with an aberrant melt curve suggestive of sequence alteration, was confirmed by bidirectional Sanger sequencing and p53 mutation bioinformatics (COSMIC / Polyphen database) were used to establish mutation frequency and its deleterious impact.
Results: The HRM assay sequence alteration detection sensitivity was 15%. HRM analysis detected aberrant melt curves in 16/45 samples; 8/10 samples (80%) had a del(17p) and 8/35 (23%) had no FISH-detectable deletion and all cases were confirmed by sequencing. p53 mutations were detected in 8% (1/12) of treatment-naïve and 33% (11/33) of relapsed / refractory patients respectively. The 4 Richter's patients had no mutations detected in blood; however, 3 of them had a mutation in LN DNA. Missense mutations affecting the DNA binding site accounted for 83% of the p53 mutations and all were both common and deleterious when compared to the COSMIC and Polyphen database respectively.
Summary and Conclusions: We developed a consensus PCR amplification and HRM protocol for p53 mutation detection. HRM methodology is a sensitive, rapid and cost-effective pre-sequencing screening assay which reduces the numbers of samples requiring sequencing with a sensitivity of 15%. Our p53 mutation detection results are comparable with reported data in the literature.

E1055

ENDOSTEAL AND VASCULAR STRUCTURES OF BONE MARROW STROMA IN CLL

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Background: Normal hematopoiesis is carried out in close association with stromal microenvironment, which forms the hematopoietic niche. Endosteal and vascular elements of bone marrow stroma regulate the development of HSC and lymphoid differentiation. The study of endosteal-vascular niche structural organization of the bone marrow allows to clarify the role of these entities in the malignancy of hemolymphopoiesis.
Aims: To determine the morphofunctional features of stromal elements of hematopoietic microenvironment that form hematopoietic niches in CLL.
Methods: Bone marrow core biopsies of 96 patients with CLL at the age 49-73 years obtained before therapy were researched. Histological, histochemical, morphometric and immunohistochemical methods were used. The statistical significance was considered with p≤0,05 (Student criterion).
Results: On the bases of CLL patients spongy bone research, in bone marrow parenchyma 3 types of lymphoid infiltration were detected: nodular (18 cases - 18,8%), interstitial (26 cases - 27%), and diffuse (52 cases - 54,2%). The distribution of bone marrow infiltration types by Rai stages was determined. So, 3-4 stage was characterized by a predominance of cases with diffuse infiltration of the bone marrow, while 0-1 stage - by a predominance of nodular infiltration. Interstitial infiltration was observed at all Rai stages (0 stage - 3 cases, 1 stage – 7 cases, 2 stage – 16 cases, 3 stage – 10 cases, 4 stage – 5 cases). Morphological signs of bone marrow stroma disorders were established at all types of infiltration. Silver impregnation showed the increase number of reticulin fibers and the formation of reticular sclerosis foci in interstitial and diffuse types of infiltration, including endosteal bone marrow areas. Microvessels density increased with the progression of bone marrow infiltration. Vessels area in diffuse infiltration increased by almost 2 times in comparison with the control group (17,9±3,7% instead of 9,1±1,2% in the control group), in interstitial infiltration increased by 1,5 (13,1±1,2%), in nodular infiltration statistically significant changes were not found (12,3±2,5%), and, what is especially important, the increase of vessels' number in subendosteal spaces was noted. Dependance of prognostic protein marker Zap70 expression from the density of microvessels was evaluated and statistically significant correlation between these parameters (R=0,7, p <0.05, N=96) was noticed. The Zap70 expression also correlated with the type of bone marrow infiltration: in all cases of diffuse infiltration of bone marrow (n=52) most leukemic cells were positive (>65%), in nodular infiltration (n=18) expression in the majority of cases was not detected, interstitial infiltration of bone marrow (n=26) differed by variable expression of Zap70. 6 cases of interstitial infiltration with lack of expression of Zap70 (less than 15%), 4 cases with high expression (50-65%), 16 cases with moderate expression (30-45%) were noted. Analysis of endosteal cells showed an increase in cells number per area unit in interstitial (1,8±0,4 against 1,4±0,2 in the control group) and diffuse (2,3±0,7) infiltration. The change of endosteal cells morphology was established too. The number of cellular elements with elongated flattened nuclei shapes was decreased, while increase in endosteal and subendosteal areas of stromal cells with large light nucleus was noticed. Expression of the molecule CD146, which marks the stromal cells endosteal and perivascular areas in diffuse infiltration was also increased and was present in subendosteal areas almost everywhere (10,2±1,3% vs. 2,5±0,3% in the control group).
Summary and Conclusions: Defects of endosteal-vascular structures of bone marrow stroma testify to the participation of the hematopoietic niche in the neoplastic transformation of B-lymphocytes in CLL.

E1056

NEW PROGNOSTIC MARKER IN CHRONIC LYMPHOCYTIC LEUKEMIA: PLASMA CCL3 (MIP-1A)

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Background: Chronic lymphocytic leukemia (CLL) is one of the most common hematologic malignancies in adulthood. Prognostic markers are still needed to aid therapy decision making. In response to BCR (B-cell receptor) activation, CLL cells secrete the chemokine CCL3 (MIP-1α) [C-C motif Ligand 3/Macrophage Inflammatory Protein-1α], which affects tumor cell-microenvironment interactions. It is found that plasma CCL3 level is a useful prognostic marker for risk assessment in CLL.
Aims: We aimed to determine if there is any correlation between CCL3 plasma levels and other prognostic markers in CLL patients, and if CCL3 has an impact on overall survival (OS).
Methods: Between May 2012-2014, 99 CLL patients and 25 control cases were included in the study. Peripheral blood samples were collected from both groups. Age, gender, Rai, Modified Rai and Binet stages, lymphocyte count, LDH level, β-2 microglobulin, CD38 expression, ZAP70 expression, del 17p status, bone marrow involvement pattern and plasma CCL3 levels were reported in the patient group. Age, gender and plasma CCL3 levels were reported in control group. Plasma CCL3 level was measured by enzyme-linked immunosorbent assay (ELISA).
Results: The median follow-up time was 18 months in both groups. Median age was 62.91±10.52 in CLL group and 67.92±12.28 in the control group. We established that plasma CCL3 levels in CLL patients were higher than in the control group (p=0.001). We divided CLL patients into two groups according to high and low CCL3, based on ROC analysis (cut off value >35 pg/ml). We found a positive correlation between high CCL3 level and β2-microglobulin (r=0.220, p=0.042). Six patients in the CLL group with high CCL3 were dead by the time of analysis, whereas no deaths occurred in the low CCL3 group. Patients lost to follow-up had higher plasma CCL3 (p=0.047). OS of patients with high CCL3 levels was found shorter. It was statistically determined that OS decreased with increased CCL3 levels (r=-0.201, p=0.046). No correlations were found between other prognostic markers. There was not any difference in CCL3 levels between the treated and not treated patients (p<0.2). In subgroup analysis, we noticed that splenomegaly was more common among patients with high CCL3 level (p=0.025).
Summary and Conclusions: Plasma CCL3 level is an important prognostic marker and is correlated with OS. The exact level of this chemokine in defining disease course is not determined yet. Further studies are warranted to confirm cut-off values of this chemokine and to make it one of the prognostic markers in CLL. CCL3/MIPI-α might also have a role in the follow-up of the targeted therapies of CLL.

E1057

IMMUNOPHENOTYPE OF CLL CASES WITH STEREOTYPED BCR IS CHARACTERIZED BY DECREASED CD23 EXPRESSION

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Background: About 1/3 of CLL cases is characterized by stereotyped motifs in BCR structure apparently representing common antigen-driven selection of malignant clones. It is conceivable that continuous antigenic stimulation would

lead to certain surface immunophenotype changes in cases with BCR stereotypy. To the best of our knowledge no studies have tested this hypothesis so far.
Aims: To compare surface marker expression in CLL cases with or without BCR stereotypy.
Methods: Out of 148 treatment-naïve participants of NORMA trial (id: NCT02110394) 89 pts with diagnosis confirmed in a single centre by standardized flow cytometry protocol and known IGHV/IGHD/IGHJ sequences were analyzed. Most of these cases had typical CLL immunophenotype with bright CD5, high CD23 (median 83,8%) and CD200 (median 98,8%) expression. In few cases with low CD23 expression (N=3) mantle cell lymphoma was further excluded by FISH with IGH/CCND1 t(11;14) probe. BCR stereotypy was determined according to ERIC recommendations. Levels of expression of surface markers (in %) in stereotyped *vs* non-stereotyped cases were compared with Mann-Whitney U test.
Results: Among 89 pts IGHV-UM cases prevailed (N=61; 68,5%). 19 (~21%) pts had stereotyped BCRs. Most common subsets included #2 (N=6), #7 (N=4), #8 (N=3) and #1 (N=2). Statistical analysis showed no differences in surface expression of such markers as CD20, CD22, CD38, CD43, CD79b, CD200, kappa or lambda among stereotyped and non-stereotyped cases. However, BCR stereotypy was associated with significantly lower % of CD23 positivity (median 64,55% *vs* 87,15%, p=.041).
Summary and Conclusions: Decreased surface expression of CD23 in CLL cases with stereotyped BCR is shown for the 1st time. Ongoing antigenic stimulation or increased TLR signaling could be possible explanations for this observation as published previously[1,2]. It is still unknown, whether underlining mechanism is downregulation of CD23 protein synthesis or increased shedding. Other B-cell activation markers, sCD23 and, possibly, IgE levels should be examined in the same manner.

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E1058

CD47 AGONIST PEPTIDES INDUCE PROGRAMMED CELL DEATH IN REFRACTORY CHRONIC LYMPHOCYTIC LEUKEMIA B CELLS VIA PLCG1 ACTIVATION
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Background: Chronic lymphocytic leukemia (CLL), the most common adulthood leukemia, is characterized by the accumulation of abnormal $CD5^+$B lymphocytes, which results in a progressive failure of the immune system. Despite intense research efforts, drug resistance remains a major cause of treatment failure in CLL, particularly in patients with dysfunctional *TP53*.
Aims: The objective of our work was to identify potential approaches that might overcome CLL drug refractoriness by examining the pro-apoptotic potential of targeting the cell surface receptor CD47 with serum-stable agonist peptides.
Methods: We performed *in vitro* cellular and molecular biology assessments in primary $CD5^+$B lymphocytes obtained from a cohort of 80 CLL patients, and we assessed, in a CLL-xenograft mouse model, the *in vivo* capacity of the CD47 agonist peptides to reduce tumor burden.
Results: Our *in vitro* approach shows that the CD47 peptide agonists enable a Ca^{2+}-mediated, caspase-independent PCD pathway that, sparing the normal T and B lymphocytes, efficiently kills CLL B cells, including those from drug-refractory patients (*e.g.*, with dysfunctional *TP53*). This PCD pathway, molecularly described here for the first time, involves a sequence of events initiated by the triggering of CD47 by serum-stable peptide agonists and the subsequent activation and phosphorylation at tyrosine 783 of the signal transduction protein PLCγ1, an over-expressed protein in CLL. Further, PLCγ1 activation leads, by means of the second messenger IP_3, to ER stress, cellular Ca^{2+}overload, mitochondrial damage, and B cell death. The *in vivo* data obtained in the CLL-xenograft mouse model demonstrated that, by inducing PLCγ1-mediated, caspase-independent PCD, the injection of CD47 agonist peptides significantly reduced tumor burden.
Summary and Conclusions: Our work provides substantial progress in (i) the development of serum-stable CD47 agonist peptides that are highly effective at inducing PCD in CLL, (ii) the understanding of the molecular events regulating a novel PCD pathway that overcomes CLL apoptotic avoidance, (iii) the identification of PLCγ1 as an over-expressed protein in CLL B cells, and (iv) the description of a novel peptide-based strategy against CLL.

E1059

FREQUENCY OF CLL-LIKE MONOCLONAL B LYMPHOCYTOSIS IN JAPANESE PEOPLE LIVING IN SAO PAULO, BRAZIL
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Background: Monoclonal B-cell lymphocytosis (MBL) is defined by the presence of <5x109/L circulating clonal B cells in healthy individuals. In the general population, the frequency of MBL varies from 5% to 12% in Western countries, depending on the sensitivity of the method. High-count MBL (MBLhi) with chronic lymphocytic leukemia (CLL)–like phenotype has been related to the progression to CLL at frequency of 1%/ per year. CLL shows clinical and epidemiological differences among different ethnic groups including a low incidence in Asian populations, even in those living in Western countries. MBL assessment in individuals of Asian ancestry might contribute to clarify the pathogenesis of CLL and its relationship with MBL.
Aims: To evaluate the frequency of MBL in healthy Japanese individuals living in São Paulo.
Methods: 195 healthy adults without racial miscegenation were studied (median 66 years; range: 40-88 years; 57% female). Peripheral blood samples collected in EDTA were analyzed in an 8-color flow cytometer. Cells were stained after "bulky-lysed" using the following antibody combinations: sIgl & CD8/ sIgk &CD56/ CD5/ CD19/ CD3/ CD38/ CD45 CD4 &CD20 and CD23/ CD10/ CD79b/ CD19/ CD5/ CD43/ CD45/ CD20. Median acquisition was of 5,000,000 events (648,250 to 7,933,086 events). A MBL clone was defined by the presence of >30 clustered events.
Results: The mean values were: hemoglobin 14.6±1.3 g/dL (11.2-18.5 g/dL), platelets 230±61x10^9/L (121-492x10^9/L), WBC 5.9±1.6x10^9/L (3.1-14.0x10^9/L), lymphocytes 1.7 ±0.5 /L (0.6-4.0x10^9 /L), T lymphocytes 1.1±0.5x10^9/L (0.3-3.4x10^9 /L), T CD4+0.7±0.4 x10^9 g/L (0.2-3.3 x10^9 g/L) and T CD8+0.3±0.2 x10^9 g/L (0.07-1.4 x10^9 g/L), CD4/CD8 ratio 2.7±1.6 (0.5 to 12.6), B cells 0.19±0.12x10^9/L (0.003-0.84x10^9 /L), sIgk/sIgl ratio 1.4±0.2 (0.6 to 3.5) and plasma cells 0.005±0.015 x10^9/L (0-0.2 x10^9). MBL was detected in 7.7 % of individuals (15/195), including 8.2% (10/122) of those cases where ≥5,000,000 cells were measured. All detected MBL clones had a CLL-like phenotype (in 9 cases a single clone was identified while 6 were biclonal). The median size of the MBL clone was of 0.06% (0.01-19.8%) of B cells and 38.1x10^3 / L (2.0x10^3-19.1x10^6/L). The frequency of MBL clones progressively increased with age: 4.6% (4/87) in individuals <65 years (GI); 6.2% (4/65) in subjects between 65 and 74 years (GII) and 16.3% (7/43) ≥75 years (GIII), while total B cells progressively decreased (mean x10^9/L) (GI=0.222±0.161, GII= 0.203±0.118 and GIII=0.144±0.09) (p≤0.002 for GIII *vs* the other groups). The oldest individuals (GIII) presented a higher frequency of MBL as compared to the others (16.3 *vs* 5.4%) and significantly lower number of total B cells (0.144±0.09 *vs* 0.214±0.144 x10^9 / L, respectively, p ≤0.001). Plasma cells also showed a tendency to be reduced with age >75 years (0.0036±0.0039 *vs* 0.0052±0.018; p=0.06). Additionaly, a trend to a positive correlation was also observed between the size of the MBL clone and age (r=0.49, p=0.07).
Summary and Conclusions: The frequency of MBL in the Japanese individuals living in Brazil is similar to that reported for Western populations but inferior to that reported by Nieto et al (12%) using a technique with a similar sensitivity. Of note, all cases corresponded to CLL-like MBL and showed a significant increased frequency (as well as clone size) with age, most cases (13/15) having clone sizes below the <0,5X109/L cutoff associated with risk of progression to CLL. (Acknowledgments: FAPESP-proc n.2010/17668-6; CAPES; Hospital Sta. Cruz and IOP/GRAACC).

E1060

CHARACTERIZATION OF NORMAL AND PATHOLOGICAL LYMPHOID POPULATIONS: VALIDATION OF A 10-COLORS FLOW CYTOMETRY PROTOCOL FOR ROUTINE DIAGNOSIS
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Background: Multiparametric flow cytometry (MFC) has developed over last years and 10-colors techniques are becoming routine, providing a great level of information. Using markers of mutually exclusive expression, one can design panels with even more markers than detectors available on the cytometer. Neverthe-

less MFC remains a complex technique needing a thorough validation before it can be used as a routine-test for diagnosis in hematology or immunology.
Aims: We developed a panel of only two tubes for screening and characterization of lymphoid abnormalities in blood or marrow samples. The "screening" tube contained 11 markers in 9 colors: CD8+kappa-FITC / CD56+lambda-PE / CD3-ECD / CD5-PC5.5 / CD2-APC / CD7-AA700 / CD20+CD4-PacB / CD45-KrO. The second tube, designed for B-cell malignancies, contains CD200-PE / CD23-ECD / CD5-PC5.5 / CD19-PC7 / CD10-APC / CD22-AA700 / CD38-AA750 / FMC7-PacB / CD45-KrO. The aim of our study was to validate the technical performances of these tubes, compared to our previous 5-colors technique, for correct characterization of lymphoid abnormalities.
Methods: The results obtained with this panel were compared with our previous method which consisted in a 3 tubes screening panel: CD45-FITC / CD4-PE / CD8-ECD / CD3-PC5 / CD2-PC7, CD45-FITC / CD56+CD16-PE / CD19-ECD / CD3-PC5 / CD7-PC7 and kappa-FITC / lambda-PE / CD19-ECD / CD5-PC5 / CD20-PC7, and 2 tubes for B-cells malignancies: CD22-FITC / CD10-PE / CD19-ECD / CD5-PC5 / CD38-PC7 and FMC7-FITC / CD23-PE / CD19-ECD / CD5-PC5. All antibodies were provided by Beckman Coulter except kappa and lambda light chains (Dako) and CD200 (BD Biosciences). Almost 100 samples were tested including normal cases, benign unbalanced lymphoid populations (*eg* HIV) and various pathological contexts: CLL, other B-cell malignancies, T-cell malignancies (*eg* Sézary cells, prolymphocytic T-cell leukemia) and blastic infiltrations. Quality assessment samples were also assayed. We used a Navios* (Beckman Coulter) cytometer and data were analyses with Kaluza* software (Beckman Coulter).
Results: The results obtained with this 2-tubes method showed no discordance with our previous technique. In addition, this new method provided a higher level of information, as more markers could be assessed together. This allowed a more accurate analyze of the lymphoid sub-populations, particularly in pathological samples. The sensibility and specificity were thereby better with this technique than with our previous 5-colors protocol. Although not expressly designed for, the screening tube detected CD45- immature cells efficiently.
Summary and Conclusions: This 10-colors technique with one 11-markers screening tube and one "Matutes"-tube for lymphocyte exploration provided very satisfying results. Hence it was adopted in our lab for routine-use and submitted to accreditation. In addition to these technical issues, it is to note that this technique saves human resources (less manipulations), cytometer resources (faster acquisition) and reagents (no marker repeated in iterative tubes), medico-economical cost being also of importance nowadays.

Chronic lymphocytic leukemia and related disorders - Clinical

E1061

CHEMOTHERAPY EXPOSURE AND SURVIVAL AMONG PATIENTS DIAGNOSED WITH CHRONIC LYMPHOID LEUKEMIA

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Background: Chronic lymphoid leukemia (CLL) accounts for approximately 30% of all lymphoid neoplasms and 12% of nodal lymphomas and is largely a disease of older people. Currently, there are around 650 new cases of CLL per year in the Netherlands.
Aims: To describe chemotherapy exposure, overall survival (OS) and progression-free survival (PFS) among patients diagnosed with CLL.
Methods: Data from the Eindhoven Cancer Registry (ECR), including data on all newly diagnosed cancer patients, was linked on an anonymized patient-level with the PHARMO Database Network that combines data from multiple linked healthcare databases including in- and out-patient drug dispensing, hospitalizations and clinical laboratory measurements. The PHARMO-ECR cohort represents approximately 10% of the Dutch population. Patients diagnosed with CLL between January 1, 1998-December 31, 2011 were included in the source population. For a subset of patients with in-patient pharmacy data available, chemotherapy exposure after initial diagnosis was assessed. OS after initial diagnosis was determined in the source population. For patients receiving chemotherapy, OS was also determined after chemotherapy treatment as well as PFS. Progression was defined based on 1) start of a different type of chemotherapeutic agent and for a subset of patients also on 2) increase in the number of blood lymphocytes by 50%, 3) decrease in Hgb level by >2 g/dl or a Hgb of <10 g/dl, 4) decrease in platelet counts by >50% or a platelet count of <100.000/ul. Survival was analyzed by the Kaplan-Meier method.
Results: In total, 624 patients were diagnosed with CLL in the study period. Mean (±SD) age was 68 (±11) years and 62% was male. For 393 of the CLL patients (63%), in-patient pharmacy data was available. Of these, 125 patients (32%) received chemotherapy: 52 patients (42%) started chemotherapy within 6 months after initial diagnosis and 73 patients (58%) started chemotherapy more than 6 months after initial diagnosis. Overall, median time from initial diagnosis until start of chemotherapy was 282 days. About 50% of the 125 patients had one treatment line and about 25% had two lines of treatment. The most common type of chemotherapy received as first and second line chemotherapy was chlorambucil (71% and 48% for 52 patients starting chemotherapy within 6 months after initial diagnosis and 75% and 58% for 73 patients starting chemotherapy more than 6 months after initial diagnosis). The second most common type of chemotherapy received as first and second line chemotherapy was R-CVP for patients starting chemotherapy within 6 months after initial diagnosis (13% and 15%) and fludarabine among patients starting chemotherapy more than 6 months after initial diagnosis (10% and 17%). Five years after initial diagnosis, 79% of all patients in the source population were still alive. The highest mortality rate was observed within the first three months (9.2 (95% CI: 5.0-15.5) per 100 person-years). Among patients receiving chemotherapy, 78% were still alive one year after chemotherapy treatment. The median PFS from end of the first treatment line was 27.4 months for patients starting chemotherapy within 6 months after initial diagnosis, and 16.8 months for patients starting chemotherapy more than 6 months after initial diagnosis.
Summary and Conclusions: About one third of CLL patients received chemotherapy, which mostly was chlorambucil. At 5 years, the majority of CLL patients were still alive (79%). Among those receiving chemotherapy, the median PFS ranged from 17 to 27 months, depending on the timing of chemotherapy.

E1062

REAL-WORLD OUTCOMES OF PATIENTS WITH RELAPSED OR REFRACTORY CHRONIC LYMPHOCYTIC LEUKEMIA WITH 17P DELETION

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Background: Patients (pts) with relapsed and refractory (R/R) chronic lymphocytic leukemia (CLL) and 17p deletion (del 17p) studied in clinical trials had poor outcomes after standard CLL treatment (tx).
Aims: To describe best response, progression free survival (PFS), and overall survival (OS) outcomes of R/R del 17p CLL pts receiving salvage tx in clinical practice.

Methods: An online medical chart review was used to collect data from 120 US oncologists/hematologists from 79 community and 41 academic sites on 408 adults with R/R del 17p CLL who started salvage tx from 2009 to 2014. Pts characteristics were collected at R/R CLL diagnosis (dx). As reported by physicians, del 17p was detected by FISH, and minimal residual disease (MRD) by bone marrow aspirate and flow cytometry or PCR. Best response on 1st and 2nd salvage tx, assessed according to local practice standards, and response duration up to response loss or end of follow-up were reported. Kaplan-Meier analyses from salvage tx initiation were used to estimate PFS and OS rates. A sensitivity analysis was conducted among the subgroup of pts who started salvage tx ≥1 year prior to study data collection (n=134).
Results: Of 408 R/R del 17p CLL pts, 54% were relapsed and 46% were refractory to primary CLL tx. Median age was 64 years and 62% were male. Among pts with reported information at R/R CLL dx, 72% had Rai stages III/IV (292/406; 78% in relapsed, 65% in refractory pts), 21% had beta-2 microglobulin ≥6 mg/L (69/322; 22%, 21%), 69% had unmutated IGHV (180/262; 71%, 66%), 27% had ≥20% cells with del 17p (98/362; 35%, 19%), and 28% had ECOG ≥2 (114/403; 29%, 28%). Median time from R/R CLL dx to salvage tx initiation was 0.4 month (range 0.0-39). Follow-up data after salvage tx initiation was available for a median of 6 months (range 0.6-71). Outcomes are presented in the table.

Table 1.

	Relapsed		Refractory	
Salvage tx	1st (N=221)	2nd (N=26)	1st (N=187)	2nd (N=28)
Best response on salvage tx				
CR with positive; negative; or unknown MRD	11%;7%;22%	0%;0%;24%	12%;9%;21%	19%;8%;15%
CCRi; CCR	4%;10%	9%;5%	4%;6%	8%;0%
PR	35%	33%	26%	27%
Stable disease; progression	9%;2%	14%;14%	9%;12%	4%;19%
Median duration of best response, weeks				
CR with positive; negative; or unknown MRD	21;14;11	-;-;4	19;16;5	4;6;10
CCRi; CCR	41;20	60;1	8;0.7	6;-
PR	4	0.1	5	7
PFS rates				
6-month (95% CI)	94% (89-97)		75% (68-81)	
1-year (95% CI) overall; subgroup analysis	71% (59-79);67% (54-77)		52% (42-62);49% (37-61)	
OS rates				
6-month (95% CI)	100% (97-100)		92% (87-95)	
1-year (95% CI) overall; subgroup analysis	97% (90-99);95% (86-98)		80% (71-87);72% (60-81)	
CR/PR=complete/partial response; CCR= complete clinical remission (i.e., no evidence of disease without bone marrow confirmation); CCRi= CCR with incomplete bone marrow recovery.				

Summary and Conclusions: In this large study describing real-world outcomes of salvage tx in R/R del 17p CLL pts, refractory pts ppeared to have lower physician-reported overall response rate, PFS, and OS after 1st salvage tx as compared to relapsed pts. Despite CRs, response durations appeared limited. Tx analyses are ongoing.

E1063

Abstract withdrawn

E1064

OUTCOMES OF ANTICOAGULANT OR ANTIPLATELET USE IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA OR INDOLENT NON-HODGKIN'S LYMPHOMA IN IDELALISIB TRIALS

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Background: Idelalisib (IDELA), a selective oral PI3Kδ inhibitor, is approved for use in relapsed chronic lymphocytic leukemia (CLL; in combination with rituximab [R]) and indolent non-Hodgkin's lymphoma (iNHL; as monotherapy). Both diseases occur mainly in the elderly, who have comorbidities that increase thrombotic risk.
Aims: This post hoc analysis characterized the use and outcomes of anticoagulant (AC)/antiplatelet (AP) therapy, which was allowed in IDELA registrational clinical trials.
Methods: In the phase 3 Study 312-116 (NCT01539512), frail patients with relapsed CLL (including those with any degree of thrombocytopenia) were randomized to receive a combination of continuous IDELA 150 mg BID or placebo (PBO) with 8 R doses. In the phase 2 Study 101-09 (NCT01282424), patients with refractory iNHL received IDELA 150 mg BID until disease progression or unacceptable toxicity. All patients provided informed consent. Grade 1, 2, and ≥3 bleeding events were analyzed using MedDRA preferred terms and CTCAE.
Results: The 2 trials included 343 patients. In the CLL study, 18 patients (16%) on IDELA +R and 31 (29%) on PBO +R had grade ≥3 thrombocytopenia at baseline. Concomitant AC/AP use was frequent (45% in each study); the most common were aspirin, enoxaparin, and warfarin. AC/AP use was more frequent in patients treated with IDELA +R *vs* PBO +R. The incidence of bleeding events was similar with IDELA, IDELA +R, and PBO +R. Of the 28 patients receiving warfarin, 9 had bleeding events (3 IDELA +R [all grade 1]; 2 PBO +R [all grade 1]; 4 IDELA patients [grade 1, n=3; grade 2, n=1). Grade ≥3 bleeding events occurred in 1 IDELA +R, 1 PBO +R, and 3 IDELA patients.

Table 1.

	CLL		iNHL
n (%)	IDELA + R n=110	PBO + R n=108	IDELA Monotherapy n=125
Patients receiving AC/AP	60 (55)	38 (35)	56 (45)
Aspirin	42 (38)	21 (19)	30 (24)
Enoxaparin	11 (10)	6 (6)	19 (15)
Warfarin	8 (7)	9 (8)	11 (9)
Patients with ≥1 bleeding event (any grade)			
Overall	15 (14)	20 (19)	17 (14)
Grade 1/2	14 (13)	19 (18)	14 (11)
Patients on AC/AP	n=60	n=38	n=56
Received AC	9	4	13
Received AP	5	3	6
Event at any time	10 (17)	6 (16)	14 (25)
Event on AC/AP	7 (12)	5 (13)	8 (14)
Patients not on AC/AP	n=50	n=70	n=69
Event at any time	5 (10)	14 (20)	3 (4)

Summary and Conclusions: AC/AP use involved 45% of the IDELA registrational trial population. Overall, rates of bleeding events were moderate and similar with IDELA +R *vs* IDELA +PBO; grade ≥3 events were uncommon. There was no specific trend with regard to AC/AP and bleeding events in the 2 arms of the CLL study. Funded by Gilead Sciences.

E1065

MODELING THE EPIDEMIOLOGY OF CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) AND WALDENSTROM'S MACROGLOBULINEMIA (WM) IN FRANCE

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Background: Chronic lymphocytic leukemia (CLL) is a slowly progressing but incurable disease and Waldenstrom's Macroglobulinemia (WM) is a rare disease, representing 6% of lymphoproliferative diseases in France. Furthermore, CLL and WM patients will eventually relapse after multiple lines of treatment, leading to a high unmet medical need.
Aims: In 2014, there were few reliable publication sources of epidemiological data for CLL and WM in France, particularly with regard to advanced stages. In addition, epidemiology registries generally provide data on disease incidence; however, no information can be directly obtained for the relapsed patient population. At the same time, several new and effective therapies will be made available to the Hematologist/Oncologist community and thus, there will be an increase in the complexity of therapeutic algorithms. To address this, we undertook an innovative modeling approach to answer the need of a greater understanding of the number of relapsed patients in need of new therapies. The objective of the model was to determine the yearly incidence of first line and later lines of therapy between 2015 and 2018 in CLL and WM patients; thus, the model provides relevant data to determine the patient population size that will be eligible for the new compounds and that could be implemented into a prospective economic model.
Methods: First, we used the "Registre Régional des Hémopathies Malignes de Basse-Normandie" (RRHMBN) to collect real world patient characteristics, such as patient subgroups and tumor staging. The RRHMBN is dedicated to Hematological malignancies and supported by official Health Authorities: "Institut National du Cancer"(INCA) and "Institut National de Veille Sanitaire" (INVS). Then, we applied these findings to the French National Network of Registers "FRANCIM" incidence and survival data. The analysis showed that in 2015, the total number of patients estimated with stage A, B or C CLL is 3612, 671 and 384 respectively. In 2018, it is expected to reach 3778, 702 and 403 patients respectively. In 2015, the total number of patients with symptomatic (20%) or asymptomatic +monoclonal gammopathy of undetermined significance (80%) WM is estimated at 252 and 1008 respectively. In 2018, it is estimated to reach 254 and 1018 patients respectively.
Results: The table below shows the yearly number of CLL and WM patients eligible for treatment in first and subsequent lines of therapy as produced by the model.

Table 1.

Category	Line of Therapy	2015	2016	2017	2018
CLL					
< 65 years (27.6%)	1st line (L)	583	589	595	600
	≥ 2nd L	463	477	485	491
	Sub-total	1 046	1 066	1 080	1 091
> 65 years Unfit (36.2%)	1st line (L)	484	489	493	498
	≥ 2nd L	323	327	326	315
	Sub-total	807	816	819	813
> 65 years Fit (36.2%)	1st line (L)	1 128	1 139	1 150	1 162
	≥ 2nd L	749	757	769	770
	Sub-total	1 877	1 896	1 919	1 932
Total	1st line (L)	2 195	2 217	2 238	2 260
	≥ 2nd L	1 535	1 561	1 580	1 576
	Total CLL population	3 730	3 778	3 818	3 836
WM					
Asymptomatic	1st line (L)	0	0	0	0
	≥ 2nd L	0	0	0	0
	Sub-total	0	0	0	0
MGUS	1st line (L)	0	0	0	0
	≥ 2nd L	0	0	0	0
	Sub-total	0	0	0	0
Symptomatic	1st line (L)	252	253	254	254
	≥ 2nd L	162	162	162	162
	Sub-total	414	415	416	416
Total	1st line (L)	252	253	254	254
	≥ 2nd L	162	162	162	162
	Total WM population	414	415	416	416

Summary and Conclusions: Our modeling approach compensates for a lack of real world information on relapsed CLL and WM. We have shown that there is a significant number of relapsed patients who are in need of new treatments in France. The model produces estimates for the number of patients in later lines in need of novel therapies by 2018 and provides efficient prospective data that can be integrated into medico-economic model development. Furthermore, these data address a current medical need for the development of new CLL and WM treatment algorithms that are required for the upcoming therapies in the relapsed setting.

E1066

Abstract withdrawn

E1067

MICRO-RNAS ASSOCIATED WITH PROGNOSIS IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) DO NOT IMPACT OVERALL SURVIVAL IN PATIENTS WHO RECEIVE NONMYELOABLATIVE ALLOGENEIC STEM CELL TRANSPLANT (NMAT)

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Background: Noncoding RNAs play an important role in the pathogenesis of CLL. Recent studies have shown that higher miR-155 levels (>56th percentile) are associated with a lower overall survival (OS) (P= 0.0122) in CLL patients treated with conventional chemotherapy regimens (Ferrajoli *et al.* Blood 2013; 122:1891). Others revealed that expression of miR-29b or miR-181b inhibits the *TCL1* oncogene (Pekarsky *et al.* Cancer Res 2006; 66:11590), whereas high Tcl1 expression correlates with aggressive CLL phenotype showing unmutated immunoglobulin variable region genes and ZAP70 positivity. However, the clinical impact of the expression of these miRs in CLL patients who undergo NMAT is unknown.
Aims: To assess the impact of miRs on overall survival in CLL patients undertaking NMAT.
Methods: We identified 28 patients with relapsed/refractory CLL who had received NMAT at our center between 2000-2010 and in whom pre-transplant serum samples collected before initiating allogeneic conditioning were available. We measured the serum levels of miR-155, miR-15a/16-1 cluster, miR-29b, and miR-181b. Total RNA was isolated from 100 µL of serum using the Total RNA Purification Kit and miRNA levels were measured by qRT-PCR (TaqMan MicroRNA assays). Twenty fmol of synthetic *C. elegans* miRNA (cel-miR-39) was spiked into each serum samples to normalize the experimental qRT-PCR data (cel-miR-39 Ct mean±SD=17.777± 0.628). Relative miR levels were calculated using the equation $2^{-\Delta Ct}$, where ΔCt=mean Ct_{miRNA} – mean $Ct_{cel\text{-}miR\text{-}39}$, and Ct=threshold cycle.
Results: Patients had a median age of 59 years (range, 45-70). Median number of prior therapies was 3 (range, 2-8). Eleven (39%) patients had a beta-2 microglobulin level of >3 mg/L. Ten of 12 (83%) patients with data that could be evaluated had unmutated immunoglobulin variable-region heavy-chain gene, and 4/19 (21%) had 17p13.1 deletion. At transplantation, 46% of patients had refractory disease. The proportion of patients who received a matched related and a matched unrelated donor was 68% and 32%, respectively. All patients received nonmyeloablative conditioning with fludarabine, cyclophosphamide, and rituximab as published previously (Khouri *et al.* Cancer 2011; 117:4679). Graft-versus-host-disease prophylaxis consisted of tacrolimus and methotrexate. Median follow-up time was 68 months (range, 43-141). The OS from the time of NMAT was evaluated as a function of tiered relative miR levels (low, below median; high, above median). Notably, none of the relative levels of the miRs evaluated were associated with 5-year OS following NMAT (Table).

Table 1.

Micro-RNA	OS associated with low-level	OS associated with high-level	P-value
miR-155	63%	50%	0.4
miR-15a	57%	57%	1.0
miR-16	43%	71%	0.2
miR-29b	52%	61%	0.7
miR-181b	60%	53%	0.9

Summary and Conclusions: Our data suggest that serum levels of miR-155, miR-15a/16-1 cluster, miR-29b, and miR-181b do not impact 5-year OS in relapsed/refractory CLL following NMAT. As such, other prognostic biomarkers are needed in this patient group.

E1068

THE OCCURRENCE OF OTHER MALIGNANCIES IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Chronic lymphocytic leukemia (CLL) is related with an increased risk of developing different malignancies due to immunologic defects associated with disease or therapy-related immunosuppression.
Aims: The aim of this study was to determine in patients with CLL type and frequency of previously diagnosed malignanat disease MD (pMD), concomitant MD (cMD), afterwards secondary MD (sMD), as well as patient's outcome.
Methods: We have retrospectively analyzed clinical data of CLL patients who were diagnosed at our institution from 1994 to 2010. Disease stage and treatment were established after collecting clinical, laboratory, and pathological data. Immunophenotypic diagnosis was verified by applying of CLL scoring system. The study involves 471 patients (311 males/ 160 females) with median age at diagnosis 64.3 (39-80).
Results: MDs were confirmed in 38 (8.1%) patients (24 males/14 females). Presenting characteristics showed WBC>30x10^9/L in 30%, Rai stage III-IV in 13%, and LDH elevation in 23 % of patients. Immunophenotyping revealed CD38 positivity in only 4/38 patients. The most frequent aditional MDs were skin neoplasms in 9 (33.3%) patients comprising 5 cases of basocellular carcinoma, 2 of planocellular and 2 cases of melanoma. Other neoplasms were colorectal carcinoma in 6 (22.2%), prostatic cancer in 4 (14.8%), lung cancer in 3 (11.1%), bladder cancer in 2 (7.4%) and larynx cancer in 2 (7.4%) patients. There was gender related distribution of MDs with the most frequent breast carcinoma in females and prostatic carcinoma in males. Among males 3 patients had double MD, colorectal carcinoma associated with basocellular, prostatic and laryngeal cancer. Regarding females, the frequency of MDs were as following: breast carcinoma had 6 patients (42.8%), uterus and cervix carcinoma 3 (21.4%), colorectal carcinoma 2 (14.2%), kidney carcinoma 2 (14.2%) and gastric carcinoma 1 (7.1%). Diagnosis of pMD noticed in 17 patients (9 males/ 8 females). Among this patients the most frequent pMD was breast carcinoma (5 patients), and afterward colorectal carcinoma (4 patients). Initial treatment of pMD was only surgery in 8 patient, in 1 patients was combined with chemotherapy, in 3 with hormone therapy, and finally in 3 patient with radiotherapy. Single radiotherapy was performed in 2 patients. Median time for appearing CLL after diagnosis of primary MD was 5 years. Regarding OS of patients with CLL and previously diagnosed MDs, 5-years survival from pMD was 68% with median OS of 23 years. In 11 CLL patients (6 males/5 females) cMD was diagnosed and majority of them (90%) had stable CLL that didn`t require medical treatment. Regarding patients with cMD, none of 11 patients received any potentially mutagenic treatment previously. The OS of patients with cMDs was 75% in the first year, with slowly dropping up to 60% in the 5-years follow up. During CLL follow-up in 10 patients (9 males/1 females) was diagnosed .sMD, which was appeared most frequently in the first 4 years (80%). Previously 5 of them received chemotherapy (fludarabine based regimen). From the diagnosis of sMD, median OS was 12 months, with 5-years survival of only 25%.
Summary and Conclusions: CLL doesn't have more aggressive nature after

the treatment of previously diagnosed neoplasm and don't affect the OS. In patients with CLL and cMD the OS mainly depends on the behavior of concomitant malignancy. Finally, diagnosis of sMDs during CLL follow-up had an effect on patient`s inferior outcome.

E1069

MINIMAL RESIDUAL DISEASE IN HAIRY CELL LEUKEMIA: DOES IT MATTER?

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Background: Hairy Cell Leukemia (HCL) is a fairly rare type of B-cell lymphoproliferative disorder. Its prognosis is good compared to most common types of leukemia but relapse remains a challenge. Minimal residual disease (MRD) assessment is routinely performed in most centers using different approaches, but its clinical usefulness is a matter of debate.
Aims: In an attempt to understand the impact of MRD evaluation in the natural history of the disease and relapse risk, we have retrospectively reviewed all patients with HCL diagnosed in our center and looked at how MRD measurements correlated with patient outcome.
Methods: MRD was assessed by flow cytometry expression of CD19, CD25, CD11c and CD103 as well as by marrow biopsy searching for minimal morphological and/or immunohistochemical evidence of disease. MRD implied that patients had achieved complete or partial remission (CR and PR, respectively) with the treatment provided. For flow cytometry MRD positivity, two cut-off values were defined: more than 0.3% or 0.01% of HCL clone. CR was defined as complete recovery of blood counts ie, hemoglobin>12g/dL in men and 11g/dL in women, neutrophils >1500/uL and platelets >100.000/uL (with exception to expected lymphopenia after chemotherapy courses), resolution of splenomegaly in case it was present at diagnosis and, if not absent, minimal morphological or immunohistochemical evidence of hairy cells in bone marrow biopsy. PR was defined as at least a 50% improvement in any of these parameters, without criteria meeting CR.
Results: We analysed data from 29 patients diagnosed between 1991 and 2013, median follow-up was 108 months (range: 23-259 months). Male prevailed (n=26). Median age at diagnosis was 54 years (range: 33-71). Most patients (89.7%) were treated with cladribine upfront (dose 0.14mg/kg x 5 days). 25 patients (86.2%) entered CR. All other 4 patients achieved PR with one cycle of cladribine. Flow cytometry MRD assessment and bone marrow histology were available in 17 and 14 patients, respectively. Median flow cytometry MRD positivity was 0.29% (range: 0-2.55%). Eight out of 17 patients had a HCL clone greater than 0.3% and MRD was negative (<0.01%) in 4 patients. Out of the 14 patients in whom MRD by histology was available, 6 proved to be positive. Median progression free survival (PFS) was 11.8 years (±11.6%). Relapse occurred in 12 patients (41.4%, n=12/29), of whom 2 had achieved PR: five patients had flow cytometry MRD >0.3%, 2 were negative for the test in question, whereas 4 had no MRD by histology. Moreover, we registered 6 relapses out of 13 patients who had a HCL clone greater than 0.01%, with the remainder maintaining CR (n=5/13) or PR (n=2/13). Of notice, we encountered only one relapse in 4 patients who had detectable MRD by both methods. We could not establish any correlation between relapse risk and MRD detection by any of the two methods used. Five-year PFS for patients with MRD >0,3% and <= 0,3% was 88.9%(±10.5%) and 62.5% (±17.1%), respectively (p=0.356). All but one patient (96.6%) continued alive at last follow-up.
Summary and Conclusions: HCL patients do not seem to behave differently in terms of relapse incidence, PFS or overall survival, in respect to the presence or not of MRD. Its assessment by flow cytometry and/or bone marrow histology did not prove to be clinical meaningful in our analysis. Currently, clinical surveillance and full blood count seem reasonable enough to accurately follow-up HCL patients. Furthermore, MRD assessments bear relevant economical costs and unnecessary stress to patients.

E1070

ONE OUT OF FOUR TREATMENT-NAÏVE CLL PATIENTS IN TAIWAN CARRY TP53 MUTATIONS: CLINICAL AND PATHOGENETIC IMPLICATIONS

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Background: Previous studies have shown significant differences between Taiwan and the West in terms of epidemiology and cytogenetic pattern of chronic lymphocytic leukemia (CLL). Recently, technology advances have led to the identification of previously unknown gene aberrations in CLL, some of which have been shown of clinical relevance. The comparison of these molecular genetic markers between Taiwan and the West may help clarifying the potential difference in the disease between these different ethnic groups.
Aims: The study aimed at establishing the molecular genetic profiles of CLL in the Taiwanese population and identifying the potential differences in this low-prevalence ethnic region.
Methods: In a cohort of 74 patients with newly diagnosed CLL at the National Taiwan University Hospital, we determined the usage and the mutational status of the Immunoglobulin Heavy chain variable (*IGHV*) genes and the presence of HCDR3 stereotypes, a frequent feature in western CLL. In addition, we also screened for the presence of mutations in the *TP53, NOTCH1, SF3B1, BIRC3, and MYD88,* genes recurrently mutated in CLL.
Results: In terms of *IGHV* gene usage, 56.4% of patients had somatic hypermutations and in 10.2% of patients the HCDR3 sequences belonged to the major stereotyped subsets, closely resembling the frequencies present in the western population. That notwithstanding, the commonly used *IGHV* genes in Taiwanese patients were different from those in Western patients as *IGHV3-07* and *IGHV3-23* were over-represented (18.4% and. 16.3%, respectively), whereas *IGHV4-34* and *IGHV4-39* were underrepresented, being absent in the current cohort. In terms of molecular genetics, the frequencies of *NOTCH1, SF3B1, BIRC3* and *MYD88* mutations were 9.7%, 9.7%, 1.6%, and 3.2%, respectively, similar to the data derived from the West. In clear contrast, the frequency of *TP53* mutation was significantly higher (24.2%) in Taiwanese CLL patients than in western populations (5-10%); such a higher frequency is compatible with the higher 17p deletion rate and inferior survival outcomes in Asian CLL patients reported previously. Similar to the data derived from the West, *TP53* mutation had a significant positive association with non-mutated *IgHV* (p=0.011), *TP53* and *SF3B1* mutations were closely associated with 17p deletion (p=0.010) and 11q deletion (p<0.001), respectively, and patients with *TP53*/17p deletion or *SF3B1* mutations had inferior overall survivals (p=0.007 and p<0.001, respectively).
Summary and Conclusions: The current study demonstrates a distinctly higher *TP53* mutation frequency that is in clear contrast with the features of CLL in the Western countries and might explain the overall inferior outcome of CLL in Taiwan, indicating a medical need for novel treatments in this region. The distinct usage of *IGHV* genes in this population may further suggest different pathogenetic mechanisms with distinct antigenic elements acting in the natural history of the disease that may predispose to genetic instability and the acquisition of dismal genetic lesions.

E1071

RETROSPECTIVE EVALUATION OF 83 PATIENTS WITH HAIRY CELL LEUKEMIA TREATED WITH 3 DIFFERENT TREATMENT MODALITIES IN THE LAST TWO DECADES: A SINGLE CENTER EXPERIENCE

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Background: Hairy cell leukemia (HCL) is a rare mature B-cell neoplasm. Various treatment modalities have been used, and until the introduction of cladribine (2-CdA), splenectomy and interferon-alpha (INF-α) were the formerly considered standard therapies for HCL, which led to clinical and hematologic responses. 2-CdA resulted in higher complete response (CR) rates and durable remissions, and became the standard treatment modalities in most of the patients.
Aims: The aim of this study was to retrospectively analyze the clinical outcome, treatment responses, toxicities and survival of HCL patients treated in our institution with 3 treatment modalities (splenectomy, INF-α, and 2-CdA) between 1991-2014.
Methods: Eighty-three patients with HCL were included this study. We analyzed the outcome of different treatment modalities as well as different lines of therapy among these patients. There were 12 patients with variant HCL (HCL-v). The response rates of the 3 first-line treatment options were evaluated. Patients were also divided into four subgroups according to the number of treatments they had received. Group 1 included patients who received first-line treatment. Group 2 consisted of patients who were treated with 2 lines of treatment; Group 3 had patients who underwent 3 lines; and Group 4 included patients with four lines of therapy. We used the criteria for response as described by the Consensus Resolution. CR and partial response (PR) together defined as overall response (OR), and any response other than a CR or PR was considered as no response (NR).
Results: Seventy-four patients (89%) were male, the median age was 49 years (range, 31-76 years), with a median follow-up duration of 54 months (range, 1-217 months). The median leukocyte counts of the HCL-v cases were 15.5 x 10^9/L and (range, 10.1-90.7 x 10^9/L). In 71 HCL cases, the median leukocyte count was 3.03 x 10^9/L (range, 0.5-9.4 x 10^9/L). The median hemoglobin level and platelet counts were 11 g/dL (range, 4-17 g/dL) and 51.5 x 10^9/L (range, 21-124 x 10^9/L), respectively. Splenomegaly was detected in 74% of the patients, with hepatomegaly and lymphadenopathy 37% and 35% of the cases, respectively. Seventy-three patients (88%) (Group 1) received 1st line treatment, and 2-CdA was administered in thirty-two (44%), twenty-one patients (29%) received INF-α and splenectomy was performed in 20 cases (27%) (Table 1). The OR rates among 2-CdA, INF-α and splenectomy as a 1st line

treatment option were 88%, 81% and 90%, respectively. Three patients (2-CdA in two, INF-α in one) died due to infection during treatment. None of the patients with splenectomy died due to post-operative complications. Twenty-nine patients (Group 2) required 2nd line treatment due to relapsed/refractory disease. Seventeen patients received INF-α, 10 patients received 2-CdA and two had splenectomy. Group 3 consisted of 9 patients, and 6 of them had INF-α, two were given 2-CdA and one patient received rituximab (RTX). As a 4th line of treatment one patient received RTX and one had fludarabine (Group 4) (Table 1). During the follow-up, 10 patients died mostly due to refractory disease and/or infections. Five patients were lost to follow-up. There were 64 infection episodes, and most common infections were bacterial infections (pneumonia in 12, tuberculosis in 8, febrile neutropenic episode in 7, soft tissue infection in 6, meningitis in one, and septic arthritis in one). Five patients had a secondary malignancy of which 3 had occurred before the diagnosis of HCL.

Table 1. Summary of the different treatment modalities. (2-CdA, cladribine; IFN-α, interferon-alpha).

Treatment	Group 1 (n=73)	Group 2 (n=29)	Group 3 (n=9)	Group 4 (n=2)
2-CdA	32	10	2	-
INF-α	21	17	6	-
Splenectomy	20	2	-	-
Rituximab	-	-	1	1
Fludarabine	-	-	-	1

Summary and Conclusions: These 3 treatment modalities were found to be comparable regarding efficacy when used in the 1st line setting. First line splenectomy was performed mostly prior to 2000, and nowadays 2-CdA and INF-α are usually chosen as the 1st line treatment in most of the patients. Infections were the most common complication which can be observed both prior to and during the treatment.

E1072

TWO DECADES OF SINGLE-CENTER EXPERIENCE REGARDING THE TREATMENT, PREDICTORS OF SURVIVAL AND CAUSES OF DEATH IN PATIENTS WITH HAIRY CELL LEUKEMIA

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Background: Treatment of hairy cell lekemia (HCL) is well established, however the predictors of survival and cause of death of these patients have not been evaluated. Also, occurence of second malignancies in HCL patients is matter of debate.
Aims: was to identify the parameters with an influence on survival as well as causes of death of HCL patients.
Methods: Study included 137 patients who HCL were diagnosed and treated at the Clinic for Hematology, CCS from January 1993 to December 2014. Eight patients were diagnosed as HCL variant. We analyzed only patients with classic HCL (129 pts) and their characteristics (age, gender, clinical and laboratory parameters, immunophenotypic expression of HCL cells in peripheral blood, histological characteristics of bone-marrow and type of therapy) as well as development of second malignancies in terms of survival.
Results: Median age at the time of diagnosis was 54 years (IQR 20), with dominance of male gender (82,9%). Regarding the therapy, one treatment-line (TL) received 67 patients (51.9%), 2 TL 34 patients (26.4%), 3 TL 10 patients (7.8%) and four or more TL 14 patients (10.9%) resulting in 80.5% of complete remissions (CR), 16.3% of partial remissions (PR) and 3.3% of non-response (NR). Four patients were followed up only, without any treatment (3%). Splenectomy was performed in 28 patients (21,7%). Purine analog (cladribine) received 106 pts (82.2%) resulting in 82,5% CR, 16.5% PR and 1% NR. Seven patients with relapse of HCL after multiple treatments with cladribine, received rituximab alone or in combination with cladribine resulting in 6 patients with CR and in 1 patient with PR. After the median follow up of 86 months, 36 deaths were documented (27.9.%). The 5- and 10- years OS was 90.2% and 70.9% respectively. Second malignancy during the follow-up of HCL developed 17 patients (13.3%). Solid tumors occurred in 13 patients while 4 patients developed second hematological malignancy. The causes of death in HCL patients were as follows: active HCL (9 patients; 25%), cardiovascular disease (11 patients with coronary disease, cardyomyopathy and heart failure; 30.6% and 4 patients with AIM; 11.1%), respiratory insufficiency (2 patients; 5.5%), second malignancy (6 patients with solid tumors; 16,8% and 2 patients with hematological malignancies; 5.5%) and concomitant diseases (2 patients with active HCL and solid tumor; 5.5%). The mean age of patients who died (63±10 years) was significantly higher than in living patients (50±11years) (p<0.001). In univariate Cox regression model unfavorable predictors of survival were: age>55y (p<0.001), hemoglobin level<100g/L (p=0.01), leukocyte count<2.8x10^9/L (p=0.001), sedimentation rate>30mm/h (p=0.011) and occurrence of second malignancy (p<0.001). Regarding the treatment, mortality of patients who did not receive cladribine was 74%, while of those who received cladribine was 18% (p<0.001).
Summary and Conclusions: The cladribine treatment is effective with the achievement of complete remission in over 80% of HCL patients. Patients with multiple relapses have benefit with rituximab treatment. Also, the long-term follow-up of HCL patients is important for evaluating the development of secondary malignancies which this study highlights as one of main causes of death in these patients.

LB2097

EVANS SYNDROME SECONDARY TO CHRONIC LYMPHOCYTIC LEUKAEMIA IS ASSOCIATED WITH ADVERSE BIOLOGICAL FEATURES AND OUTCOME

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Background: Secondary Evans syndrome (ES) is characterised by simultaneous or sequential development of autoimmune haemolytic anaemia (AIHA) and immune thrombocytopenia (ITP), with a positive direct antiglobulin test in the presence of an underlying aetiology. ES secondary to chronic lymphocytic leukaemia (CLL) is an extremely rare occurrence, and its prevalence is unknown. Conversely, isolated AIHA and ITP are much more common in patients with CLL, occurring in 7-10% and 2-5% of patients, respectively.
Aims: Very few epidemiological and clinical data are available on ES secondary to CLL. In this study, we collected clinical and biological data of patients with ES secondary to CLL, and we compared them with patients developing isolated ITP or AIHA.
Methods: We analysed the records of consecutive patients with CLL diagnosed in two major Institutions from Veneto region between 2000 and 2014. All patients met the CLL diagnostic criteria of National Cancer Institute Working Group. To be included, patients with ES had to fulfill the international standardized criteria proposed both for ITP and AIHA.

Figure 1. Overall survival in patients with chronic lymphocytic leukaemia not developing autoimmune cytopenias (AIC) (740), compared to patients with isolated AIHA (63), isolated ITP (32) or Evans syndrome (25).

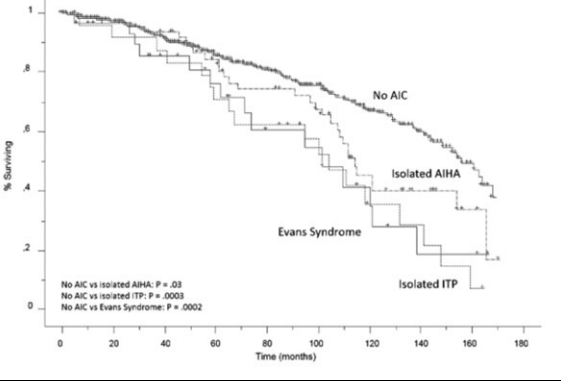

Figure 1.

Results: Overall, 860 patients with CLL were identified, and 25 (2.9%) developed ES. Additional 32 patients (3.7%) developed isolated ITP, and 63 isolated AIHA (7.3%). Demographic and clinical characteristics at CLL presentation (age, gender, Binet stage, median lymphocyte count) of patients developing either ES, ITP or AIHA were similar among the three groups, and not different from patients not developing autoimmune cytopenias (AIC). Of the three AIC, isolated ITP was more frequently diagnosed concurrently to CLL presentation (34% of patients developing this complication), as compared to ES (20%) or AIHA (13%). ZAP70 expression was high (cut-off 20%) in 79% of patients with ES, being significantly higher than in patients with no AIC (50%, p=0.01), but similar to patients with isolated AIHA or ITP. Immunoglobulin heavy chain variable region gene (*IGHV*) was un-mutated in 86% of patients with ES, which was statistically higher than in patients with no AIC (41%, p=0.001), but also compared to patients with ITP (60%, p=0.04) or AIHA (62%, p=0.04). Further-

more, del(17)(p13) and TP53 gene mutations were significantly more frequent in patients with ES (23% and 33% of tested patients, respectively) than in patients with no AIC (5% for both, p=0.003). In terms of overall survival (OS), patients developing ES had a significantly inferior OS than patients with no AIC (Figure 1), but similar to patients with isolated AIHA or ITP. No impact on OS was observed for patients with ES when divided according to time of onset of AIC (simultaneous ITP and AIHA, ITP before AIHA or vice versa). However, patients with ES diagnosed concurrently to CLL presentation had a significantly inferior OS compared to patients developing ES later in the course of disease (5 years OS 40% vs 74%, p=0.006). Treatment of ES was challenging, due to the quite high rate of primary resistant forms, and frequent recurrences, often requiring chemo-immunotherapy.

Summary/Conclusion: We showed that ES secondary to CLL is an infrequent but not so rare occurrence, that similarly to AIHA and ITP was significantly associated with unfavourable biological prognostic factors like ZAP70 expression, and un-mutated *IGVH* status. Furthermore, del(17)(p13) and TP53 gene mutations were highly prevalent among patients with ES. Consequently, patients with ES had poorer OS compared to patients not developing AIC, and OS was even poorer when ES occurred concurrently to CLL diagnosis.

Chronic myeloid leukemia - Biology

E1073

DETECTION OF BCR-ABL GENE MUTATIONS IN CHRONIC MYELOID LEUKEMIA USING SENSITIVE LOW-DENSITY MICROARRAY APPROACH

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Background: Chronic myeloid leukemia (CML) is a hematopoietic stem cell disease characterized by the Philadelphia (Ph) chromosome and BCR-ABL fusion gene coding for oncogenic tyrosine kinase. Imatinib mesylate, a tyrosine kinase inhibitor (TKI) is used as a first line therapy in newly diagnosed CML patients. However, imatinib resistance, usually due to BCR-ABL kinase domain point mutations, remains an important problem in treatment of CML patient. Different methods are used to analyze BCR-ABL point mutations, the common ones are direct sequencing and allele-specific polymerase chain reaction (AC-PCR). Direct sequencing is not very sensitive and can reveal 20-30% of mutant sequence in the background of wild-type DNA. AC-PCR is patient material and labour consuming while analysing a large number of point mutations.
Aims: Development of rapid and sensitive microarray-based approach for routine clinical diagnostics of BCR-ABL kinase domain point mutations.
Methods: A low-density microarray for the analysis of BCR-ABL point mutations has been designed and manufactured. Cytogenetically prepared fixed cells as well as fresh blood samples of CML patients were taken after informed consent. The procedure included RNA isolation and reverse transription-polymerase chain reaction (RT-PCR) followed by hybridization on microarray with immobilized probes able to discriminate between mutant and wild-type sequence. The amplified DNA was labelled via incorporation of fluorescent triphosphates in newly synthesized DNA strand. Fluorescent hybridization pattern was analyzed using portable laser detector. To increase the sensitivity of the assay locked nucleic acid (LNA) oligonucleotides were used to inhibit an amplification of wild-type DNA.
Results: Fifteen mutations associated with imatinib resistance in 85% of CML cases were included in the analysis: T315I, E255K, E255V, E255D, E255R, M351T, G250A, G250E, Y253H, Y253F, F359I, F359C, F359L, F359V, M244V. The microarray approach was validated using control CML samples with known mutations or synthesized mutant DNA matrice. In total, 62 CML patients were genotyped using the approach: 40 patients had chronic phase (CP) of the disease, 18 had accelarated phase (AP) and 4 patients had blast crisis (BC) manifestation. Mutations were found in 15% (6/40) of patients with CP, 72% (13/18) of patients with AP and 100% (4/4) of BC patients. Additionally, 2 of BC patients carried simultaneously 2 mutations. The following mutations have been found: T315I, E255K, E255V, E255D, M351T, G250A, G259E, Y253H, F359I, F359L, M244V. Most frequent mutations were T351I, M351T and M244V. The microarray genotyping data were confirmed by Sanger sequencing of selected samples. The sensitivity of the approach was experimentally tested. The method allowed detecting 2% of mutant DNA in the background of wild-type DNA. All CML patients carrying mutations developed imatinib resistance.
Summary and Conclusions: A low-density microarray-based approach has been developed to identify mutations in tyrosine-kinase domain of BCR-ABL gene, leading to resistance against target therapy with imatinib. The method includes RT-PCR following by allele-specific hybridization with immobilized oligonucleotide probes. Fifteen BCR-ABL mutations which determine 85% of imatinib resistant cases are included in the assay. The sensitivity of the method allows detecting at least 2% of cells carrying mutations among cells with wild-type BCR-ABL gene. The feasibility of the assay for routine clinical practice has been demonstrated by genotyping of CML samples.

E1074

ANTIPROLIFERATIVE AND APOPTOTIC EFFECTS OF PONATINIB AND ITS EFFECTS ON MACROMOLECULAR CHANGES IN IMATINIB-SENSITIVE AND RESISTANT CHRONIC MYELOID LEUKEMIA (CML) CELL LINES: A MECHANISTIC APPROACH

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Background: Although effective treatment strategies have been invented for CML therapy, CML patients have still developed resistance against tyrosine kinase inhibitors used in the clinic. The most aggressive reason of resistance in CML therapy is T315I mutation. Frontline therapeutics, imatinib, nilotinib, and dasatinib cannot be effective in CML patients with T315I mutation. Ponatinib, approved in 2012 by FDA, is an effective inhibitor of BCR/ABL, even with

the mutation T315I. However, the exact mechanism of ponatinib in CML has been still unknown.
Aims: In this study, we aimed to examine apoptotic and antiproliferative effects of ponatinib, and also its effects on macromolecules in imatinib-sensitive K562 human CML cell lines and 3 μM-imatinib-resistant K562/IMA3 CML cell lines generated at our lab. In other words, we aimed to determine the potential mechanisms and structural metabolic changes activated by ponatinib-treatment in CML cell lines.
Methods: K562/IMA3 cells were previously generated by selecting them under increased concentrations of imatinib. The cells are resistant to 3 μM imatinib. Cytotoxic effects of ponatinib on K562 and K562/IMA3 cells were determined by MTT assay while apoptotic effects were assessed by examining the changes in caspase-3 enzyme activity, loss of mitochondrial membrane potential (MMP), and the localization of phosphatidylserine on the plasma membrane by Annexin-V staining. Cytostatic effects of ponatinib were determined by flow cytometry after propidium iodide staining. Changes in macromolecules were determined by Fourier Transform Infrared (FTIR) spectroscopy as the intensities of the secondary derivatives of the absorption bands.
Results: IC50 values of ponatinib for 72 h in K562 and K562/IMA3 cells were found 1,72 nM and 28,5 nM, respectively. In order to examine the apoptotic effects of ponatinib, K562 and K562/IMA3 cells were treated increasing concentrations of ponatinib (10-100 nM) for 72 h. Caspase-3 enzyme activity changed 1,82- to 2,38-fold; and 1- to 1,45-fold in K562 and K562/IMA3 cells, respectively, as compared to untreated controls. Ponatinib treatment also caused changes in MMP by 1,3- to 3,56-fold; and 1- to 6,6-fold in K562 and K562/IMA3 cells, respectively, as compared to untreated controls. Additionally, ponatinib increased apoptotic cell population 1,96- to 8,47-fold in K562 cells in response to 10-100 nM ponatinib; and also 7,75- and 9,64-fold in K562/IMA3 cells exposed to 50- and 100 nM ponatinib, respectively. Furthermore, cell cycle analyses revealed that ponatinib arrested K562 and K562/IMA3 cells at G1 phase, and it also arrested K562/IMA3 cells treated with the highest concentration at G2/M phase. Moreover, FTIR results showed that the transcription rate observed as 994/969 cm^{-1} ratio (RNA/DNA ratio) decreased 13.79% in K562/IMA3 cells. The glycogen/DNA ratio indicated by 1023/1021 cm^{-1} ratio decreased in K562 cells by 8.15% whereas it increased by 16.67% in K562/IMA3 cells. The intensity ratio of 1172/1011 cm^{-1} is accepted as the ratio of cholesterol esters/DNA and it decreased in both types of the cells. The order of the cellular DNA is expressed as 1069/1053 cm^{-1} decreased in K562 cells, whereas increased in K562/IMA3 cells.
Summary and Conclusions: Under the light of these results, ponatinib could affect strongly both imatinib-sensitive and resistant CML cells via cytotoxic, cytostatic, and apoptotic ways in a dose-dependent manner. Moreover, our results indicate that increasing concentrations of ponatinib causes significant structural and organizational changes in both imatinib-sensitive and -resistant K562 cells.

E1075

COMPARATIVE ANALYSIS OF BCR-ABL AND VEGFR2 INHIBITORY ACTIVITIES OF PONATINIB, AXITINIB, AND PF-114

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Background: Resistance to tyrosine kinase inhibitors (TKIs) in patients (pts) with chronic myeloid leukemia (CML) is frequently caused by mutations in the BCR-ABL kinase domain. The T315I gatekeeper mutation (BCR-ABL^{T315I}) confers uniform resistance to all approved TKIs except ponatinib, which has demonstrated deep and durable responses in heavily pretreated pts without or with BCR-ABL mutations, including T315I. However, at a starting dose of 45 mg, ponatinib treatment is also associated with an increased risk of arterial occlusive events. Ponatinib also inhibits a number of other kinases that may contribute to its adverse event profile, including VEGFR2. Recently, axitinib, a commercially available VEGFR2 inhibitor, was reported to have preclinical activity against BCR-ABL^{T315I}, and PF-114, a close analog of ponatinib, was reported to have T315I activity while exhibiting reduced inhibitory activity against VEGFR2.
Aims: To compare the anti-BCR-ABL and -VEGFR2 activities of axitinib and PF-114 to that of ponatinib, and where possible, relate these potencies to clinical PK parameters to address the potential for these compounds to be clinically effective.
Methods: The potencies of ponatinib, axitinib, and PF-114 were assessed using Ba/F3 cells whose viability was dependent on native BCR-ABL, BCR-ABL^{T315I}, or VEGFR2. IC_{50}s for ponatinib and axitinib were compared to "clinically effective" plasma concentrations (C_{eff}), which were derived using average steady-state levels in patients treated at recommended starting doses (C_{ave} 101 nM and 49 nM for 45 mg qd ponatinib and 5 mg bid axitinib, respectively), and corrected for the functional effects of protein binding that we measured *in vitro* (3.6-fold for ponatinib and 49-fold for axitinib). Analysis of crystal co-structures was used to assess detailed molecular ligand-protein interactions.
Results: Consistent with previously reported results, ponatinib potently inhibits the activity of native and T315I-mutant BCR-ABL, as well as VEGFR2, with IC_{50}s ≤5 nM (Table). These IC_{50}s are substantially below the C_{eff} of ponatinib in patients (28 nM). Axitinib is a similarly potent inhibitor of VEGFR2, with an IC_{50} (2 nM) that is close to its C_{eff} (1 nM). However, axitinib has substantially lower activity against T315I and native BCR-ABL (IC_{50}s 365 and 635 nM, respectively). PF-114 inhibits native BCR-ABL (13 nM), though less potently than ponatinib, and has substantially lower activity against not only VEGFR2, but also BCR-ABL^{T315I} (IC_{50}s 201 and 538 nM, respectively). A plausible explanation for this loss of potency is the introduction of an atom substitution into PF-114 that creates a repulsive molecular interaction with a conserved structural element in the hinge-binding region of the proteins.

Table: IC_{50} in Ba/F3 Cell Viability Assay (nM)

	Native BCR-ABL	BCR-ABL^{T315I}	VEGFR2
Ponatinib	1	5	3
Axitinib	635	365	2
PF-114	13	538	201

Summary and Conclusions: Neither axitinib nor PF-114 has a preclinical profile that would suggest greater potential clinical benefit than ponatinib. Concentrations of axitinib required to inhibit T315I and native BCR-ABL substantially exceed effective concentrations achievable in patients, and those required to inhibit its clinically validated target VEGFR2. In generating PF-114, structural modifications of ponatinib, designed to reduce its VEGFR2 activity, also result in reduced potency against native BCR-ABL, and especially against BCR-ABL^{T315I}. A dose ranging trial aimed at evaluating the benefit/risk of ponatinib treatment for patients with CML is planned.

E1076

CD26 ANTIGEN IS SPECIFIC FOR LEUKEMIC STEM CELLS IN CHRONIC MYELOID LEUKEMIA AND DISTINGUISHES PATIENT GROUPS WITH DIFFERENT RATIOS OF LEUKEMIC AND HEALTHY STEM CELLS

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Background: Putative leukemic stem cells (LSC) persist in chronic myeloid leukemia (CML) patients during treatment and constitute risk of relapse. To study LSC and develop a targeted therapy, it is necessary to define these cells. Herrmann *et al.* (Blood 2014) recently proposed that CML LSC are defined by expression of CD26. We therefore set to further confirm these results on a larger group of patients.
Aims: To analyze the stem cell population in a wider cohort of chronic phase CML patients based on CD26 expression and to analyze the disease burden in sorted CD26- and CD26+stem cells.
Methods: For 25 consecutive *de novo* patient samples, we FACS sorted CD45+34+38-/dim population into CD26- and CD26+cells fractions and analyzed the Ph burden in both fractions by FISH. Additionally, we re-analyzed the FACS data and CD26 expression on SC using a more stringent CD45+34+38- gate. Detection of BCR-ABL1 in sorted sub-fractions was performed using single-cell nested RT-PCR.

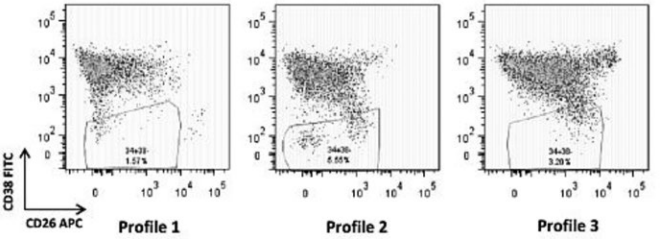

Figure 1.

Results: We FACS sorted CD26- and 26+SC populations and analyzed the percentage of Ph+cells in both fractions by FISH. This showed 96.2±9.3% (mean±SD) Ph+cells in the supposed CD26+LSC fraction, and 40,0±42,0% (mean±SD) Ph+cells in the CD26- fraction, regarded as HSC, which, however, did not correspond with the results by Herrmann *et al.* We suspected that the Ph positivity in CD26- fraction originated from cells with slightly higher CD38 expression (thus being CD38dim non-SC), included in the sorting to provide enough cells for FISH analysis. Therefore, we performed a re-analysis of all our FACS data with more stringent CD38- gate and re-analyzed the CD26 expression in the pure CD38- SC population. This revealed three clear CD26 SC expression profiles among the patients: 1 – dominant CD26- population; 2 – equal CD26- and +populations; 3 – missing CD26- population. The re-analysis results therefore supported our previous theory; where, for example, the patients analyzed with almost 100 % FISH Ph+cells in sorted CD26- fraction had no or very few (0-11%) HSC after the re-analysis (Profile 3). This confirmed that the FISH positivity in sorted CD26- fraction originated from the CD38dim/26- non-stem cells and proved the concept that CD26 expression

reflects Ph positivity only in the true hematopoietic CD34+38- compartment. To provide a further proof, we analyzed 3 samples using a nested RT-PCR protocol for single cell analysis. Here, we sorted 3-5 consecutive sub-fractions (each constituting 5-20 cells) for both CD26- and +populations, ranging from most CD38neg to CD38dim. CD26+population proved positive in all sub-fractions, whereas in CD26- population, a weak BCR-ABL1 positivity could be detected only for sub-fractions previously defined for re-analysis as CD38dim.
Summary and Conclusions: We have confirmed that CD26 based differentiation of CML HSC and LSC (CD45+34+38-) is applicable irrespective of CD26 expression profiles, showing different HSC/LSC ratios, and proved the concept that CD26 expression reflects Ph positivity only in strictly gated true hematopoietic CD34+38- compartment. The results also show that simultaneous sorting of pure CD26+LSC and CD26- HSC fractions from one patient will often be unfeasible due to low cell number in either population. These findings extend our knowledge on SC in CML and are important for further study and eradication attempts of LSC.

E1077

SPHINGOSINE-1-PHOSPHATE RECEPTOR 2/GQ/PHOSPHOLIPASE C AXIS IS A NOVEL MECHANISM TO OVERCOME NILOTINIB RESISTANCE IN T315I MUTATION EXPRESSING 32DCL3 MURINE CELLS

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Background: Chronic myeloid leukemia is characterized by the Philadelphia chromosome (Ph), encoding chimeric BCR-ABL with constitutive tyrosine kinase activity. Although tyrosine kinase inhibitors (TKIs) provide great success in the treatment, the development of resistance against TKIs is a main problem in CML patients. Despite the expression of mutant forms of BCR-ABL such as T315 mutation is a common reason for the development of nilotinib resistance, the alterations in sphingolipid signaling pathway is an alternative significant BCR-ABL1-dependent resistance mechanism. Some sphingolipid members such as ceramide have apoptotic effects while others like sphingosine-1 phosphate (S1P) cause the development of drug resistance and induce cell growth. Recently, we showed that sphingosine kinase-1 (SK-1)/sphingosine-1 phosphate (S1P)/ sphingosine-1 phosphate receptor 2 (S1P2)-mediated drug resistance is related to inhibition of protein phosphatase 2A (PP2A), causing increased stability of BCR-ABL1 (Salas *et al*. Blood 2011) However, specific signaling cascade involved in this process remains unkown.
Aims: Determine the details of S1P2/PP2A mediated-TKI resistance in a mechanistic manner by identifying intermediate players between S1P2 and PP2A.
Methods: BCR-ABL1 expressing 32Dcl3 cells, 32D-p210$^{Bcr-Abl}$(wt) and 32D-p210$^{Bcr-Abl}$(T315I) were used. The antiproliferative effects of nilotinib, SK-1 inhibitor (PF-543), S1P2 inhibitor (JTE-013), phospholipase C inhibitor (U-73122) and nilotinib/PF-543 and nilotinib/JTE-013 combinations on wt and resistant cells were determined by MTT assay. Isobologram analysis was performed using CompuSyn program. The mRNA and protein levels of BCR-ABL1, SK-1 and S1P2 were checked by qRT-PCR and western blotting. Resistant cells were also transfected with Gq peptide. The effect of U-73122/okadaic acid (PP2A inhibitor) and Gq peptide/ okadaic acid combinations on BCR-ABL1 levels were evaluated by western blot.
Results: Vector transfected control cells do not give any response to nilotinib while IC_{50} values were 8 and >500 nM for wt and resistant cells, respectively. IC_{50} values for JTE-013 were calculated as 20 and 40 μM while that for PF-543 were 8 and 30 μM, respectively. Nilotinib/PF-543 and nilotinib/JTE-013 combination studies showed strong synergistic effects on both cell types as indicated by MTT assay and isobologram results. Although there is no significant changes in BCR-ABL1 levels for wt and resistant cells, SK-1 and S1P2 levels increased in resistant cells. Combination studies caused significant decreases in BCR-ABL1 protein levels in resistant cells comparing to untreated control, PF-543 or JTE-013 alone treatments. Overall, these data suggest that nilotinib resistance is not related to BCR-ABL1 overexpression, but related to SK-1/S1P2 axis. Although U-73122 and Gq peptide treatments decreased BCR-ABL1 protein, their combination with okadaic acid restored BCR-ABL1 protein levels. Therefore, SK-1/S1P2-mediated nilotinib resistance could be overcome by activating PP2A through the inhibition of Gq and phospholipase C, which are the newly identified players in this signaling.
Summary and Conclusions: Our data suggest that BCR-ABL1 levels decreased by activating PP2A via Gq and phopholipase C inhibition, which is a novel mechanism reported for the first time. This proposed mechanism of nilotinib resistance could be a potential novel therapeutic target to overcome nilotinib resistance.

E1078

SINGLE NUCLEOTIDE POLYMORPHISMS IN APOPTOSIS PATHWAY ARE ASSOCIATED WITH RESPONSE TO IMATINIB THERAPY IN CHRONIC MYELOID LEUKEMIA

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Background: The mechanism of action of imatinib is known to involve the Fas-mediated apoptosis pathway. Consequntly inter-individual variations in this apoptosis pathway might be associated with imatinib response or resistance.
Aims: This study attempted to focus on 8 genotypes in the apoptosis pathway including *FAS* (rs1800682, rs2229521, rs2234767 and rs2234978), *FASLG* (rs763110), *CASP10* (rs13006529), and *APAF1* (rs1439123, rs2288713) and analyzed their association with treatment outcomes including molecular response with 4.5 log reduction (MR4.5), following imatinib therapy in 187 Korean CML patients.
Methods: Candidate genotypes were selected as non-synonymous SNPs in exon regions with a minor allele frequency over 0.01 or based on the literature review. The candidate SNPs were primarily evaluated for adequacy of Hardy-Weinberg Equilibrium (HWE) using the Chi-square test. HWE and genotype frequencies were calculated using Haploview software version 4.2 (Broad Institute, Cambridge, MA). For validation of the genetic effect, we performed internal validation using a bootstrap algorithm.
Results: The GG/GA genotype in *FAS* (rs2234767) showed a higher rate of MR4.5 than the AA genotype (at 5 years 59.7% *vs* 37.4%, p=0.013). Using a bootstrap procedure for internal validation we confirmed that FAS (rs2234767) correlates with MR4.5 (p=0.050). Multivariate analysis confirmed that the *FAS* genotype (rs2234767) is an independent surrogate for MR4.5 (p=0.019, HR 0.43, 95% CI [0.22-0.87]).
Summary and Conclusions: In conclusion, the Fas/FasL signaling pathway may represent the major pathway that mediates apoptosis in CML treated with imatinib. SNP markers in the apoptosis pathway including *FAS* genotype (rs2234767) can be potential surrogates for predicting deeper molecular response after imatinib therapy.

E1079

THE ROLE OF MICRORNAS IN RESPONSE TO NEW TARGETED THERAPIES IN CHRONIC MYELOID LEUKEMIA

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Background: Imatinib is, at present, the first-choice treatment for chronic myeloid leukemia (CML) patients. Despite the impressive success of treatment obtained with this drug, some patients show incomplete response or resistance to Imatinib. The BCR-ABL oncoprotein, resulting from the *BCR-ABL* fusion gene, characteristic of CML, is able to activate multiple signaling pathways, including the NF-kB, mTOR and the proteasome pathways, which may provide new therapeutic targeted alternatives in cases of resistance to Imatinib. Furthermore, the altered expression of microRNAs, small RNA molecules that regulate gene expression, may influence the sensitivity and/or acquisition of resistance to therapy.
Aims: Our goal was to evaluate the influence of the expression levels of miR-21, miR-125b, and miR-155 in CML cell lines sensitive and resistant to Imatinib, as well as the therapeutic potential of new drugs such as Bortezomib, Parthenolide, and Everolimus.
Methods: In this work we used two CML cell lines, one sensitive to Imatinib (K562 cells) and another resistant to Imatinib (K562 RC cells). Cell viability was assessed by rezasurine assay and cell death by flow cytometry (annexin V and propidium iodide) and through optic microscopy (May-Grünwald-Giemsa staining). The expression of BAX, BCL-2, phosphorylated NF-kB, ubiquitin conjugates, and cell cycle analysis was performed by flow cytometry. The expression levels of AKT and AKT activation were analyzed by Western Blot. MiRNAs expression was performed by qRT-PCR using commercial kits.
Results: Initially we observed that K562 RC cells had increased expression of miR-21 and miR-125b as well as decreased expression of miR-155 in relation to K562 cells. Bortezomib, Parthenolide and Everolimus were able to induce a

decrease in cell viability in a time-, dose- and -dependent manner. Parthenolide and Bortezomib induce a stronger cytotoxic effect on K562 cells resistant to Imatinib, while Everolimus shows a higher cytotoxic effect on K562 cells sensitive to Imatinib. These results may be related to the differential expression of miRNAs observed in the cell lines. These compounds induce cell death predominantly by apoptosis and also induce cell cycle arrest. Furthermore, Bortezomib induced a decrease in NF-κB levels and an increase in ubiquitin levels in both cell lines and may represent a possible therapeutic alternative for CML patients resistant to Imatinib. Moreover, it was found that Parthenolide influences NF-κB levels in a dose and cell type-dependent manner, since small doses induce a decrease of NF-κB in Imatinib-sensitive cells, while high doses induce a decrease of NF-κB in the resistant cell line and an increase in the sensitive cell line. Additionally, it was also observed that treatment with these compounds was also able to modulate miRNAs expression levels, which can influence therapy response.

Summary and Conclusions: In summary, miR-21, miR-125b and miR-155 expression levels could provide new biomarkers predictive of response to TKI and/or new targeted drugs such as Bortezomib, Parthenolide and Everolimus in CML. Moreover, these drugs may be new therapeutic approaches in CML, specially Bortezomib and Parthenolide, namely as an alternative therapy in CML patients with resistance to Imatinib. *This work was supported by CIMAGO and R. Alves is supported by the FCT fellowship FRH/BD/51994/2012.*

E1080

A STUDY OF CELL CYCLE KINETICS AND OF KU80 EXPRESSION AS A MARKER OF GENETIC INSTABILITY IN BONE MARROW MESENCHYMAL STEM CELLS OF PATIENTS WITH CHRONIC MYELOID LEUKEMIA

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Background: In chronic myeloid leukemia (CML), there is a well-characterized lesion of the haemopoietic stem cell; however, mechanisms underlying disease initiation and progression are unclear. Therefore, the role of bone marrow (BM) stromal cells in disease pathogenesis merits further investigation. In myelodysplastic syndromes, BM mesenchymal stem cells (MSCs) were shown to have a proliferation defect in comparison to BM MSCs of healthy individuals.
Aims: We first examined BM MSCs of CML for cytogenetic abnormalities. We then studied kinetics of BM MSCs from CML patients and compared them to those of BM MSCs from healthy controls. We also measured expression of the Ku80 protein, a protein involved in non-homologous end joining of double strand DNA breaks, as a marker of genetic instability. We compared Ku80 expression among BM MSCs of CML patients, adipose tissue (healthy tissue) MSCs of CML patients and BM MSCs of healthy individuals.
Methods: Samples of seven (7) CML patients (6 diagnosed in chronic phase and 1 in blast crisis) were collected before onset of therapy, along with samples of 4 healthy controls. MSCs were isolated from the mononuclear cell fraction of BM or adipose tissue via a limited number of passages, removing non-adherent-to-plastic cells. Purity of MSCs from haemopoietic cell contaminants (>94%) was confirmed by flow cytometry; mesenchymal stem cells were positive for CD90, CD73 and CD105 and negative for CD34, CD45 and CD14. Karyotyping was performed in BM MSCs of CML patients. BrdU and 7-AAD staining of BM MSCs to detect S-phase and apoptotic cells, respectively, was performed via standard protocols and stained cell populations were analyzed with flow cytometry. Cell lysates of BM and adipose tissue MSCs were used in standard SDS electrophoresis. Western blotting with the use of Ku80 and actin antibodies was performed.
Results: Karyotypes of BM MSCs of CML patients were normal. Percentage of S-phase cells and apoptotic cells was compared between patient BM MSCs (20,4% S-phase, 2,7% apoptosis) and control BM MSCs (19,3% S-phase, 4,6% apoptosis). Mean values (t-test) did not have a statistically significant difference (p=0,88% for S-phase percentage and p=0,92% for apoptosis). The ratio of expression Ku80/actin, calculated by densitometry of Western blotting bands, did not differ to a statistically significant degree among: patient BM MSCs, adipose tissue MSCs and control BM MSCs (one-way ANOVA for 3 groups, p=0,28).
Summary and Conclusions: Bone marrow mesenchymal stem cells in CML are known to lack the BCR-ABL fusion gene, but their properties and possible contribution to disease pathogenesis have not been thoroughly researched. This is the first study of kinetics of BM MSCs in CML in the literature. We have shown that BM MSCs display a normal karyotype and have a proliferative and apoptotic potential equivalent to MSCs of healthy individuals. We have additionally shown that expression of Ku80, a marker of genetic instability (double strand DNA breaks), does not significantly differ between BM MSCs of patients and those of adipose tissue MSCs or MSCs of healthy donors. Further research is warranted on properties of MSCs in CML, also assessing whether these properties can be exploited in bone marrow transplantation strategies.

E1081

BCR-ABL EXPRESSION IS REDUCED IN THE PRIMITIVE PRECURSOR FRACTION FROM CML TKI-TREATED PATIENTS.

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Background: Insensitivity to imatinib is a property exhibited by a population of leukemic rare, primitive stem cells that are present in most chronic myeloid leukemia (CML) patients, even in those with sustained undetectable molecular residual disease (UMRD). It remains unclear whether such patients are definitively cured or whether leukemic stem cells (LSCs) that persist in their bone marrow represent a risk of disease recurrence. This suggests that the characterization of LSCs by functional assays could be of interest in clinical practice.
Aims: To evaluate the burden of LSCs in TKI-treated patients.
Methods: We evaluated the presence of LSCs in patients with CML at different levels of molecular response induced by imatinib or nilotinib, and patients naïve of treatment. All patients gave their informed consent. Bone marrow-derived $CD34^+$cells were used as initiators for 2-week CFU-C and 6-week long-term culture-initiating cell (LTC-IC) assays. Their phenotype was defined using RT-qPCR on individual (8-10 colonies per patient) and pooled colonies (24-30 colonies per patient).
Results: In samples from newly diagnosed patients, BCR-ABL mRNA expression was measured in individual colonies from CFU-C assays (mean %BCR-ABL/ABL=24.0, SD=7.3, n=4). However, in LTC-IC assays, BCR-ABL mRNA only became detectable when measured in pools of 3-15 colonies (mean %BCR-ABL/ABL =6.6%, SD=9.6, n=3). BCR-ABL mRNA levels were highly variable in LTC-IC-derived pooled CFU-Cs, but still lower than in 2-week CFU-Cs (ANOVA, $p<0.05$) (Figure 1a). In patients under TKIs treatment, BCR-ABL transcripts were only detectable in LTC-IC-derived pooled CFU-Cs from patients with a high burden of disease (n=7, %BCR-ABL/ABL in peripheral blood=1.1-7.7%), whereas no BCR-ABL transcripts were detected either in individual colonies from patients with a high burden of disease, nor in individual or pooled colonies from patients with deeper responses (n=5). We observed a tendency of reduction in BCR-ABL/ABL ratios in LTC-IC-derived CFU-Cs in accordance with a reduction in disease burden, measured at peripheral blood and in response to TKIs treatment (Figure 1b). On the other hand, the number of colonies (expressed as the square root of no. colonies per 1,000 initiating cells) obtained in LTC-IC assays was higher in patients showing a deep molecular response (defined as at least $MR^{4.0}$) than for patients with a high burden of disease (at diagnose or with at least BCR-ABL/ABL of 1%) (ANOVA, $p<0.01$).

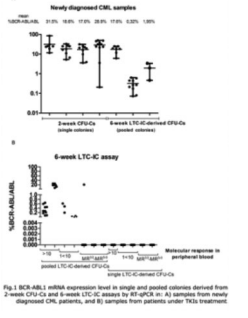

Figure 1.

Summary and Conclusions: We observed a reduction in BCR-ABL/ABL ratios in LTC-IC-derived pooled colonies compared to CFU-Cs in newly diagnosed patients. Given that these patients have not received any TKI treatment, this seems to be an intrinsic characteristic of primitive precursors, unrelated to exposure to TKI selective pressure. On the other hand, as the burden of disease was reduced by TKI treatment, lower BCR-ABL/ABL ratios were detected in the primitive fraction, suggesting that the ability to detect rare residual LSCs in TKI-treated patients will be limited by BCR-ABL expression levels. Despite that measuring on pooled colonies may be masking a dilution effect, the same tendency has been reported for HSCs and progenitor fractions isolated by sorting (Kumari et al, Blood 2012, 119:530-539), further suggesting that LSCs are not oncogene-addicted. Therefore, the results obtained in this work support the hypothesis that evaluation of minimal residual disease in candidates for discontinuation studies would benefit from the measurement of Ph^+primitive precursors cells at DNA level, so as to accurately assess LSCs persistence.

E1082

TRIM32 E3 LIGASE AS A TARGET IN CHRONIC MYELOID LEUKAEMIA

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Background: Chronic myeloid leukaemia (CML) is a clonal myeloproliferative disorder characterised by the presence of the BCR-ABL fusion gene. The gold standard treatment for CML is the tyrosine kinase inhibitor (TKI) imatinib. Although imatinib and second generation TKIs induce durable responses, TKI resistance is a major issue and additional therapeutic options are still required. Proteasome inhibitors have shown efficacy in the treatment of haematological malignancies which has validated the ubiquitin proteasome system (UPS) as a therapeutic target; there is now interest in targeting of specific components of the UPS, such as E3 ligases and deubiquitinating enzymes. We have shown previously that the proteasome inhibitors, bortezomib and carfilzomib, potently induce apoptosis in imatinib-sensitive and -resistant CML cell lines and primary CD34+CML cells, suggesting that CML cells may be sensitive to targeting of other UPS components. Gene expression profiling of CML CD34+cells compared to normal CD34+cells using UPS-specific microarrays (PIQOR) identified that the E3 ligase TRIM32 was overexpressed in CML (p=0.02). TRIM32 is a member of the TRIM family of RBCC proteins; it contains a RING finger domain, one or two B-boxes, and a predicted coiled-coil domain. Studies have shown TRIM32 to be involved in the ubiquitination and degradation of the tumour suppressor proteins abelson interactor (ABI)-2 and p53.
Aims: The aim of this study was to examine the expression of TRIM32 in CML cell lines and investigate its effects on ABI-2 and p53.
Methods: TRIM32 expression in KCL22 and LAMA84 imatinib sensitive and resistant CML cell lines was analysed by qPCR and Western Blotting. ShRNA-mediated gene knockdown was used to analyse the effects of TRIM32 on cell viability (*CellTiter-Glo*®) and cell cycle (flow cytometry analysis of propidium iodide stained cells). Following knockdown, qPCR and western blotting were used to validate the change in TRIM32 expression. Effects of TRIM32 knockdown on ABI-2 and p53 were analysed by Western Blotting.
Results: Increased expression of TRIM32 was validated at both the gene and protein level in CML cell lines compared to normal cells. Following knockdown of TRIM32 expression with shRNA, a 14.8±8.8% decrease in cell viability was determined with a statistically significant decrease (p= *0.009*) observed in KCL22-sensitive cells. This decrease in cell viability was associated with an increase in the subG0 population in each CML cell line. TRIM32 knockdown resulted in an increase in ABI-2 and an increase in p53 protein levels. Furthermore we show that combining knockdown of TRIM32 with imatinib and nilotinib treatment increases the sensitivity of CML cell lines to theTKIs.
Summary and Conclusions: In this study we demonstrate that TRIM32 is overexpressed in CML cells at both the RNA and protein levels. Knockdown of TRIM32 results in decreased cell viability and increased apoptosis. This was associated with an increase in expression of the tumour suppressors ABI-2 and p53. TRIM32 knockdown also increased sensitivity of CML cells to TKIs. Loss of ABI-2 has been previously shown to contribute to BCR-ABL+leukaemia progression and suppression of p53 has been seen to enhance survival and proliferation of CML. These studies suggest that TRIM32 may be a relevant therapeutic target in CML and further study is warranted.

E1083

SELECTION OF RUNX1-MUTATED CLONE ASSOCIATED WITH RELAPSE AND BLAST CRISIS IN CHRONIC MYELOID LEUKEMIA PATIENT AFTER ALLO-HSCT AS REVEALED BY TARGETED ENRICHMENT AND DEEP SEQUENCING

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Background: Advanced phase of chronic myeloid leukemia called blastic phase or blast crisis (CML-BC) is incurable, despite success of targeted therapy for the chronic phase CML (CML-CP). CML-BC is characterized by successive increase in amount of blast cells in blood and bone marrow but pathogenesis is still poorly understood. Majority of genomic studies are focused on detecting clonal abnormalities, which arise during the progression of the disease and are present only in advanced phase of the disease. However, recent findings revealed, that in addition to the t(9;22) translocation, CML-CP at diagnosis may already harbor a variety of sporadic genetic aberrations affecting the prognosis and treatment.
Aims: We aimed at employing targeted deep-sequencing strategy to dissect the underlying genetic cause of clonal evolution and rapid progression to CML-BC in a patient who relapsed after allogeneic haematopoietic stem cell transplantation (HSCT), developed lymphoid CML-BCs and died within 18 months.
Methods: Targeted enrichment strategy (NimbleGene SeqCap EZ Choice custom gene panel comprising almost 1000 genes) was applied followed by massive-parallel sequencing using the Illumina HiSeq1500 system and chemistry. The data were processed by the CASAVA. Reads were mapped to the reference genome (hg19 build) using Burrows-Wheeler Aligner 6.2 and processed using Genome Analysis Toolkit. The detected variants were annotated using Annovar. Alignments were viewed with Integrative Genomics Viewer.
Results: Female patient (30-yrs old) was diagnosed with CML in 12/2005 (low Sokal and Hasford score and no detectable clonal cytogenetic abnormalities (CCA) additional to Philadelphia chromosome). Within 6 months from the diagnosis patient underwent allo-HSCT and achieved 100% chimerism and complete cytogenetic remission (CCyR) but not a major molecular response (MMR). After 4 months, patient relapsed with lymphoid BC with no detectable *BCR/ABL1* mutation at that time. Despite intensive chemotherapy and imatinib patient developed within few months 2nd BC and Y253H mutation in *BCR-ABL1* was detected. Then dasatinib was added to chemotherapy and patient obtained partial remission. Unfortunately, 14 months after transplantation patient developed 3rd BC with detectable *BCR-ABL1* T315I mutation and died. Since there were no CCA detected at any time point, we screened four sequential DNA samples (peripheral blood leukocytes): one from the time of diagnosis and three samples from three consecutive BCs with targeted deep sequencing. We detected a nonsense R320* *RUNX1* mutation, resulting in carboxyl-terminal truncated RUNX1 protein in all 4 samples. Importantly, this mutation was already present in the diagnostic sample, although the prevalence of mutated clone was low (1%). In the BC samples *RUNX1*mut. clone rised and ranged between 90% to 50% of CML blasts (Figure 1). We have also confirmed both *BCR-ABL1* mutations previously detected with standard sequencing. Interestingly appearance of T315 clone in the 3rd BC was associated with lower frequency of *RUNX1* mutated clone (Figure1). No *BCR-ABL1* gene mutation was detected by NGS sequencing in diagnostic sample nor in the 1st BC. Additionally, missense mutation in *ASXL1* (Q1102N) was detected in all samples, consistent with observation in acute myeloid leukemia (AML),where mutations in *ASXL1* frequently co-occurs with *RUNX1*.

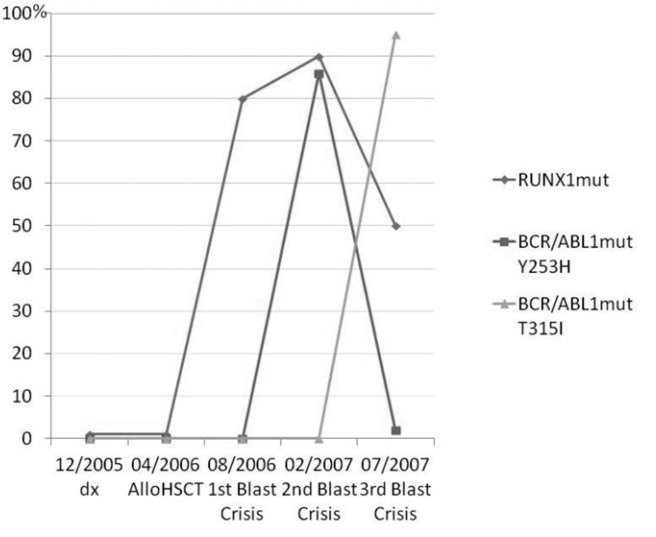

Figure 1. Frequency of RUNX1-mutated and BCR-ABL1-mutated clones during progression and subsequent CML-BCs.

Summary and Conclusions: Deregulation of RUNX1 transcription factor by chromosomal translocation or mutation are common events in haematological malignancies, especially in AML. In CML-BC *RUNX1* mutations have been reported in few studies. We have shown that *RUNX1* mutation may be already present in CML -CP at diagnosis and may potentially occur as a pre-leukemic event prior to t(9;22) translocation (as also recently described by Schmidt *et al.*, Leukemia 2014; 28(12); 2292-9). Genetic screening of a large cohort of patients at diagnosis may answer if presence of CML cells with *RUNX1* mutation at diagnosis confers a negative prognostic factor.

E1084

NANOPORE SEQUENCING TO RAPIDLY CHARACTERISE LEUKEMIA-SPECIFIC GENOMIC TRANSLOCATION BREAKPOINTS

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Background: The identification of leukemia associated fusion genes is critical for the correct diagnosis, choice of therapy and molecular monitoring of hematological malignancies. Although usually inferred by cytogenetic analysis, the precise identification of fusion genes typically requires specific PCR amplification from cDNA and careful positioning of primers to enable detection of all possible mRNA variants. Identification of gene fusions from genomic DNA is

technically much more demanding since for many genes the breakpoints are dispersed over large regions, for example >100kb in the case of *ABL1*. Nevertheless, the identification of genomic breakpoints provides some potential advantages, for example patient-specific molecular monitoring using genomic DNA enables the cellular burden of residual disease to be estimated much more accurately than RT-PCR based methods. Furthermore, genomic DNA assays facilitate much more sensitive detection of residual disease. We have previously described long range PCRs (up to 10 kb) that amplify genomic breakpoints of two fusions, *BCR-ABL1* and *FIP1L1-PDGFRA*, with subsequent breakpoint identification by more focused PCR and Sanger sequencing. It is expected that new sequencing technologies will simplify this process, ideally allowing *ab initio* identification of any fusion gene present from genomic DNA. The Oxford Nanopore MinION is a miniature sensing system currently adapted for DNA single molecule sequence analysis. A DNA strand passes through a nanopore which registers a change in current allowing determination of nucleotide sequence. The MinION device is currently being made available by Oxford Nanopore though an early access program.
Aims: To assess Nanopore technology for sequencing long range PCR products to identify precise translocation breakpoints.
Methods: Long range PCR products encompassing genomic fusions were amplified from *BCR-ABL1* (n=4) and *FIP1L1-PDGFRA* (n=5) positive samples. PCR products were processed according to the Oxford Nanopore genomic DNA protocol which involves end repair, dA-tailing, ligation of adapter, HP motor and tether. *FIP1L1-PDGFRA* fragments were sequenced using R6 flow cell chemistry and *BCR-ABL1* fragments using R7 chemistry. Samples were run sequentially with washing between samples. Reads were aligned to the reference genome and split at their 'soft' or 'hard' clip sites using an in-house programme. The resulting daughter reads were subjected to another round of alignment in order to finely map fusion breakpoints.
Results: A median of 40 reads (range 7 - 458) passing internal quality control were obtained per sample, of these 26% (11%>100%) were 2D reads *i.e.* were read in both directions and therefore of higher quality. Median read length of 2D reads was 2980bp (range 318-7729) and consistent with PCR fragment sizes. Only 2D reads were considered in subsequent analyses. During the initial alignment phase 61% (33-100%) aligned to within 50bp of one of the amplification primer sequences. The location of the breakpoints determined by Nanopore sequencing were fully concordant with breakpoints determined by focused PCR and Sanger sequencing in five samples, the remainder had insufficient read depth.
Summary and Conclusions: We conclude that LR-PCR and Nanopore sequencing provides a rapid approach to characterize genomic breakpoints in leukemia. Nanopore technology is rapidly improving and recent developments promise improved accuracy, particularly for 2D reads.

E1085

THE STUDY OF FUNCTIONAL ACTIVITY OF CD34 CELLS IN CML PATIENTS WITH DIFFERENT RESPONSE TO IMATINIB THERAPY

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Background: It is believed that the reason of the leukemic clone cell resistance to treatment with tyrosine kinase inhibitors in CML patients is mutations in the genome of an early bone marrow progenitor cells that are CD34-positive. Such cells, regardless of treatment, acquire ability to proliferation and differentiation. This leads to the re-expansion of the CD34-positive cells.
Aims: was to determine the features of CD34 antigen expression in bone marrow (BM) and peripheral blood (PB) in CML patients with different response to imatinib therapy using the results of hematopoietic cells culturing and the data of flow cytometry.
Methods: We studied the BM samples of 41 CML patients in chronic phase of disease treated with imatinib for at least 12 months. To determine the functional activity of hematopoietic cells of patients *in vitro* culturing of BM mononuclear cells in semisolid agar ("Difco", USA). To assess the phenotypic characteristics of hematopoietic cells of patients the method of direct cytometry was used. For this purpose, BM mononuclear cells were stained with monoclonal antibodies (Becton Dickinson, USA).
Results: The results of cultivation suggest that in patients with an optimal response to imatinib therapy the number of colonies was 1.8 times lower than the number of those in the group of patients with suboptimal response to therapy. In turn, in patients with failure of imatinib therapy the number of colonies was the highest and was 2.1 times higher than the patients with optimal response. In the case of clusters was recorded tendency to increase this indicator in patients with suboptimal response and treatment failure. In addition, the patients with inefficiency and suboptimal response to imatinib therapy had the significantly higher proliferative potential than in patients with optimal response. This may indicate that early progenitor cells dominated the more differentiated cells which form clusters in the culture. In turn, in the BM of patients who have increasing numbers of leukemic clone cells proliferation processes dominate over the processes of differentiation that may be the cause of the increase of the number of colonies in semisolid agar *in vitro.* The results of cytometry analysis showed that in patients with the acquisition of resistance to imatinib the number of $CD34^+$cells increases in the BM and PB. It should be noted, in patients with different response to therapy the number of CD34 antigen in BM and PB was significantly different. This may indicate that during the progression of CML and during the acquisition of resistance to the drug by leukemic clone cells the pool of early progenitor cells not only expands, but also, due to the weakening of contacts between the cells of the microenvironment and leukemic clone cells, the early progenitor cells fall in blood flow in large numbers, promoting a poorly differentiated hematopoietic cells in PB of patients. A correlation analysis was conducted to identify the correspondence between the number of CD34 cells in the BM and in PB of patients treated with imatinib. These results indicate a direct correlation between the number of colonies and clusters in semisolid agar *in vitro* and the number of CD34 cells in the patient BM (R=0,83 and R=0,58, $p<0.05$).
Summary and Conclusions: Thus, the number of CD34 cells in the BM of patients increases with the acquisition of leukemic clone cells resistance to imatinib. The correlation between the number of CD34 cells and the number of cell aggregates in semisolid agar *in vitro*, which may be predictive method for determining the future course of CML, shows the prognostic value of method for determining the CD34 cells in the BM of patients; and a parallel increase of their number in PB will allow to develop express methods for the detection of individual patient response to imatinib therapy.

E1086

TYROSINE KINASE INHIBITOR THERAPY INDUCES ALTERATIONS OF CD137 AND CD137L EXPRESSION IN CHRONIC MYELOID LEUKEMIA PATIENTS

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Background: CD137 (4-1BB, TNFRSF9) is a member of the TNFR superfamily that provides expansion and survival signals to T cells. In addition to the ability to costimulate T cells, CD137 ligand (CD137L) also signals back into antigen presenting cells, promoting their activation and differentiation. Recently, CD137 has been proposed as a therapeutic target to improve and sustain anticancer immune response. Expression of CD137 or CD137L was described in T leukemia and B lymphoma activated cell lines, respectively, and soluble CD137L has been found in sera of leukemia patients. However, the role of these costimulatory molecules in hematologic malignancies is still unknown.
Aims: The aim of the present study was to evaluate CD137/CD137L role in Chronic Myeloid Leukemia (CML) patients treated with tyrosine kinase inhibitors (TKI).
Methods: In this study, approved by the Hospital and University Center of Coimbra ethics committee, peripheral blood mononuclear cells from 60 patients with CML, treated with imatinib mesylate and 39 blood donors were analyzed for membrane expression (CD137 and CD137L) and intracellular cytokine (IFNγ and TNFα) content in T (CD3+, CD4/CD8), B (CD19) and NK (CD3-CD56+) cells using flow cytometry. *In vitro* 4-hours stimulation with LPS or PMA/ionomycin was performed in order to evaluate the impact on CD137/CD137L expression. Soluble CD137 was analyzed by ELISA in patients sera and supernatants of stimulation experiments. Gene expression profiling for cytokines (IFNG, IL1B, IL2, IL4, IL6, IL10, IL12, TNFA, TGFB), granules (perforin, granzyme and granulysin), caspases (CASP3 and CASP9) and reference genes (ATP5B, SDHA and UBC) was performed for all samples. Additionally, miRNA profiling was performed by RT-qPCR.
Results: Soluble CD137 was significantly increased in sera and supernatants of *in vitro* stimulated PBMCs from CML patients. CD137 and CD137L were found in T (CD4 and CD8), B and NK cells from CML patients. CD137 was mainly expressed in CD8+T cells and CD137L was significantly detected in CD4+T cells, but was also found in B and NK cells. Expression of CD137 and CD137L positively correlated with TKI dose. These results were accompanied by the increased production of IFN-γ. Gene expression of IFN-γ, granzyme B and perforin was found to be upregulated in TKI-treated patients, compared to TKI-*naïve* patients and healthy controls. Analysis of miRNAs that were predicted as targeting TNFRSF9 and TNFSF9, revealed a significant increase of hsa-mir-886-3p. The analysis of miRNAs associated with CD137 signaling pathway and the induction of IFN-g via the ADAP-CBM signaling module (Lck, Fyn, ADAP, CARMA/CARD11, Bcl-10, MALT1, c-Jun and IFN-γ) showed no correlation in TKI treated CML patients.
Summary and Conclusions: Taking together, our results suggest that CD137/CD137L signaling should be further investigated in CML patients undergoing TKI therapy to improve and sustain anticancer immune response.
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E1087

METABOLIC CHARACTERISTICS OF IMATINIB RESISTANT CHRONIC MYELOID LEUKEMIA WITH T315I MUTATION

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Background: Imatinib resistant chronic myeloid leukemia (CML) cells made by treatment of low dose imatinib in long period has known metabolic phenotypes as more glycolysis and fatty acid synthesis compared with imatinib sensitive cells. But metabolic characteristics of T315I mutant CML cells which accounts for ~20% of the total burden in CML patients remains unknown.
Aims: Differenced metabolic characteristics of T315I mutant in CML cells could provide opportunity as therapeutic target. Two human CML cell lines, KBM5 (imatinib-sensitive) and KBM5-T315I (imatinib-resistant) were analyzed to assess metabolic phenotypes, including cell proliferation, oxygen consumption, lactate production and redox state.
Methods: Metabolic phenotype between KBM5 and KBM5-T315I without imatinib has been identified as the therapeutic target by molecular biologic methods including measurement of cell viability, western blot and realtime-PCR.
Results: Unlike previously reported imatinib resistant cells, we found that genetically mutated T315I cells showed decreased cell proliferation rate, lactate production, fatty acid synthesis and Glucose-6-phosphate dehydrogenase (G6PD), which is enzyme in the pentose phosphate pathway by maintaining the level of the co-enzyme nicotinamide adenine dinucleotide phosphate (NADPH), compared with KBM5 without imatinib. This result correlated with decrease of cellular reactive oxygen species (ROS) and mRNA expression of ROS scavengers, including SOD1, 2, catalase and enzymes related gulutathione, which is an important antioxidant. Although basal mitochondrial oxygen consumption had no difference between KBM5 and KBM5-T315I, mitochondrial maximal capacity and Isocitrate dehydrogenase 2 (IDH2) was higher in T315I mutant.
Summary and Conclusions: Thus, we described changed specific metabolic markers in T315I mutant in CML. Differenced metabolic characteristics of T315I mutant might be considered for finding therapeutic target of imatinib resistance.

E1088

MISSPLICED GSK3B TRANSCRIPT IS UNIVERSALLY EXPRESSED AND CANNOT BE USED TO PREDICT BLAST CRISIS TRANSFORMATION IN CHRONIC MYELOID LEUKAEMIA

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Background: GSK3β (glycogen synthase kinase 3β) has been shown to be misspliced in its kinase domain in granulocyte/macrophage precursors (GMP) in blast crisis (BC) samples of chronic myeloid leukaemia (CML). The misspliced GSK3β could enhance β-catenin expression and self-renewal ability of CML BC progenitors. Moreover it was also demonstrated that the misspliced GSK3β was not detected by sequencing in more primitive CML blasts or normal samples.
Aims: Here we investigated the presence of the misspliced form of GSK3β transcript in a broader population of peripheral blood white cell samples from CML chronic phase (CP), blast crisis (BC), *de novo* Ph+acute leukaemia, healthy volunteers and *in vitro* immortalised cell lines.
Methods: Peripheral blood white blood cells were separated and RNA was extracted and cDNA synthesised. Long range RT-PCR was performed to amplify the full length of the GSK3β coding region. Nested PCR steps were subsequently used to amplify exons 1-6 and exons 5-12. In addition, nested PCR steps were performed to amplify exons 6-10 and exons 10-12. PCR products were then run on an agarose gel. Products on the agarose from selected samples were purified from the gel and cloned into p-GEMTeasy vector to be sequenced. Allele specific real-time RT-PCR primers were designed to quantify the transcript level of misspliced GSK3β (exon 8 and 9 deleted). The specificity of the allele specific PCR primers were confirmed by PCR product direct sequencing using both forward and reverse primers. The misspliced GSK3β transcript levels were normalised to the levels of dominant GSK3β transcripts (GSK3β with exon 9 deleted). The misspliced GSK3β to dominant GSK3β transcript ratio in KYO-1 cells was considered as an arbitrary unit of 1.0.
Results: It was found the misspliced GSK3β transcripts could be detected in CML CP (6/12), BC (12/13), *de novo* Ph+acute leukaemia (7/9), the CML cell lines KYO-1, LAMA84, the T cell leukaemia line Jurkat, and the hepatoma cell line HepG2. The dominant GSK3β transcript in all samples tested was GSK3β with exon 9 deleted. It was showed by using allele specific real-time RT-PCR that CD34+cells separated from newly diagnosed CP CML had greater levels of misspliced GSK3β than matched mononuclear cells (n=6, p=0.028, Wilcoxon signed-rank test). For 9 cases of CML who had transformed to BC, the misspliced GSK3β levels at BC stage were greater than those at the CP stage (p=0.008, Wilcoxon signed-rank test). However no significant difference was found between CML CP (n=50), CML BC (n=34) and *de novo* Ph+acute leukaemia (n=15). Furthermore, at diagnosis of CP (prior to imatinib treatment), there was no significant difference in misspliced GSK3β levels between patients who achieved complete cytogenetic response after 12 months treatment (n=26), those who had no cytogenetic response (n=23) and those who subsequently progressed to BC (n=6).
Summary and Conclusions: The misspliced GSK3β transcript can be detected at various levels in a wide range of cells from CML CP, CML BC and healthy volunteers. Transcript levels of the misspliced GSK3β do not appear to be useful as a prognostic marker to predict the progression of CML.

E1089

DISCOVERING ALTERNATIVE TARGETS IN CHRONIC MYELOID LEUKEMIA (CML): DETERMINATION OF EXPRESSION LEVELS OF BIOACTIVE SPHINGOLIPID GENES IN NEWLY DIAGNOSED AND DRUG-RESISTANT CML PATIENTS

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Background: Bioactive sphingolipids, found in cellular membranes, are important molecules that have vital roles affecting several cellular events such as proliferation, apoptosis, senescence, cell cycle arrest, angiogenesis, and transformation. Ceramide, the central molecule of bioactive sphingolipid metabolism, is synthesized *de novo* by the activity of ceramide synthases (CERS1-6), whereas clearence of ceramide is carried out by the activity of glucosylceramide synthase (GCS) and sphingosine kinase 1 (SK1). While ceramide is a powerful pro-apoptotic molecule, glucosylceramide generated from ceramide via GCS, and S1P generated from ceramide via SK1 are powerful anti-apoptotic molecules. Previous studies from our lab revealed that increased ceramide or decreased GCS and SK1 levels in tyrosine kinase inhibitor (TKI)- sensitive and –resistant CML cell lines result in trigger of apoptotic pathways while increased intracellular GCS and SK1 levels trigger the pathways related to cell survival. Additionally, for the first time, we revealed that sensitivity of CML cell lines against TKIs can be increased via targeting bioactive sphingolipids. So far, there is no study about the roles of bioactive sphingolipids in CML patient samples.
Aims: In this study, our goal was to determine the potential relation between bioactive sphingolipids and disease progression in CML patients newly diagnosed, TKI-treated and shown hematological response (HR), and also TKI-resistant. Our additional goal was to confirm our previous data obtained in CML cell lines.
Methods: Bone marrows were harvested from CML patients with different disease profiles, and mononuclear cells were isolated by lysis buffer. cDNA was synthesized by reverse transcription from RNAs isolated from the cells. Expression levels of CERS1, -2, -3, -4, -5, and -6, GCS, SK1, and BCR/ABL genes were analyzed via quantitative real-time PCR. In this study, totally 66 CML patients including 33 newly diagnosed, 14 imatinib-treated, 2 nilotinib-treated, 3 dasatinib-treated, 6 imatinib-resistant, 1 nilotinib-resistant, 1 dasatinib-resistant, 1 dasatinib&nilotinib-resistant, 2 imatinib&nilotinib-resistant, and 3 blastic phase were involved.
Results: Our results revealed that expression levels of CERS1-6 genes were higher in the patients treated with TKIs and shown HR than the patients newly diagnosed or TKI-resistant. GCS and SK1 expression levels were markedly greater in TKI-resistant patients than that of newly diagnosed or TKI-treated and shown HR. Moreover, comparison of the expression levels of the genes mentioned above in bone marrow samples harvested from the particular patients at once in six months periods showed that while CERS1, -2, -3, and -6 expressions increased, GCS and SK1 expressions decreased by the time in the patients treated with TKIs and shown HR. Additionally, GCS and SK1 expressions were found increased, whereas expression levels of CERS genes decreased in the patients developing TKI-resistance.
Summary and Conclusions: The results of this study revealed for the first time that there might be a strong relation between TKI-sensitivity or resistance and the expression levels of bioactive sphingolipid genes in CML patients. More importantly, our results demonstrated that expression levels of bioactive sphingolipid synthesis genes might be decisive markers for predicting TKI-resistance as well as novel targets for more effective therapy in CML patients. Our project was supported by The Scientific and Technological Research Council of Turkey with the Project number 111S392.

Chronic myeloid leukemia - Clinical

E1090

COMPARISON OF TYROSINE-KINASE INHIBITORS AS FIRST-LINE TREATMENT FOR CHRONIC MYELOID LEUKEMIA: RESULTS OF A NETWORK META-ANALYSIS INCLUDING 6314 PATIENTS

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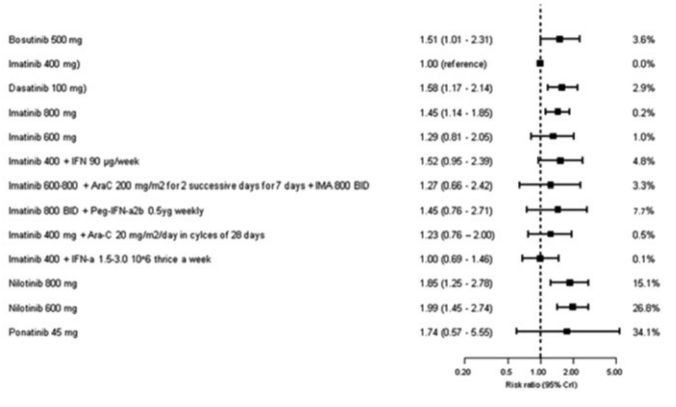
Background: The best treatment strategy for patients with chronic myeloid leukemia (CML) is still a matter of debate. Since the introduction of the BCR-ABL tyrosine-kinase inhibitor (TKI) imatinib in 1998, CML patients have experienced a significant improvement in overall survival. Prior to the usage of TKIs, the 10-year survival rate was 20%, therefore imatinib 400 mg nowadays is recommended as standard treatment. Currently, published evidence of second generation TKIs also recommends dasatinib and nilotinib as first-line therapy. However, direct head-to-head comparisons of second generation TKIs, which are important for a clinical decision-making, are lacking.
Aims: To assess the benefits and risks of different initial treatment strategies with TKIs for patients with CML in chronic phase and to provide patients and physicians with a high-level evidence for treatment decisions.
Methods: Data sources: We developed sensitive search strategies for CENTRAL, MEDLINE, and conference proceedings (searched from 1990 to 12/2014). Study selection and data extraction: Randomized controlled trials that evaluated in at least one arm a regimen including a TKI as first-line therapy in patients with CML in chronic phase. Two authors independently assessed studies for eligibility. We extracted data and assessed quality of trials in duplicate. The primary outcome was major molecular response (MMR). Secondary outcomes included complete cytogenetic response (CCyR), overall survival and adverse events. Data synthesis: Effect measures were risk ratios (RR) for complete MMR, CCyR and adverse events, and hazard ratios (HR) for OS. We pooled data using network meta-analysis. Direct comparisons within trials were combined with indirect evidence from other trials by using a Bayesian random-effects model. We evaluated both, the full network including all trials reporting the outcome and a core network including only those regimens that were evaluated in at least two trials. Results are reported relative to imatinib 400 mg with a RR <1 and HR >1 indicating superiority of imatinib 400mg. This project was funded by the Federal Ministry of Education and Research, grant number: 01KG1112.
Results: The systematic search resulted in a database including 1,114 potentially important references. Of those, 16 final RCTs from 209 publications were identified and included into the network-analysis. These trials assessed the effects of 14 various treatment arms and 18 comparisons for 6,314 patients with CML. Nilotinib was associated with the highest chance to be best (41.9%) in terms of MMR at 12 months (nilotinib 600 mg RR 1.99, 95%>credibility interval (CrI) 1.45 to 2.74 and nilotinib 800 mg RR 1.85, 95%>CrI 1.24 to 2.78, respectively) in the full network meta-analysis (see figure 1). This effect is even more pronounced in the core network with a probability to be best of 83.1%. Dasatinib 100 mg (RR 1.58, 95% CrI 1.16 to 2.13) and imatinib 800 mg (RR 1.45, 95% CrI 1.13 to 1.85) might be superior to imatinib 400 mg as well. All other treatment strategies and the results for CCyR showed no statistically significant difference to imatinib 400 mg. There are few events for OS, and thus results are not conclusive (*i.e.* wide 95% CrI). Results regarding adverse events will be reported in due course. Between-trial heterogeneity was negligible in all three analyses (tau-square 0.01, 0.02, and 0.05, respectively).

Table 1.



Summary and Conclusions: The comparison of different first-line treatment strategies for CML patients in this network meta-analysis shows a significant and relevant nilotinib advantage over standard imatinib 400 mg treatment. This analysis provides the currently best available evidence of different initial treatment strategies for chronic phase CML patients and therefore adds valid and important information for both, patients and physicians. However, some of the regimens were only evaluated in one trial with a limited number of patients, leading to imprecise results.

E1091

PREDICTIVE FACTORS FOR TREATMENT-FREE REMISSION IN CHRONIC MYELOID LEUKEMIA PATIENTS WITH IMATINIB-TREATED, UNDETECTABLE MOLECULAR RESIDUAL DISEASE: RESULTS FROM THE KID STUDY

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Background: Since the recent several reports have shown that imatinib (IM) discontinuation can be employed in patients who had enough IM therapy and undetectable molecular residual disease (UMRD) durations prior to IM discontinuation, treatment-free remission (TFR) has emerged as a goal in chronic myeloid leukemia (CML) treatment. Although UMRD with a sensitivity of 4.5 log for at least 2 years is required for a trial of TFR and factors associated with a higher rate of sustained UMRD after IM cessation have found, precise identification of the minimum requirement for a safer, successful TFR and the underlying mechanism of sustained UMRD thus remains elusive. In addition, the possibility of the second attempt to discontinue IM in patients with second sustained UMRD who experienced loss of MMR after first IM discontinuation was suggested.
Aims: The aim of this study is to identify predictors for safer, successful IM discontinuation and to explore additional contributing factors for sustained molecular responses.
Methods: CP CML patients who were treated with IM for more than 3 years and had undetectable levels of BCR-ABL1 transcripts determined by quantitative reverse transcriptase polymerase chain reaction for at least 2 years were eligible for Korean Imatinib Discontinuation (KID) study. In this analysis, the patients with at least 6 months of follow-up were included, with the exception of the patients who received IM for post-transplant relapse. In case of relapse, defined as loss of MMR on 2 consecutive assessments, IM therapy was re-introduced. If the patients re-achieved and maintained second UMRD for at least 2 years, second IM discontinuation was allowed.
Results: A total of 92 patients (52 females, 40 males) with follow-up of ≥6 months were analyzed, with a median age of 49 years (range, 21-77). After a median follow-up of 20.2 (range, 6.6-52.1) months after IM discontinuation, 46 patients (50%) lost UMRD. Of the 46 patients who lost UMRD but not MMR exhibited different patterns of BCR-ABL1 kinetics; 7 patients spontaneously re-achieved UMRD and 2 patients showed fluctuation of BCR-ABL1 transcript under the level of 0.1% on IS, while the other 37 patients lost MMR by 2 consecutive analyses. Probabilities for sustained MMR at 12 months and 24 months were 61.7% and 58.3%, respectively. All 37 patients who lost MMR were re-treated with IM therapy for a median of 12.3 months (range, 0.3-41.4 months). Among them, 30 patients re-achieved MMR at a median of 4.2 months (range, 0.5-11.1 months) after resuming IM therapy. In the univariate analysis, positivity of digital PCR at screening, IM duration and UMRD duration before IM discontinuation, and aggravation or newly development of musculoskeletal pain had a higher probability of sustained MMR. In addition, three patients who re-achieved and maintained second UMRD for 23.9, 25.3, and 25.3 months, respectively entered into the second IM discontinuation, among whom 2 patients re-lost MMR after 2 months and 1 patients sustained MMR during 6 months.
Summary and Conclusions: Our data suggest the predictive factors for sustained MMR which may provide additional information to guide clinical decisions on the TFR study and the possibility of the second attempt to discontinue IM. Overall, positivity of digital PCR at screening, both UMRD and IM duration, and

IM withdrawal symptom were predictive factors for successful IM-off. Further studies on underlying mechanisms about immunological control, minimal residual leukemia and stem cell biology during TKI-off study should be conducted.

E1092

SUPERIORITY OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION TO NILOTINIB & DASATINIB FOR ADULT PATIENTS WITH CHRONIC MYELOGENOUS LEUKEMIA IN THE ACCELERATED PHASE

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Background: In the tyrosine kinase inhibitor (TKI) era, imatinib is often the first-line therapy for chronic myeloid leukemia (CML) patients in either the chronic or the accelerated phases. However, even though second-generation TKIs (TKI_2) including dasatinib and nilotinib are appropriate options for patients with disease progression to the accelerated phase following imatinib therapy, allogeneic hematopoietic stem cell transplantation (allo-HSCT) is the only curative therapy. Nevertheless, the second-generation TKIs (TKI_2) including nilotinib and dasatinib can induce a faster and stronger molecular response and may achieve better OS and PFS than first-generation TKIs. Meanwhile, great progression has been achieved in human leukocyte antigen (HLA)-mismatched/haploidentical HSCT recently, the clinical results of which are comparable to that of matched sibling HSCT. Now, almost every patient can have a donor. We recently reported that haploidentical HSCT affords a 4-year OS rate of 73.3% for CML in AP. However, the efficacy of TKI_2 has not been compared with that of HSCT.
Aims: This study retrospectively analyzed the data from the Chinese CML alliance database to compare the efficacy of TKI_2 and HSCT for the treatment of CML in AP.
Methods: This study retrospectively analyzed the efficacy of TKI_2 and HSCT for the treatment of CML in the accelerated phase. Ninety-three CML patients registered in the Chinese CML alliance database from February 2001 to February 2014 were enrolled. Patients were divided into the TKI_2 (n=33) and allo-HSCT (n=60) groups. Twenty-six patients toke Nilotinib as initial TKI_2, 7 patients toke Dasatinib as initial TKI_2. Eleven patients exchanged to the alternative TKI_2 after failure to one TKI_2. Sixty patients received HSCT; 22 (36.7%), 35 (58.3%), and 3 (10%) patients underwent allo-HSCT from an HLA-matched sibling donor, HLA mismatched/haploidentical donor and unrelated donor respectively.
Results: All patients in HSCT group engrafted. Overall, 69.7% of patients had hematological response, 48.5% had cytogeneic response, 45.5% had MMR. to at least one of TKI2.. Sity (100%) patients achieved CHR and cytogenetic response in HSCT group patients. The 5-year overall survival rate was significantly lower in the TKI_2 group than the allo-HSCT group (42.9% vs. 86.4%, P=0.002). The 5-year event-free survival rate was significantly lower in TKI_2 group than the allo-HSCT group (14.3% vs. 76.1%, $P<0.001$). The 5-year progression-free survival rate was significantly lower in the TKI_2 group than the allo-HSCT group (28.6% vs.78.1%, P <0.001). Multivariate analysis showed that male sex and TKI_2 therapy were predictors of poor OS, while hemoglobin <100 g/L and TKI_2 therapy were predictors of poor EFS and PFS.
Summary and Conclusions: These results indicate allo-HSCT may be superior to nilotinib and dasatinib for adults CML patients in the accelerated phase. Prospective trial is needed in the future.

E1093

ASSESSING RISK FACTORS OF ELECTRONICALLY MEASURED MEDICATION NON-ADHERENCE IN CML PATIENTS: AN OBSERVATIONAL LONGITUDINAL DESIGN–A SUBANALYSIS OF THE TAKE-IT STUDY

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Background: Medication non-adherence (MNA) to tyrosine kinase inhibitors (TKIs) occurs in around 30% of chronic myeloid leukemia (CML) patients. Electronically measured MNA to TKIs is associated with less favorable outcomes. There are no proven strategies for improving adherence or identifying patients at risk for non-adherence in CML. Modifiable risk factors for non-adherence may guide intervention development yet have mainly been studied for imatinib alone, based primarily on adherence measured by pharmacy refill records, pill counts and subjective measures such as collateral and self-reports. Electronically measured adherence to imatinib was reported primarily in the landmark study by Marin *et al*, while electronically measured adherence to nilotinib and dasatinib has not yet been reported.
Aims: (1) To determine the period prevalence of electronically-assessed MNA to TKIs; (2) To assess which factors are associated with MNA to TKIs.
Methods: We used baseline data of the TAKE-IT study (NCT01768689), a prospective Israeli multicenter (N=4) pre-post adherence intervention study in CML. A convenience sample of 56 adults with chronic phase CML receiving imatinib, nilotinib or dasatinib, for ≥3 months before study inclusion were included. Sample characteristics were as follows: median age, 60.5 [IQR 19] years; prior disease duration, 43 [IQR 81] months; current TKI treatment duration, 34 [IQR 61] months; line of TKI treatment: 1st=62.5%, 2nd=28.5%, 3rd=9%; type of TKI: imatinib 59%, nilotinib 18%, dasatinib 23%; optimal response at baseline according to the ELN(2013) guidelines, 93%; membership in a CML advocates (support) group, 50%. Period prevalence of TKIs adherence was assessed using electronic monitoring during a 3 month period and expressed as a percentage of the prescribed doses taken over 3 months. Selected socio-demographic, patient, treatment, condition and healthcare system-related (according to WHO taxonomy) risk factors of TKI MNA were assessed (For overview see Table 1). Simple linear regression was used to identify risk factors associated with TKIs MNA. Statistical significance was set at p<0.05.
Results: Median period prevalence of TKI adherence was 97.7% (IQR: 6.6%; range: 48%>100%). We found the following *sociodemographic factors*, *i.e.* younger age, Arab ethnicity (perhaps reflecting health inequalities), living alone; *patient related factor*, *i.e.* non-membership in a CML advocates group; *condition related factor*, *i.e.* prolonged duration of CML; *treatment related factors*, *i.e.* advanced line of treatment, Nilotinib treatment; and *healthcare system factor*, *i.e.* study center, to be significant predictors of TKI MNA (see Table 1).

Table 1.

Risk factor evaluated for TKI non-adherence	WHO taxonomy of adherence risk factors	p-value for association with TKI non-adherence* in CML (univariate analysis)
Younger age [@]	Socio-demographic	**0.005**
Arab Ethnicity (vs. other) [@]		**0.003**
Secondary education not completed (vs. secondary or tertiary education completed) [@]		0.85
Living alone (vs. living with partner or parent) [+]		**< 0.001**
Not a member of CML advocates (support) group [+]		**0.017**
No previous education on TKI adherence [+]	Patient-related	0.136
Prolonged disease duration [@]	Condition-related	**0.03**
Nilotinib (vs other TKIs) [+]	Treatment -related	**< 0.001**
Increasing treatment line (3rd) [@]		**0.02**
Duration of current TKI [@]		0.06
Hematologist's professional experience [+]	Healthcare system-related	0.2862
Study center (proxy for practice patterns) [+]		**0.007**

Table 1: Risk factors of electronically measured non-adherence to tyrosine kinase inhibitor treatment in chronic myeloid leukemia

* Electronically measured adherence over a 3 month period.
[+] Modifiable risk factor.
[@] Non-modifiable risk factor.
CML, chronic myeloid leukemia; TKI, tyrosine kinase inhibitor

Summary and Conclusions: TKI adherence was high yet showed large variability. We identified several multi-level risk factors of TKI MNA, most of them congruent with the chronic care literature. Half of the risk-factors identified are modifiable and can thus guide intervention development in the future, optimally targeting the different domains of the WHO taxonomy. Of special interest is decreased adherence with Nilotinib, taken twice daily while fasting, as opposed to imatinib and dasatinib. This may be a proxy for increased treatment complexity, or for non-adherent patients receiving advanced lines of treatment due MNA-driven failure of 1st line treatment. This must be taken in the context of conflicting findings from previous studies comparing adherence between various TKIs. Study center may be a proxy for practice patterns or differences in case mix treated. Non-membership in a patient's advocate group as risk factor for MNA is an interesting novel finding. Such groups might provide informational

and other types of social support favoring adherence. In conclusion, our study quantifies MNA to different TKI regimens and provides guidance for identifying patients at risk, as well as ideas regarding which factors could be targeted for intervention.

E1094

A CRITICAL COMPARISON OF SOKAL, EURO, AND EUTOS RISK SCORES IN CHRONIC MYELOID LEUKEMIA PATIENTS TREATED WITH TYROSINE KINASE INHIBITORS

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Background: In the era of molecular hematology, the prognosis of BCR-ABL+chronic myeloid leukemia (CML) is still based on three prognostic systems including few clinical and hematological variables: age, platelet count, blast cells, eosinophils, basophils (percentage in peripheral blood) and spleen size (assessed by manual palpation; maximum distance in cm from costal margin). The three prognostic systems are the Sokal score (Sokal et al, Blood 1984;63:789-799), the EURO score (Hasford et al, J Natl Cancer Inst 1998;90:850-858), and the EUTOS score (Hasford et al, Blood 2011;118:686-692), based on the analysis of international, multicentric series of newly diagnosed, chronic phase (CP) CML patients, treated with conventional chemotherapy (Sokal), interferon-alfa (IFNa)-based regimes (EURO), or imatinib-based regimes (EUTOS), respectively.
Aims: In all the main studies exploring the efficacy of tyrosine-kinase inhibitors (TKIs), at least one of these scores has been used: all three scores were able to predict the response and/or the outcome, but the three scores are different, and there is no agreement on which score may be more useful and should be adopted. The aim of our analysis was to identify which score has been more frequently used and which one is able to give more consistent results.
Methods: We reviewed the most relevant reports of the 13 important clinical studies, published in peer-reviewed journals between 2003 and 2014. Moreover, we analyzed 559 patients enrolled within 3 multicentric prospective studies conducted by the GIMEMA CML WP (NCT00514488, NCT00510926, observational trial CML023).

Table 1. Cytogenetic and molecular response according to Sokal, EURO and EUTOS score in 13 selected clinical studies, published in peer-reviewed journals between 2003 and 2014.

Sokal score								
Study	No pts	Treatment	CCyR by	Rates by risk (L, I, H)	P	MMR by	Rates by risk (L, I, H)	P
IRIS	383	Ima 400	1 year	76,67,49%	.001	1 year	66,45,38%	.007
Korea	257	Ima 400	1 year	94,nr,80%	.02	1.5 year	54,nr,30%	.03
ENESTnd	283	Ima 400	2 years	90,77.59%	nr	2 years	53,44,32%	Nr
GIMEMA	208	Ima 400	5 years	92,82,65%	.02	nr	nr	Nr
Hammersmith	277	Ima 400	8 years	94,87,77%	.001	8 years	94,87,77%	.001
TOPS	476	Ima 400/800	1 year	75,65,63%	ns	nr	nr	Nr
UK Spirit	142	Das 100	2 years	Nr	.09	2 year	nr	.04
ENESTnd	282	Nil 600	2 years	91,87,81%	nr	2 year	75,75,51%	Nr
BELA	252	Ima 400	nr	nr	nr	1 year	28,22,28%	Ns
BELA	250	Bos 500	nr	nr	nr	1 year	53,31,37%	.15
TIDEL	84	Ima 600	nr	nr	nr	2 year	80,67,46%	.02
GERMAN IV	101 4	Ima 800 or 400 alone or with IFNa or low dose Ara-C	nr	nr	nr	Time to MMR	nr	.001
EURO score								
Study	No pts	Treatment	CCyR by	Rates by risk (L, I, H)	P	MMR by	Rates by risk (L, I, H)	P
DASISION	260	Ima 400	1 year	76,72,64%	nr	1 year	36,28,16%	nr
DASISION	259	Das 100	1 year	94,94,78%	nr	1 year	56,45,31%	nr
TIDEL	82	Ima 600	nr	nr	nr	1 year	58,33,10%	.005
EUTOS score								
Study	No pts	Treatment	CCyR by	Rates by risk (L, H)	P	MMR by	Rates by risk (L, H)	P
GIMEMA	209	Ima 400	5 years	85,69%	.04	nr	nr	nr
Hammersmith	272	Ima 400	8 years	88,87%	.35	8 years	68,66%	.15
MDAnderson	465	Ima 400/600, das 100, Nil 600/800	6 years	93,81%	.02	6 years	85,81%	.48
UK Spirit	142	Das 100	2 years	nr	.11	2 years	88,87%	.35

Legend: nr = not reported; ns = not significant; L = low, I = intermediate, H = high

Results: The Sokal score was used in 12 studies, the EURO score in 2 studies, the EUTOS score in 4 studies (Table 1). The results are difficult to interpret, because sometimes only the p-values for differences (not the response rates), and sometimes only the response rates (not the p-value) were reported. The relationship between the risk and survival was analyzed in few studies: the Sokal score was able to predict the survival in three studies (Hammersmith, Czeck registry and Swedish registry), the EURO in only one study (Swedish registry), and the EUTOS in no studies. Therefore, we tested all three scores in 559 newly diagnosed CP-CML patients who were enrolled in GIMEMA studies with imatinib front-line. No score was able to predict the early molecular response. The complete cytogenetic response (CCyR) and the major molecular response (MMR) rates by 1 year were better predicted by Sokal (p=.006 and. 001) and EURO (p=.002 and .002) scores than by EUTOS score (p=.009 and .010). The rate of deep molecular response ($MR^{4.0}$) by 6 years was better predicted by Sokal score (p<.001) than by EURO (p=.019) or EUTOS (p=.031) score. The 6-year overall survival was better predicted by Sokal (p=.002) and EURO (p=.003) score than by EUTOS score (p=.160).
Summary and Conclusions: There are no studies supporting the superiority of any risk score over the others. The EUTOS score could be the first choice because based on TKIs-treated patients. However, the Sokal score may still be considered the reference risk score, because it has been validated in many more independent studies, with more consistent results. All the three scores could be refined introducing other biological variables (*i.e.* clonal chromosome abnormalities in Ph+cells). However, more progress in CML biology is needed to move from the era of clinical/hematologic prognosis to an era of molecular-based prognosis.

E1095

SWITCHING TO NILOTINIB IS ASSOCIATED WITH DEEPER MOLECULAR RESPONSES IN CHRONIC MYELOID LEUKEMIA CHRONIC PHASE WITH MAJOR MOLECULAR RESPONSES ON IMATINIB; STAT1 TRIAL IN JAPAN

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Background: Nilotinib is a second-generation tyrosine kinase inhibitor (TKI) that exhibits significant efficacy as first- or second-line treatment in patients with chronic myeloid leukemia (CML). Superior rates of deeper molecular responses were achieved with nilotinib vs. imatinib in patients newly diagnosed with CML in chronic phase (CML-CP) in the ENESTnd trial. In addition, the 24-month analysis of the ENESTcmr study demonstrated that switching to nilotinib after a minimum of 2 years on imatinib led to increased rates of deeper molecular responses vs. remaining on imatinib. Significantly more patients treated with nilotinib achieved undetectable BCR-ABL1 by 24-month (32.7% on nilotinib vs. 16.5% on imatinib; P =0.005).
Aims: To evaluate the efficacy of nilotinib in patients with CML-CP who have achieved a major molecular response (MMR) with imatinib.
Methods: Patients with CML-CP who had achieved MMR but still had persistent BCR-ABL positivity by real-time quantitative polymerase chain reaction (RQ-PCR) on imatinib were eligible. Nilotinib will be taken twice daily (600 mg/day) for 2 years. Thirty-five institutions in STAT study group participated. The study was conducted in accordance with the principles of the Declaration of Helsinki. Informed consent was written by all patients according to institutional guidelines. The study was approved by all institutional review boards and registered with ClinicalTrials.gov (number UMIN000005903). The primary objected of this multicenter phase II, single-treatment arm, open-label clinical study was to identify the MR4.5 rate at 24 months after the initiation of nilotinib treatment. The molecular response was evaluated according to the International Scale [IS] RQ-PCR upon study entry and every 3 months thereafter. IS RQ-PCR was performed in a central laboratory using a Molecular MD One-Step qRT-PCR BCR-ABL kit (BML Inc., Kawagoe, Japan). At least 32,000 control gene (ABL) were required for scoring deep molecular responses.

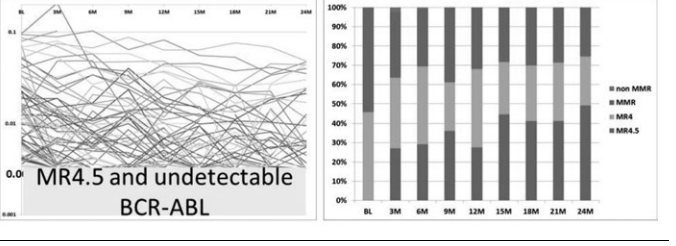

Figure 1-2.

Results: Between July, 2011 and December, 2012, 80 Japanese patients were recruited, and 74 patients were evaluated in the study. The median age was 54.5 years. The ratio of men to women was 52:22. The median duration of CML in patients was 68.9 months. The median duration of imatinib treatment was 66.0 months. All patients showed MMR at the time of entry into the study and the median time to MMR on imatinib therapy was 20.2 months. The proportion of patients achieving deeper molecular responses (MR4, MR4.5)

increased over time (Figure 1). The rates of MR4 in the evaluable patients were 69% and 73% at 12 and 24 months, respectively, and the rates of MR4.5 were 28% and 49% at 12 and 24 months, respectively (Figure 2).
Summary and Conclusions: Switching to nilotinib led to deeper molecular responses in patients with minimal residual disease on long-term imatinib therapy. The results of our study suggest that switching to the more potent, selective tyrosine kinase inhibitor nilotinib is beneficial in patients with minimal residual disease after long-term imatinib therapy. Achievement of MR4.5 after switching to nilotinib may enable a greater proportion of CML-CP patients to be eligible for future discontinuation studies.

E1096

THE LYMPHOCYTE DYNAMICS PREDICTS THE BETTER RESPONSE FOR DASATINIB TREATMENT IN PATIENTS WITH NEWLY DIAGNOSED CHRONIC MYELOID LEUKEMIA IN CHRONIC PHASE

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Background: Currently, as a second generation tyrosine kinase inhibitor, dasatinib is one of the key treatment options for chronic myeloid leukemia (CML) patients. Increase in lymphocyte counts, especially in the large-granular lymphocytes (LGLs), has been well investigated in patients treated with dasatinib as a second line therapy, suggesting the correlation of increased LGL number with the favorable outcome. However, lymphocyte dynamics in CML patients treated with dasatinib as a first line therapy has yet to be clarified.
Aims: Elucidate the association between the lymphocyte dynamics after dasatinib introduction and a favorable treatment response in newly diagnosed patients with CML in chronic phase (CML-CP).
Methods: An open-label, multicenter, prospective clinical trial; D-First study (ClinicalTrials.gov NCT01464411), enrolling patients with newly diagnosed CML-CP was implemented to receive dasatinib 100mg/day. All patients were followed-up for at least 18 months. BCR-ABL1 transcripts were quantified at 1, 3, 6, 9, 12, 15, and 18 months after initiation of dasatinib in all but one, harboring the atypical undetectable BCR-ABL1 transcript. A deep molecular response (DMR) was defined as BCR-ABL1 IS <0.01%. The incidence of lymphocytosis was diagnosed if the number of peripheral blood (PB) lymphocyte counts exceeded 3,600/μL on two occasions or more, after at least 4 weeks of dasatinib initiation. Flow cytometry analysis was performed on each PB sample to examine the phenotype of lymphocyte fractions.
Results: Of the 52 patients registered to the D-First study, 14 patients developed lymphocytosis and 29 patients achieved DMR by 18 months. The median time from dasatinib induction to the first incidence of lymphocytosis was 7 months (range 1 – 16). The majorities of increased lymphocytes were morphological LGLs and were highly differentiated CD56+CD57+natural killer cells (NK) or cytotoxic T lymphocytes (CTL) in most cases. Cumulative DMR rates by 18 months were not significantly different between patients with lymphocytosis and those without (50% *vs* 62%, P=0.53). However, total lymphocyte counts at 1 month were significantly higher in patients with DMR by 18 months compared to those without DMR (median 1,709/μL vs. 1,118/μL; P=0.018). Furthermore, NK and CTL counts were significantly higher in patients with DMR compared to those without DMR (P=0.009 for NK and P=0.005 for CTL). Using the receiver operating characteristic curve, the optimal threshold in total lymphocyte, NK and CTL values at 1 month were calculated, indicating moderate correlations (area under the curves were 0.70 in total lymphocyte, 0.72 in NK and 0.74 in CTL) with the highest Youden's index at 1,583/μL, 467/μL and 367/μL, respectively. When the patients were divided into 2 groups according to those calculated thresholds, the cumulative DMR rates were significantly better in higher value group compared to lower value groups (P=0.03 in total lymphocyte, Figure 1 upper left; P=0.013 in NK, Figure 1 upper right and P=0.007 in CTL, Figure 1 lower left). In contrast, regulatory T cell (Treg) counts were significantly lower at 12 and 15 months in patients achieved DMR.

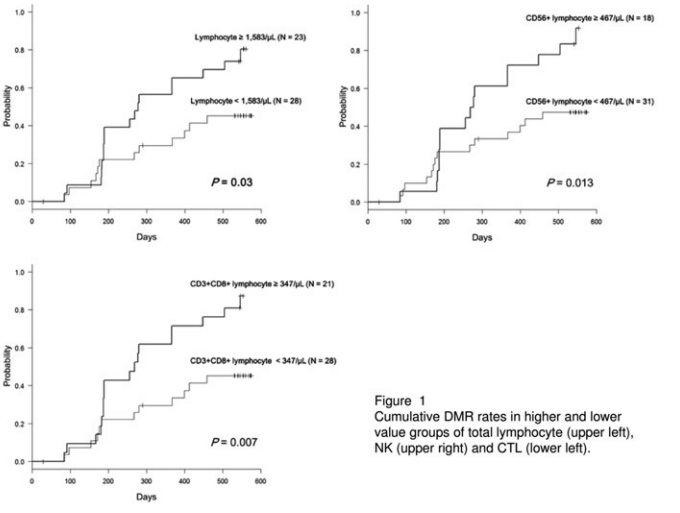

Figure 1
Cumulative DMR rates in higher and lower value groups of total lymphocyte (upper left), NK (upper right) and CTL (lower left).

Figure 1.

Summary and Conclusions: Increased counts of total lymphocyte, NK and CTL at 1 month after the initiation of dasatinib predicted better DMR rate in newly-diagnosed CML-CP patients. Treg inhibition was also obvious in patients with DMR at later time points, suggesting the dual effects of dasatinib on immune system through the cytotoxic lymphocytes activation and Treg deregulation in different periods.

E1097

EVALUATION OF A QUANTITATIVE MULTIPLEX BCR-ABL1 ASSAY ALIGNED DIRECTLY TO THE WHO PRIMARY STANDARD REFERENCE MATERIALS TO REPORT EXPRESSION ON THE INTERNATIONAL SCALE

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Background: Detection of BCR-ABL1 e13a2 or e14a2 transcripts (major breakpoint, M-BCR) of translocation t(9;22) (also known as the Philadelphia chromosome) is important in CML monitoring tumor burden. The International Scale (IS) was established to standardize reporting relative to a common baseline. As newer TKI therapies create deeper responses, analytical sensitivity has become a critical topic in investigations into TKI discontinuation, where researchers require an assay that calls a molecular response (MR) of ≥4.5 logs below baseline (*i.e.* MR4.5 or 0.0032%IS).
Aims: Asuragen has developed a multiplexed RT-qPCR assay for the simultaneous amplification and detection of two BCR-ABL1 fusion transcripts (e13a2 and e14a2) and ABL1 (an endogenous control) using total RNA extracted from human leukocytes. We evaluated the assay's ability to meet current performance requirements for ease of use, sensitivity, and traceability to a higher order standard reference material.
Methods: We tested whether this prototype BCR-ABL1 fusion quantification assay could be an alternative to current clinical diagnostic tools for monitoring CML patients for minimal residual disease. We compared patient test results obtained with the Asuragen assay to our current clinical laboratory developed test (LDT) to define correlation and concordance between the two methods. Clinical and cell line RNA samples were used to study additional performance characteristics of the Asuragen assay, including sensitivity (7-member human RNA panel over 3 days), specificity (cell line RNAs and *in vitro* transcripts), linearity (7-point human RNA dilution), precision (3-member human RNA panel over 5 days), and clinical accuracy (20 human RNA specimens over 2 runs, assessed against the LDT). EDTA blood-based RNA specimens were evaluated for purity ($OD_{260/280}$ >1.6) and concentration (100-500 ng/uL). Asuragen's BCR-ABL1 assay included all required RT and qPCR reagents in the kit. The RT and qPCR were performed and analyzed on the ABI 7500 Fast Dx with software version 1.4. RNA was provided as 7 different blends of BCR-ABL1 and ABL1 Armored RNA Quant® (ARQ) standards and controls to calibrate and control the system. A single four-point standard curve using ARQ blends mimicked the WHO Primary BCR-ABL1 reference materials. Assay-specific software was used to automatically generate results reported in MR and %IS values.
Results: We found positivity in samples ≤MR4.5 (≥0.0032%IS) with human CML positive RNA, indicating good analytical sensitivity. Despite deep analytical sensitivity, the system maintains analytical specificity (non-M-BCR, non-CML, and non-leukemic specimens produce true negative results). Linearity was

demonstrated from LOQ to <MR1 (>10%IS). Technical replicates yielded acceptable precision, including good %CV around the key clinical decision points of 0.1%IS (MR3, MMR) and 10%IS (MR1, near PCyR). The Asuragen assay showed good intra-laboratory correlation. Moreover, using assay comparison criteria proposed by Müller *et al.* (Leukemia 2009), the Asuragen assay was considered comparable to our current LDT, suggesting good clinical accuracy (when treating the prior result in the LDT as truth).

Summary and Conclusions: The BCR-ABL1-targeting prototype improves workflow with its streamlined reagent formulation, all inclusive contents, and multiplex assay format. It facilitates assessment on the IS without conversion (through integrated ARQ materials traceable to the WHO Primary reference set), and generates results sufficient for studies in deep molecular responses.

E1098

IMATINIB TREATMENT IN CHRONIC MYELOID LEUKEMIA PATIENTS IN EARLY AND LATE CHRONIC PHASE: CURRENT INCIDENCE OF CYTOGENETIC REMISSION AND A VERY LONG-TERM AN INTENTION-TO-TREAT ANALYSIS

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Background: Imatinib (IM) is remarkably effective in treating newly diagnosed patients with chronic myeloid leukemia (CML) in chronic phase (CP). The long term outcome is extremely important to assess the treatment efficacy and stability. To date, most of the available data come from studies in which some of the patients were censored for diverse reasons. In the IRIS trial, at 8 year, 55% of patients were still on IM and the overall survival (OS) was 85%. Other published reports have shorter follow-up.
Aims: To evaluate the results of long term treatment in CML patients in a setting where all events were recorded.
Methods: A total of 235 adult patients with CML in CP started IM from July 2000 till April 2007. Early CP was in patients with <1 year of pretreatment before IM**.** At the time of therapy initiation 37% (86/235) patients were in early CP. Common (ComCI) and current (CCI) cumulative incidences of complete cytogenetic response (CCyR), progression-free survival (PFS) and overall survival (OS) were evaluated.
Results: Baseline demographics characteristics: median age: 42 years (extremes 18-66 years); male sex: 49%; high Sokal, high Euro and high EUTOS scores: 16%, 7% and 6%, respectively; clonal chromosomal abnormalities (CCA) in Ph+cells: 7%. Median follow-up: 123 (9-162) months; still on IM treatment: 54%; switched to TKI2: 34%; alive: 81%. The reasons for IM discontinuation were: lack of efficacy (31%), death (12%), toxicity and other reasons (4%); 4% of patients were lost to follow-up. ComCI of CCyR in early CP and late CP was 89% and 77%, respectively (p<0,0001). The CCI of CCyR in early CP was higher than in late CP at all observation time. Switching to TKI2 allowed to increase CCI in all group after 5 years therapy. The 12-year OS for early CP and late CP was 90% and 74%, respectively (p=0,017); the 12-year PFS for early CP and late CP was 94% and 77%, respectively (p=0,006).

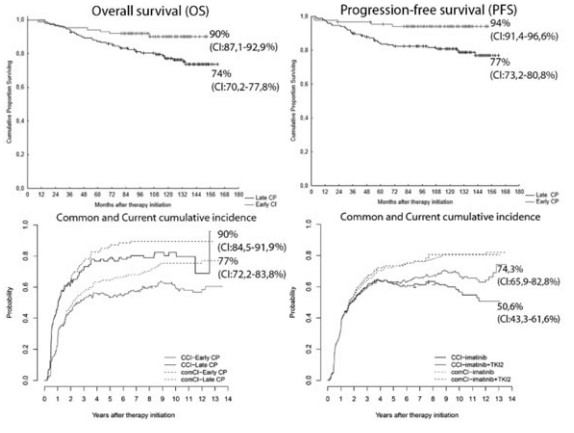

Figure 1.

Summary and Conclusions: The available data on the very long-term outcome of newly diagnosed CP CML patients treated frontline with IM are limited to a company sponsored study (IRIS study). Until now, there are no available data on the very long-term outcome of late CP CML patients treated with IM. This study provided an unbiased overview of the long-term IM therapeutic effects in early and late CP CML. IM more effective in early CP then in late CP CML. Achieving and maintaining CCyR correlated with disease duration before IM initiation. Switching to TKI2 allows to increase achieving and maintaining CCyR in early and late CP CML

E1099

CARDIOVASCULAR (CV)-RELATED HOSPITALIZATION IN PATIENTS WITH CHRONIC-PHASE CHRONIC MYELOID LEUKEMIA (CP-CML) IN SIMPLICITY, A PROSPECTIVE OBSERVATIONAL STUDY

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Background: SIMPLICITY is an ongoing observational study of CP-CML patients (pts) designed to understand the use of first-line (1L) imatinib (IM), dasatinib (DAS) or nilotinib (NIL) in the United States (US) and Europe (Eu) outside clinical trials (NCT01244750). Previous SIMPLICITY data (ASH 2013) showed pts had 3.3 (±2.8) comorbidities (mean±standard deviation [SD]) at start of 1L tyrosine kinase inhibitor (TKI; N=949); ≥1 baseline comorbidity reported in >75% of pts, with >3 comorbidities in majority. 40.6% had cardiovascular (CV) comorbidities at start of 1L TKI. Baseline comorbidity did not affect initial TKI selection, although cautions regarding risks for specific adverse events (*e.g.* cardiac and pulmonary) have been described for individual TKIs in pts with pre-existing conditions.
Aims: This analysis focuses on the frequency of CV-related hospitalizations in SIMPLICITY pts and describes these events by 1L TKI and TKI received at time of hospitalization. Demographics and clinical characteristics of hospitalized pts are compared with the total SIMPLICITY population.
Methods: SIMPLICITY includes three prospective pt cohorts treated with IM, DAS or NIL as 1L therapy since 2010 and a historical cohort treated with IM since 2008. Among hospitalizations reported through 22 Sept 2014, CV-related hospitalizations were identified using preferred MedDRA terms in the CV category. Based on events reported, events were categorized: valvular disease; arrhythmia; cardiac failure; cardiac ischemic disease; and pericardial disorder. TKI exposure was calculated from total duration on specified TKI regardless of initial TKI.
Results: 1460 pts were enrolled prospectively on IM (n=415), DAS (n=416) or NIL (n=376), and data collected retrospectively for IM (n=253). Over half of pts were male (54.7%) and over half were from the US (66.7%). Median age (interquartile range [IQR]) at 1L TKI was 56.0 (45.0–67.0) years and 31% of pts were ≥65 years. Overall, 329 pts (22.5%) were hospitalized and 46 pts (3.2%) had CV hospitalizations (retrospective IM: n=21; prospective IM: n=6; DAS: n=8; NIL: n=11). Multiple CV hospitalizations were reported in 21.7% of CV hospitalized pts. Of pts with CV hospitalizations, median (IQR) age at 1L TKI was 70.8 (57.1–74.8) years; 60.9% were ≥65 years. Most pts were male (60.9%) and from the US (82.6%); 62% were on 1L TKI at first admission. Median follow-up from start of TKI to hospitalization was 370 days (retrospective IM, 484; prospective IM, 847; DAS, 105; NIL, 239 days) compared with 29.3 months for the total SIMPLICITY population. Most frequent causes of CV hospitalization were cardiac ischemic disease (34.6%), arrhythmia (30.8%) and cardiac failure (26.9%).The rate of CV hospital admissions per 100 pt years exposure was 1.46 for all hospitalized pts and was highest in the NIL-treated cohort (NIL: 2.61 compared with 0.77 [retrospective IM], 1.15 [prospective IM] and 1.15 [DAS]). The mean (±SD) duration of hospitalization was 5.9 (±7.2) days.
Summary and Conclusions: In SIMPLICITY, few patients overall were hospitalized for CV-related events. Pts with CV-related hospitalizations were older than the total SIMPLICITY population. The highest rate of CV hospitalizations per 100 pt years was in NIL-treated pts, while the observed rates for DAS and IM patients appeared similar. Analysis of pre-existing CV co-morbidities in hospitalized patients will be described.

E1100

ESTIMATED GLOMERULAR FILTRATION RATES OF CHRONIC MYELOID LEUKEMIA (CML) PATIENTS TREATED WITH TYROSINE KINASE INHIBITORS (TKIS) IN DASATINIB TRIALS: DASISION (CA180-056), CA180-034, AND CA180-035

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Background: As the survival of patients with CML has increased with the introduction of BCR-ABL-targeted TKIs, more attention is being paid to the potential long-term effects of chronic treatment. There is evidence that suggests individual TKIs may affect metabolic pathways such as glucose control, lipid levels, and thyroid function (Breccia 2014, *Leuk Res*). In addition, imatinib has been associated in two single center studies with decreased estimated glomerular filtration rate (eGFR) over time, not reported with either dasatinib or nilotinib

(Marcolino 2011, *Ann Oncol*; Yilmaz 2013, *Blood*, abstr 1488).
Aims: The objective of this analysis was to evaluate the long-term effects of BCR-ABL-targeted TKIs on renal function in patients from three dasatinib phase 3 clinical trials: newly diagnosed patients with CML in chronic phase (CP) from DASISION, imatinib-intolerant or -resistant CML-CP patients from CA180-034, and imatinib-intolerant or -resistant patients with CML in accelerated/blast phase (AP/BP) or Ph+acute lymphoblastic leukemia (ALL) from CA180-035.
Methods: This pooled trial analysis was conducted using final data reports from each study: 5-year data from patients treated with first-line dasatinib 100 mg once daily (QD) or imatinib 400 mg QD in DASISION (NCT00481247; N=519), 7-year data from patients treated with second-line dasatinib 100 mg QD/50 mg twice daily (BID)/140 mg QD/70 mg BID in CA180-034 (NCT00123474; N=670), and 5-year data from patients treated with dasatinib 140 mg QD/70 mg BID in CA180-035 (NCT00123487; N=609). Modification of Diet in Renal Disease (MDRD) values for eGFR were calculated for patients aged ≥18 years using the formula: eGFR (mL/min/1.73 m²)=175 x (serum creatinine)$^{-1.154}$ x (age)$^{-0.203}$x (0.742 if female) x (1.212 if African American).
Results: In DASISION, newly diagnosed patients started on imatinib and dasatinib had a similar mean eGFR at baseline (89 mL/min/1.73 m^2). The mean eGFR with imatinib dropped approximately 9 mL/min/1.73 m² within the first 2 weeks of treatment, and then stabilized over the remaining length of the analysis (Figure 1). Conversely, there was a small increase in mean eGFR with dasatinib at early time points that then returned to baseline levels for the remainder of the analysis. In patients from CA180-034 and CA180-035 previously treated with imatinib, the baseline mean eGFR (79 mL/min/1.73 m^2 for dasatinib 100 mg QD in CA180-034 and 82 mL/min/1.73 m^2 for dasatinib 140mg QD in CA180-035) was lower than that observed in DASISION (89 mL/min/1.73 m^2). There was no difference in mean eGFR over time across all dasatinib doses tested in patients from CA180-034. In CA180-035, where all patients had advanced CML or ALL, a decrease in eGFR of approximately 10 mL/min/1.73 m^2 in the year following dasatinib initiation was observed without any long-term changes.

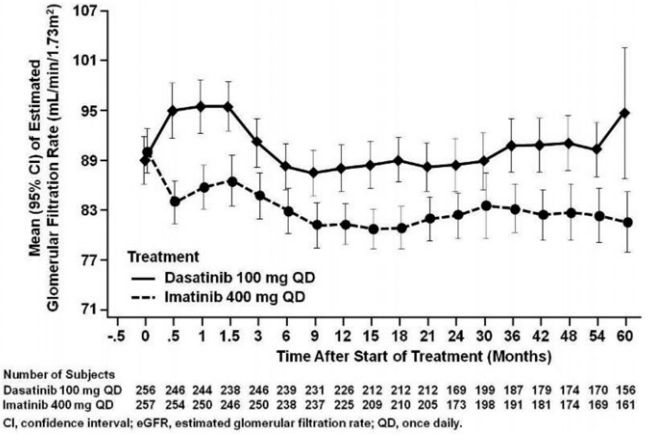

Figure 1. eGFR Over Time After the Start of Treatment in DASISION.

Summary and Conclusions: The renal profile of newly diagnosed CML patients or imatinib-intolerant/resistant CML or Ph+ALL patients did not worsen with long-term dasatinib treatment, regardless of CML phase. In DASISION, the mean eGFR with imatinib decreased soon after treatment initiation to a level consistent with the National Kidney Foundation (NKF) chronic kidney disease stage 2 (NKF Guidelines, 2002). Further analyses are ongoing to determine if there are any subpopulations of CML patients at risk, or other confounding factors, for developing renal dysfunction on TKIs.

E1101

NILOTINIB IN JAPANESE PATIENTS WITH CHRONIC MYELOID LEUKEMIA (CML) WITH SUBOPTIMAL MOLECULAR RESPONSE (MR) TO FRONTLINE IMATINIB: SENSOR FINAL 24-MO ANALYSIS AND BIM POLYMORPHISMS SUBSTUDY

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Background: Nilotinib (NIL) elicits rapid, deep MR as first- or second-line treatment of patients (pts) with CML in chronic phase. However, the best treatment for pts with suboptimal MR to first-line imatinib (IM) is not yet known. *BIM* polymorphisms (eg, *BIM* deletion [del] polymorphism at intron 2 or a single nucleotide polymorphism [SNP; C465C>T at *BIM* exon 5]) reduce rates of MR and increase the frequency of newly detected *BCR-ABL1* mutations with IM treatment, but little is known about how *BIM* affects pts receiving NIL.
Aims: SENSOR (NCT0104387) evaluated the efficacy and safety of switch to NIL in Japanese pts with suboptimal MR (per 2009 European LeukemiaNet criteria) to first-line IM. Here, we present final 24-mo data and results from a *BIM* polymorphisms substudy.
Methods: In this multicenter study conducted in Japan, pts with suboptimal MR to frontline IM (complete cytogenetic response but not major molecular response [MMR] after ≥18 mo of IM) received open-label NIL 400 mg twice daily for up to 24 mo. Laboratory tests were performed centrally. *BCR-ABL1* transcript levels (using the International Scale with *ABL1* as a control gene) were assessed each mo (mo 1-3) and every 3 mo thereafter. *BCR-ABL1* mutation status was assessed by direct sequencing at baseline and retrospectively analyzed at all these time points in pts without mutations detected. *BIM* polymorphisms were evaluated using a validated assay during or after treatment. Progression-free survival (PFS) and overall survival (OS) were estimated using the Kaplan-Meier method.

Table 1.

		n (%)
Evaluable pts for polymorphism status		40
BIM del	del hetero	3 (7.5)
	No del homo	37 (92.5)
BIM SNP	C/T	10 (25.0)
	C/C	30 (75.0)
BIM del or SNP	del hetero and/or C/T	12 (30.0)
	No del homo and/or C/C	28 (70.0)

		n	MR at 24 mo, n (%) [95%CI] MMR	MR^4	MR$^{4.5}$
All pts		45	30 (66.7) [51.0-80.0]	5 (11.1) [3.7-24.1]	3 (6.7) [1.4-18.3]
Pts evaluable for polymorphism status		40			
BIM del	del hetero	3	3 (100) [29.2-100.0]	0 [0.0-70.8]	0 [0.0-70.8]
	No del homo	37	24 (64.9) [47.5-79.8]	5 (13.5) [4.5-28.8]	3 (8.1) [1.7-21.9]
BIM SNP	C/T	10	8 (80.0) [44.4-97.5]	0 [0.0-30.8]	0 [0.0-30.8]
	C/C	30	19 (63.3) [43.9-80.1]	5 (16.7) [5.6-34.7]	3 (10.0) [2.1-26.5]
BIM del or SNP	del hetero and/or C/T	12	10 (83.3) [51.6-97.9]	0 [0.0-26.5]	0 [0.0-26.5]
	No del homo and/or C/C	28	17 (60.7) [40.6-78.5]	5 (17.9) [6.1-36.9]	3 (10.7) [2.3-28.2]

		n	*BCR-ABL1* Point Mutations or Splicing Abnormalities, n (%) Yes	No
Evaluable pts for polymorphism status		40		
BIM del	del hetero	3	2 (66.7)	1 (33.3)
	No del homo	37	24 (64.5)	13 (35.1)
BIM SNP	C/T	10	8 (80.0)	2 (20.0)
	C/C	30	18 (60.0)	12 (40.0)
BIM del or SNP	del hetero and/or C/T	12	10 (83.3)	2 (16.7)
	No del homo and/or C/C	28	16 (57.1)	12 (42.9)

Results: Of 45 pts enrolled in the main study (Dec 2009-Feb 2012), 6 pts discontinued (3 had adverse events [AEs], 1 had disease progression, and 2 withdrew consent). Median treatment duration was 22.1 mo (range, 0.1-22.7 mo). Rates of MMR, MR^4, and MR$^{4.5}$at 24 mo were 66.7% (95% CI, 51.0%–80.0%), 11.1% (95% CI, 3.7%–24.1%), and 6.7% (95% CI, 1.4%–18.3%), respectively. The cumulative incidence of MMR, MR^4, and MR$^{4.5}$by 24 mo was 75.6%, 13.3%, and 6.7%, respectively. Only 1 pt progressed or died; this pt had a newly detected T315I mutation, progressed to blast crisis at 5.4 mo, and died at 9.4 mo. Estimated 24-mo PFS and OS were 97.8% (95% CI, 85.3%–99.7%) and 97.8% (95% CI, 85.3%–99.7%), respectively. Of 40 pts evaluated for *BIM*

polymorphisms, 3 pts (7.5%) were heterozygous (hetero) for the *BIM* del, 10 pts (25.0%) had the C/T SNP, and 12 pts (30.0%) were hetero for the *BIM* del and/or had the C/T SNP. Pts achieved MMR and acquired *BCR-ABL1* point mutations or splicing abnormalities regardless of polymorphism status, but no pts with these *BIM* polymorphisms achieved MR^4 or $MR^{4.5}$(Table). Headache (28.9%) and rash (26.7%) were the most common drug-related nonhematologic AEs. The most common new or worsening laboratory abnormalities were increased bilirubin (95.6%) and increased alanine aminotransferase (80.0%). New or worsening hematologic abnormalities were decreased lymphocytes (53.3%) and decreased hemoglobin (31.1%).

Summary and Conclusions: Upon switch to NIL, 66.7% of pts achieved MMR at 24 mo, and 75.6% achieved MMR by 24 mo. Safety results were consistent with those from other NIL studies. Pts achieved MMR regardless of *BIM* polymorphism status, but no pts with *BIM* polymorphisms achieved deeper MR. Larger studies are required to determine how polymorphism of *BCL-2*-related genes, including *BIM*, may affect clinical response to NIL in pts with CML.

E1102

EFFICACY OF NILOTINIB VS HIGH-DOSE IMATINIB VS SUSTAINING STANDARD-DOSE IMATINIB IN EARLY CHRONIC PHASE CML PATIENTS WHO HAVE SUBOPTIMAL MOLECULAR RESPONSE TO FRONTLINE IMATINIB

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Background: In chronic myeloid leukemia (CML), achievement of optimal responses by time point has improved long-term outcomes. In IRIS study, patients who achieved major molecular response (MMR) at 18 months had event-free survival (EFS) benefit, compared to those who achieved complete cytogenetic response (CCyR) without MMR. However, the best treatment for these patients is still not confirmed. By the previous studies, sustaining standard-dose of imatinib (IM) is expected to yield less than 20 percent of additive MMR.

Aims: In this study, we investigated the efficacy of nilotinib (NIL) versus high-dose IM versus sustaining standard-dose IM for CCyR patients with suboptimal molecular response to frontline IM therapy.

Methods: Early chronic phase (CP) CML patients who have achieved CCyR but no MMR after at least 18 months and up to 24 months (18 to 24 months) on first-line IM therapy at a daily dose of 400 mg were divided into 3 treatment groups; NIL 400mg BID (800 mg/day; group 1) *vs* IM 400 mg BID (800 mg/day; group 2) *vs* IM 400mg QD (400mg/day; group 3). Group 1 and 2 patients were selected in RE-NICE multicenter study and group 3 patients were selected with the same inclusion criteria of RE-NICE. The efficacy endpoints are MMR rate by 12 months and MMR rate and undetectable molecular residual disease (UMRD) rates by 36 months. Safety profiles of each group were compared. Patients showing lack of response (lack of complete hematologic response (CHR) at 6 months, increasing WBC, no major cytogenetic response (MCyR) at 24 months), loss of response (loss of CHR or MCyR) or intolerance to treatment were allowed to switch to other treatment.

Results: With a data cut-off date of 12 Jan 2015, a total of 84 patients were evaluated; 29 patients in NIL group (group 1), 29 patients in high-dose IM group (group 2) and 26 patients in standard-dose IM group (group 3). With a median follow-up of 36 months (range, 1-63), all patients in group 1 remained in nilotinib treatment, 18 patients in group 2 switched to NIL 400mg BID due to intolerance (n=4) and lack of response (no MMR; n=14). In group 3, with a median follow-up of 13 months (range, 1-130), 16 patients switched to other treatment due to intolerance (n=5) and lack of response (no MMR; n=10, treatment failure; n=1) and 1 patient loss of follow-up after 9 months follow-up. Up to now, three patients lost CCyR (1 in group 1, 1 in group 2 and 1 in group 3). With an initial treatment 10 in 29 (35%), 8 in 29 (28%) and 5 in 26 (19%) patients achieved MMR by 12 months, and 20 in 29 (69%), 10 in 29 (34%) and 10 in 26 (38%) patients achieved MMR by 36 months in group 1, group 2 and group 3 respectively. Overall, 3 patients in group 1 (3/29, 10%) achieved confirmed UMRD. Overall 3 years probability of MMR was higher in group 1 than the other two groups (81.0% *vs* 64.6% *vs* 64.0%, group 1, 2, 3 respectively, group 1 *vs* 2, P=0.098, group 1 *vs* 3, P=0.020, group 2 *vs* 3, P=0.443). Compare to other groups, the patients in group 2 showed higher toxicities, such as leukopenia, anemia, thrombocytopenia, edema, fatigue, dyspnea and hypophosphatemia.

Summary and Conclusions: Nilotinib 400mg twice daily treatment showed better efficacy than high-dose or same standard-dose imatinib for the treatment of patients who have suboptimal molecular response to initial standard-dose imatinib. Additionally, a switch to nilotinib in suboptimal molecular responder to imatinib would also be preferable option in terms of tolerability. Updated data with longer follow-up duration will be presented in the meeting.

E1103

OUTCOMES OF CHRONIC PHASE CHRONIC MYELOID LEUKEMIA PATIENTS WITH 10%≤AND >10% BCR-ABL1 (IS) TRANSCRIPT LEVELS AFTER 3 MONTHS OF GENERIC IMATINIB TREATMENT

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Background: The molecular response at 3 months of tyrosine kinase inhibitor (TKI) therapy for patients with chronic myeloid leukemia (CML) has prognostic significance and, a *BCR-ABL1* transcript level >10% on the international reporting scale (IS) at 3 months is associated with inferior outcomes. This finding was confirmed by many groups with the original imatinib (OI) (Glivec®; Novartis, East Hanover, NJ, USA), but never been tested for generic imatinib (GI). GI was first introduced in Turkey in August 2012, and afterwards most of the patients with chronic phase CML (CML-CP) were administered up-front GI according to the reimbursement policy in Turkey.

Aims: The first aim of this study was to evaluate the *BCR-ABL1 (IS)* transcript levels at 3 months of GI treatment, and to demonstrate whether early molecular response (EMR) had a prognostic impact on outcome among CML-CP patients who receive GI in the first-line setting. The second aim was to compare these findings to that of OI, in order to determine whether there was any difference between group of patients receiving these two imatinibs.

Methods: The testing for *BCR-ABL1 (IS)* was available in our institute after January 2010, so the study cohort consisted of 90 CML-CP patients who were diagnosed between 2010 and 2014. Patients' demographics, Sokal risk scores and follow-up periods were retrospectively noted from the patients' files. Imatinib response was evaluated according to the criteria recommended by the European LeukaemiaNet (ELN). The cumulative major MR (MMR) and complete cytogenetic response (CCyR) rates were calculated. Patients were divided into two groups according to imatinib they consume; patients receiving OI were Group A, and GI were Group B. Also these groups were further divided into two regarding the *BCR-ABL1 (IS)* transcript levels at 3 months of imatinib as 10%≤and >10%.

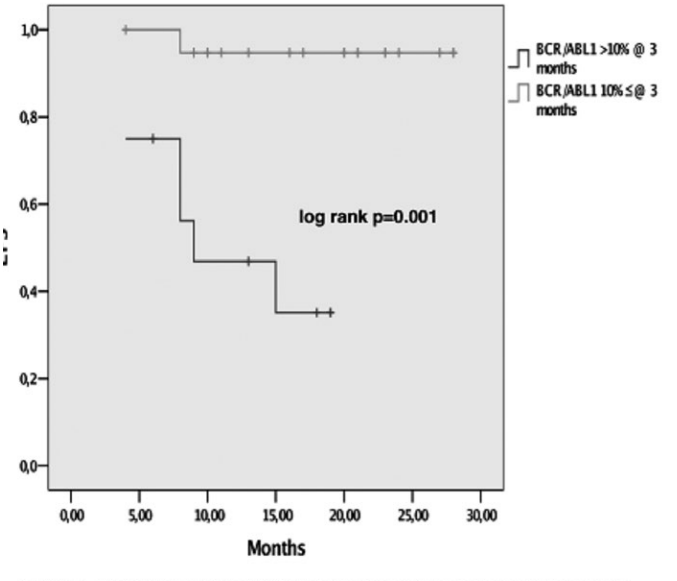

Figure 1. Event-free survival (EFS) difference between patients receiving generic imatinib with 10%≤ and >10% BCR-ABL1 (IS) transcript levels at 3 months

Figure 1.

Results: There were 47 patients in Group A and forty-three in Group B. The two groups were equally balanced regarding age, gender and Sokal risk scores

(Table 1). But patients in Group A had significantly longer median follow-up than patients in Group B (32.5 months vs. 13 months, *p<0.001*). *BCR-ABL1 (IS)* transcript levels at 3 months were available in 43 patients in Group A and thirty-two in Group B. There were 31 patients in Group A with a *BCR-ABL1 (IS)* transcript level 10%≤at 3 months whereas twelve patients had *BCR-ABL1 (IS)* transcript levels >10%. In Group B, 20 patients had a *BCR-ABL1 (IS)* transcript level 10%≤at 3 months and twelve had *BCR-ABL1 (IS)* transcript levels >10% (Table 1). The cumulative CCyR and MMR rates in patients with *BCR-ABL1 (IS)* level 10%≤at 3 months were significantly superior than patients with *BCR-ABL1 (IS)* level >10% both in Group A and B (Table 1). Also there were significantly more patients switching to 2nd generation TKIs (2GTKIs) due to resistance in both groups among patients with a *BCR-ABL1 (IS)* level >10% at 3 months (Table 1). Two groups did not significantly differ regarding cumulative CCyR and MMR rates as well as the number of patients with *BCR-ABL1 (IS)* levels 10%≤and >10%. In Group B, among patients with *BCR-ABL1 (IS)* levels 10%≤at 3 months (n=20), sixteen had MMR but the remaining 4 patients had a median follow-up of 8 months (range, 4-9 months) and 3 of them had CCyR and one had partial CyR. Among patients receiving GI, event-free survival (EFS) was significantly superior in patients with *BCR-ABL1 (IS)* level 10%≤at 3 months than patients with *BCR-ABL1 (IS)* level >10% (p=0.001) (Figure 1).

Table 1.

Table 1. Patient characteristics and treatment outcomes. (F, female; M, male, MMR, major molecular response; CCyR, complete cytogenetic response; PCyR, partial cytogenetic response; CHR, complete hematologic response; 2GTKI, second generation tyrosine kinase inhibitor) *Forty-three patients in Group A and 32 in Group B had a *BCR/ABL1* transcript level available at 3 months. ΦThe remaining 4 patients had a median follow-up of 8 months (range, 4-9 months) and 3 of them had CCyR and one had PCyR.

	Patients on front-line original imatinib Group A (n=47)			Patients on front-line generic imatinib Group B (n=43)			p value
Gender, n (M/F)	29/18			30/13			0.673
Median age, years (range)	42 (16-83)			46 (17-75)			0.411
Sokal risk score, low/intermediate/high (%)	54/33/13			41/38/21			0.388
Median follow-up, months (range)	32.5 (5-58)			13 (3-29)			<0.001
Patients with optimal response, n (%)	40 (85)			37 (80)			0.673
	*BCR/ABL1 (IS) at 3 months**			*BCR/ABL1 (IS) at 3 months**			
	10%≤, n 31	>10%, n 12	p value	10%≤, n 20	>10%, n 12	p value	
Cumulative CCyR rate, n (%)	30 (97)**	7 (64)***	0.001	19 (95)**	4 (34)***	<0.001	**0.150 ***0.229
Cumulative MMR rate, n (%)	30 (97)**	5 (45)***	<0.001	16 (80)+**	2 (17)***	0.001	**0.955 ***0.187
# of patients need to switch to 2GTKIs due to resistance, n	NA°	4°°	0.001	1°	5°°	0.011	°0.123 °°0.680

Summary and Conclusions: *BCR-ABL1 (IS)* transcript levels 10%≤at 3 months in CML-CP patients receiving generics had superior outcomes in terms of cumulative CCyR and MMR rates as well as EFS when compared to patients with *BCR-ABL1 (IS)* level >10%. And these findings were consistent with the literature displaying similar results in patients receiving Glivec.

E1104

PONATINIB EFFICACY AND SAFETY IN PATIENTS WITH A HISTORY OF STEM CELL TRANSPLANTATION (SCT) IN THE PACE TRIAL

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Background: Ponatinib is a potent oral BCR-ABL tyrosine kinase inhibitor (TKI) approved for patients with refractory chronic myeloid leukemia (CML) or Philadelphia chromosome–positive acute lymphoblastic leukemia (Ph+ALL) and those with the T315I mutation in BCR-ABL1.
Aims: This retrospective analysis describes the efficacy and safety of ponatinib (starting dose 45 mg once daily) in patients with a history of SCT prior to enrollment in the ongoing phase 2 PACE trial.
Methods: Patients with CML or Ph+ALL who were resistant or intolerant to dasatinib or nilotinib or who had the T315I mutation were enrolled in PACE (NCT01207440). All patients gave informed consent. The PACE trial excluded patients who underwent SCT <60 days before the first ponatinib dose, patients with evidence of ongoing graft-versus-host disease (GVHD), and patients who had GVHD requiring immunosuppressive therapy. Of 449 patients, 40 (9%) had a history of SCT.
Results: Of 40 patients with a history of SCT, 12 (30%) had chronic-phase (CP)-CML, 8 (20%) had accelerated-phase (AP)-CML, 11 (28%) had blast-phase (BP)-CML, and 9 (23%) had Ph+ALL at study entry (PACE enrollment). Median age was 48.5 years and median time since diagnosis was 3.8 years at the time of the first dose. Most patients (73%) had received ≥3 TKIs, either before or after SCT, prior to ponatinib treatment. Twelve patients (30%) had T315I detected at baseline (1 CP-CML, 2 AP-CML, 5 BP-CML, and 4 Ph+ALL patients). Twenty-five patients (63%) had an SCT ≤2 years (but ≥60 days) before starting ponatinib, and 15 (38%) had an SCT >5 years before starting ponatinib. Patients may have received intervening therapy between SCT and ponatinib. As of 6 October 2014, median follow-up for these 40 patients was 12.6 months after start of ponatinib; median duration of ponatinib treatment was 5.9 months. Median dose intensity was 39.9 mg/d and 45% of patients had their dose reduced. The primary endpoint in CP-CML (major cytogenetic response by 12 months) was achieved by 4 (33%) of 12 CP-CML patients. The primary endpoint in AP-CML, BP-CML, and Ph+ALL (major hematologic response by 6 months) was achieved by 3 (38%) of 8 AP-CML patients, 3 (27%) of 11 BP-CML patients, and 2 (22%) of 9 Ph+ALL patients. Major molecular response at any time was achieved in 1 (8%) of 12 CP-CML patients, 1 (13%) of 8 AP-CML patients, 2 (18%) of 11 BP-CML patients, and 1 (11%) of 9 Ph+ALL patients. Vascular adverse events occurred in 6 (15%) of 40 patients and included myocardial infarction (n=2), pulmonary embolism (n=2), angina pectoris (n=1), cerebral ischemia (n=1), coronary artery disease (n=1), portal vein thrombosis (n=1), superficial thrombophlebitis (n=1), and transient ischemic attack (n=1). Additional data regarding SCT and other treatments before ponatinib will be presented.
Summary and Conclusions: Ponatinib treatment induced responses in a substantial proportion of heavily pretreated Ph+patients with a history of SCT. Vascular adverse events were observed in 15% of patients. Benefits and risks should be considered when using ponatinib in patients with a history of SCT.

E1105

LONG-TERM BOSUTINIB FOR PHILADELPHIA CHROMOSOME–POSITIVE (PH+) ADVANCED CHRONIC MYELOID LEUKEMIA (CML) AFTER PRIOR TYROSINE KINASE INHIBITOR FAILURE

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Background: Advanced (ADV) Ph+CML patients (pts) have worse outcomes *vs* chronic CML pts.
Aims: In this first report of bosutinib (BOS) activity in this cohort as fully enrolled, we evaluate long-term efficacy and safety of BOS in ADV pts ≥4 y *vs* ≥1 y from last enrolled pt.
Methods: Ongoing phase 1/2 BOS study in 79 accelerated phase (AP) and 64 blast phase (BP) pts with prior tyrosine kinase inhibitor (TKI) failure. Informed consent was obtained from all pts.

Table 1.

Newly Occurring AEs*								
	Year 1		Year 2		Year 3		Year 4	
n	AP N=79	BP N=64	AP N=34	BP N=5	AP N=19	BP N=3	AP N=15	BP N=2
Diarrhea	67	41	0	0	0	0	0	0
Cardiac	12	7	1	1	1	1	2	0
Vascular	4	7	4	0	1	0	1	0
*Not experienced by same patient previously (denominator=patients on treatment each y [1 y = 52 wk])								

Results: For AP and BP pts, 18% and 3% remained on BOS at 4 y (vs 48%; 13% at 1 y; 1 y=48 wk); 57% and 28% newly attained or maintained baseline overall hematologic response (OHR) by 4 y (most by 12 mo); 40% and 37% attained/maintained major cytogenetic response (MCyR) by 4 y (most by 12 mo). Kaplan-Meier probabilities of maintaining OHR in responders at 4 *vs* 1 y were 49% *vs* 78% (AP) and 19% *vs* 28% (BP); Kaplan-Meier MCyR probabilities at 4 *vs* 1 y were 49% *vs* 65% (AP) and 21% *vs* 21% (BP). AP and BP pts were treated for median 10.2 (range, 0.1–88.6); 2.8 (0.03–55.9) months. Most common AEs were gastrointestinal (AP, 96%; BP, 83%), primarily diarrhea (85%; 64%), which was typically low grade (grade 1/2: 96%; 93%), transient (median duration/any grade AE: 2 [range, 1–910] d; 2 [1–211] d); no pt discontinued due to this AE. Newly occurring AEs arose mostly in y1; new cardiac/vascular AEs occurring in y4 were pericardial effusion, sinus bradycardia/1st degree atrioventricular block (same pt), and hypertension (all n=1); those in y1 (>2 pts either cohort) were pericardial effusion (AP, n=4; BP, n=1), tachycardia (n=2; n=4), hypertension (n=3; n=2; Table). Serious AEs occurred in 56% AP and 58% BP pts, most commonly pneumonia (n=9; n=5). 11 AP and 13 BP pts died within 30 d of last dose; 2 BOS-related (AP). Treatment discontinuations were mostly due to progressive disease (AP, ≤1/>1 y,

n=10/13; BP, n=29/3) and AEs in AP (n=16/5); death (n=6/0) and symptomatic deterioration (n=6/0) in BP.
Summary and Conclusions: Durable response was seen in ~50% AP responders (~25% BP responders at y1, for whom BOS may be bridge to transplant); toxicity was manageable with long-term treatment.

E1106

PATIENT CHARACTERISTICS AND ADVERSE EVENTS (AES) OF TYROSINE KINASE INHIBITORS (TKIS) FOR THE TREATMENT OF CHRONIC MYELOID LEUKEMIA (CML) IN REAL WORLD SETTINGS

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Background: The growing number of TKIs available for CML increases complexity in choosing therapy in non-research settings.
Aims: This study aims to determine patient (pt) characteristics & emergent AEs that might underlie treatment choices.
Methods: TKI treatment episodes for adult CML pts from 1/2008 - 7/2014 were identified in the MarketScan Commercial, Medicaid, and Medicare supplemental databases. Pts were required to have ≥6 mos of enrollment prior to each TKI episode and ≥1 pharmacy or medical claims for one of the TKIs on or after the first CML diagnosis date. Analyses were retrospective and descriptive. Charlson comorbidity index (CCI) and frequency of other key comorbid conditions prior to each TKI episode were used to characterize patient baseline status (BL). Corresponding treatment-emergent events were captured using the same diagnosis or treatment codes indicative of the conditions. Vascular occlusive (VO) conditions include one or more of the following: myocardial infarction, congestive heart failure (CHF), thrombotic events, acute coronary syndrome, peripheral vascular, cerebrovascular, or coronary artery diseases. Median duration of treatment was assessed using the Kaplan-Meier method.
Results: 4,166 TKI treatment episodes for CML were identified. Median follow up was 13 months. Imatinib (IM) was most commonly used 1st line, dasatinib (DAS) and nilotinib (NIL) 2nd line, and bosutinib (BOS) and ponatinib (PON) 3rd line. BOS pts had the most previous TKI episodes and were furthest from initial diagnosis. They were the oldest, had the highest CCI, the most histories of VO conditions, kidney diseases, and pleural effusion at baseline, but the lowest rates of serious emergent AEs and discontinuation. With younger age and lower CCI, PON pts had the highest incidences of treatment emergent VO events and fluid retention, with the shortest treatment duration and highest rates of discontinuation.

Table 1.

	IM n=1,898	DAS n=1,180	NIL n=953	BOS n=87	PON n=48
Age (median)	55	52	53	56	51.5
# Prior TKIs (mean)	0.8	1.2	1.2	2.4	2.0
Time since initial diagnosis (median mos)	9	10	11	25	18
CCI (mean)	5.5	5.0	5.4	6.2	5.4
BL /emergent AE (%)					
VO conditions	31/5	30/6	31/5	43/5	40/15
CHF	11/3	10/4	14/3	23/1	15/6
Dysrhythmias	19/4	18/6	24/9	39/5	35/8
Fluid retention	16/6	17/6	18/5	36/3	33/10
Renal diseases	13/4	11/4	16/3	20/3	19/6
Diabetes	24/6	24/4	23/5	23/2	25/4
Pleural effusion	7/3	10/8	14/4	33/1	29/2
COPD	22/4	22/6	23/3	26/2	23/0
Abdominal pain	32/7	36/7	37/9	45/2	46/10
Diarrhea	14/6	16/5	17/3	24/13	31/8
Treatment Duration (median mos)	16	17	15	13	5
Discontinuation (%)	27	19	24	15	29

Summary and Conclusions: BOS appeared to be associated with lower risks of serious AEs compared to other TKIs and suited to elderly pts with high orbidities. It will be important to confirm the findings with larger sample sizes.

E1107

THE EFFECT OF NILOTINIB IN CHRONIC MYELOID LEUKEMIA TREATMENT DOSE ON FERTILITY AND TERATOGENICITY IN A HEALTHY MOUSE MODEL

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Background: Chronic myeloid leukemia (CML) is a hematopoietic pluripotent stem cell disease where myeloid cells lead to uncontrolled proliferation. Current treatment of Ph (+) CML is based on the inhibition of tyrosine kinase inhibitors (TKI), especially second-generation drugs. Majority of CML patients are male and 46% of them are between 20 and 64 years of age. Therefore, it is conceivable that inhibition of c-kit or PDGFR by TKI may have deleterious effects on spermatogenesis or folliculogenesis, resulting in male or female subfertility. In the first part of this study we showed the suppression of folliculogenesis and prevention of spermatogenesis during the long-term nilotinib treatment.
Aims: The aim of this part of our study is to determine the effect of nilotinib on fertility and teratogenicity that is used routinely to treat CML.
Methods: Here we present the results of testicular and ovarian changes after nilotinib administration to five- week old male and female C57bl6 mice. Mice received 0.4 mg of nilotinib per day dissolved in the drinking water for 2 months. Control group received only drinking water. Treatment dose was determined according to the clinical studies regarding the plasma concentrations (20 mg/kg, orally).
Results: There were no differences in the fertility and live birth index, the sex ratio or the frequency of survival to the time of weaning and also no evidence of teratogenicity in the fetuses. Low birth weight fetuses were seen in the nilotinib receiving female group whether the interruption of the drug during the pregnancy. The pregnancy rates of the couples according to the case of the using nilotinib or not were shown in Table 1. The pregnancy rates were reduced to 75% in the case of male mice used nilotinib; to 60% in the case of female mice used nilotinib; and finally to 25% in the case of both female and male mice used nilotinib in the cohort.

Table 1. The pregnancy rates of the couples

Pregnancy Rate	Nilotinib Female (n=12)	Control Female (n=12)
Nilotinib Male (n=12)	25%	75%
Control Male (n=12)	60%	100%

Summary and Conclusions: Although our results indicate that mice achieved the pregnancy whether they used nilotinib, the pregnancy rates reduced significantly compared to the control group in the study, especially if both female and male mice used nilotinib together. According to limited information, the potential consequences of the drug on developing fetus is still matter of debate, all female mice have given birth to healthy baby in our study. In the third part of our ongoing study, we are investigating the long-term effect of nilotinib on baby mice.

E1108

DYNAMICS OF RESPONSE AND IMPACT OF SOKAL SCORE IN 3 MONTHS MOLECULAR "WARNING" CML PATIENTS. A RETROSPECTIVE STUDY OF GRUPPO TRIVENETO LMC

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Background: Response to TKI is considered the strongest predictor of long-term outcome in CML patients. As known, effective treatment overcomes the negative impact of most prognostic factors, including Sokal score. Different studies have demonstrated that early molecular response is strictly correlated with outcome: infact, missing the 10% BCR-ABL landmark at 3 months predicts inferior long-term survival.
Aims: We investigated the dynamics of 3 months molecular "warning" CML patients at 6 and 12 months landmarks; secondly, we sought to evaluate the impact of Sokal score in the context of this unfavourable group
Methods: A total of 51 patients with BCR-ABL levels >10% at 3 months were identified for the analysis from a cohort of 350 consecutive CML patients treated with front-line standard dose imatinib (400 mg daily). "Optimal", "warning" and "failure" responses were stated according to ELN2013 recommendations. Complete cytogenetic response (CCyR) was defined as 0% Ph+metaphases; major molecular response (MMR) was defined as BCR-ABL <0.1%IS. TTF was measured from the start of imatinib to the date of any of the following events: progression to accelerated or blastic phase, death for any cause at any time, primary or secondary hematologic, cytogenetic or molecular resistance leading to imatinib discontinuation. PFS was measured from the start of imatinib to the date of progression to accelerated or blastic phase or death for any cause at any time. Survival probabilities were estimated by the Kaplan-Meier method and compared by log rank test; differences among variables were evaluated

by the Fisher's exact test or by Cochran–Mantel–Haenszel test
Results: The median age of patients was 56 years (range 25–81), with 33 males and 18 females. The median follow-up was 45.3 months (range 7–110). The distribution of patiens according to the Sokal score was: 11 (21.6%) in the low risk, 24 (47.1%) in the intermediate and 16 (31.4%) in the high risk group, respectively. At 6 months, only 3 of 40 evaluable patients, 1 in each group (11.1%, 5% and 9% respectively), improved their 3 months molecular response, achieving an optimal response. 20 failures, defined as more than 10%IS BCR-ABL transcript level, were recorded (1, 11 and 8 in low, intermediate and high risk group respectively, P=0.008 comparing low *vs* other risk groups). At 12 months, 21 of 25 evaluable patients were considered failures; only 1 patient gained an optimal response. 27.2%, 50% and 50% of low, intermediate and high Sokal score patients failed to achieve a CCyR at any time (P=0.047), while 72.7%, 45.8% and 31.3% of patients obtained a MMR (P=0.104), respectively. Median time to CCyR and MMR was 12, 12.5, 24.5 months (P=0.21), and 30, 25 and 22 months (P=0.21) for low, intermediate and high risk group responding patients. Imatinib discontinuation rate, albeit remarkable in all Sokal groups, was not significantly different (66.7% *vs* 78.3% *vs* 93.8% respectively, P=0.22), as well as PFS (P=0.349), TTF (P=0.321) and OS (P=0.812). 9.1%, 12.5% and 25% of low, intermediate and high Sokal risk patients progressed to AP/BC phase, respectively.
Summary and Conclusions: These data suggest that although non-low Sokal score CML patients may experience a higher probability of early failure, long term outcomes of molecular "warning" patients seem not to be significantly influenced by Sokal risk. Therefore, 3-months BCR-ABL transcript level higher than 10% IS appears to overcome non only the negative, but also the positive prognostic impact of Sokal score. These observations warrant further confirmation in larger studies.

E1109

EVALUATION OF A CENTRALIZED MOLECULAR MONITORING OF CHRONIC MYELOID LEUKEMIA THERAPY IN SOUTH KOREA

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Background: For the treatment of chronic myeloid leukemia (CML), higher efficacy of tyrosine kinase inhibitors (TKI) has required accurate molecular monitoring by standardized real-time quantitative polymerase chain reaction (RQ-PCR). Although the standardization of RQ-PCR test has become particularly important to increase the accuracy, each participating laboratory should establish a laboratory-specific conversion factor through regular exchange of a series of samples. However the process is very complicated and periodic validation is needed to keep the stabilization of original conversion factors of laboratories. Otherwise the measurement of *BCR-ABL1* transcript by single standardized laboratory can reduce many unnecessary processes and is able to increase accuracy of result.
Aims: In this study, we evaluated the Korean centralized RQ-PCR program and the result of molecular monitoring of CML patients in routine clinical practice.
Methods: Twenty four Korean university hospitals were agreed on a centralized assay performing in the Catholic University of Korea and the program was started in October, 2012. EDTA tubes containing peripheral blood were shipped to central laboratory within 24 hours and standardized RQ-PCR assays were performed. To validate the result, we analyzed *BCR-ABL1* transcript level and compared with *ABL1* copy number for quality control of each samples.
Results: Between October 2012 and November 2014, a total of 4,638 samples from 1,545 CML patients were tested to measure the ratio of *BCR-ABL1* to *ABL1* transcript level in international scale (IS). The *ABL1* copy number was more than 100,000 copies in 3,770/4,638 (81.3%), 32,000 and 100,000 copies in 784 (16.9%), 10,000 and 32,000 in 43 (0.9%), and less than 10,000 copies in 41 (0.9%) samples. Of 1,203 patients who were tested more than 2 times, 1,094 (90.9%) had appropriate copies of *ABL1* for defining MR of each patient. However, 109 (9.1%) in undetectable level were less than 100,000 ABL1 copies which is inappropriate copies of *ABL1* for defining $MR^{5.0}$. Of 1,545 patients, 1,064 (69%) had $MR^{3.0}$. Among them, 515 (33.1%) and 514 (33%) were in $MR^{4.5}$and $MR^{5.0}$ respectively. Of patients achieving at least $MR^{3.0}$, 98% (n=1,043) and 87% (n=925) of patients had ≥32,000 and ≥100,000 *ABL1* copies respectively, and these were in the minimal requirement of *ABL1* copy number for defining $MR^{4.5}$and $MR^{5.0}$.

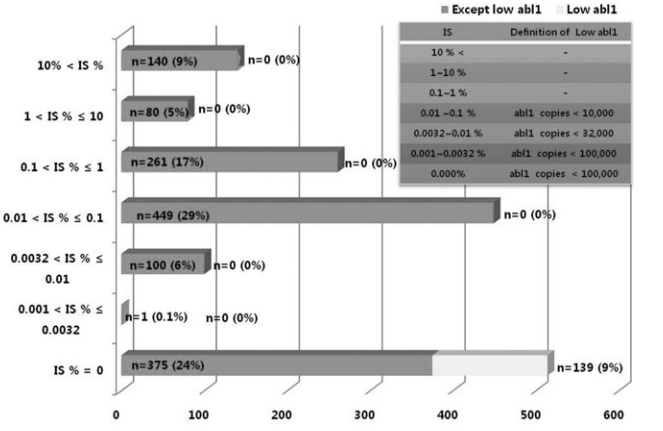

Figure 1. Molecular response and Quality of RQ-PCR assay measuring by *ABL1* copy number (n=1,545).

Summary and Conclusions: Molecular monitoring using a centralization program of *BCR–ABL1* assay may provide stable and qualified results. Through this program, more than 98%of samples met the minimum requirement of ABL1 copy numbers for at least $MR^{4.5}$sensitivity and it can support for TFR studies in the future.

E1110

PREDICTIVE FACTORS TO ACHIEVE DEEP MOLECULAR RESPONSE AND LONG-TERM OUTCOMES IN CHRONIC MYELOID LEUKEMIA PATIENTS TREATED WITH TYROSINE KINASE INHIBITORS. A SINGLE CENTER EXPERIENCE IN ARGENTINA

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Background: Few data is available about long-term follow-up treatment and outcomes with Tyrosine Kinase Inhibitors (TKI) in South American patients (pts). Response to TKI is known as the most important predictor of outcome in Chronic Myeloid Leukemia (CML). Early achievement of responses has also been determined by many authors as predictor of long-term outcomes. However, major molecular response (MMR) is not a failure criterion at any time point.
Aims: To identify predictive factors for achievement of deep molecular response (MR) and improvement of failure-free survival (FFS). Secondary objective was to determine whether late MMR acquirement impacts on FFS.
Methods: Pts were treated with various TKI modalities between 2000 and 2014. Cytogenetic analyses were performed with standard G-banding. Molecular diagnostics for residual BCR-ABL transcripts followed the procedures and definitions of Cross et al; and were performed in a standardized and accredited laboratory. Confirmed $MR^{4.0}$was defined as ≥4.0 log reduction of BCR-ABL (detection ≤0.01%) on the international scale and determined by Rq-PCR in two consecutive analyses. Overall survival (OS) was defined as the time between diagnosis and death from any cause. FFS accounts for failure to achieve response as defined by ELN, lost of complete cytogenetic response (CCyR), accelerated phase and blast crisis. Adherence monitoring was assessed by treating physician and reported in medical record.

Results: Of 119 pts with diagnosed chronic phase CML, median age was 48 years (range, 16-82), 52% were males. The percentage of pts with low Sokal risk score were 82%, 25% of pts had been previously treated with interferon. Median follow-up was 89 months (mo) (range, 3-306). A total of 103 pts were treated with 1st line imatinib (IM) 400mg daily and constitute the basis of the analysis. The proportion of pts treated with IM who achieved CCyR or BCR-ABL <1% within 6 mo and MMR within 12 mo was 84% and 38% respectively. Median-time to achieve MMR and $MR^{4.0}$was 2.6 and 3.6 years respectively. At 60 mo, FFS was 91% and OS 98%. Pts still on initial IM were 79% after 8 years. By logistic regression analysis CCyR/BCR-ABL <1% within 6 mo, MMR within 12 mo and adherence resulted independent predictors for achieving $MR^{4.0}$ (*OR, 95%CI* and *p value* are detailed in Table 1). In pts who acquired CCyR/BCR-ABL <1% within 6 mo, MMR within 12, 24 mo and adherence >90%, FFS at 60 mo was higher than those who did not (Table 1). The sub-analysis determined that pts ≤30 years had lower adherence than older pts (76% *vs* 94% *p.* 0.02).

Table 1. Predictors of Deep Molecular Response and FFS analysis in Pts With CML treated With IM.

Variables	n (%)	Univariate analysis		Multivariate analysis		Univariate analysis	
		MR 4.0 (%)	p Value	OR	95% CI	5-yr FFS (%)	p Value
Within 6 mo							
CCyR/BCR-ABL < 1%	78 (84)	77	0.003	5.6	(1.6-19)	95	0.001
No CCyR	15 (16)	40				71	
Within 12 mo							
MMR	31 (38)	84	0.006	5.3	(1.4-21)	100	0.01
No MMR	50 (62)	54				82	
Within 24 mo							
MMR	54 (64)	80	0.03	2.5	(0.9-7.2)	100	0.006
No MMR	31 (36)	58				75	
Adherence							
> 90%	86 (91)	78	0.003	7.0	(1.3-37)	92	0.00001
< 90%	9 (9)	33				79	

Responses at each time point were compared using χ^2 test or Fisher exact test, as appropriate. Multivariate models (logistic regression) were performed, considering MR4.0 as dependent variable and CCyR/BCR-ABL < 1% within 6 mo, MMR within 12 mo, MMR within 24 mo and adherence as predictive variables. Failure-Free Survival (FFS) was estimated using the Kaplan-Meier method and compared by log-rank test.

Summary and Conclusions: In our long-term follow-up population, CCyR/BCR-ABL <1% within 6 mo, MMR within 12 mo and adherence constitute early independent predictors for achievement of deep MR consistent with the published literature. Although good responses are obtained with IM, some pts still progress, this is the reason why it is necessary to identify those who will relapse so as to consider a change in treatment to another TKI at a convenient time point. Better results in terms of FFS were observed for pts who acquired CCyR/BCR-ABL <1% within 6 mo, MMR within 12 mo and adherence >90%. Nevertheless MMR within 24 mo does not seem to affect long-term outcomes and further investigations, in a larger pt population, are needed to determine when MMR must be considered a failure criterion. In terms of adherence, young adults are particularly at risk and a new strategy may be needed in this special subgroup of pts. Lack of adherence can prevent the long-term molecular objective of treatment. These results emphasize the validity, feasibility and harmonization of molecular monitoring and treatment of CML worldwide.

E1111

NEW TOOL FOR MONITORING MOLECULAR RESPONSE IN CHRONIC MYELOID LEUKEMIA

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Background: Chronic myeloid leukemia (CML) treatment monitoring using PCR based peripheral blood testing provides improved test sensitivity over cytology but suffers from inadequate standardization due to variation inherent in the existing PCR methodologies. Standardized Nucleic Acid Quantification BCR-ABL (SNAQ-BCRABL) is a novel competitive template based peripheral blood b2a2/b3a2 transcript abundance method. It uses mixtures of b2a2 or b3a2 and GusB competitive templates and melting curve analysis to provide desired quality controls to correct for procedural variation.
Aims: A pilot study was conducted in CML patients to evaluate the imprecision and linearity of two established real-time qPCR laboratory developed test (LDT) and SNAQ for monitoring BCR-ABL.
Methods: Thirty six CML patients treated at our institution were enrolled in this pilot study. Their peripheral blood sample were analyzed at MD Anderson Molecular Diagnostic Laboratory and Cancer Genetics Institute for BCR-ABL by LDT (L1 and L2) and SNAQ (S1 and S2) test respectively. The LDT methods used TaqMan real-time assays to quantify BCR-ABL and ABL targets in cDNA synthesized from WBC RNA isolated from the blood specimens. The SNAQ method likewise used cDNA synthesized from WBC, but differs at the PCR step by the addition of known quantities of competitive templates (b2a2 or b3a2 and GusB) to the cDNA prior to PCR amplification and melting curve analysis. AccuGenomics's SNAQ software imports the melting curve data, performs quality control and curve fitting analysis to generate a BCR-ABL report. The LDT methods report %BCR-ABL/ABL results using International Standard, and the SNAQ results were reported as %BCR-ABL/GusB.
Results: Each test result (n= 36) was ranked against all the other samples tested by the same method. The Pearson correlation between SNAQ and LDT was met with correlations of 0.96, 0.96, 0.97 and 0.94 with L1 x S1, L1 x S2, L2 x S1 and L2 x S2 respectively. ANOVA of log %BCR-ABL interlaboratory results indicated a significant difference between LDT methods ($p<0.0000001$), but not with the SNAQ methods between labs ($p=0.98$) (Figure1A). Imprecision was estimated using the Bland-Altman method and the plot indicated that sample results from L2 had 1 outlier, which was excluded from analysis. All three L1 difference plots (Figure1B & C) had a significant trend ($p<0.007$), requiring regression correction prior to estimating variation. The linear regression analysis, indicated L2 LDT was a large source of method bias.Post hoc analysis of method agreement showed the SNAQ method did not generate any outliers and had a 95% limit of agreement of ±3-fold between laboratories, whereas L1 and L2 LDT method had significant differences despite reporting in international scale, with 95% limit of agreement of 2 to 20-fold.

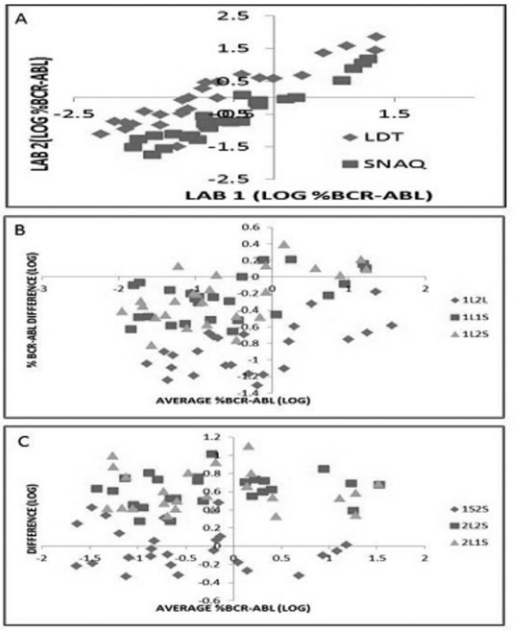

Figure 1.

Summary and Conclusions: In this pilot study, SNAQ methodology performed well suggesting it might be able to overcome some of the limitations encountered by some of the LDT currently in clinical practice. Additional studies with more patients and correlation with clinical outcomes are required to confirm this observation.

E1112

MULTICENTER STUDY OF COMORBIDITY IN CANARIAN PATIENTS WITH CHRONIC MYELOID LEUKEMIA TREATED WITH TYROSINE KINASE INHIBITORS

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Background: In February of 2009 the Canarian registry of chronic myeloid leukemias (CML) was created in order get a better insight of treatment response, behavior and outcome in our region. For that purpose data from patients treated in the 7 hospitals of the Canary Islands was collected.
Aims: The purpose of the study was to investigate the cardiovascular risk associated with the use of Tyrosine Kinase Inhibitors (TKI) in the CML treatment.
Methods: Since 2009**,** 127 patients have been included. The series consisted of 65 (51.18%) males and 62 (48.8%) females with a mean age at diagnosis of 54.64±16.37. 75 patients (59%) received Imatinib, 37 (29%) received Nilotinib and 15 (12%) Dasatinib. We analyzed cardiovascular risk factors such as hypertension (HTA), diabetes (DM), body mass index (BMI) and smoking. We estimated the Framingham risk score (FRS) and researched the series retrospectively for atherosclerotic events.
Results: Among risk factors to be considered in our series, we revealed that 55 patients (43.31%) were receiving concomitant drugs for hypertension, 34 patients (26.77%) had a concomitant DM, and 9 patients (7.09%) were smok-

ers. According to WHO classification, 12 patients were classified as normal weight (BMI<25), 32 patients were overweight (BMI 26≤30), 36 were obese (BMI>30) and 47 unclassified because we couldn't retrieve the BMI for the whole series. At time of diagnosis 87 patients (68.5%) were classified as low risk FRS category (<10% chance of suffering an atherosclerotic event), 19 patients (14.96%) as intermediate risk (10-20%) and 21 patients (16.54%) as high risk (>20%). There were 9 patients (7.09%) who presented an atherosclerotic event after treatment with a TKI of which 1 patient used Dasatinib (1/15) (Cerebrovascular accident), 4 used Imatinib (6.67%) (2 aortic stenosis, 1 aortic aneurism with bypass and 1 peripheral arteryoclusive disease (PAOD) with ischemic ulceration) and 4 used Nilotinib (10.81%) (1 anterior ischemic optic neuropathy, 1 intermittent claudication, 1 aortic stenosis accompanied with intermittent claudication and 1 toe amputation). There was no significant difference in the incidence of the atherosclerotic events between Imatinib and Nilotinib. Dasatinib patients were excluded due to the small series. The 9 patients with atherosclerotic events had high blood pressure levels, 2 of them had the hypertension under contol and the rest (7 patients) had an uncontrolled hypertension. There was a significant difference in incidence of the events between patients with low risk FRS category (33.33%) and intermediate-high risk FRS (66.66%) regardless the treatment (p=0.025). There was no significant difference in patients with imatinib between low risk FRS (57.33%) and intermediate-high risk FRS (42.67%), where 2 of the patients having atherosclerotic events were classified as low risk FRS and the other 2 were classified as high risk FRS. But in patients with nilotinib is a significant difference between patients classified as low risk FRS (64.86%) and intermediate-high risk FRS (35.10%) (p=0.005), where the 4 patients presenting atherosclerotic events were classified as high risk FRS.

Summary and Conclusions: Atherosclerotic risk factors in patients with CML should be considered before the treatment with TKIs. Those patients with high risk factors to which is decided treatment with Nilotinib despite the risk score should also be closely monitored.

E1113

THE RATE OF BCR-ABL DECLINE AS AN OPTIMIZED PREDICTOR OF OUTCOME FOR PATIENTS WITH CHRONIC MYELOID LEUKEMIA IN CHRONIC PHASE ON TREATMENT WITH TYROSINE KINASE INHIBITORS

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Background: About 70% of chronic myeloid leukemia (CML) patients achieved early molecular response (*BCR-ABL*^{IS} ≤10% at 3-months) that led to 5-years overall survival close to 95%. Nonetheless, CML patients remain heterogeneous group and several studies in recent years were aimed to personalize treatment based on individual patients' characteristics. Our group previously put forward a hypothesis about the prognostic value of individual *BCR-ABL* decline rate in the first three months of CML therapy[1,2]. The ratio *BCR-ABL* at 3 months to baseline had chosen as 0.1 as best cut-off value (sensitivity 83.33, CI 62.6-95.3; specificity 66.67, CI 34.9-90.1) to predict MMR at 12 months.

Aims: The aims of this study were to validate our prognostic method in larger group of patients.

Methods: Fifty-five patients (median age, 52 years; range 19-84; 24 male and 31 female) with chronic phase CML were included in the study. Distribution of Sokal risk groups were as follows low-30 / intermediate-15 / high-10. Six patients had EUTOS high-risk. Forty-two patients started treatment with Imatinib 400 mg/day, 12 patients started with Nilotinib 600 mg/day and 1 patient started with Dasatinib 100 mg/day. Median *BCR-ABL*^{IS} transcript levels was 41.38% at diagnosis, range 3.39-3185.36%. The ratio of *BCR-ABL* levels at 3 months to baseline for each patient was calculated. In addition, we calculated ratio of *BCR-ABL* levels at 3 months to *BCR-ABL* levels at 1 month for 13 patients. Comparison was made of the predictive sensitivity to achieve early molecular response at 3 months (10% by IS). We estimated relative risk (RR) the probability of achieving MMR, depending on the method of stratification of patients. Statistical analysis was conducted with Fisher exact test.

Results: Twenty-six out of 34 patients (76.5%) with ratio of *BCR-ABL* levels at 3 months to baseline below than 0.1 achieved MMR at 12 months, while only 9 of 21 patients (42.9%) with ratio more than 0.1 had optimal response (RR=0.41 (0.20 – 0.84); p=0.03). Ratio of *BCR-ABL* levels at 3 months to 1 month also showed good results with the same (0.1) cut-off value – 5 out of 6 patients (83.3%) with ratio *BCR-ABL* at 3 months to 1 month below than 0.1 achieved MMR, while only 1 patient (14.3%) with ratio more than 0.1 achieved optimal response (RR=0.19 (0.03 – 1.19); p=0.05). Application of early molecular response at 3 months (10% by IS) yielded worse discrimination results: 34 of 47 (72.3%) patients with *BCR-ABL* level ≤10% at 3 months had achieved MMR at 12 months, whereas 2 of 8 (25%) patients with *BCR-ABL*>10% had MMR at 1 year (RR=0.96 (0.62 – 1.49); p=0.78). Moreover, application of our ratio cut-off value among patients with *BCR-ABL* level ≤10% at 3 months allowed us to revealed additional 6 high-risk patients have not reached MMR at 1 year of therapy, Figure1.

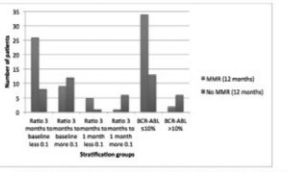

Figure 1. The patient numbers of achieving MMR at 12 months of therapy in various stratification groups.

Figure 1.

Summary and Conclusions: Our study demonstrated that the individual *BCR-ABL* decline rates from baseline to 3 months and to 1 month might be useful prognostic markers that allowed detecting more patients at risk who had no MMR at 1 year of treatment with using *ABL* as control gene. Also, the study showed that the individual ratio of *BCR-ABL* levels might be studied as more predictive landmark for change of TKI treatment even among patients that have *BCR-ABL* levels ≤10% at 3 months.

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E1114

IMATINIB TREATMENT DURATION EFFECT ON ESTIMATED GLOMERULAR FILTRATION RATE IN CHRONIC MYELOID LEUKEMIA PATIENTS

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Background: Imatinib (IM) represents an important therapeutic option in Chronic Myeloid Leukemia (CML). There are various clinical trials suggesting safe IM discontinuation in CML patients with sustained major molecular response (MMR), however, life-long therapy with IM is still the consensus recommendation. Therefore, there is increasing interest in identifying long-term therapy associated toxicity of this treatment. There is lack of evidence regarding the effect of long-term IM treatment on renal function.

Aims: To study the possible effect of IM therapy duration on renal function impairment.

Methods: We studied a group of 48 patients (25 men, 23 women), 47 in chronic phase (CP) CML, 40 (83%) of these patients with deep molecular response ($MR^{4.5}$), 1 patient with accelerated disease, receiving IM in the course of their disease. Exclusion criteria was chronic renal disease at baseline (estimated glomerular filtration rate (eGFR) ≤60 ml/min/1.73m^2). The median age was 51 (range 25-78) at the time when IM was started. The median duration of IM treatment was 76,8 months (4,2 – 149,6), median total follow-up was 84,1 months (4,2 – 152,6). 36 patients (75%) were previously treated with interferon (IFN), median duration of treatment 29,4 months (4,2-278,8). At present, 6 of these patients are receiving IFN together with IM treatment. Laboratory data (creatinine, urea) and eGFR using CKD-EPI equation were obtained periodically from the start of IM treatment. Clinical information about hypertension (HTA) and diabetes (DM), relevant for renal function assessment was collected.

Results: The mean baseline eGFR was 96,27±17 ml/min/1.73m^2, mean eGFR at last observation 77,55±25 ml/min/1.73m^2, mean decrease between the baseline and last eGFR 18,71 ml/min/1.73m^2. During the follow-up, 5 patients developed Acute kidney injury (AKI), defined as increase in creatinine ≥0,3mg/dl, all of them during the first month of IM treatment. There was the normalization of creatinine and eGFR values in only 2 of these patients. 10 patients developed chronic kidney disease, median age of this group was 64 (31-78), 1 patient was diabetic and 6 patients had history of HTA. eGFR decreased significantly with IM treatment duration (p<0,05). Age, HTA, DM or previous IFN treatment were not significantly related to eGFR decrease.

Summary and Conclusions: Although IM is well tolerated and highly efficient, its long-term effect on eGFR decrease and its mechanism are not well established. We proved that IM treatment duration may be associated with important eGFR decrease. Patients receiving IM should be monitored closely with regular eGFR calculation.

E1115

CHRONIC MYELOID LEUKEMIA: IS THE REAL PROGNOSIS OF NEWLY DIAGNOSED PATIENTS REALLY SO GOOD?

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Background: Precise, mature data about real-world treatment efficacy in modern era of chronic myeloid leukemia (CML) are still missing. Moreover, true incidence of persistent side effects of tyrosine kinase inhibitors (TKI) is rarely reported in clinical trials.
Aims: To give a real picture of TKI treatment by analyzing detailed, prospective database of all consecutive CML cases.
Methods: Data regarding all patients treated in the academic institution with the catchment area of about 2 million people were analyzed according to the European LeukemiaNet recommendations (Guilhot, Blood 2012). Diagnostic and treatment protocols follow ELN and EUTOS recommendation/standardization. TKI side effects were assessed according to CTCAEv4.
Results: Two hundred and fourteen patients (median age 58 years, range 18-92; 53.7% of males) with newly diagnosed CML in 2005 – 2014 underwent the analysis: 194 patients (90.7%) in chronic phase (CP), 11 (5.1%) in accelerated phase, 8 (3.7%) in blast crisis, and in 1 (0.5%) case the phase was unknown. In total, 41 (19.2%) patients died during the follow-up (median 3.8 years, range 0.1-9.8): 22 patients due to CML activity, 13 patients due to causes probably not related to CML, and in 6 cases the reason was unknown. The first line therapy given to patients diagnosed in CP (Sokal high risk in 29.9%) was as follows: imatinib, N=152 (78.4%); nilotinib, N=24 (12.4%); dasatinib, N=6 (3.1%); other, N=7 (3.6%); none, N=5 (2.5%) due to death (N=3) and lost to follow-up (N=2) before the treatment start. The median follow-up of 182 patients in CP treated with 1st line TKI was 45.3 months (range, 5.2-115.8). Estimated cumulative incidences of complete cytogenetic responses and major molecular responses at 48 months were 92.2% and 89.2%, respectively. Estimated OS (defined as the time from the start of TKI therapy to the death, with no censoring at the time of therapy change) at 48 months was 89%. Estimated PFS, FFS, EFS, and ATFS at the same time point were 89.9%, 74.5%, 67.4%, and 66.7%, respectively. In total, 38.5% of patients permanently discontinued the first line TKI. The reasons for discontinuation in group treated with imatinib (N=61/152; 40.1%) were resistance in 32/61 patients (52.4%), intolerance (13/61; 21.3%), and other reasons (16/61; 26.2%; in 9 cases the reason was participation in discontinuation trials, and in 5 patients non-CML related deaths). Reasons for nilotinib discontinuation (6/24; 25%) were as follows: resistance (N=1), intolerance (N=3), and other (N=2). Subsequent therapy after imatinib discontinuation included dasatinib (N=19), nilotinib (N=16), and other (N=15). After cessation of nilotinib, patients were treated with dasatinib (N=2), imatinib (N=2), and other therapy (N=2). Analysis of imatinib and nilotinib clinical non-hematological toxicity incidence during the time revealed significant proportion of clinically relevant events of grade 2-4, and their persistence during the whole follow-up (Table 1).

Table 1. Incidence of clinical non-hematological toxicities during the time of imatinib and nilotinib first line therapy.

1st line imatinib (N=152)						
Grade	M0-3 N=152	M3-6 N=142	M6-12 N=135	M12-24 N=120	M24-48 N=97	M48+ N=58
1	71 (46.7%)	76 (53.5%)	68 (50.4%)	67 (55.8%)	48 (49.5%)	32 (55.2%)
2	19 (12.5%)	23 (16.2%)	23 (17.0%)	24 (20.0%)	27 (27.8%)	15 (25.9%)
3	15 (9.9%)	7 (4.9%)	5 (3.7%)	4 (3.3%)	8 (8.2%)	6 (10.3%)
4	-	1 (0.7%)	1 (0.7%)	-	-	-
Total	105 (69.1%)	107 (75.4%)	97 (71.9%)	95 (79.2%)	83 (85.6%)	53 (91.4%)
1st line nilotinib (N=24)						
Grade	M0-3 N=24	M3-6 N=24	M6-12 N=20	M12-24 N=12	M24-48 N=4	M48+ N=0
1	11 (45.8%)	14 (58.3%)	11 (55.0%)	1 (8.3%)	1 (25.0%)	-
2	3 (12.5%)	2 (8.3%)	4 (20.0%)	1 (8.3%)		-
3	2 (8.3%)	1 (4.2%)	1 (5.0%)	4 (33.3%)	1 (25.0%)	-
4	-	-	-	-	-	-
Total	16 (66.7%)	17 (70.8%)	16 (80.0%)	6 (50.0%)	2 (50.0%)	-

Summary and Conclusions: About 10% of CML patients is diagnosed in advanced disease and many newly diagnosed patients still die from leukemia. Moreover, more than one third of patients in CP have changed the first line therapy, mainly due to resistance, or intolerance. ATFS is a valuable parameter covering all situations of treatment change. In patients who continue on originally chosen TKI, there is the evidence of clinically relevant adverse events persistence in many of them, which can influence quality of life and can contribute to drug non-compliance. More detailed analysis will be presented.

E1116

SIGNIFICANCE OF ANEMIA IN PATIENTS WITH CML AT DIAGNOSIS AND DURING THE TREATMENT WITH IMATINIB OUTSIDE THE CLINICAL TRIALS: CAMELIA REGISTRY EXPERIENCE

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Background: Attention of physicians caring for CML patients is seldom focused on anemia as it was not shown to be an independent prognostic factor and it may have different causes during the course of disease. The relevance of anemia in routine clinical setting outside the clinical trials has not been sufficiently covered.
Aims: To evaluate the prognostic and clinical importance of anemia in CML patients treated with imatinib using data from the international CML CAMELIA Registry.
Methods: Retrospective analysis of all files of CML patients treated with first-line imatinib in first chronic phase was performed. For the purpose of the study anemia was defined by hemoglobin level lower than 120g/l. Single center survey of quality of life using simple questionnaire in 32 CML patients with and 31 without anemia was performed. For statistical testing Mann-Whitney test and Fisher exact test, for survival analysis Kaplan-Meier method with log-rank test were used.
Results: Anemia was identified at diagnosis in 211 (45%) from the total cohort of 469 patients in first chronic phase of CML treated with imatinib. Anemia was not associated with initial cytogenetic findings (p=0.946) or age (p=0.125), but strong correlation was found with higher risk scores in all prognostic systems (Sokal, Hasford, EUTOS; p<0.001). Patients with anemia had significantly higher numbers of WBC (median=175.6 *vs* 48.0 p<0.001) and more frequent splenomegaly (p<0.001). Response to the treatment was similar: major molecular response was achieved in 72.5% and 68.7% of patients without and with anemia, respectively. However, when assessing overall survival with the endpoint death caused by CML, anemia was associated with worse outcome (log-rank test p=0.013). There were 23 deaths caused by CML among 31 dead patients with anemia (74.2%) but only 11 out of 28 deaths (39.3%) in the group of patients without anemia (p=0.009). During the treatment with imatinib anemia was identified in 91 (19.4%) patients at one or more occasions. There was no correlation with the anemia or risk scores at diagnosis. There was a trend towards association of anemia with age over 60 years (24.4% *vs* 16.9%; p=0.063). Anemia during the treatment was not associated with response to treatment, but has significant impact on quality of life: patients spent more time in bed (p=0.014), less time outside the flat or house (p<0.001), they suffered more from dyspnea (p=0.022) and there were trends for lower Karnofski score (p=0.057), unemployment (p=0.077) or sport capacity (p=0.079).
Summary and Conclusions: Anemia at diagnosis in CML patients from CAMELIA Registry was a frequent finding and was associated with the high-risk features. Despite the fact that response to treatment was not compromised with anemia at diagnosis, a significant association was found with CML-related death rate. During the treatment with imatinib, anemia was found in about 20% of patients, affected more frequently elderly patients and had negative impact on their quality of life.

E1117

EFFICACY AND SAFETY FOR DASATINIB IN EARLY CHRONIC PHASE CML PATIENTS WITH LATE SUBOPTIMAL RESPONSE TO FRONTLINE IMATINIB. PRELIMINARY RESULTS FROM DASAPOST STUDY

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Background: The additional benefit of achieving major molecular response (MMR) in patients with Complete Cytogenetic Response (CCyR) response is still under debate, and therefore, patients with CCgR without MMR after 12 months of treatment are considered as a "warning" by European LeukemiaNet (ELN) recommendations. Several clinical trials have shown how patients treated with imatinib front line and classified as late warning responders can benefit from treatment changed to nilotinib in terms of improving molecular response. However, there are no data regarding to treatment change to dasatinib in this group of patients
Aims: To evaluate the efficacy and safety of treatment change to dasatinib in patients treated with imatinib first line with late suboptimal response (patients with CCyR without MMR after at least 18 months of treatment) by the ELN 09 recommendations
Methods: We are presenting preliminary results of the first 18 patients enrolled in the phase II DASAPOST study (NCT01802450). Main inclusion criteria were patients treated with late suboptimal response by the ELN09 (CCyR without MMR after 18 months of treatment). Previous treatment with imatinib 600mg (but not 800mg) was allowed. Median exposure to imatinib before dasatinib was 5.1 years (1.8-12.2). Sokal risk groups % (L/I/H) was 22.5%, 55% and 22.5%. Median age was 56 years (34-77). Primary end point was the achievement of MMR after 6 months of dasatinib. Secondary endpoints were to assess the efficacy of dasatinib in terms of depth and kinetics of molecular response, as well as the relationship of response with lymphocyte alterations. Responses evaluations were performed following indications of the ELN. All BCR-ABL/ABL (IS) measurements were centralized in an EUTOS laboratory.
Results: Clinical: Eighteen patients have been enrolled in the study. Median follow up at data cut-off was 262 days (21-380). Three out of 18 (16%) patients had discontinued dasatinib due to side effects (pancreatitis, pleural effusion and low grade, persistent side effects (fever, arthralgias, anemia and asthenia). 16/18 patients have been evaluated at 3 months, 12 at 6 months and 6 at 12 months. Cumulative incidences by ITT of MMR calculated by competing risks by 3 and 6 months were 50 and 83%. However, for patients who reached the 6 months assessment frequencies of MMR and MR4.5 were 85% and 42% respectively. No patient have lost CCyR while 1 patient in MR4.5 lost MMR. 1 patient had reduced dasatinib dose to 70mg due to congestive heart failure (patient achieved and maintained undetectable molecular response). Immunological: Lymphocyte counts were done before and after dasatinib intake at baseline, at 3 and 6 months, observing an increment of counts post intake in most patients. At baseline the median increase post intake was 1,79 fold (0,98-3,2). There was no significant association between this increment and MMR at 3M (MMR at 6 months was not studied, as most patients obtained this response at that timepoint).
Summary and Conclusions: Our study shows, for the first time to our knowledge, that in patients treated with Imatinib and late suboptimal (warning) responses, switch to Dasatinib induced MMR in 83% of the patients, although 16% discontinued treatment because of toxicity. No association was found between lymphocyte "mobilization" post intake and response. Dasatinib appears to have a good benefit/ risk ratio in this type of patients. More details on the immunologic studies will be provided.

E1118

OPTIMIZATION OF THERAPEUTIC DOSES OF RADOTINIB FOR CHRONIC MYELOID LEUKEMIA BASED ON EXPOSURE-RESPONSE RELATIONSHIP ANALYSES

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Background: BCR-ABL1 tyrosine kinase inhibitors (TKIs) have been administered as fixed doses for adult patients with chronic myeloid leukemia (CML). However, due to the wide inter-individual variability in the pharmacokinetics of TKIs and increasing evidence supporting the relationship between drug exposure and the efficacy and toxicity of these agents, there may be potential benefits of TKI dose individualization based on body size. Radotinib is a selective second generation BCR-ABL1 TKI and a phase 2 study was previously conducted in patients with TKI failed CP CML.
Aims: Using the data from the phase 2 study, radotinib exposure-efficacy and -safety relationship analyses were conducted to explore the dosing methods that will potentially improve the efficacy and safety profiles of radotinib.
Methods: The efficacy and safety data were collected for 12 months after the initiation of radotinib therapy from a multi-center phase 2 study conducted in 77 CP CML patients resistant and/or intolerant to other TKIs. All patients received radotinib 400 mg twice daily until a dose-limiting toxicity (DLT) appeared, after which the dose was reduced to 300 mg twice daily. The relationships between the body weight-adjusted dose (Dose/wt) and the probability of achieving major cytogenetic response or experiencing DLT were explored using a logistic regression method. The analyses were repeated using body-surface-area-adjusted dose (Dose/BSA). Upon a stratification of the patients based on Dose/wt or Dose/BSA, time-to-first DLT curves were compared using a Kaplan-Meier method.
Results: *Efficacy.* No significant associations were found between radotinib Dose/wt or Dose/BSA and major cytogenetic response at Months 1, 3 and 6. *Safety.* Positive correlations were observed between radotinib Dose/wt and the probabilities of first DLT occurrence at Months 3 (p=0.002), 6 (p=0.003), 9 (p=0.004), and 12 (p=0.007). Similar positive correlations were observed for Dose/BSA. Statistically significant differences were evident in the Kaplan-Meier curves of DLT between various Dose/wt groups, particularly between the groups of Dose/wt <6 mg/kg and Dose/wt ≥6 mg/kg (p=0.008) with the median time to first DLT being 259 and 83 days, respectively. At the cut-off of 6 mg/kg, the patient weighs 66.7 kg. A 2-tier weight-based dosing method was recommended to reduce the probability of DLT: radotinib 300 mg or 400 mg twice daily for patients weighing ≤65 kg or >65 kg, respectively.
Summary and Conclusions: The probability of DLT increased without improvement in efficacy as the Dose/wt or Dose/BSA of radotinib increased. Therefore, a lower initial radotinib dose of 300 mg twice daily is recommended for CP CML patients weighing ≤65 kg. A randomized clinical trial would be needed to confirm the efficacy and safety of this alternative dosing regimen.

E1119

EFFICACY AND SAFETY OF DASATINIB VS. IMATINIB IN LATIN AMERICAN SUBPOPULATION FROM THE DASISION TRIAL IN PATIENTS WITH NEWLY DIAGNOSED CHRONIC MYELOID LEUKEMIA (CML) IN CHRONIC PHASE (CP)

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Background: Kantarjian *et al.* reported in the phase 3 DASISION TRIAL that 519 patients with newly diagnosed CML-CP from 108 centers in 26 countries were randomized to receive dasatinib (n=259) or imatinib (n=260). Of these, 23 % (120) were from the Latin America (LA) region (23 % Argentina, 9 % Chile, 3 % Colombia, 15 % Peru, 19 % Brazil and 30% Mexico).
Aims: The main objective was to evaluate if LA results from DASISION TRIAL can be considered similar to those obtained in the overall study.
Methods: DASISION (CA180-056; NCT00481247) is a signed IC 60 month-Open Label Multinational randomized phase 3 trial comparing dasatinib 100 mg QD versus imatinib 400 mg QD in patients with CML-CP diagnosed within 3 months who had not received previous treatment for CML. All exposure, safety, and efficacy results described here were analyzed on the 120 LA patients (all randomly assigned to receive dasatinib (n=63) or imatinib (n=57)) in comparison to the total patients treated (including Latin American patients), here referred to as "all randomized". The efficacy and safety were assessed by using data obtained during the initial 3 years period of the trial.
Statistical Methods: Comparison of the rates was performed for p=0.05 (two-tailed). Response (efficacy variables) and AEs rates were estimated with their 95% confidence intervals (CIs) when needed. The difference in rates between the 2 treatment groups was tested by using Pearson Chi square Test. PFS and OS by treatment group were estimated via the Kaplan-Meier product-limit method. Due to the fact that the 120 LA patients is a subset of the planned total enrolled population, every statistical analysis of LA was considered exploratory.
Results: Treatment was discontinued due to disease progression in 4,8 % and

7% of the cases, unacceptable toxicity in 3,2% and 8,8% of the cases in dasatinib and imatinib groups respectively. Baseline characteristics and demographics of LA patients were relatively well balanced between the two treatment groups and were similar to the overall study data with exception of baseline high Hasford risk score in LA patients that was higher in both (dasatinib and imatinib) treatment groups. At 36 months the rates of cumulative cCCyR, MMR and PFS, OS and AEs rates for patients in either arm in LA patients and in all randomized patients can be seen in Table below. AEs in LA and in all randomized patients will be described.

Table 1.

Rates (% of patients) of Efficacy Variables and Safety				
Variable	Latin America (LA)		DASISION TRIAL (All Patiens)	
	dasatinib (n=63)	imatinib (n=57)	dasatinib (n=259)	imatinib (n=260)
cCCyR within 12 months (a)	79.4	64.9	76.8	66.2 **
cCCyR within 24 months (a)	82.5	73.7	80.3	74.2
cCCyR within 36 months (a)	82.5	73.7	82.6	77.3
MMR within 12 months (b)	44.4	28.1	52.1	33.8 **
MMR within 24 months (b)	69.8	43.9 **	64.5	50.0 **
MMR within 36 months (b)	73.0	49.1 **	69.1	56.2 **
PFS within 36 months (c)	91.9	89.7	91.0	90.9
OS within 36 months (d)	91.9	92.7	93.7	93.2
DRAEs (e)	84.1	98.2	84.5	88.0
(a) confirmed Complete Cytogenetic Response; (b) Major Molecular Response; (c) Progression Free Survival; (d) Overall Survival; (e) Drug Related Adverse Events. dasatinib vs. imatinib: **: p < 0.01.				

(1) Kantarjian H, Shah N, Hochhaus A, et al. Dasatinib versus imatinib in newly diagnosed chronic-phase chronic myeloid leukemia. New England Journal of Medicine. June 2010; 362:2260-70.
(2) Kantarjian H, Shah N, Cortes J, et al. Dasatinib or imatinib in newly diagnosed chronic-phase chronic myeloid leukemia: 2-year follow-up from a randomized phase 3 trial (DASISION). February 2012;119:1123-1129.

Summary and Conclusions: The results reported here suggest that the efficacy profiles of dasatinib vs. imatinib in LA patients are similar to those seen in the analysis of all patients worldwide. Exploratory comparisons of efficacy in LA patients of dasatinib vs. imatinib arms yielded similar trends as observed in all patients, having numerically higher rates of cumulative long-term response rate values of cCCyR and MMR in dasatinib treatment Group. Both treatment groups also experienced high rate values of PFS and OS after a 3-year follow-up. Since small sample size in LA limits the strength of conclusions for efficacy and safety, further exploration is needed to confirm any potential differences compared with the total treated population in order to increase accuracy.

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E1120

THE EFFICACY AND SAFETY OF GENERIC IMATINIB IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA (CML) AFTER SWITCHING FROM GLIVEC: UPDATED DATA FROM CERRAHPAŞA CML COHORT

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Background: Imatinib is the standart of care in patients with chronic myeloid leukemia (CML). Generic imatinib (GI) has been approved in the treatment of CML in many countries including Turkey. We previously published our experience regarding the efficacy and safety of GI in patients with CML who started tyrosine kinase inhibitor (TKI) treatment with original imatinib (OI) [Glivec] but then had to switch to GI due to reimbursement policy [Eskazan AE, *et al.* Leuk Lymphoma. 2014;55:2935-7]. Among our patient cohort, the efficacy and safety of GI were comparable to those of OI. However the median duration of GI exposure in that study was 12 months, shorter than the OI treatment duration prior to switching.
Aims: The aim of this study was to update the efficacy and safety data of GI among our chronic phase CML (CML-CP) patient cohort when used sequentially after OI treatment with an extended follow-up.
Methods: Our study cohort consisted of one hundred and forty-five patients with CML-CP who were followed under OI with a median of 55 months (Figure 1). Patients on OI first switched to GI due to reimbursement policy after August 2012, and 80 patients switched to GI whereas sixty preferred to receive OI and pay the price difference from their own pockets. After a median follow-up of 12 months, the data was first analyzed in October 2013, and the generics were found to be at least non-inferior to the OI regarding efficacy and tolerability when used subsequently [Eskazan AE, *et al.* Leuk Lymphoma. 2014;55:2935-7]. We updated the data of this study after an additional follow-up of 16 months in February 2015.
Results: Since the first analysis, 74 patients received GI with a median duration of 15.5 months, and there were four patients who switched to 2nd generation TKIs (2GTKIs) due to resistance and 4 patients were lost to follow-up. The median of GI exposure in these patients after the first switch was 27 months (range, 6-32 months). In the OI group, 60 patients received Glivec with a median of 61 months and 13 months after the first switch and the first analysis, respectively. There were 3 patients who switched to 2GTKIs (2 due to resistance, one due to grade IV hepatitis), one patient was lost to follow-up, and one patient quit OI due to a planned pregnancy. Twenty-seven patients receiving OI switched to GI during the follow-up after a median of 10 months, and at the time of the analysis, the study cohort consisted of 121 patients of which 28 were still on OI whereas ninety-three were receiving GI. All of these 121 patients had durable major molecular respose (MMR), and none of the 27 patients who switched from OI to GI lost their responses during the follow-up. There were no imatinib dose reductions due to toxicities in both arms, and 4 patients had non-hematological adverse events (AEs) (myalgia in 3 and gastrointestinal in one) in the GI group whereas in the OI group there were 3 patients (myalgia in 2 and one patient had both myalgia and hepatitis) with non-hematological AEs. Among the twenty-seven patients who switched from OI to GI, two had grade I myalgia after the switch.

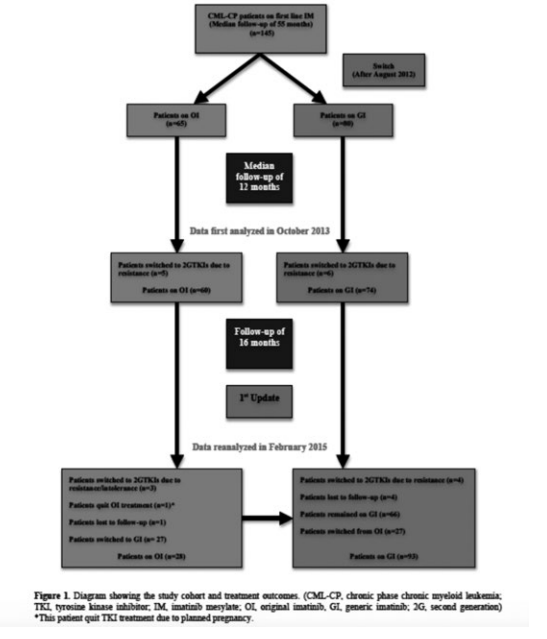

Figure 1. Diagram showing the study cohort and treatment outcomes. (CML-CP, chronic phase chronic myeloid leukemia; TKI, tyrosine kinase inhibitor; IM, imatinib mesylate; OI, original imatinib; GI, generic imatinib; 2G, second generation) *This patient quit TKI treatment due to planned pregnancy.

Figure 1.

Summary and Conclusions: With an extended follow-up, generics were still found to be comparable to Glivec regarding both efficacy and safety when used subsequently.

E1121

GENERIC IMATINIB IN NEWLY DIAGNOSED CHRONIC MYELOID LEUKEMIA (CML) PATIENTS IN CHRONIC PHASE: UPDATED DATA FROM A TURKISH CML COHORT

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Background: Generic imatinib (GI) has been approved in the treatment of patients with chronic myeloid leukemia (CML)in many countries including Turkey. Since there were limited data and some concerns about the efficacy of

generics, we previously published our experience regarding the efficacy and safety of GI when used in the up-front setting [Eskazan AE, *et al.* Br J Haematol. 2014;167:139-41]. Although the duration of GI exposure in this study was relatively short, the efficacy and safety of GI were at least non-inferior to those of original imatinib (OI) [Glivec].

Aims: The aim of this study was to update the efficacy and safety data of GI among our chronic phase CML (CML-CP) patient cohort in the frontline setting with a prolonged follow-up.

Methods: We first analyzed the data of CML-CP patients who received up-front Glivec and GI in February 2014 [Eskazan AE, *et al.* Br J Haematol. 2014;167:139-41] (Figure 1). There were 36 patients who started the tyrosine kinase inhibitor (TKI) treatment had with OI, and twenty-six with GI. After median follow-up of 8.5 months and 20 months of GI and OI, respectively, we did not find significant difference regarding efficacy and AEs between these 2 groups. During the follow-up, 8 patients switched from OI to GI due to reimbursement policy. We wanted to update the data of the initial cohort with an additional follow-up of 12 months, and we also recruited new patients to the study.

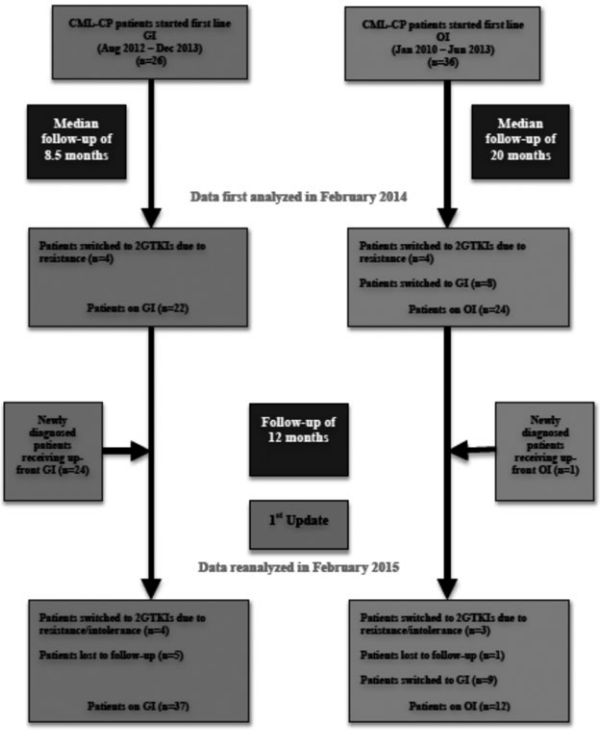

Figure 1. Diagram showing the study cohort and treatment outcomes. (CML-CP, chronic phase chronic myeloid leu TKI, tyrosine kinase inhibitor; IM, imatinib mesylate; OI, original imatinib, GI, generic imatinib; 2G, second generation

Figure 1.

Results: During the follow-up, 24 newly diagnosed patients were recruited in the GI group whereas one patient started receiving OI (Figure 1). Nine patients from the OI group switched to GI group due to reimbursement policy after a median follow up of 24 months (range, 11-45 months). They were all in major molecular response (MMR) at the time of the switch, and after a median of 7 months (range, 3-15 months) of GI expose, they all maintained their responses. In the GI group, 20 patients from the initial analysis were still receiving GI after a median of 20 months (range, 10-28 months), of which 15 had MMR, 4 had complete cytogenetic response (CCyR), and one had partial CyR. Among the 24 newly diagnosed patients receiving GI, sixteen (67%) were male, and the median age was 55 years. Low, intermediate and high Sokal risk score rates were 47%, 47% and 6%, respectively. The median duration of GI treatment was 8 months (range, 2-28). There were 11 patients with a follow-up of ≤6 months of which ten had complete hematologic response (CHR). Among the 8 patients with a follow-up of >6 and <12 months, 5 had CCyR and three had MMR. Five patients had a follow-up duration of ≥12 months (median, 22 months), and all of them were in MMR. During the follow-up, patients who needed to be switched to 2nd generation TKIs (2GTKIs) due to resistance in the GI and OI groups were 3 and two, respectively. When the two groups were compared regarding AEs, in the OI group the most common non-hematologic AEs (all grades) were myalgia (n=8) and peripheral edema (n=4). The most common hematologic AEs were thrombocytopenia (n=4) and neutropenia (n=1) observed among patients in the OI group. In the GI group, the most common non-hematologic AEs were peripheral edema (n=6), myalgia (n=5) and skin reactions (n=3). The most common hematologic AEs in the GI group were thrombocytopenia (n=3) and neutropenia (n=3). There was a patient in the GI group who was switched to a 2GTKI due to grade III skin reaction. Also one patient from the OI group switched to 2GTKI due to grade IV hepatitis. There were 5 patients in the GI group who were lost to follow-up, and one patient was lost to follow-up in the OI group. At the time of the analysis, after a median follow-up of 16 months (range, 3-28 months), there were 37 patients who were on up-front GI. Twelve patients who were still on frontline OI after a median follow-up of 31 months (range, 19-55 months) were all in MMR.

Summary and Conclusions: GI was still non-inferior to OI regarding efficacy and tolerability when used in the up-front setting among patients with CML-CP with an extended follow-up.

E1122

ANALYSIS OF THE RELATIONSHIP BETWEEN DOSE AND BCR-ABL HALVING TIME IN CP-CML PATIENTS TREATED WITH PONATINIB OR IMATINIB

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Background: Ponatinib is a potent pan-BCR-ABL tyrosine kinase inhibitor (TKI). The phase 2 PACE study demonstrated that ponatinib is highly active in heavily pretreated CP-CML patients, 58% of whom received ≥3 prior TKIs. Preliminary evidence in the phase 3 EPIC study suggests that ponatinib has improved efficacy over imatinib in newly diagnosed CP-CML, but with a higher rate of arterial occlusive events at the doses studied. Fixed starting doses of ponatinib (45 mg qd) and imatinib (400 mg qd) were used in these studies, but the allowance for treatment interruptions and dose reductions, coupled with frequent assessment of *BCR-ABL* levels, enabled high-resolution analysis of dose-response relationships.

Aims: To inform design of trials to optimize ponatinib dose, here we use a novel approach, BARD (*BCR-ABL* Response-Dose association), to evaluate associations between ponatinib dose and changes in *BCR-ABL* levels across different lines of therapy.

Methods: *BCR-ABL* transcript levels were measured every 1-3 months in blood samples from CP-CML patients in the PACE (N=267) and EPIC (N=154 [ponatinib] and 152 [imatinib]) studies, which had median follow-ups of 34.2 and 5.1 months, respectively. The change in *BCR-ABL* levels (expressed as 1/doubling time [1/DT]) and average daily dose (based on patient daily dosing records) were calculated for every measurement interval (window). 1/DT values were analyzed for differences by t-test. An exponential growth/decay model was used to transform the average 1/DT values, to *BCR-ABL* halving times. We performed a series of analyses to demonstrate that including *BCR-ABL/ABL* transcript values >10% IS does not strongly affect our conclusions; we will present additional data using GUS to normalize the data.

Results: In newly diagnosed patients (EPIC), across all dose levels, BARD analysis showed that *BCR-ABL* halving times induced by ponatinib and imatinib were 13.8±1.2 and 27.1±2.4 days, respectively, consistent with the more rapid molecular responses observed in the ponatinib arm. Within windows that included only continuous dosing at starting dose levels, *BCR-ABL* halving times were significantly more rapid with 45 mg ponatinib than 400 mg imatinib ($p<0.0001$) (Table). Moreover, compared with 400 mg imatinib, *BCR-ABL* halving times were also more rapid when average ponatinib dose levels were <15 mg ($p>0.05$), 15 to <30 mg ($p<0.02$), and 30 to <45 mg ($p<0.0001$) (Table). In heavily pretreated patients (PACE), across a similar timeframe as EPIC, increased doses of ponatinib were also associated with a trend towards more rapid decreases in *BCR-ABL* levels, although the rate of decrease induced by 45 mg ponatinib (halving time 28.1±4.8 days) was reduced compared with that observed in newly diagnosed patients. Importantly, across the entire PACE study, average daily doses of ponatinib as low as 10 mg were associated with net decreases in *BCR-ABL* levels.

Table 1.

Table. *BCR-ABL* Response Dose Association (BARD) from EPIC

Drug	Dose range (mg)	Windows* (N)	*BCR-ABL* halving time (Days)
Ponatinib	<15	40	20.0 ±6.9
	15 to <30	60	16.6 ±3.4
	30 to <45	136	14.3 ±2.1
	45	184	12.0 ±1.3
Imatinib	400	374	24.9 ±2.3

*Number of *BCR-ABL* measurement intervals when average dose was within the indicated range

Summary and Conclusions: In newly diagnosed patients, ponatinib doses as low as 15 mg induced more rapid decreases in *BCR-ABL* levels than 400 mg imatinib. The magnitude of *BCR-ABL* decreases induced by ponatinib in heavily pretreated patients was reduced compared with newly diagnosed

patients; nonetheless, ponatinib doses as low as 10 mg were still associated with disease control overall. These analyses have helped inform the design of studies aimed at optimizing the benefit/risk of ponatinib treatment for patients with CML.

Gene therapy, cellular immunotherapy and vaccination

E1123

IL-10/IL-17 DOUBLE-PRODUCING T CELLS: NEW IMMUNOSUPPRESSIVE INSIGHT IN ACUTE MYELOID LEUKEMIA

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Background: Acute myeloid leukemia (AML) is the most common form of acute leukemia in adults and current treatments remain unsatisfactory. Serious infections, resistance to therapy and relapses are the main causes of mortality among these patients. The high frequency and severity of infections, even before or during any chemotherapy, are probably due to a deep dysfunction of adaptive immunity directly induced by the disease. T helper 17 cells (Th17) play a key part in inflammatory response and autoimmune diseases, reducing or promoting tumor growth and protecting against bacterial and fungal pathogens. However, the role of Th17 cells in AML has not yet been clarified.
Aims: In our study we examine the relationship between Th17 cells, leukemic cells, infections and immunocompetence.
Methods: After obtaining the patient's informed consent, peripheral blood was collected from 30 newly diagnosed AML patients without infections and from 30 age-matched healthy volunteers (HV). Mononuclear cells (PBMCs) were separated by density gradient centrifugation and CD4+cells isolated by negative immunomagnetic depletion. Cells were cultured in complete medium, primed for 24 h with IL-6 and for 5 h with PMA and ionomycin. An unstimulated control was included for each experiment. After stimulation, cells were fixed, permeabilized and immunophenotyped for intracellular IFN-γ, IL-4 and IL-17A expression using the human TH1/TH2/TH17 phenotyping kit. For Treg analysis, naïve PBMCs were stained with anti-CD4 FITC, anti-CD25 APC-Cy7 and then fixed, permeabilized and stained with anti-FoxP3 APC. Appropriate isotype controls were included for each sample. For cytokine secretion analysis, stimulated CD4+cells were analyzed using human IL-17 and IL-10 secretion assay-detection kits. For co-culture assays, leukemic CD33+blast cells magnetically isolated from patients and allogeneic CD4+cells obtained from HV were co-seeded in 1:1, 1:5 and 1:10 ratios and stimulated as previously described. At the end of stimulation, T cell immunophenotypic and cytokine secretion analysis were performed. Naïve CD4+cells were also stimulated for 24 h with *C. Albicans* peptides, then depleted of IL-17-secreting cells and cultured for a further 24 h in complete medium supplemented with *C. Albicans* peptides. T cells were then analyzed for intracellular IFN-γ expression using the human TH1/TH2/TH17 phenotyping kit. A sample stimulated with *C. Albicans* for 48 h without depletion of IL-17-secreting cells was added as control.
Results: In AML patients, compared to HV, we observed a strong increase in Th17 cells that simultaneously released immunosuppressive IL-10, together with a reduced frequency in Th1 and Th2 cells. Through the culture of AML-derived CD4+cells with an infectious antigen (*C. Albicans*), we demonstrated that Th17 cells selectively determined an immunosuppressive state, in terms of a reduction in IFN-g production (Figure A). Moreover, all the changes observed in T cells, and in particular in Th17 cells, were induced *in vitro* by CD33+leukemic cells, as observed after co-cultures of healthy CD4+cells and AML peripheral blasts (Figure B) and confirmed by restoration of the healthy cytokine pattern after purification of patient T cells from CD33+cells.

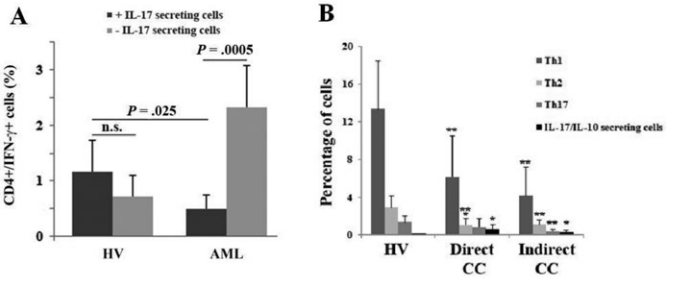

Figure 1.

Summary and Conclusions: Our results show that altered Th17 cells actively cause an immunosuppressive state in AML patients that may, considering the long life of these cells, persist long enough to promote infections and probably tumor escape. Th17 cells could thus represent a new target to improve AML immunotherapy.

E1124

HUMAN MEMORY-LIKE NATURAL KILLER CELLS ABLE TO KILL NEOPLASTIC CELLS AS A TOOL FOR A NOVEL APPROACH OF ANTI-TUMOR CELL THERAPY

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Background: Several strategies have been under investigation for enhancing antitumor activity in patients affected by a high risk solid or hematological malignancies. The poor prognosis of these patients and the need to sustain antitumor surveillance early after HSCT prompted the development of immune-based therapies including the use of natural killer (NK). Currently, strategies to prepare NK cell products include stimulation with IL-2 or other cytokines without any pre-activation. The efficacy of these approaches is restricted by short-term persistence and limited survival and effector function after adoptive transfer of the NK cells into a patient. Recent studies in mice have shown that *in vitro* activation with appropriate cytokines result in differentiation of long-lived NK lymphocytes with memory-like properties.
Aims: Aim of this study was to investigate optimal culture conditions to induce human memory-like NK cells both in autologous and allogeneic setting and to analyze their lytic capacity against tumor cells (TC) or leukemia blasts (LB) derived from onco-hematological patients.
Methods: The optimal culture conditions for *ex vivo* activation of human memory-like NK cells were established in allogeneic setting in 10 donors/recipients pairs, using NK cells derived from HSCT donors and tested against leukemia blasts (LB) derived from transplanted patients affected by acute myeloid or lymphatic leukemia. For induction of *memory like* NK cells, NK cells isolated from mononuclear cells of HSCT-donor by NK cell isolation Kit (Miltenyi Biotec) were pre-activated for 16 hours with different concentrations of IL-12/IL-15/IL-18, maintained in culture 7-10 days with IL-2 or IL-15 and then further activate or not prior cryopreservation and evaluation of cytotoxic activity. NK cells were alternatively activated overnight (ON) with IL-2 (control NK). During the various phases and at the end of culture cell recovery, NK receptors and anti-tumor cytotoxic activity of memory-like and control NK cells, were evaluated.
Results: Donor-derived NK *memory like* displayed high levels of cytotoxic activity against patients LB (mean: 53.7%, range 26-82 at effector:target ratio of 30:1), while the levels of lysis against patients' non malignant cells was always less than 10% at E:T ratio of 30:1. Interestingly, this approach of activation with IL-12/IL-15/IL-18 makes NK cells endowed of high levels of anti-tumor cytotoxic activity when compared with control NK cells activated ON with IL-2 alone (mean 25%, range 12-33 at E:T ratio of 30:1). A surface marker phenotype specific to memory-like NK cells has not yet been clearly identified in mice, while in humans few studies have established some correlation between surface phenotype of human NK-memory like and IFN-gamma production. In agreement with these studies we confirmed that human cytokine induced memory-like NK cells demonstrated increased expression of CD94, NKG2A, NKp46 and CD69 compared with control NK cells from the same donor. Preliminary experiments in murine model, obtained after transfer of *memory like* and control NK cells derived from two different HSCT donors, suggested that *memory like* NK cells persisted longer in the mice compared with control NK cells. However, these data have to be confirmed in a larger number of experiments, together with the ability of the transferred cells to maintain their anti-tumor activity. Further experiments are in progress to determine the possibility of *in vitro* inducing *memory like* NK cells, able to efficiently kill TC, in autologous setting, starting from NK cells isolated from patients affected by solid tumors.
Summary and Conclusions: In the present study, we identified human NK cells that exhibit enhanced anti-tumor cytotoxic activity after short-term pre-activation with IL-12/IL-15/IL-18, followed by 7-10 days of *in vitro* culture with IL-2 or IL-15 as compared with control NK cells. After confirmation that these cells can persist and maintain their effector functions *in vivo* in both autologous and allogeneic settings, this approach could be translated into future clinical trials of adoptive NK cell therapy for cancer patients.

E1125

HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS: REVIEW OF 28 CASES

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Background: Hemophagocytic syndrome (HLH) is characterized by prolonged and excessive activation of antigen-presenting cells (macrophages, histiocytes) and $CD8^+$T cells. This generates a severe hyperinflammatory condition and organ damage including fever, cytopenia, splenomegaly, coagulophaty and/or hypertrigliceridemia. HLH includes two different conditions: primary (or genetic HLH) and secondary (or adquired), caused by infections, malignancy or autoimmune disorders. Histiocyte Society criteria have been widely used for diagnosing HLH, however not all of them are usually present at the initial presentation. HLH is a life-threatening disease which must be suspected and treated as early as possible.
Aims: HLH is a rare and often fatal disease. Its incidence and main form of presentation are not yet well known. This study aimed to evaluated the etiology, clinical features and prognosis of HLH in our community.
Methods: A retrospective analysis was conducted through the medical files of all patients with diagnosis of HLH between 2004 and 2014 in two different hospitals. Clinical features, age, diagnostic criteria proposed by the Histiocyte Society, etiology, treatment and evolution were analyzed. In our study 7 out of 28 patients did not meet the requested criteria due to the rapidly fatal evolution of the disease.
Results: A total of 28 patients with a median age of 46.5 years were identified (0,2 – 84 years), 19 male and 9 female (ratio 2.1/1). 12 were infection associated forms, 7 malignancy related, 5 due to autoimmune diseases and 4 were of unknown etiology. Only secondary forms were analyzed. On the 21 patients who met the diagnostic criteria, the clinical manifestations were fever (100%), splenomegaly (66.6%), adenopathies (61.9%), ictericia (28.5%), edema (28.5%) and neurological manifestations (19%). The laboratory findings were hyperferritinemia (100%), cytopenias affecting two or more lines (90.5%), hypertriglyceridemia (66.6%), liver enzyme alteration (52.4%), hypofibrinogenemia (47.6%), coagulopathy (38%), hypoproteinemia (38%), and hyponatremia (28.5%). Hemophagocytosis was observed in 19 cases in bone marrow, only 1 case in the liver. The activity of natural killer lymphocytes was measured in 9 cases, and it was reduced in 5. Comparing the treatment regimens, 13 were treated according to protocol HLH-04, in 8 of the underlying disease, a supportive treatment was given to 7. The mortality due to HLH complications was 38% (8 patients), for non related complications 4.7% (3 patients). 52% experienced full recovery (11 patients).

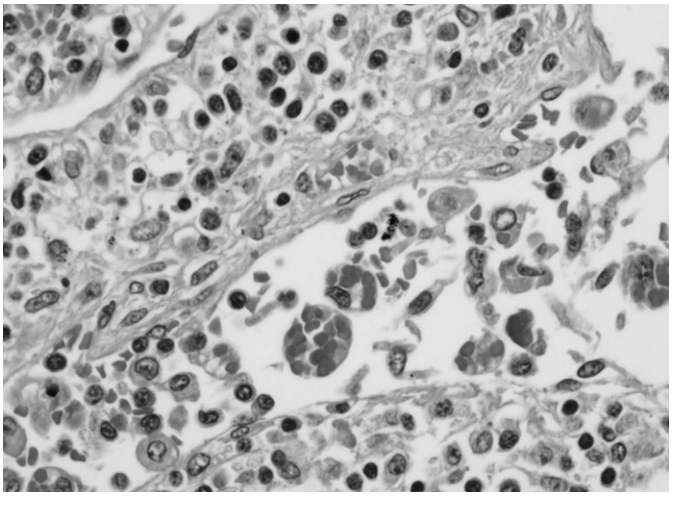

Figure 1.

Summary and Conclusions: The incidence of HLH in our population can be estimated between 2,1 and 2,8 cases /million. The majority were male (ratio 2.1/1). No primary forms were found in this analysis. The most common trigger was infection which is consistent with the studies found in the literature. Outstanding clinical manifestation were persistent fever and splenomegaly. Laboratory data indicated that the most prevalent abnormality were hyperferritinemia and cytopenia. The most used treatment regimen protocol was HLH-04 which obtained acceptable tolerance and results.

E1126

HLA-PARTIALLY MATCHED CELLULAR THERAPY (STEM-CELL MICROTRASPLANTATION) IN ACUTE MYELOID LEUKEMIA AND MYELODISPLASTIC SYNDROMES

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Background: Non-engraftment alloreactive cellular therapy has been proposed as a treatment for acute myeloid leukaemia (AML). A host-*versus*-tumour effect induced by graft-rejection has been suggested.

Aims: To evaluate feasibility and safety of the microtransplantation in AML and myelodisplastic syndrome (MDS) and estimate response rate, infections incidence and the development of GVHD and alloreactivity.
Methods: We have performed 25 HLA-partially matched donor leukocyte infusions (PM-DLI) infusions in 9 elderly patients (64-72 years old) with AML (6), CMML (2) or RAEB (1) from 11 partially mismatched donors (9 haploidentical). One or two apheresis of related donor peripheral mononuclear cells were collected after G-CSF mobilization. The median number of MNC, CD34+, CD3+and NK cells infused per course were: $3.16x10^8$/kg (range 1.37-$5.51x10^8$/kg), $3.29x10^6$/kg (range1.42-$6.58x10^6$/kg), $1.15x10^8$/kg (range 0.35-$2.42x10^8$/kg), $0.94x10^7$/kg (range 0.50-$1.55x10^7$/kg), respectively. Patients received each PM-DLI following a conventional chemotherapy cycle (IA, HDAC, MEC) or hypomethylant agent course. All patients except one received at least two PM-DLI in each microtransplant. Expected NK cell alloreactivity, chimerism and minimal residual disease were analyzed.
Results: The procedure was well tolerated, with a mild and transient "haploimmunostorm syndrome" (fever, rash, diarrhea). Only the two patients with CMML received corticosteroid. One patient suffered from early infusional reaction that was resolved with support treatment. None of the patients showed acute or chronic graft-versus-host disease (GVHD) or donor engraftment in chimerism tests. No significant infections were observed. All AML/RAEB patients achieved complete remission (CR). Four patients relapsed at 7, 9, 10 and 15 months after the infusion; two of them achieved a second sustained complete remission with another PM-DLI from a different donor (one of them had developed anti-HLA antibodies). Currently, with a median follow-up of 11 months (range 1-25 months), six patients are alive and three died (one with CMML during the induction treatment and two due to leukemia progression).
Summary and Conclusions: Microtransplantation is a well tolerated procedure, infectious complications are insignificant and the remission rates are encouraging, in the absence of engraftment or GVHD. Patients can undergo a second microtransplantation from a different donor. Leukocyte infusions can be safely administered following a hypomethylant agent course instead of conventional chemotherapy. Despite of the promising results, larger patient cohorts are necessary to assess the efficacy of this novel therapeutic strategy for hematologic malignancies.

E1127

Abstract withdrawn

E1128

UNTOUCHED GMP-GRADE PURIFIED ENGINEERED IMMUNE CELLS

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Background: Engineering T cells with receptors to re-direct the immune system against cancer has most recently been described as one of the scientific breakthroughs. However, a main challenge remains the GMP-grade purification of immune cells selectively expressing the introduced receptor in order to reduce potential side effects due to poorly or non-engineered immune cells.
Aims: By taking advantage of a model γδT cell receptor (TCR) naturally interfering with endogenous TCR expression we aimed to develop a GMP-grade method to obtain pure but untouched receptor engineered immune cells.
Methods: We designed the optimal retroviral expression cassette to achieve maximal interference with endogenous TCR chains. Following retroviral transduction, non-and poorly engineered immune cells were efficiently depleted with GMP-grade anti-αβTCR-beads. Next, the engineered immune cells were validated for TCR expression, function and potential allo-reactivity *in vitro* against a panel of tumor cell lines and primary tumors and in two humanized mouse models.
Results: The untouched enrichment of engineered immune cells translated into highly purified receptor engineered cells with strong anti-tumor reactivity both *in vitro* but also *in vivo* in two humanized mouse models. Importantly, this approach also eliminated residual allo-reactivity of engineered immune cells. Our data demonstrate that even with long-term suboptimal interference with endogenous TCR chains such as in resting cells, allo-reactivity remained absent and tumor control preserved.
Summary and Conclusions: All together, we present a novel GMP-grade enrichment method of untouched engineered immune cells, which is potentially applicable to all receptor-modified cells even if interference with endogenous TCR chains is far from complete.

E1129

EFFICIENT AND STABLE GENE TRANSFER INTO MESENCHYMAL STEM CELLS

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Background: Mesenchymal stem cells (MSC) are attractive tool for gene therapy due to their intrinsic properties. These cells produce long-living progeny, differentiate into all mesenchymal lineages, and are easy to manipulate *ex vivo*. Lentivectors have been shown to facilitate efficient transduction of rarely proliferating cells.
Aims: The aim of this study was to evaluate the efficiency and stability of MSC and progenitor-cell transduction by lentivectors *in vitro* and *in vivo*.
Methods: For in-vitro gene transfer, murine Dexter type long-term bone marrow cultures (LTBMC) were infected 2 weeks after initiation by either a standard LeGO-G2 vector (encoding eGFP gene under SFFV promoter) or a modified, promoter-deprived vector (LeGO-G2pd) that was in some experiments barcoded. The frequency of colony-forming unit fibroblast (CFU-F) was analyzed in adherent cell layers (ACL) of LTBMC 2 weeks after infection. ACL were trypsinized, and 500 cells/well were seeded in 96-well plates in aMEM with 20% FBS and 5 ng/ml bFGF. Infected LTBMC from each group were implanted under the renal capsules of syngeneic recipients for the hematopoietic ectopic foci formation. DNA was isolated from ACL, CFU-F, the inner cell mass of the foci and the corresponding bone shells. The percent of marked samples was assessed by PCR. For in-vivo gene transfer mice were injected with lentivirus intrafemorally. The percent of marked ACL, CFU-F and foci formed was calculated 2-6 months after injection of lentivirus.
Results: Results are shown in Table. The percentage of cells in ACL marked by lentivirus with eGFP expression was 17.6±5.9. About 25% of CFU-F from those ACL were eGFP positive. Over half of all ectopic foci and corresponding bone shells but only 5% of CFU-F from those foci were eGFP positive. Unlike in LTBMC no eGFP fluorescence was detected in ectopic foci and CFU-F derived from them. eGFP containing samples were revealed only by PCR. It has been suggested that eGFP itself is immunogenic *in vivo* and CFU-F expressing eGFP are eliminated by immune system of the recipients. So SFFV promoter was deleted from the vector and LTBMC were infected with viruses lacking eGFP expression. All bone shells developed from these implanted LTBMC were marked. The abrogation of eGFP expression led to the increase of marked CFU-F percentage within the ectopic foci up to 55%. The infection of LTBMC with barcoded library provided the information of individually marked CFU-F from infected ACL. Ectopic foci obtained from the infected LTBMC were also marked. As ectopic foci are formed only by true MSC able to self-renew the presence of foreign gene in the foci and CFU-F derived from them indicates the possibility to transfer the genes of interest into MSC. It is important for the gene not to be immunogenic. *in vivo* infection of MSC and CFU-F with vector w/o SFFV promoter was effective as over half of LTBMC and ectopic foci carried foreign gene.

Table 1.

Lentivector/method of infection	Percent of marked ACL	Percent of marked CFU-F from ACL	Percent of ectopic foci with marked bone shells	Percent of ectopic foci with marked cells	Percent of marked CFU-F in ectopic foci
eGFP/LTBMC infection	100	26.9±5.9	59.3±12.2	50.9±18.5	5.4±1.3
eGFP without promoter/LTBMC infection	100	4.0±1.8	100	33	55±5.6
eGFP without promoter/in vivo injection	55.7±24.1	4.3±1.4	57	14	6.4±1.8
Bar code/ LTBMC infection	100	60.9±5.2	75	74	0

Summary and Conclusions: Our data suggest that MSC and stromal precursor cells can be effectively and stably transduced *in vitro* and *in vivo* by lentivectors. The genes of interest should not be immunogenic. Thus MSC can be used as a reliable delivery system in gene therapy settings.

E1130

IDENTIFICATION OF PREDICTIVE MARKERS OF IMMUNO-SENESCENCE WITH FOCUS ON TUMOR SUPPRESSIVE GENES: HEALTHY DONORS VERSUS PATIENTS DIAGNOSED WITH A LYMPHOPROLIFERATIVE DISEASE

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Background: Aging is associated with the functional alteration of multiple organs including our immune system, thus affecting immune surveillance that prevent the development of cancer. This is illustrated by the high median age of most of the malignant hemopathies (MDS: 75y, AML: 70y, MM: 70y, NHL: 67y, CLL: 72y). In addition, aging is affecting other mechanisms of natural protection against cancer, resulting in poor DNA repair, telomere shortening, chromosomal instability, altered intercellular communication, senescent environment and loss in apoptosis-regulating genes. However, little is known in terms of genetic or epigenetic modifications of tumor suppressive genes (TSG) in lymphocytes subsets.
Aims: 1.To identify predictive markers of immuno-senescence in T/B and NK lymphocytes subsets, in a population of elderly patients with or without lymphoproliferative diseases (LPD): 2.To study tumor suppressor genes expression

in lymphocytes subsets, according to age and diseases; 3.To Study the impact of these factors on therapeutic responses and overall survival.
Methods: Healthy donors belonging to different ages groups and patients with lymphoproliferative diseases (LPD) are recruited for immune status evaluation after signed informed consent forms accepted by our ERB. Lymphocytes subsets were analysed by flow cytometry (CD4, CD4RA, CD4RO, CD4/CD25/FOXP3, CD8, CD19, CD16, CD56, CD197, CD27) before any treatment. Each lymphocytes subset was isolated by the MASC isolation technology for further molecular investigations. Tumor suppressor genes (TP53, PRDM1 and others) were quantified by RT-PCR on each purified lymphocytes subset.
Results: 21 healthy donors and 17 LPD (CLL and NHL) are currently prospectively investigated and stratified according to ages and diseases. Absolute lymphocytes count was not significantly different among the different groups. In terms of innate immunity, we found a significant lower number of NK celles (CD56+) betwenn younger (<50y) and older (p=0 ,002) and between healthy donors and patients (p=0,001). The functional tests are still on going. The CD4+/CD8+T cells ratio was significantly increased in older patients (p=0,008). Among CD4+T cells, CD4 memory and particularly T cenral memory lymphocytes were significantly increased. CD3-/CD4+was also increaded. Quantitative RT PCR analysis demonstrated a significant reduction in the TP53 gen expression in all pruified lympocytes subgroups (CD4+, CD8+, CD19+, CD57+) when matched healthy donors (>50yr) was compared with LPD patients (p=0,02). Thiese TP53 values were inversely correlated with the expression of RPDM1 gene. Patients recruitment is still on going and follow-up is too short to answer the tihrd objective.
Summary and Conclusions: our preliminary observations in a small series of donors confirm that 1) both innate and adoptive immunities are affected by aging: 2) Immuno-senescence is even more pronounced in patients compared to matched healthy donors. The reason remains to be investigated. 3) down-regulation of tumor suppressor gene such as TP53 is present in all lymphocytes subsets and is correlated with aging.

E1131

CHRONIC EXPOSURE TO INTERFERON-ALPHA DRIVES MEDULLAR LYMPHOPOIESIS TOWARDS T CELL DIFFERENTIATION IN MICE
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Background: Interferon-α (IFNα) is an antiviral, immunomodulatory and antiproliferative cytokine which is produced in response to a variety of infectious agents including viruses and bacteria. It constitutes a key component of natural immunity linking innate and adaptive immune responses. In line with these functions, IFNα has been utilized in the treatment of chronic viral infections and diverse neoplastic conditions including hematological malignancies and solid tumors. However, these activities are counterbalanced by the induction of peripheral pancytopenia, which frequently limits its clinical use.
Aims: Although a large amount of information exits about the beneficial and deleterious effects of IFNα the modulation of hematopoiesis by IFNα still remains poorly understood. This study aimed to investigate the consequences of long-term IFNα treatment on blood cell homeostasis using a gene therapy vector expressing this cytokine.
Methods: In this work, we analyzed the hematopoietic changes occurring in mice subjected to chronic IFNα exposure. This was achieved by transducing the liver of C57/BL6 mice with an intravenous injection of an adenoassociated vector encoding IFNα (AAV-IFNα) to obtain sustained high serum levels of the cytokine. Furthermore, the consequences of chronic IFNα exposure on lymphoid differentiation was assessed by transferring bone marrow cells from IFNα-treated mice to Rag-/- mice. To understand the way IFNα modulates the commitment of HSCs, we analysed by quantitative real time-PCR the expression levels of key transcription factors (TFs) involved in multipotent progenitors (MPPs) lineage specification in total bone marrow (BM) cells, in purify Lin-cKit+(LK) cells and in differentiated cells (Lin+).
Results: Chronic IFNα exposure by AAV-IFNα injection induces a dramatic change in the composition of the leukocyte population in the peripheral blood and in the bone marrow. Here we found that (long-term hematopoietic stem cells) LT-HSCs and (short-term) ST-HSCs are dramatically reduced in IFNα treated animals causing a progressive and lethal pancytopenia indicative of the exhaustion of the HSCs compartment. Moreover, long term IFNα treatment guides multipotent hematopoietic progenitor cells toward a T cell fate. IFNα directly downregulates both *in vivo* and *in vitro* the expression of B cell TFs in lymphocyte precursor cells and this effect alters the differentiation of these cells driving them to the production of T lymphocytes.
Summary and Conclusions: In conclusion, our results demonstrate that long-term exposure to IFNα exerts a complex impact on hematopoiesis, it compromises the stemness of hematopoietic stem cells (HSCs) but also redirects the function of the hematopoietic precursors cells triggering an unique genetic program in these cells favoring the generation of T cells while blocking the development of B cells.

E1132

Abstract withdrawn

Hematopoiesis, stem cells and microenvironment

E1133

REQUIREMENT FOR PHOSPHOLIPASE C GAMMA 1 (PLCG1) IN DEVELOPMENT AND MAINTENANCE OF HEMATOPOIETIC STEM- AND PROGENITOR CELLS
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Background: Hematopoietic stem cells (HSC) play a crucial role in the maintenance of hematopoiesis, balancing self-renewal capacity and differentiation potential to form more committed progenitor cells. HSC homeostasis is highly regulated by a complex network of signaling pathways and transcription factors. Phospholipase C gamma 1 (Plcg1) is known as a key regulator of calcium signaling which plays an important role in proliferation and differentiation of immune. Upon activation by receptor and non-receptor tyrosine kinases, such as T-cell receptor or JAK2, Plcg1 regulates the hydrolysis of phosphatidylinositol 4,5-biphosphate, leading to the activation of various downstream pathways. Several studies have reported Plcg1 to be essential for erythropoiesis during murine embryonic development as well as for granulopoiesis in zebrafish models. Recently, our group provided first evidence for Plcg1 regulating maturation of adult erythropoiesis. However, no previous study has investigated whether Plcg1 is required for development or maintenance of hematopoietic stem cells.
Aims: In this study we aim to investigate the functional role of Plcg1 signaling in fetal liver cells and adult hematopoietic stem- and progenitor cells using RNA interference technology.
Methods: Fetal liver cells (FLC) were isolated from embryos at stage E13.5 while adult immature hematopoietic cells (Lin-Sca1+KIT+CD48-CD150+) were sorted from 6-8 weeks C57/BL6 mice. Cells were transduced with either (non-targeting) control shRNA or two different validated shRNAs targeting Plcg1. All shRNAs were either GFP-labeled or selectable by puromycin. Colony-forming potential was measured in methylcellulose medium and immunephenotype was analyzed by flow cytometry. Functional potential of HSPCs was measured *in vivo* using a short-term colony-forming unit spleen assay (CFU-S12) as well as a long-term competitive repopulation assays. Engraftment capacity was measured by a homing assay.
Results: Following Plcg1 knockdown, colony-forming capacity was significantly reduced in fetal liver cells and adult HSC (when compared to non-targeting control). While fetal liver cells showed a significant erythroid maturation defect upon Plcg1 knockdown in our previously published data, hematopoietic stem cells revealed no maturation defect or lineage bias following inactivation of Plcg1. Immunophenotypic analysis of colonies confirmed presence of all lineages at reduced total numbers. To assess for HSC function we performed colony-forming spleen assay and competitive repopulation studies *in vivo*. Here, loss of Plcg1 affected the functional potential of HSCs and fetal liver cells with significant impairment of their colony-forming potential and repopulation capacity. This was indicated by a drop in whole bone marrow chimerism below 10% at week 16-20 post-transplantation. Again, lineage commitment was not affected, while a reduction in HSPC abundance was observed. Inactivation of Plcg1 did not affect homing of transplanted HSCs but effectively reduced proliferative potential.
Summary and Conclusions: Taken together, our data provide first evidence that Plcg1 is required for HSPC homeostasis, since its genetic inactivation negatively affects the functional capacity of HSCs. Ongoing experiments investigate the effects of Plcg1 on cell cycle activity and induction of apoptosis and, aim to establish a mechanistic model to explain the observed phenotype.

E1134

THE UTILITY OF FLUORESCENCE LIFETIME IMAGING IN ROUTINE BONE MARROW SMEARS
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Background: After excitation by a photon, a fluorophore will drop to the ground state with some delay, according to exponential decay rates. This delay is called lifetime. The new technique "fluorescence-lifetime imaging microscopy (FLIM)" creates the image contrast with the help of the fluorescence lifetime values (transformed in pseudo-colors) at each pixel of the two-dimensional microscopic image and does not use the local concentration of the fluorophores or the emitted spectrum. Now we can visualize microscopic structures based on differences of their fluorescence decay rates, which depend on the physicochemical properties of the molecules, even when fluorescence is emitted at the same wavelength. FLIM is a non-invasive technique, using low-potency lasers. Therefore

it has been widely used in experimental settings with cell cultures or small organisms. There are only very few reports on its application for diagnostic purposes in routinely collected samples, such as bone marrow smears.
Aims: The aim of this pilot study was to investigate the utility of the FLIM technique for diagnostic purposes in routinely collected bone marrow smears.
Methods: We used non-stained routine bone marrow smears of 15 patients after fixation with formaldehyde vapor. Images were obtained with a confocal Zeiss Upright LSM780-NLO microscope equipped with a 63x oil immersion objective and a HPM-100-40 Hybrid detector (Becker & Hickl), Image size was 512 x 512 pixels. The specimens were excited by a 405 nm pulsed diode laser (80 MHz). In order to create equivalent images of the cytologic smears, pseudo-colors were attributed to different lifetime ranges. Images were compared with the standard May-Grünwald-Giemsa (MGG) stained smears.
Results: In every case we obtained highly contrasted FLIM images, with clearly distinguishable cellular elements. Photobleaching was rare. The obtained chromatin textures were somehow similar to that of the MGG images and permitted to recognize the different types of hemopoietic cells. Erythrocytes were characterized by the short lifetimes of their hemoglobin component. Cytoplasm and the protein background showed intermediate lifetime values. Fluorescence lifetime of granulopoetic nuclei was considerably longer than in nuclei of erythroblasts. Leukemic blasts of several types of acute leukemia showed considerable variation, generally long lifetime values. In one case of Chediak-Higachi disease, the pathologic cytoplasmic granula were clearly distinguishable. Besides, in that case, intracytoplasmic bacteria could be identified, which were not well visible in the MGG stained smears.
Summary and Conclusions: The FLIM technique can be applied in routinely acquired diagnostic bone marrow smears. No staining is needed. The images are well contrasted, and permit proper identification of the cellular elements. Different lifetime values of nuclear chromatin distinguish erythropoetic and granulopoetic lineage, thus suggesting relevant physicochemical differences of the nuclear organization. Supported: FAPESP, CNPq

E1135

CORD BLOOD STEM CELLS BUT NOT ADULT STEM CELLS AFTER TRANSPLANTATION OVEREXPRESS STEMNESS AND REPROGRAMMING GENES PARTIALLY OVERLAPPING THE SIGNATURE OF INDUCED PLURIPOTENT STEM CELLS (IPS)

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Background: Hematopoietic stem cells (HCT) undergo tremendous expansion and amplification during Hematopoietic Cell Transplant (HTC). To cope with this challenge, HSC must activate several genes including those responsible for self-renewal.
Aims: To prove or disprove whether HSC decline or not in their proliferative potentiality after HCT, we analyzed the expression of genes involved in self-renewal and reprogramming in CD34+cells obtained from bone marrow cells after the engraftment has been achieved.
Methods: Ninety-three genes, mainly involved in HSC regulation plus 30 gene involved in epigenetic regulation, were analyzed in CD34+cells isolated from: (i) Cord Blood (CB), (ii) normal Bone Marrow (BM), (iii) BM taken after umbilical CB Transplant (UCBT), BM taken from (iv) adult or (v) children transplanted with adult HSC. Expression data were compared among the five groups and further with those obtained from iPS (induced pluripotent stem cells).
Results: Among the 93 genes analyzed, the following genes: *DPPA2, LIN28, NANOG, NESTIN, OCT4, SOX1* and *SOX2* were found highly over-expressed in CD34+cells isolated after UCBT with respect to CB or BM CD34+cells. The level of expression of the above mentioned genes found in CD34+cells after UCBT was similar to iPS cells. However, relevant differences in the expression of several other genes were found between CD34+cells post-UCBT and iPS. For instance, PTEN expression was 2 logs higher in UCBT CD34+cells than in iPS. Protein analysis on CD34+cells confirmed the RNA data. Remarkably, over-expression of genes overexpressed in CD34+cells after UCBT was not observed in CD34+taken from BM after any other type of adult cell transplantation. Moreover, we found about 2 logs over-expression of genes involved in epigenetic control in CD34+cells taken from BM after UBCT when compared to native CB CD34+cells.
Summary and Conclusions: CD34+cells taken from BM after UCBT overexpress fundamental genes involved in self-renewal and somatic cell reprogramming thus, partially acquiring the signature of iPS. These features are unique since no other CD34+cell taken either from CB or adult BM or after any hematopoietic adult cell transplantation shows similar pattern of gene expression. These findings open new perspectives: (i) toward a better understanding of transplantation biology, (ii) toward governing gene expression in iPS to render them safer for therapeutic purposes; (iii) in designing new methods to expand HSC in more efficient and consistent manner.

E1136

STROMAL CELL-DERIVED FACTOR-1 PLAYS IMPORTANT ROLES IN THE REGULATION OF HUMAN EARLY B- AND T/NK-LINEAGE LYMPHOID DIFFERENTIATION IN DIFFERENT MANNERS

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Background: Stromal cell-derived factor-1 (SDF-1) is shown to be essential for B-lymphoid differentiation in mice. However, role for SDF-1 in human lymphopoiesis remains undefined. We previously reported that telomerized human stromal cells support the differentiation of human hematopoietic progenitors to $CD45RA^+CD7^+CD10^-$ T/NK- and $CD45RA^+CD7^-CD10^+CD19^+$B-lineage lymphoid precursors (Br J Haematol. 157:674-86, 2012). Because the stromal cells produced SDF-1, we examined a role of SDF-1 in human early lymphoid differentiation, using our coculture system.
Aims: In this study, we investigated whether and how SDF-1 regulates early lymphoid differentiation from human hematopoietic progenitors to $CD45RA^+CD7^+CD10^-$ and $CD45RA^+CD10^+CD19^+$lymphoid precursors in the presence of stromal cells.
Methods: $CD34^+lin^-CD45RA^-CD38^{lo}$ hematopoietic progenitors were purified from cord blood and cultured for 21 days either on *hTERT*-transduced human bone marrow stromal cells or with conditioned medium (CM) collected from cultures with stromal cells, in the presence of SCF, Flt3L, and TPO. To block the binding of SDF-1 to CXCR4 receptor, anti-CXCR4 blocking antibody (Ab) was added to the cultures. In some experiments, $CD34^+lin^-CD45RA^-CD38^{lo}$ cells were cultured with CM for 14 days and $CD45RA^+CD7^+CD10^-$ or $CD45RA^+CD7^-CD10^+$cells were isolated and incubated on stromal cells with anti-CXCR4 Ab or isotype control.
Results: In the cultures on stromal cells, anti-CXCR4 Ab significantly inhibited the generation of $CD45RA^+CD7^+$as well as $CD45RA^+CD10^+$lymphoid precursors from $CD34^+lin^-CD45RA^-CD38^{lo}$ hematopoietic progenitors. Anti-CXCR4 Ab predominantly inhibited B-lineage differentiation in the cultures with CM. We next examined the effect of anti-CXCR4 Ab on $CD45RA^+CD7^+CD10^-$ lymphoid precursors by culture on stromal cells. In control cultures, the number of $CD45RA^+CD14^-$ lymphoid cells including $CD10^+$cells increased during 10 days of cultures. However, in the presence of anti-CXCR4 Ab, the generation of $CD45RA^+CD14^-$ lymphoid cells including $CD10^+$cells from $CD45RA^+CD7^+CD10^-$ cells was suppressed, and few or no $CD45RA^+CD14^-$ cells were detected at day 10 after culture. Significant numbers of $CD14^+$monocytic cells were generated in both cultures. In the culture of $CD45RA^+CD7^-CD10^+$cells on stromal cells, anti-CXCR4 Ab inhibited their differentiation to $CD45RA^+CD10^+CD19^+$proB cells.
Summary and Conclusions: These data suggest that SDF-1 is important for B-lineage differentiation regardless of presence of stromal cells but critical for lymphoid differentiation from human early hematopoietic and $CD7^+$lymphoid precursors in contact with stromal cells. These findings indicate that SDF-1 plays key roles in early B- and T/NK-lineage lymphoid differentiation in different ways.

E1137

COMPARISON OF SELF-RENEWAL EXPRESSION IN STROMAL IN VITRO AND IN VIVO MICROENVIRONMENT MODELS – CRITICAL DIFFERENCES AND THEIR IMPACT ON HEMATOPOIETIC SUPPORT FUNCTIONS

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Background: The intricate relationship between the bone marrow (BM) microenvironment and hematopoietic stem cells (HSCs), both normal and malignant, is well established. Recent evidence highlights the importance of the BM niche in governing stem cell behavior. Translational research is dependent on understanding this relationship, utilizing both 2D and 3D *in vitro* co-culture experiments aiming to recapitulate this environment prior to *in vivo* studies. The use of radiation and anti-proliferative agents on stromal cells is essential in long-term co-culture. The effects of these treatments on hematopoietic supportive function remain poorly understood.
Aims: (1) To define patterns of gene and protein expression of self-renewal pathway components in *in vitro* co-culture models, using mesenchymal stem cells (MSCs) and stromal cell lines; (2) To compare *in vitro* and *in vivo* models, using CD34+CML-engrafted NSG mice. We hypothesized that radiation, anti-proliferative agents and confluency changes would be integral in altering hematopoietic supportive function leading to incomparable results across models.
Methods: Human BM samples were collected from healthy donors and CML patients (n=13) following informed consent. An unselected population of mononuclear cells was isolated to allow expansion of MSCs by plastic adherence. MSC immunophenotype was confirmed as described (Mennan et al, 2013). Primary CD34+CML cells were transplanted into sublethally irradiated (2.5Gy) 8week old NSG mice (n=6). Mice were euthanized after 16weeks. Engraftment was assessed using anti-human CD45 FACS analysis. Sorted CD45+cells were evaluated for BCR-ABL translocation by FISH. *In vitro*, M210B4, SLSL and HS5

stromal cell lines were evaluated±irradiation (40Gy) or±mitomycin (10ng/mL); varying confluency states were assessed. Gene expression of self-renewal pathway components and downstream targets were analysed by Fluidigm qRT-PCR. Protein expression was studied by IF and western blot.
Results: Between experimental models, significant variation in expression of self-renewal pathway components was observed. Expression did not significantly vary between normal and CML MSCs. Ageing MSC passage altered these components, with upregulation in Notch (NOTCH2 p=0.0005, JAG1 p<0.0001, HES1 (ns)) between 4 subsequent passages. Unlike *in* vivo models, there is limited expression of NOTCH4. The Hedgehog (Hh) pathway increased with ageing MSC passage (SMO p=0.0002, GLI1 p=0.05). As expected, irradiation altered self-renewal expression in a time and cell-type dependent manner, with most variation in M210B4. There was progressive downregulation of Hh receptors, SMO (p=0.0005) and PTCH1 (ns). Within the Notch pathway, there was initial upregulation followed by significant downregulation, particularly in NOTCH2 (p=0.001). Ligands and downstream targets were unaffected. Mitomycin did not alter expression. Progressive confluency significantly increased NOTCH1-4 expression in SLSL (p=0.03). However, unaffected ligand and downstream targets suggested this was not due to cis-inhibition. Protein analysis confirmed significant changes. Expression of both Hh and Notch varied considerably from 2D *in vitro* models compared to *in vivo* murine models suggesting further analysis is required in CML co-culture models.
Summary and Conclusions: Our data highlights the diversity between *in vitro* co-culture models and the need to critically appraise these confounding factors when developing 3D models and interpreting results *in vivo*. This will allow for improved translation to clinical research.

E1138

BONE MARROW-DERIVED MSCS STIMULATED BY IFN-Γ INHIBITED THE GROWTH OF TOXOPLASMA GONDII VIA UP-REGULATION OF GBP1
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Background: Mesenchymal stromal cells (MSCs) are a heterogeneous cell population endowed with multi-lineage differentiation potential and extensive immunomodulatory properties. MSCs have been successfully used for prevention and treatment of immune disorders such as graft-versus-host disease. Emerging preclinical studies suggest that MSCs might also protect against infectious challenge. *Toxoplasma gondii* is an apicomplexan protozoan parasite with a broad host range that is capable of causing significant disease in humans and animals.
Aims: This study aimed to rule out the potential mechanism of human MSCs against *T. gondii*.
Methods: Human bone marrow-derived MSCs (hMSCs) were pretreated for 24h with a series of concentrations of IFN-γ and then infected with *T. gondii* strains of variant virulences (virulent RH/GFP and avirulent PLK/RED). RNA-seq and westernblots were used to analyze gene and protein expression patterns of hMSCs under IFN-γ-stimulated and unstimulated conditions. The intracellular parasites (with fluorescence labeled) were counted microscopically at multiple time points postinfection. The short hairpin RNA (shRNA) expression was used to generate RNAi of GBP-1, GBP-2 and GBP-5.
Results: Human MSCs stimulated with IFN-γ were capable to inhibit the growth of *T. gondii* (eg: at IFN-γ 10 ng/ml, the inhibition rates are 26.5% (RH/GFP) and 37.5% (PLK/RED) at 12 hr postinfection) in a dose-dependent manner. Compared with the unstimulated MSCs (controls), IFN-γ treatment groups of 5, 10, 20 ng/ml inhibited *T. gondii* (RH/GFP) growth by percent of 17.7±7.9, 26.5±6.2, 30.0±7.7 (mean±SD, n=4) at 12 hr postinfection and the inhibition rates are 48.5±9.1%, 66.5±8.0% and 68.5±6.1% at 24 hr postinfection, respectively. In the same concentrations of IFN-γ, the inhibition effect of MSCs on PLK/RED strain was stronger than that on RH/GFP strain. Furthermore, After 48 hr postinfection, the ratio between parasites per parasitophorous vacuole (PV) containing rosettes and single parasites in IFN-γ-stimulated MSCs was significantly reduced compared with that in the unstimulated MSCs (p<0.01, p<0.01, p<0.001 for RH/GFP at IFN-γ 5, 10, 20 ng/ml, respectively). We also observed that the resistance in hMSCs does not depend on IDO (p=0.59 for RH/GFP and p=0.45 for PLK/RED at IFN-γ 20ng/ml 24 hr postinfection). RNA-seq data showed that IFN-γ-inducible p65 guanylate-binding proteins (GBPs) might play pivotal roles in the inhibition of *T. gondii* growth. Reads per kilobase-pairs per million (RPKM) mean values of GBP1, 2, 5 in IFN-γ-stimulated MSCs are 1093.3, 443.3, 348.2, respectively. RNAi knockdown of GBP1 (but not GBP2, GBP5) in hMSCs resulted in significantly recovery of *T. gondii* growth from inhibition stimulated by IFN-γ at 24 hr postinfection (p<0.01 and p<0.01 for RH/GFP and PLK/RED at 20 ng/ml level of IFN-γ, compared to shRNA control).
Summary and Conclusions: Human MSCs pre-stimulated with IFN-γ inhibited the growth of *T. gondii* in a dose-dependent manner via up-regulation of GBP-1 expression.

Hodgkin lymphoma - Clinical

E1139

SALVAGE TREATMENT OF PATIENTS WITH RELAPSED/REFRACTORY HODGKIN LYMPHOMA WITH BRENTUXIMAB VEDOTIN: THE GREEK EXPERIENCE
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Background: Patients with Hodgkin lymphoma (HL) who progress after autologous hematopoietic stem cell transplantation (ASCT) have a dismal prognosis. Therapeutic efficacy of conventional chemoradiotherapy is of little benefit and only allogeneic stem cell transplantation (allo-SCT) is associated with prolonged progression free (PFS) and overall survival (OS) in a proportion of eligible patients. Monoclonal antibody drug conjugate Brentuximab Vedotin (BV) has recently received approval for patients with HL who relapsed after, or are not candidates for auto-SCT.
Aims: To retrospectively study the outcome of patients with HL who were treated with BV in Greece. The primary objective of our study was the estimation of overall response rate as well as the PFS and OS of patients treated with BV.
Methods: From June 2011 until August 2014, eighty patients with relapsed/refractory HL received treatment with BV in Greece. Forty-three males and 37 females with a median age of 32 years, range (18-77) were included in this retrospective analysis. The median time from diagnosis to treatment with BV was 35 months, range (5-245). Among the 80 patients, 61 (76%) received BV due to relapse/progression after auto-SCT, 2 (2.5%) after failing both auto- and allo-SCT, whereas 19 patients were treated with BV before auto-SCT, since they were considered non-eligible for the procedure due to advanced age or refractory disease. Fifty-four out of 80 (67%) patients had disease refractory to induction treatment. The median number of treatments administered before BV was 4, range (1-7). Refractoriness to treatment administered before BV was observed in 52 out of 80 (65%) patients. BV was administered at a dose of 1.8mg/kg every 3 weeks until disease progression or for a maximum of 16 cycles.
Results: At the time of this analysis, the median number of BV cycles administered was 6, range (1-6). Three patients died from progressive disease after cycle 1, and therefore they were excluded from statistical analysis. The best response achieved after BV was complete (CR) and partial remission (PR) in 14 (18%) and 31 (40%) patients, respectively. Nine (12%) patients had stable disease (SD), while 23 out of 77 (30%) patients progressed during treatment with BV. With a median follow up period of 9.5 months (range, 1-35) the median PFS and the median OS were 10.5 and 31 months respectively (Figure 1). In multivariate analysis: 1) refractoriness to treatment administered before BV was associated with significantly reduced probability of achieving response to BV [Odds Ratio=2.7, (95% CI 1.0 to 7.2), p=0.04], and 2) response to BV was associated with significantly increased PFS [HR=0,14, (95% CI, 0,06 to 0,29), p <0,0001] and OS [HR= 0,24, (95% CI, 0,07 to 0,77), p=0,017].

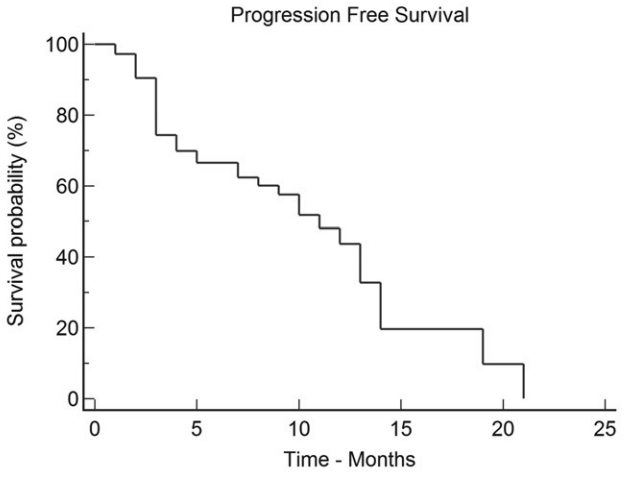

Figure 1.

Summary and Conclusions: To our knowledge, this study is the second largest study reported so far after the pivotal phase II trial which included 102 patients and resulted in the accelerated approval of BV. Although BV is a promising therapeutic option with an overall response rate of 58%, the median PFS is only 10 months when BV is administered as a single agent. The therapeutic efficacy of BV should be explored in different time settings such as, as salvage before auto-SCT or as maintenance after SCT in order to consolidate established responses.

E1140

EXPRESSION OF PD-I LIGANDS FOR CLASSICAL HODGKIN LYMPHOMA PROGNOSIS

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Background: Classical Hodgkin lymphoma (cHL) is presente by some cancerous cells from germinal center B-cells and generality from non-malignant reactive cells. Clinical outcome of cHL could be directly depended on pathways between Hodgkin and Reed-Sternberg (HRS) cells and other cells in their microenvironment. There is still much to learn in significance of the microenvironment in cancerous tumors and cHL especially. So, the main purpose of study to evaluate importance of microenvironment expression.
Aims: To determine value of PD-L1 as a marker at prognosis and its association with clinical outcome in cHL patients.
Methods: 49 patients with cHL were included in the analysis with stages IIA – 23, IIB – 3, III-IV – 23. Patients were treated by ABVD or BEACOPP 14/esc as the first line therapyand radiotherapy by indications. cHL samples were received from lymph node biopsies. PD-L1, PD-L2 expression levels were analyzed using real-time RT-PCR.
Results: For 49 patients OR rate after the first-line therapy was 93.9 % (47/49) with a CR of 73.5 % (36/49) and PR – 22.4 % (11/49). Progression of the disease during the therapy was observed in 2 patients. Among the patients who achieved a CR during the follow up (median – 24 months; range 12–36) – 9 had relapses. PD-L1 positive were 73.5% (36/49) of cHL cases and 26.5 % (13/49) were PD-L1 negative. PD-L1 expression level was higher in nodular sclerosis cHL and advanced stages of disease (p=0.19). PD-L2 expression level was not associated with histological cHL variant, disease stage and clinical outcome. ROC analysis revealed that PD-L1 expression level in tumor is an important marker which is associated with clinical outcome of cHL patients (Se=87.5 %; Sp=64.5 %; AUC=0.75; p=0.002). High PD-L1 expression was associated with reduced progression-free survival (PFS) in cHL patients. The 2-year PFS rate for cHL patients with high PD-L1 expression was 47% compared to 95% for low or absent of PD-L1 expression.
Summary and Conclusions: PD-L1 showed promising activity as a marker at prognosis in patients with cHL. High expression of the PD-L1 is associated with unfarouble clinical outcome in cHL patients.

E1141

THE ARROVEN STUDY (MA25101): POST-AUTHORISATION OBSERVATIONAL SAFETY STUDY OF BRENTUXIMAB VEDOTIN IN RELAPSED/REFRACTORY HODGKIN LYMPHOMA AND SYSTEMIC ANAPLASTIC LARGE CELL LYMPHOMA

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Background: Brentuximab vedotin, a CD30-targeted antibody-drug conjugate, has conditional approval in Europe for the treatment of adult patients with relapsed/refractory (R/R) CD30+Hodgkin lymphoma (HL), and systemic anaplastic large cell lymphoma (sALCL). The European Medicines Agency requires a post-authorisation safety study to further evaluate brentuximab vedotin's safety profile. ARROVEN is an ongoing, multi-centre, international, prospective, observational study of R/R CD30+HL and sALCL patients treated with brentuximab vedotin as part of routine clinical care. Currently available data will be presented.
Aims: To evaluate the occurrence of serious adverse events (SAEs) and AEs of special interest (AESI) in patients actively treated for R/R HL or sALCL with brentuximab vedotin in routine practice; and to identify and describe potential risk factors for peripheral neuropathy.
Methods: R/R CD30+HL or sALCL patients aged ≥18 years who are planned to start or recently commenced brentuximab vedotin as part of routine clinical care, are eligible. Data are extracted from information routinely recorded in the medical record and no study visits or examinations are required. Follow-up information and safety data are collected in conjunction with all routine visits, every 3 months until death, withdrawal of consent, or loss of follow-up. The frequency, intensity and relationship to treatment is evaluated for all SAEs and AESIs considered related to brentuximab vedotin; peripheral neuropathy, neutropenia, infections, hyperglycaemia, and hypersensitivity reactions.
Results: Between June 2013 and January 2015, 62 patients (mean age 47.6 years [range 19–82]) out of a target of 500 were enrolled across 21 sites; 39 (63%) had HL, and 18 (29%) had sALCL (5 [8%] unknown). Patients received a median of 4 treatment cycles (range 1–12). AEs were reported in 45 (73%) patients. The most common AEs (n≥4) were peripheral neuropathy (n=18), infections (n=14), neutropenia (n=13), hypersensitivity reactions, and lethargy (each n=4). There were no reported hyperglycaemia events. In the 18 patients with peripheral neuropathy, all 21 events reported were categorised as 'sensory, motor, or other'; peripheral neuropathy risk factors were not evaluable at the time of analayis. Grade ≥3 AEs were reported in 24 (39%) patients; grade ≥3 AEs in >1 patient were infections (n=8), neutropenia (n=6), and peripheral neuropathy (n=2). Seven (11%) patients reported grade 4 toxicities. These included infection (n=4), progression with sepsis (n=2), neutropenia (n=2), thrombocytopenia (n=1), and tumour lysis syndrome (n=1, sALCL patient). SAEs were reported in 21 (34%) patients. This included 11 patients with drug-related SAEs; the most common (n≥2) were infection (n=9), and peripheral neuropathy (n=4). No cases of progressive multifocal leukoencephalopathy, Stevens-Johnson syndrome, toxic epidermal necrolysis, or acute pancreatitis were reported. Two patients discontinued brentuximab vedotin, 1 due to grade 5 multi-organ failure during cycle 1, and 1 due to grade 3 left pleural effusion and grade 5 bronchopneumonia during cycle 4. There were 4 on-study deaths in total, due to pneumonia (n=3) and disease progression (n=1).
Summary and Conclusions: The severity and frequency of reported toxicities in R/R HL and sALCL patients treated with brentuximab vedotin are consistent with the known safety profile of brentuximab vedotin, and the pivotal phase 2 studies. These data indicate that brentuximab vedotin is manageable and tolerable in the conditionally approved indications.

E1142

BRENTUXIMAB VEDOTIN (BV) AN EFFECTIVE TREATMENT FOR TRANSPLANT INELIGIBLE PATIENTS WITH RELAPSED/REFRACTORY (R/R) HODGKIN LYMPHOMA (HL)

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Background: Patients with R/R HL, who are refractory to ≥2 lines of salvage chemotherapy, are generally considered ineligible for ASCT and have a very dismal 2-year PFS. Furthermore, patients relapsing after ASCT need effective treatment to eventually proceed to alloSCT. BV is a safe and highly effective treatment in R/R HL after ASCT and 75% overall responses have been reported.
Aims: The aim of this retrospective study was to assess the efficacy of BV in

inducing remission and enable R/R HL patients to proceed to ASCT or alloSCT.
Methods: Patients with R/R CD30-positive HL, treated with BV between January 2011 and April 2014 for failure of at least two prior therapies, when ASCT was not considered a treatment option (Group A), or for failure following ASCT (Group B), were included in this retrospective analysis. BV was given at the standard dose of 1.8 mg/kg iv every 21 days in an outpatient setting. The primary endpoint of this study was to evaluate efficacy of BV to enable patients to proceed to ASCT or alloSCT. Secondary endpoints included BV toxicity, Progression-free Survival (PFS) and Overall Survival (OS).
Results: Twenty R/R HL patients for Group A (ASCT-ineligible) and 25 patients for Group B (ASCT failures) were identified. Main characteristics at start of BV were as follows:

Table 1.

Characteristics	Group A (n=20)	Group B (n=25)
Males/Females	9/11	9/16
Median age (range) yrs	38 (20-76)	39 (18-64)
B Symptoms	7	9
Stage III-IV	10	11
Extra ±nodal involvement	9	11
Bulky disease (>7 cm)	7	4
Median number prior regimens (range)	4 (2-12)	4 (2-9)
Refractory disease after frontline therapy	15	17

A median of 6 BV cycles (range, 3 to 19) were administered. Overall response rate in Group A and B was 75% and 64%, respectively; CR was documented in 8 patients (40%) in Group A and 5 patients (20%) in Group B. Best response was reported after a median of 3 cycles (range, 2-9). Three pts in Group A and one in Group B, achieving a negative PET scan, by continuing BV therapy, had PD and were considered transplant ineligible; one patient in Group B refused alloSCT. BV enabled 7 patients in Group A (achieving CR: 5, PR:1, SD:1) and 15 patients in Group B (achieving CR:3, PR: 8, SD:4) to receive a transplant procedure. Nine patients (20%) had grade ≥3 adverse events (2 sensory peripheral neuropathy, 1 pneumonia, 6 neutropenia), no patient discontinued treatment due to toxicity. After a median follow-up of 14 months (range, 1-36), median PFS was 5 months and median OS was still not reached. For patients achieving CR or PR after BV and receiving ASCT (n=5) or alloSCT (n=12), median PFS was 8 months. At 2 years OS was 70%, 53% and 60%, for Group A, Group B and the entire patient population, respectively.
Summary and Conclusions: These data confirm that BV is effective in heavily pretreated R/R transplant ineligible HL patients, who have generally limited conventional treatment options and a low median OS. BV may overcome chemorefractoriness and enable patients to receive ASCT or allo-SCT, by omitting the significant toxicity of multiagent chemotherapy regimens.

E1143

THE PROGNOSTIC SIGNIFICANCE OF ELEVATED LEVELS OF SERUM FERRITIN PRIOR TO AUTOLOGOUS STEM CELL TRANSPLANT IN PATIENTS WITH HODGKIN LYMPHOMA

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Background: Some studies have shown that serum ferritin levels correlated with tumor mass and closely followed disease activity in patients with hematologic malignancies such as malignant lymphoma and acute leukemia. Studies have also shown that elevated serum ferritin levels in cancer patients are associated with a poor prognosis. Studies suggest that elevated pretransplantation serum ferritin levels are associated with increased mortality and a lower transplantation-related survival after allogeneic hematopoietic stem cell transplantation and after autologous hematopoietic stem cell transplantation (ASCT) for malignant lymphoma.
Aims: The aim of this study was to determine the relationship between pretransplantation ferritin levels and outcome following ASCT for Hodgkin lymphoma.
Methods: We performed a retrospective analysis the predictive value of pretransplantation serum ferritin (drawn within 60 days preceding transplant admission) of 136 consecutive adult patients who underwent ASCT for Hodgkin lymphoma (HL) at the Clinica Marly between January 1995 and December 2014, was available for 136 patients (64%) who form the patient population for this report. The normal range for ferritin in our laboratory is 10 to 500 ng/mL.
Results: The median pretransplantation ferritin for patients in this study was 658 ng/mL (range: 10 – 6352). Baseline ferritin 912 ng/mL as the cut off point that best correlated with poor survival. Univariable analysis demonstrated that number of prior chemotherapy regimens, disease status, and elevated ferritin were significantly associated with overall survival (OS). On multivariate analysis, a pre transplantation ferritin 912 ng/mL was associated with significantly lower overall (OS; P: 0.025) and Disease Free Survival (DFS ; P= 0.002). Ferritin 912 ng/mL was associated with a higher incidence of relapse (P=0.004) and relapse mortality (P= 0.001). The median follow-up for surviving patients is 44 months (range: 12 - 74). Multivariable analysis demonstrated that elevated ferritin (P= .0022, HR 1.78, 95% CI 1.35 – 2.26) and, number of prior chemotherapy regimens (P= 0.032, HR 1.63, 95% CI 1.12- 2.45) were significant adverse prognostic factors for OS. Elevated ferritin (P= .021, HR 1.60, 95% CI 1.08-2.38) and disease status (P= .003, HR 2.05, 95% CI 1.45-2.71) were also adverse factors for Disease Free Survival.
Summary and Conclusions: Elevated levels of serum ferritin of 912 ng/mL, number of prior chemotherapy regimens, disease status, may be an important marker for predicting poor survival outcomes following ASCT for Hodgkin lymphoma. The present study cannot distinguish between a causal relationship of elevated ferritin, through iron stores, on the malignancy and ferritin being a surrogate for extent or aggressiveness of malignancy. Our results indicate the need for studies designed to correlate an elevated ferritin with iron overload and survival outcomes.

E1144

AUTOMATED IMAGE ANALYSIS REVEALED A HIGH VARIABILITY OF CD30+HRS CELLS DENSITY IN CLASSICAL NODULAR SCLEROSIS HODGKIN LYMPHOMA

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Background: Hodgkin lymphoma, classic type (cHL) tissue is characterized by an extreme disproportion between tumor (Hodgkin-Reed-Sternberg, HRS) cells ranging from 0.1% to 10% and surrounding non-malignant microenvironment cells. Last years brought an explosion of novel information about close HRS-microenvironment cross-talk, which promotes a tumor growth. Numerous studies tested the predictive value of microenvironment cells density using immunohistochemistry (IHC) methods and many of them were focused on lymphoma-associated macrophages (LAM). High LAM density at time of diagnosis was found to be an inferior prognostic factor. However, no studies were performed on HRS cells density and HRS:LAM ratio analysis.
Aims: To assess an impact of HRS cells density and HRS:LAM relationship on disease development/prognosis in cHL, nodular sclerosis subtype using novel automated scanning system of the total tumor sample area.
Methods: We analyzed cHL tissues, obtained at time of diagnosis, in fourteen patients with intermediate and advanced stages (KS IIB-IV). Six patients represented a relapsed/refractory (RR) cohort, eight patients were selected as age-sex matched relapse-free (RF) controls. Paraffin-embedded biopsies were prepared from pre-selected high-quality diagnostic samples stained with hematoxylin-eosin for morphology analyses and using CD30 (HRS) and CD68 (LAM) for cell-density analyses. Tissue array analyses were performed using the TissueFAXS (TissueGnostics, Vienna, Austria) system that combines detailed morphologic information offered by microscopy with the scientific accuracy of multi-channel flow cytometry. Data were analyzed with TissueQuest software. Each sample was previewed by scanning software and only significant tumor tissue was manually gated for further analyses, excluding fibrotic (≥10 %), necrotic or residual lymphatic structures. This area was considered as a Total Tumor Sample (TTS) area.
Results: Mean TTS area covered 17.7±11.9 mm2 with mean total number of cells 457,257±397,599. The mean TTS was not different in RF (17.9 mm2) compared to RR cohort (19.2 mm2, p=0.86). There was a trend for higher cell density in RF compared to RR cases (27,679 *vs* 20,861 cells per mm2, p=0.09). Density of CD30+HRS cells was 2.4 fold higher in RF than in RR patients (429 *vs* 180 cells per mm2, p=0.055). Although mean density of CD68+LAM was comparable in RF and RR group (2,372 and 2,716 cells per mm2, respectively, p=0.66), a CD30:CD68 ratio was higher in RF (0.31) than in RR (0.07, p=0.044) patients. The number of LAM per one HRS cell was 33.1 in RR, whereas only 12.1 in RF group (p=0.10). Our data showed that automated analysis of a TTS area may overcome technical limitations caused by a tissue selection, heterogeneity in HRS and bystander cell distribution or tissue fibrosis/necrosis comparing to analysis of small tissue samples (2-3 mm2).
Summary and Conclusions: Cell distribution in cHL tissue is highly variable, automated analysis of may bring more accurate cell-density results with lower variance. We describe differences in HRS and total cell density in diagnostic biopsies of relapse/refractory and relapsed- free cHL patients. Higher HRS:LAM ratio in relapsed patient presume more potent HRS-microenvironment interaction with the possible prognostic role.
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E1145

HODGKIN LYMPHOMA PATIENTS WITH HIGH NUMBER OF MACROPHAGES IN TUMOR TISSUE - RISK PROFILE AND TREATMENT OUTCOME

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Background: The treatment approach in Hodgkin Lymphoma (HL) is risk adopted. Thus, patients have to be stratified into certain risk groups based on clinical stage and risk factors before selecting the appropriate therapy. In the past decade, many studies were performed with the aim to identify reliable prognostic biomarkers for HL. Among potentially very powerful biomarkers is considered the number of macrophages in tumor tissue, with negative prognostic impact of their high content in tumor.
Aims: The aim of this study was to thoroughly evaluate high risk group of HL patients according to number of macrophages in tumor tissue, in order to identify patients who potentially require more effective treatment approach.
Methods: The retrospective study was performed on selected patients with newly diagnosed classical HL, initially treated with ABVD in the period 2000-2008. The selection criteria was based on ROC curve analysis which determined 8 as the most appropriate cut off value for number of CD68+macrophages in 1000x magnification high power field (hpf). The analysis was performed on 61 patients with high number (higher or equal 8) of CD68+macrophages/hpf. The examined parameters in survival and multivariate analysis were presence of bulky disease, ESR≥50 mm/h, B symptoms, elevated LDH and high IPS score (3-7).
Results: The median follow up was 71 months (range 2-162 months). The median age was 31 yrs (range 17–84). Five-years overall survival (OS) was 68,9% and 5-years event free survival (EFS) was 54,1%. In univariate analysis patients with ESR≥50 mm/h, B symptoms and elevated LDH had significantly shorter OS (p=0.047, p=0.011, p=0.045, respectively), while there was a trend toward worse OS in patients with bulky disease (p=0.058). EFS was significantly shorter in patients with ESR≥50 mm/h, B symptoms, elevated LDH and high IPS (p=0.047, p=0.044, p=0.017, p=0.033, respectively). Multivariate analysis identified presence of B symptoms as the independent risk factor for poor OS (p=0.018) and elevated LDH as the independent risk factor for poor EFS (p=0.023).
Summary and Conclusions: The further stratification in high risk groups of HL patients could be potentially useful for selecting the adequate treatment approach. More effective treatment approach is potentially required in patients with high number of CD68+macrophages with present B symptoms and/or elevated LDH. Still, these have to be confirmed through randomized clinical trials.

E1146

LYMPHOMA SURVIVORSHIP AND CARDIOVASCULAR DISEASES: DETECTION OF EARLY CARDIAC DYSFUNCTION

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Background: Improvements in the treatment of Lymphomas have resulted in an increasing number of long term survivors. This patient's population is at risk of developing late therapy related complications as cardiovascular diseases and secondary cancers.
Aims: In our institution since September 2014 the Hodgkin's (HL) and aggressive non Hodgkin's Lymphomas (NHL) long term survivors are followed up in a dedicated clinic. This approach allows clinicians to better appreciate and monitor not only signs and symptoms of the known hematologic disease but also of other medical problems that can affect people undergone previous radiation therapy and/or chemotherapy (survivorship). An additional target of this project is to define a program of early detection of otherwise asymptomatic cardiovascular, endocrine, respiratory, neurologic and oncologic disease. In this work we focus on the preliminary results of screening of early cardiovascular disturbances.
Methods: We collected data regarding cardiovascular diseases in 216 patients and performed electrocardiographic and echocardiografic evaluation in 79 patients selected mainly according to the age, cardiovascular risk factor, time from therapies, type and amount of antiblastic (antracyclines) and mediastinal radiotherapy received.
Results: We analyzed data regarding 216 patients coming in our clinic from 15 September 2014 to 16 February 2015, 119 were affected by HL and 97 by NHL. One hundred fifteen are females, 101 males; median age at observation is 53 (range 22-89). All of them are in complete response for lymphoma for at least 5 years from the completion of curative therapy. Thirty seven patients (17%) had known cardiovascular problems developed after lymphoma treatment (some of them had more than one defects): 9 isolated arterial hypertension, 14 ischemic cardiac disease, 4 valvular insufficiency, 1 dilatative cardiomyopathy, 5 arrhythmia, 2 cardiac failure, 1 aortic ectasia, 2 cerebrovascular ischemia. Electrocardiographic and echocardiografic evaluation has been performed in 79 patients selected mainly according to the age, cardiovascular risk factor, time from therapies, type and amount of antiblastic (antracyclines) and mediastinal radiotherapy received. Thirty eight out of 79 (48%) showed previous unknown cardiac disturbance: particularly 21 presented diastolic relaxation abnormality, 15 valvular insufficiency and 5 aortic sclerosis of low-moderate grade, 1 has low grade myocardic thickness and 3 arterial hypertension. The median age of patients with cardiovascular abnormalities discovered during the screening is 49 (range 32-70), 28 of them had received therapy for HL and the other 10 for NHL.
Summary and Conclusions: Our analysis confirms that an high percentage of patient survived to lymphomas can develop cardiovascular diseases. Their monitoring can detect silent dysfunctions as diastolic relaxation abnormality considered an early sign of cardiomyopathies.

Indolent Non-Hodgkin lymphoma – Clinical

E1147

THE CHANGING FACE OF GASTRIC MALT-LYMPHOMA: THE VIENNA UNIVERSITY EXPERIENCE

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Background: In the past, the stomach has been thought to be the most common site of origin for extranodal marginal zone B-cell lymphoma of the mucosa associated lymphoid tissue (MALT-lymphoma), with the large majority of cases being associated with Helicobacter pylori (HP) infection.
Aims: As changes in clinicopathological characteristics and incidence have been suggested by scattered reports, we have assessed characteristics and outcome of patients with gastric MALT lymphoma from a tertiary referral center.
Methods: Between 1999 and January 2015, a total of 320 consecutive patients with MALT lymphoma were diagnosed, treated and followed at our institution. We have retrospectively re-assessed all patients with gastric MALT-lymphoma from this cohort in terms of stage, HP-infection, pathological characteristics, treatment and outcome.
Results: In total, 116 / 320 patients (36%) with MALT lymphoma were of gastric origin, while the large majority of patients had extragastric MALT lymphoma. Only 4 /116 patients (3%) also had a diffuse-large B-cell lymphoma component along with MALT lymphoma. Using a standardized staging regimen, we found 25 pts (22%) to have multiorgan-involvement, including 3 (2.6%) with bone marrow involvement, while 91 had localized disease (78%). Out of 99 patients with a fully tested HP-status, 34 (34%) were negative for HP and 65 positive. Interestingly, 9 of these patients had been diagnosed before 2004 (9/49, 18%) and 25 between 2004-2015 (25/50, 50%). In total, 40% of patients assessed had monoclonal gammopathy, and plasmacytic differentiation was present in 31%. In addition, 29% of patients were found to have an underlying autoimmune disease, with chronic autoimmune thyroiditis being most common. In terms of treatment, 76 patients (66%) had antibiotics as first line therapy, 31 systemic therapy (27%), while the remaining patients had surgery, radiation or wait and see. After a median follow-up of 63 months, 85% (99/116) are alive, while 17 (15%) have died. Transformation to DLBCL was only seen in one patient after therapy, while 2 developed clonally unrelated nodal DLBCL. HP-status was not associated with relapse following first line therapy (p=0.066), and there was no difference in estimated time to progression between the two cohorts. Interestingly, 14 HP-negative patients were treated with antibiotics alone, resulting in 5 CR and 1 PR (46%), 5 stable disease and 3 progressions; results of antibiotic treatment in HP negative patients were not significantly different for estimated time to progression (p=0.1) and progression following first line therapy (p=0.38).
Summary and Conclusions: These results suggest that the rate of gastric MALT lymphoma amongst the cohort of MALT lymphomas as a whole is decreasing, while the rate of HP-negative gastric MALT lymphomas was much higher (34%) than stated in the literature in the past. In addition, cautious interpretation of our data suggests an increase of HP-negativity over the last 10 years. Surprisingly, a relevant percentage of patients can be managed with antibiotic therapy alone without compromising patients`prognosis. Further studies on the pathogenesis and therapy of HP-negative patients are needed in order to optimize therapy and avoid overtreatment. In addition, our data suggest that the risk of transformation to DLBCL in the course of the disease is minimal.

E1148

INTERIM ANALYSIS OF A PHASE 1B STUDY EVALUATING THE SAFETY OF GS-9820, A SECOND-GENERATION PI3K DELTA-INHIBITOR, IN RELAPSED/REFRACTORY LYMPHOID MALIGNANCIES

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Background: In B-cells, phosphatidylinositol 3-kinase delta (PI3Kδ) mediates cell survival and proliferation, and its activity is critical for retaining B cells in lymphoid tissues. Idelalisib inhibits PI3Kδ in B-cell malignancies, and is indicated for the treatment of 1st line 17p deleted and relapsed chronic lymphocytic leukemia (CLL) in combination with rituximab. Idelalisib also demonstrated high response rates in relapsed follicular lymphoma (FL) and small lymphocytic leukemia (SLL). GS-9820 is a second-generation, PI3Kδ-inhibitor and was evaluated in B-cell malignancies including CLL and non-Hodgkin's lymphoma (NHL).
Aims: The primary objective was to determine the maximum tolerated dose (MTD) and to assess safety including the incidence and severity of elevated transaminase levels, diarrhea and pneumonitis which are observed with idelalisib.
Methods: Subjects with relapsed B-cell malignancies and measurable lymphadenopathy (LAD) with ≥1 prior therapy received GS-9820 at doses of 50, 100, 200, or 400 mg, orally, twice daily (BID). The dose escalation stage (n=12) had a 3+3 design and measured safety, efficacy and pharmacologic properties. Additional subjects (n=27) enrolled in the dose expansion cohort at 400 mg BID. Antitumor activity was evaluated every 2 months including CT scans, with adjustments for redistribution lymphocytosis, consistent with PI3Kd inhibition. Nodal PR (nPR) is defined as ≥50% reduction in LAD. Subjects received GS-9820 until disease progression or unacceptable toxicity.
Results: As of July 2014, 15/39 subjects remain on treatment. Reasons for discontinuation include disease progression (11), death (4) (all unrelated to GS-9820), adverse events (AE) (14) and other (9). Response rates assessed by independent review committee were; overall 33.3% (95%CI (19.1-50.2)), for CLL subset (n=22) 33.3%, for NHL subset (n=17; 8 MCL, 4 DLBCL) 28.6%. nPR in CLL subset was 84.6%. The median duration of response was not reached and maximum duration of response was up to 11.9 months. No dose limiting toxicities were reported. AEs (subject incidence >20%) were cough, diarrhea, dyspnea, fatigue, peripheral edema and rash. Severe adverse events (grade 3 or 4) reported by >1 subject were pneumonia (5, 12.8%), pyrexia (4, 10.3%) sepsis (2, 5.1%) and diarrhea (2, 5.1%). AEs considered related to study drug by the investigator (subject incidence >10%) were pyrexia, dysgeusia, diarrhea and increase in AST or ALT. Three subjects had elevations in transaminase levels at grade 3 or 4 in the 400 mg BID dose cohort. No subjects reported pneumonitis and 8 subjects reported pneumonia[a].
Summary and Conclusions: Interim analysis of this phase 1b study of GS-9820 demonstrates clinical efficacy and safety comparable to idelalisib. Based on the similarity to idelalisib, no further clinical development is planned. [a]pneumonia included AEs of pneumonia, viral pneumonia, organizing pneumonia.

E1149

LONG TERM SAFETY AND ACTIVITY OF CLADRIBINE IN MUCOSA ASSOCIATED LYMPHOID TISSUE LYMPHOMA (MALT LYMPHOMA)

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Background: The purine analogue 2-chloro-deoxyadenosine (2CDA, cladribine) +/- rituximab (R) was successfully tested in MALT lymphoma patients achieving remission rates of 80-100%. However, studies using 2CDA in other indications have reported the potential for prolonged hematological side effects and secondary malignancies. To date, there have been no data on long term effects of 2CDA in MALT lymphoma patients.
Aims: In view of this, we have retrospectively analysed long term safety and activity of 2CDA in MALT lymphoma patients.
Methods: From 1998-2004, 49 patients were treated with either 2CDA alone (0.12 mg/kg i.v. d1-5, every 28 days) or R-2CDA (R 375 mg/m2 i.v. d1, 2CDA 0.1 mg/kg s.c. d1-4, every 21 days) for a maximum of six cycles. All patients were treated within clinical trials and had undergone a standardised follow-up (FUP) protocol (three-monthly for the first two years, then six-monthly for the first five years, annually thereafter) minimizing a potential bias in the detection of late sequels and relapses. This retrospective analysis had been approved by the Ethical Board of the Medical University of Vienna.
Results: Patients included in this analysis had received either 2CDA alone (n=17, 35%) or combined with R (n=32, 65%). The median age at treatment start was 60 years. Response to treatment was 100% for 2CDA and 78% for R-2CDA, respectively and the median number of applied cycles was four and six, respectively. All documented relapses (n=17) occurred within a median time of 13 (interquartile range; 10-42) and 15 (IQR; 8-33) months, suggesting long term remissions in patients "outlasting" the initial post-treatment period. After a median FUP of 55 months (IQR; 42-70) three of 49 patients aged 59, 70 and 77 had developed prolonged pancytopenia starting 1, 3 and 83 months after treatment. All patients had a bone marrow biopsy and in one patient myelodysplastic syndrome (MDS) was diagnosed. Two of three patients died with ongoing panzytopenia after 14 and 89 months while the third recovered to normal blood counts. Noteworthy, all three patients had received the maximum number of six cycles and in all 2CDA was not the sole treatment administered. Thus additional triggers for cytopenia/MDS cannot be ruled out. In one patient, R-CHOP was given for relapse before MDS occurred while the other two were pretreated with abdominal radiation or radioimmunotherapy. At the time of analysis 35/49 (71%) are alive with 25/35 (71%) in ongoing complete remission, two (6%) in ongoing stable disease, and eight (23%) free of lymphoma after 2nd line. Fourteen patients (29%) have died including two patients who died after only one cycle of treatment due to unrelated reasons and one patient who withdrew consent after four cycles. As reported, two patients died

with pancytopenia 14 and 89 months after initiation of treatment. In addition four patients died from secondary malignancies (gastric cancer, B-cell lymphoma, SCLC, skin cancer) after median 48 months (range; 12-93) and five due to cancer-unrelated reasons after median 55 months (range; 11-124). Median overall survival was not reached (Figure 1: Survival curve for MALT lymphoma patients receiving cladribine treatment, n=49).

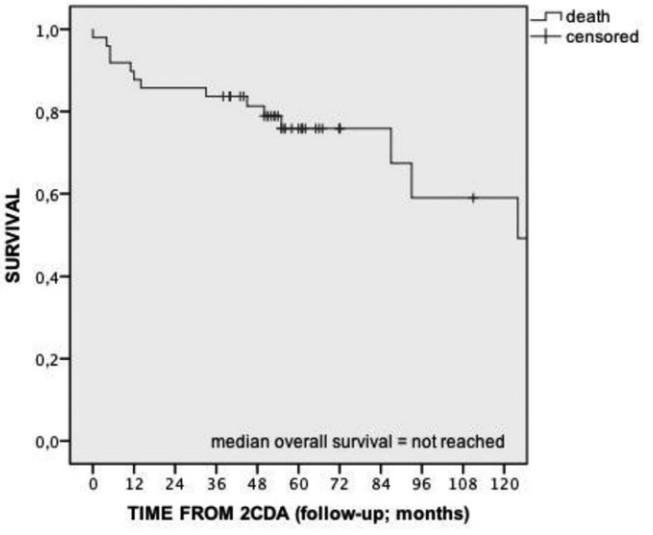

Figure 1.

Summary and Conclusions: After a median FUP of 55 months, 8/49 patients (16%) treated with 2CDA for MALT lymphoma have developed a secondary malignancy. In most of these cases, however, a causative role of 2CDA is relatively unlikely given the nature and time of development of these cancers. In addition, 3/49 patients had prolonged pancytopenia/MDS. While the number of patients in our series is relatively small, these data suggest that with the high rate of remissions and the fact that the median survival was not reached after more than 5.5 years 2CDA might be safely applied also in terms of long term toxicities. However, prolonged pancytopenia may occur particularly in older patients.

E1150

NON-GASTRIC MALT LYMPHOMAS (NG-MALT). CLINICAL CHARACTERISTICS AND OUTCOME IN A SERIES OF 185 PATIENTS: ASSESSING PARAMETERS OF PROGNOSTIC SIGNIFICANCE

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Background: NG MALT lymphomas may arise at any anatomic site. Recent data suggest that primary localization may have an impact on patients' clinical course and outcome.
Aims: To investigate whether anatomic localization is associated with specific disease features and outcome in a large series of NG MALT lymphoma patients and to identify possible prognostic parameters.
Methods: 185 patients, from 6 Hematology Units in Greece, with biopsy-confirmed NG-MALT lymphoma, were retrospectively analyzed. Survival probability was estimated using the Kaplan-Meier method.
Results: The main characteristics of the studied population are given in table 1. Fourteen different sites of involvement were recorded. The most common were skin (22%), salivary gland (SG) (20%), ocular adnexa (OA) (13%) and lung (12%). Seventy-two% presented with limited stage disease, while 78% were classified as low risk according to IPI. A history of autoimmune disorders was present in 24%, with Sjogren's syndrome being the most common. Gastroscopy was performed in 87 pts; a MALT lymphoma was identified in 18% and *H.pylori* (HP) (+) gastritis in 16%. Bone marrow was infiltrated in 12%. Treatment was not uniform, comprised mainly of systemic therapies [chemotherapy (CT) in 22%, Rituximab (R)-CT in 34%, R monotherapy in 9%, IFN-a (3%)], local therapies (surgery in 13%, radiotherapy in 9%, R locally in 1%), while 6 pts (3%) were placed on follow up observation. *Correlations of anatomic site with specific disease features:* 1. SG was more often associated with advanced disease stage (p=0.001). 2. SG and thyroid had more often an autoimmune background (p<0.001). 3. OA was strongly associated with *HP*(+) gastritis (p=0.002). 4. Small intestine and lung had more frequently paraproteinemia (p=0.001). After a median follow-up time of 74 months (1-369) the median failure free (FFS), overall (OS) and disease specific survival (DSS) were 14,8, 25,5 and 25,5 years, respectively. Small intestine, lung and multiple MALT site disease (>2) had a shorter median FFS compared to all other MALT localizations (92 *vs* 197,7 months) (p=0.02). Patients could be grouped into 3 different risk categories according to their disease localization: Group 1 (skin, large intestine, thyroid, SG and liver) had the most favorable FFS (median not reached), group 2 (head and neck localization, breast, kidney and OA) had an intermediate prognosis (median FFS 178 months), while group 3 (lung, small intestine, thymus, bladder, brain and multiple MALT sites) were associated with the worst outcome (median FFS 81,8 months) (p=0.013). Age<60 years was a favorable factor for FFS (not reached *vs* 145,8 months) (p=0.034). IPI was a strong prognostic factor for FFS: IPI 0/1 (median FFS not reached), IPI 2/3 (median FFS 37,9 months), IPI 4 (median FFS 8,6 months) (p<0,0001).

Table 1. Patients' Characteristics.

Characteristics	#of patients (%)
Localization	
Skin	40 (22)
Sg	38 (20)
Oa	24 (13)
Lung	23 (12)
Head And Neck	21 (11)
Small Intestine	11 (6)
Thyroid	6 (3)
Kidney	5 (3)
Large Intestine	4 (2)
Breast	3 (2)
Thymous	2 (1)
Bladder	2 (1)
Brain	1 (0,5)
Liver	1 (0,5)
>2 MALT Sites	4 (2)
Age Median, *Years* (Range)	60 (20-87)
Clinical Stage	
I	111 (61)
II	20 (11)
III	4 (2)
IV	47 (26)
Elevated LDH (N=164)	11 (7)
IPI (N=166)	
Low	130 (78)
Low/Intermediate	16 (10)
High/Intermediate	18 (11)
High	2 (1)
Autoimmune Background (N=115)	28 (24)
Bone Marrow Infiltration (N=138)	17 (12)
Gastroscopy (N=87)	
Normal Findings	55 (63)
Hp(+) Gastritis	14 (16)
MALT Lymphoma	16 (18)
Cancer	2 (2)
Paraproteinemia (N=91)	21 (23)
Failure Free Survival	
Median	14,8 years
5-Year/10 Year	69%/58%
Overall Survival	
Median	25,5 years
5-Year/10-Year	90%/80%
Disease Specific Survival	
Median	25,5 years
5-Year/10-Year	95%/93%

N= number of pts assessed

Summary and Conclusions: NG MALT lymphomas display a highly favorable clinical course and survival. The present study shows that certain anatomic sites were highly associated with specific disease features. In addition lung, small intestine and multiple MALT sites of involvement carried the most adverse prognosis. Furthermore, age<60 and low IPI were associated with improved FFS.

E1151

FLUDARABINE TREATMENT FOR WALDENSTRÖM MACROGLOBULINEMIA-ASSOCIATED NEUROPATHY – 20 YEARS EXPERIENCE OF A SINGLE CENTER

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Background: Waldenström macroglobulinemia (WM) is a malignant indolent incurable lymphoproliferative disease characterized by the association of the evidence of a lymphoplasmacytic proliferation and a monoclonal component of IgM. WM-related polyneuropathy (WM-PN) is its most frequent neurological complication. WM-PN diagnosis is difficult and underestimated due to the heterogeneity of the mechanisms and of clinical presentations.The presence of neuropathy is widely accepted as an indication for treatment in WM but the choice and time of the appropriate therapy can be challenging due to different toxicity profile of each option, the non-curable characteristic of the disease and the heterogeneous response to treatment of different subtypes of neuropathy
Aims: The aim of our study was to present our local experience of the use of fludarabine, as a single agent or in combination with rituximab or cyclophosfamide (fludarabine-based chemotherapy) for the treatment of Waldenström macroglobulinemia associated neuropathies.
Methods: Seventy patients with IgM monoclonal immunoglobulin and neuropathy were addressed to our hematology department in the last 20 years: 27 patient were diagnosed with monoclonal gamapathy on undetermined significance (MGUS) median age 67 years, 39 patients were diagnosed with WM, median age 74 years and 4 patients with other low grade non Hodgkin lymphoma, median age 66 years. Treatment was proposed to 32 WM patients, from whom 17 with fludarabine based chemotherapy. Our primary objective was to evaluate the neurological response to treatment by clinical evaluation, electromyography and the 10 points INCAT disability score, the secondary objectives were the treatment-free survival (TFS) and the hematological response.
Results: Of 17 patients (IPSSWM low- intermediate- high: 3-12-2), 13 started treatment for neurological impairment only and 4 patients had in addition hematological treatment criteria. All patients were treated with 4 to 6 cycles of chemotherapy adapted to tolerance: 1 received RFC (rituximab-fludarabine-cyclophosphamide) regimen, 11 received RF regimen, 5 received fludarabine alone. Neurologically, 5 patients had pure axonal neuropathy; 11 patients presented demyelinating neuropathy and 2 patients demyelinating neuropathy with secondary axonal lesions. In 8 patients anti MAG antibodies were detected and 11 patients presented severe forms of neuropathy. The neurological response after treatment: 11 patients presented clinical improvement, 5 patients presented stable clinical disease, 1 patients dissociated response, the INCAT score had significant improvement, more than 2 points, in 7 patients, and 1 point improvement in 4 patients. EMG registered only partial amelioration in 3 cases of axonal neuropathy and 1 case of non severe demyelinating neuropathy. The hematological response: median peak reduction 75% (min 33%, max 100%). The median TFS for neurological or hematological criteria was not reached, the mean TFS 92.5 months [min 68.3, max 116.7].
Summary and Conclusions: The fludarabine based chemotherapy for WM-PN allows good control of the symptomatology and long treatment-free survival. Further trials are needed to establish the optimal moment to treat in the evolution of the neuropathy.

E1152

PHARMACOKINETIC DATA, CLINICAL CHARACTERISTICS AND OUTCOME IN FOLLICULAR LYMPHOMA PATIENTS IN MAINTENANCE WITH RITUXIMAB: AN ANALYSIS OF THE FONDAZIONE ITALIANA LINFOMI (FIL)

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Background: Rituximab (R)-chemotherapy induction followed by R maintenance is the current standard of care for patients with follicular lymphoma (FL). In recent years, several studies have investigated the optimal dose and schedule of R based on pharmacokinetic (PK) data. These studies have proposed a presumptive 'active' level of 25 µg/ml. However, scanty data are available with regard to the PK of R during maintenance and its possible relationship with the patients' characteristics and outcome.
Aims: Here, we report the preliminary analysis of PK data correlated with patients' clinical characteristics and outcome during R maintenance.
Methods: Patients with grade 1, 2 or 3a FL in maintenance therapy with iv R (375 mg/m^2) administered every 2 (patients treated front-line) or 3 months (after salvage immuno-chemotherapy) were investigated. R plasma trough concentrations (C_{tr}) and the area under the curve (AUC) were determined using a sensitive validated ELISA assay. PK data were then correlated with the patients' clinical characteristics and outcome.
Results: From March 2013 to March 2014, 101 patients have been recruited by 12 FIL centers. The median age was 60 years (IQR 30-84) and 50 (48.5%) were males. According to body mass index (BMI) 44 (43%) patients were overweight and 10.8% were obese. At study entry, 92 patients were in first complete response (CR), 19/92 in second CR and 9 in partial response (PR), of whom 2 after salvage therapy. In 85/101 (group 1) and in 16/101 (group 2) patients, R was administered every 2 or 3 months, respectively. After a median follow-up of 23 months from the start of R maintenance, 91 patients were in CR, 3 in PR and 7 had relapsed. The median C_{tr} level was 32 µg/ml (0.0 µg/ml – 200 µg/ml) in all patients, 33 µg/ml (0 µg/ml-188 µg/ml) in patients treated with R every 2 months and 22.8 µg/ml (0 µg/ml-200 µg/ml) in patients treated every 3 months, respectively (p=0.7). A higher median R C_{tr} level (36 µg/ml *vs* 22 µg/ml, p=0.04) and a higher AUC (61.23 *vs* 41.9, p=0.011) were found in females. No correlation between C_{tr}, AUC and BMI were documented, but a trend towards higher levels were found in patients with a BMI <30 (C_{tr}=40.38 µg/ml *vs* 31.96 µg/ml, p=0.23; AUC=54.19 *vs* 42.55, p=0.13). This preliminary analysis did not show any relationship between the quality of response (CR *vs* PR), the outcome (response *vs* relapse) and the C_{tr} levels and AUC; however, in a subanalysis of group 1, more patients with lower C_{tr} relapsed during R maintenance (38.8 µg/ml *vs* 33.9 µg/ml, p=0.01) and, notably, 4/6 who relapsed had C_{tr} levels under 25 µg/ml.
Summary and Conclusions: Our study confirms the favorable R PK profile in female patients and in the 2 months administration scheme. At present, probably because of the relatively short follow-up and low number of events during maintenance, no relationship between PK data and outcome has so far emerged and a longer observation time is required.

E1153

HYPOGAMMAGLOBULINEMIA AT LONG-TERM AFTER RITUXIMAB/ CHEMOTHERAPY TREATMENT FOR LYMPHOMA, AND USE OF INTRAVENOUS IMMUNOGLOBULINS FOR RECURRENT INFECTIONS.

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Background: Rituximab (R) is a monoclonal antibody against CD20 that causes suppression of B-lymphocytes and may induce hypogammaglobulinemia, with the subsequent increased risk of infections. B-cells usually recover within 6-12 months after R administration. Actually, many patients can be exposed to rituximab during prolonged periods of time, either following maintenance or because of retreatment.
Aims: This retrospective study sought to evaluate (at long term) the kinetics of gammaglobulins during the first 4 years after R treatment, the influence of prolonged R exposition (following maintenance and/or second/third line treatment) and its impact on the use of intravenous immunoglobulins (IVIG).
Methods: We identified 194 patients who received R or R-chemotherapy for lymphoma between 2005 and 2011 in our Lymphoma Unit. Patient clinical histories were evaluated with special interest in: type of R-chemo received, maintenance with R, use of other anti-CD20 monoclonal antibodies and treatment at relapse. Quantitative serum immunoglobulin levels (sIg) prior to and at the end of first line treatment and every 12 months was recorded, as well as need for IVIG replacement therapy and relevant infectious events.
Results: median age of 67 years, 41 % female. Lymphoma types: aggressive (58%), indolent follicular (29%), indolent non-follicular (13%). First R-containing treatment: R (10%), R-purine analogs (6%) and R-chemo (84%). During the 4-year study period, second-line treatment was administered in 71 patients (R: 13%, R-purine analogs: 13%, R-chemo: 52%, chemo without R: 22%) and, 53 and 10 patients received R as maintenance or Ibritumomab as consolidation, respectively, in first or subsequent line of therapy. 85 patients had a total of 125 infectious adverse events: 53 during R or R-chemo (36% grade 1-2), 18 during R-maintenance (all but 1 grade 2) and 54 off R-therapy (39% grade 1-2). Median lymphocyte count, gammaglobulin, and levels of IgG, IgA and IgM are showed prior and post-therapy and at +12, +24 and +48 months:

Table 1.

	Lymphocytes (x10e9/L)	Gammaglobulin (g/L)	IgG (mg/dl)	IgA (mg/dl)	IgM (mg/dl)
Pre-Therapy	1.37	9.4	897	188	76
Post-Therapy	1.05	7.5	712	136	48
+12 months	1.31	8.4	778	148	54
+24 months	1.42	8.4	765	143	56
+48 months	1.62	8.8	824	168	64

Of note, prior treatment with R, 14% of patients had reduced levels of gammaglobulins (<6.6 g/L) and 24.5% of patients had a level of IgG <700 mg/dL. In the whole population, median levels of IgG, IgA and IgM were significantly reduced at the end of the first treatment (p<0.0001, Wilcoxon test for paired

samples), and also at months +12, +24 and +48. However, the kinetic of levels of IgG-IgA-IgM was significantly different according to R exposition. Patients receiving only one schema of R or R-containing chemo showed a faster recovery with normal values of IgG, IgA and IgM at +12 months. Interestingly, patients who received more than one treatment of R-chemo or R-maintenance after R-chemo had a prolonged reduced levels of all gammaglobulins that remained under normal values even at +48 months (figure 1). Even though 30% of cases continued with reduced levels of IgG at +48 months, only 8 patients (4%) ultimately required IVIG replacement.

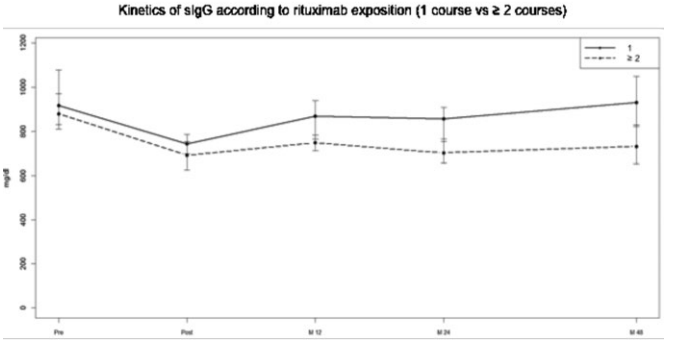

Figure 1.

Summary and Conclusions: Lymphoma patients receiving more than one treatment of R or R-containing chemo and those receiving R-maintenance are at increased risk of long-term hypogammaglobulinemia. With the increasing use of R, it is important to monitor sIg levels even though the incidence of symptomatic hypogammaglobulinemia that prompted IVIG administration is infrequent.

E1154

UPDATED RESULTS FROM A MULTICENTER, OPEN-LABEL, DOSE-ESCALATION PHASE 1B/2 STUDY OF SINGLE-AGENT OPROZOMIB IN PATIENTS WITH HEMATOLOGIC MALIGNANCIES, INCLUDING WALDENSTRÖM MACROGLOBULINEMIA

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Background: Oprozomib (OPZ) is an oral epoxyketone proteasome inhibitor that has shown promising antitumor activity in a phase 1b/2 study (NCT01416428) in patients with hematologic malignancies, including Waldenström macroglobulinemia **(**WM; Siegel *et al*., *Blood* 2014;124: abstract 1715). In the phase 1b portion of the study, the maximum tolerated dose of single-agent OPZ when administered once daily on days 1, 2, 8, and 9 of a 14-day cycle (2/7 schedule) or on days 1–5 of a 14-day cycle (5/14 schedule) to patients with hematologic malignancies was 300 mg/day and 240 mg/day, respectively.
Aims: We present updated efficacy and safety results from patients with WM enrolled in the phase 2 portion of the study.
Methods: Adult patients with hematologic malignancies who have relapsed after receiving ≥1 line of therapy are eligible for enrollment. The primary objective of the phase 2 portion of the study (the target enrollment for the phase 2 portion is 66 patients with WM) is to determine the best overall response rate (ORR; minimal response or better). In the phase 2 portion of the study, single-agent OPZ is being administered once daily on days 1, 2, 8, and 9 of a 14-day cycle (2/7 schedule: 240 mg/day during cycle 1; 300 mg/day thereafter if well tolerated) or on days 1–5 of a 14-day cycle (5/14 schedule: 240 mg/day). All patients provided informed consent. Efficacy and safety results are presented for patients with WM in the phase 2 portion of the study.
Results: As of November 3, 2014, a total of 129 patients with hematologic malignancies were enrolled in the phase 1b/2 study. Of those patients, 21 had WM and were enrolled in the phase 2 portion of the study and treated with OPZ. Four patients with WM were enrolled in the 2/7 dosing schedule; 17 patients with WM were enrolled in the 5/14 schedule. The median age of patients was 64.5 years (range, 63–67 years) in the 2/7 schedule and 62 years (range, 44–85 years) in the 5/14 schedule. The median number of prior regimens was 2.5 (range, 1–8) in the 2/7 schedule and 3 (range, 1–7) in the 5/14 schedule. Preliminary median treatment duration was 6.6 weeks (range, 0.3–8.1 weeks) in the 2/7 schedule and 8.1 weeks (range, 0.7–31.6 weeks) in the 5/14 schedule. All 21 patients with WM in the phase 2 portion were response-eligible. The ORR in the 2/7 schedule was 25% (1 of 4 patients; 1 partial response); the ORR in the 5/14 schedule was 53% (9 of 17 patients; 5 partial responses and 4 minimal responses). The most common adverse events (AEs) are shown in the Table. Grade 3 AEs occurring in >1 patient included nausea and vomiting (2 patients each); there were no grade 4 AEs. Antiemetic medication was administered to 3 patients (75%) in the 2/7 schedule and 17 patients (100%) in the 5/14 schedule. Four patients in each schedule (100% in the 2/7 schedule; 24% in the 5/14 schedule) received antidiarrheal medication. No on-study deaths occurred in patients with WM. No patients enrolled in the 2/7 schedule and 7 patients (41%) enrolled in the 5/14 schedule discontinued treatment due to an AE. Three patients (75%) in the 2/7 schedule and 11 patients (65%) in the 5/14 schedule had ≥1 dose reduction due to an AE.

Table 1. AEs occurring in ≥30% of WM patients (by schedule)

	2/7 schedule (n=4)		5/14 schedule (n=17)	
AE, n (%)	Any grade	Grade 3–4	Any grade	Grade 3–4
Nausea	3 (75)	1 (25)	14 (82)	1 (6)
Diarrhea	3 (75)	0 (0)	11 (65)	1 (6)
Constipation	1 (25)	0 (0)	10 (59)	0 (0)
Fatigue	1 (25)	0 (0)	9 (53)	0 (0)
Vomiting	2 (50)	1 (25)	6 (35)	1 (6)
Decreased appetite	0 (0)	0 (0)	6 (35)	0 (0)
Gastroesophageal reflux disease	2 (50)	0 (0)	2 (12)	0 (0)

Summary and Conclusions: Single-agent OPZ has promising antitumor activity in patients with WM. Enrollment in both treatment schedules is ongoing. Updated results will be presented at the meeting.

E1155

WHICH GUIDELINES TO TRUST? CRITICAL APPRAISAL OF CLINICAL PRACTICE GUIDELINES FOR MANTLE CELL LYMPHOMA SHOWS HIGH VARIABILITY AMONG RECOMMENDATIONS.

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Background: Guidelines are evidence-based tools supporting everyday clinical practice. Therefore, several national and international scientific and governament institutions regularly produce guidelines. Unfortunately, major inconsistencies are usually found among basic recommendations, which limits both applicability and harmonization. Some excellence institutions overcame such a hurdle developing high-quality local clinical pathways. Nevertheless, guideline representation through an ontology-based format may permit a greater insight into inconsistencies and ambiguities.
Aims: In a pilot phase of a guideline harmonization project sustained by the Italian Society of Hematology, we tested the comparability of guidelines for frontline treatment of mantle cell lymphoma (MCL).
Methods: We applied the ADAPTE standard to search, appraise and compare guidelines: those published in English language after the year 2010 were selected. Clinical practice guidelines (CPG) reporting evidence levels and grading of recommendations were listed separately from coonsensus statements and clinical pathways. CPG quality was assessed by the standard tool AGREE. Finally, we extracted implicit ontologies out of the recommendations and compared them across guidelines.
Results: We retrieved 11 documents from 7 countries, including 5 clinical practice guidelines (CPG), 2 clinical pathways, 1 consensus statement and 1 hybrid document. Four different grading systems for guidelines were adopted, only 2 of them using GRADE system. AGREE score for the 5 CPG ranged from 25% to 57%, the weakest areas being applicability, editorial independence and stakholders. The two CPG adopting GRADE system scored highest at AGREE evaluation. Major inconsistencies among guidelines regarded the criteria used to choose a therapy plan. Analysis of ontologies allowed us to classify the decisional criteria suggested for prompting frontline choices into 7 classes: age, performance status, comorbidity, biologic aggressiveness (blastoid variant, proliferation index), clinical presentation (indolent disease, symptoms, nodal disease), prognostic score (MIPI, IPI), tumor burden (included stage). Each guideline incorporated only a small subset of the above decisional criteria and overlap among guidelines was minimal. Rituximab-bendamustine and Rituximab-CHOP were recommended as frontline therapy for transplant inelegible patients by all the guidelines, however, only 2 of them transparently indicated a preference ranking among therapies. Recommendations to other chemoimmunotherapies (R-CVP, R-Chl, R-FC, R-CVAD/R-MA) were highly heterogeneous. Rituximab maintenance was recommended by five out of five guidelines updated in 2014, but the recommendation was usually not restitricted to patients treated with frontline RCHOP. Radiotherapy was consistently recommended for stage I-II disease, usually as consolidation after chemotherapy. Indication to ASCT consolidation in younger patient with advanced disease was not always stringent, however it was in almost all the CPGs. Suggested induction regimen included high-dose aracytin, except for one CPG suggesting the use of Rituximab-bendamustine. Only one CPG and one algorithm reported criteria for consolidation allogeneic SCT in patients with a first complete remission. Three guidelines

provided recommendations for management of compromised patients. No guideline suggested the use of frontline regimens containing bortezomib, ibrutinib or idelalisib.
Summary and Conclusions: Inconsistency among guidelines does not reside only in paucity of comparative high-quality evidence, rather, in lack of transparent methods for linking evidence to recommendations, in disagreement on ontologies and in empiric management of uncertainty. Guideline harmonization projects may prompt an improvement of guideline quality and allow a more critical adoption by physicians.

E1156

CIRCULATING ADAMTS-13 IS REDUCED IN PATIENTS WITH WALDENSTROM'S MACROGLOBULINEMIA AND IS ASSOCIATED WITH INCREASED IGM LEVELS AND DISEASE FEATURES BUT NOT WITH THE LEVELS OF VON WILLEBRAND FACTOR

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Background: ADAMTS-13 is a protease, which cleaves the cell bound large ultrapolymeric von Willebrand factor (vWF) strings. Circulating ADAMTS-13 is primarily synthesized and released from hepatic stellate and endothelial cells. Acquired functional deficiency of ADAMTS-13 results in excessive platelet aggregation and disseminated vWF/platelet-rich thrombus formation. Hivert et al (Blood 2012) and our group (ASH 2013, # 3194) have shown that increased levels of vWF, the only known substrate of ADAMTS-13, are associated with poor prognosis in patients with symptomatic WM.
Aims: Our aim was to investigate the possible association of ADAMTS-13 antigen (Ag) levels with features of WM and possible biologic implications of the ADAMTS-13 / vWF interaction in WM patients' prognosis.
Methods: Our study included 42 patients with symptomatic WM who were treated and followed in the Department of Clinical Therapeutics of the University of Athens (Greece), 22 patients with asymptomatic WM and 19 healthy controls of matched gender and age. ADAMTS-13 antigen levels were measured in stored serum, collected at diagnosis and before initiation of therapy, using a commercially available kit (R&D Systems, Minneapolis, MN, USA).
Results: The median age of patients with symptomatic WM was 65 years (range: 37-83 years) and 54% were males. Anemia (Hb<11.5 g/dL) was present in 78%, platelet counts <100x10^9/L in 17%, beta2-microglobulin >3 mg/dl in 56%, while 7.5% had serum LDH ≥250 U/L and 58% had serum albumin <3.5 g/dL. Median serum IgM was 3340 mg/L (range 246-9563 mg/dL). According to ISSWM, 22% had low, 43% intermediate and 35% high risk WM, respectively. Reasons to initiate therapy included cytopenias in 42%, B-symptoms in 15%, hyperviscosity in 12%, neuropathy in 10% and other reasons in 21%. Primary therapy based on rituximab was given in 93% of the patients and 54% achieved at least 50% reduction of IgM. Median ADAMTS-13 levels in patients with symptomatic WM were 848 ng/ml (range 471-1622 ng/ml), in those with asymptomatic WM were 875 ng/ml (range 280.5-1466 ng/nl) and in healthy controls were 1170 ng/ml (range 770-1598 ng/ml). Thus, there was a significant difference of ADAMTS-13 levels between patients with either symptomatic or asymptomatic WM and normal individuals (p<0.001 and p=0.002 respectively) but there was no difference between symptomatic and asymptomatic WM (p=0.934). There was a negative correlation between levels of ADAMTS-13 and levels of IgM (r=-0.544, p<0.001), beta2-microglobulin (r=-0.376, p=0.01) and the infiltration of the BM by lymphoplasmatic cells (r=-0.338, p=0.03),and a positive correlation with serum albumin levels (r=0.373, p=0.016). Accordingly, in patients with hyperviscosity syndrome, serum ADAMTS-13 was lower (p=0.04). There was no association of ADAMTS-13 levels and vWFAg levels, in patients with either symptomatic or asymptomatic WM (p>0.5 for both). Thus, a direct link between increased vWFAg levels, which are found in patients with WM, and compensatory ADAMTS-13 levels cannot be supported by our data. The levels of ADAMTS-13 had no prognostic association with overall or progression free survival in patients with symptomatic WM.
Summary and Conclusions: This is the first evaluation of ADAMTS-13:Ag levels in the serum of WM patients. Serum ADAMTS-13:Ag levels are lower compared to healthy controls and inversely correlate with IgM levels, beta2-microglobulin and the degree of bone marrow infiltration but do not increase in compensation to increased levels of vWF Ag.

E1157

DOES AGE ADJUSTED CHARLSON COMORBIDITY SCORE STRONGLY PREDICT OUTCOME OF THE PATIENTS WITH FOLLICULAR LYMPHOMA TREATED WITH IMMUNOCHEMOTHERAPY?

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Background: The clinical outcome of patients with follicular lymphoma (FL) has been improved since the introduction of rituximab. However, the management and optative outcome of patients with lymphoma is frequently complicated by their comorbid conditions.
Aims: The aim of this study was to evaluate possible influence of comorbidities on the overall survival (OS) of patients with FL, using correlation between clinical outcome and comorbid status expressed through Cumulative Illness Rating Scale (CIRS) and age adjusted Charlson comorbidity index (aaCCI).
Methods: A total of 140 patients (84 female/56 male) from age of 20-74 years (median 52 years) were analyzed. Majority of patients (94.3%) were in advanced clinical stage, III and IV according to the Ann Arbor classification. Bulky disease was present in 35.7% patients, B symptoms in 67.1%, leukemic phase of disease in 7.1% of patients. Bone marrow infiltration had 67.1% patients and histopathological grade (Gr) 3 of disease had 25.6% of patients. Regarding the Follicular Lymphoma International Prognostic Index 2 (FLIPI2), low score was present in 4.4% patients, intermediate in 47.8% and high score in 47.8%. According to CIRS (minimal score 14), 58.5% of patients were without comorbid condition (CIRS 14) and 41.5% had at least one comorbidity (CIRS≥15). In a view of aaCCI, 63.1% patients had aaCCI 0-2 and 36.9% had aaCCI≥3.
Results: All patients were treated with immunochemotherapy including R-CHOP protocol (rituximab, cyclophosphamide, doxorubicin, vincristine, prednisolone) applied in 92.1% of patients and R-CVP protocol (rituximab, vincristine, prednisolone) applied in 7.9%. Partial and complete remissions were achieved in 92.1% patients, and 7.9% did not respond to initial medical treatment. There was no statistical difference regarding OS between patients who were treated with R-CVP protocol comparing to patients treated with R-CHOP (Log Rank $\chi^2$1.436, p>0.05). According to the lymphoma grade, there was difference in 5–years survival between patients with Gr 1-2 and Gr 3 (Log Rank χ^2=5.291, p=0.06), 84% in patients with Gr 1-2 comparing to 68% in those with Gr 3. The patients with B symptoms had poorer outcome with 5–years survival of 78%, comparing to the patients without B symptoms whose 5–years survival was 90% (Log Rank χ^2=1.672, p<0.05). Leukemic phase of lymphoma was not in correlation with the worst outcome (Log Rank χ^2=0.04, p>0.05), neither was bulky disease (Log Rank χ^2=1.672, p>0.05). The FLIPI2 was highly predictive in our group of patients (Log Rank χ^2=20.361, p<0.01). OS in patients with CIRS≥15 after 1, 2 and 5 years was 92%, 87% and 80% respectively, and 98%, 95% and 89% for those with CIRS 14. Regarding aaCCI, OS in patients with aaCCI≥3 after 1, 2 and 5 years was 94%, 82% and 76% respectively, and 97%, 93% and 88% for those with aaCCI 0-2. Multivariate analysis among CIRS, aaCCI, lymphoma histopathological grade and FLIPI2, revealed that only FLIPI 2 was independent prognostic parameter associated with worse OS (HR=18.18, 95% CI 2.359-140.134).
Summary and Conclusions: In the rituximab era prognostic index FLIPI2 still remains the most predictable value for OS in FL patients, while histopathological Gr 3 illustrated the different, more biologically aggressive form of disease. Among FL patients, aaCCI was associated with poorer outcome and should be calculated in this population.

E1158

TOXICITY AND EFFICACY OF BENDAMUSTINE IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) AND INDOLENT LOW GRADE NON-HODGKINS LYMPHOMA (INHL); A MULTI-CENTRE OBSERVATION OF REAL LIFE EXPERIENCE IN THE UK

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Background: Bendamustine has shown resurgence as a new 'old' drug effective in both CLL and iNHL with a favorable side-effect profile (Rummel MJ et al, 2013, Lancet, 381:1203 & Fischer K et al, 2012, J Clin Oncol, 30:3209). Data outside of the trial setting is lacking.
Aims: To analyze the response rate and toxicity of bendamustine in a large heterogeneous cohort of patients treated in the United Kingdom.
Methods: We collected data on 161 patients treated with bendamustine over 2 years across 5 hospitals in the UK. Toxicity was assessed by red cell & platelet transfusion, hospital admissions, G-CSF use during admission and dose reduction. Univariate analysis of each factor assessed association with response, toxicity, progression-free survival (PFS) and overall survival (OS). PFS and OS were assessed by Cox regression.
Results: Mean age was 71 years (47-89). Diagnosis: CLL (n=108) and iNHL (n=53). Bendamustine was front line therapy in 57 patients (35%), relapse therapy in 104 (65%). ECOG performance status (PS) was 0-1 in 108 (67%)

patients (range 0-3). Treatment was part of a trial in 19 patients (12%). Median dose in CLL was 70mg/m2 and in iNHL 90mg/m2 (range 45-120), used as a single therapeutic agent in 20%, and combined with rituximab in 76%. Supportive therapy with septrin, anti-fungal and G-CSF was used in 64 (40%), 43 (27%) and 47 (29%) patients respectively. Improved response to bendamustine in the whole cohort significantly correlated with front-line use (p=0.02) and lower PS (p=0.01), but not age. In the CLL cohort, first line bendamustine overall response rate (ORR) was 90%, complete response (CR); 51%. In the CLL relapsed setting ORR was 76%, CR was 18%. Deletion of 17p and 11q did not impact on ORR. In the iNHL cohort, first line bendamustine ORR was 100%, CR was 53%. In the iNHL relapsed setting the ORR was 81%, CR of 28%. Age, bulky disease, stage or bone marrow involvement did not affect ORR in iNHL. Red cell transfusions were administered in 67 patients (43%), with higher PS predictive of this (p=0.03). Of these patients, only 35 (53%) received irradiated blood. Platelet transfusions were administered in 23 patients (15%). Admission during treatment occurred in 82 patients (53%), with male sex predictive of this (p=0.04). G-CSF administration during these admissions occurred in 28 patients (19%), with a higher number of co-morbidities predictive of this (p=0.008). Dose reduction after cycle 1 occurred in 17 patients (11%). Median PFS in all patients was 17 months, median OS was 29 months. In CLL median PFS was 16 months, median OS was 28 months. Deletion of 17p had no influence on OS (p=0.11) or PFS (p=0.25). In iNHL, median PFS was 21 months, median OS was 66 months (see figure 1). Poor performance status (p <0.001) and treatment in relapsed setting (p=0.04) predicted inferior PFS. In multivariate analysis higher performance status (p <0.001), female gender (p=0.04) and lower eGFR (p=0.004) predicted poorer OS.

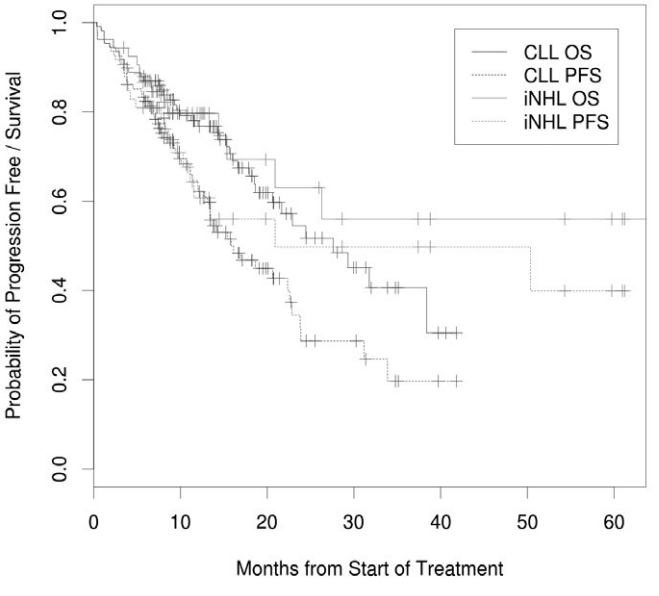

Figure 1. Overall PFS and OS for iNHL and CLL.

Summary and Conclusions: Our data suggests bendamustine is an effective therapy for CLL and iNHL. It delivers comparable efficacy to published trial data even in non-trial patients, particularly in front-line therapy. Of note, response in CLL was not affected by cytogenetic status. Similarly, response in iNHL was not affected by bulky disease or marrow involvement. In our study we found a high incidence of hospital admissions (53%) and blood product use. Several factors including gender, performance status, co-morbidities and renal function were found to independently correlate with toxicity. This study may help to improve patient selection for this chemotherapeutic agent.

E1159

MODELING THE EPIDEMIOLOGY OF FOLLICULAR LYMPHOMA (FL) IN FRANCE

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Background: As of 2014, there were few reliable publication sources of epidemiology data for Non-Hodgkin Lymphoma (NHL) in France, particularly with regard to indolent forms such as Follicular Lymphoma (FL). In addition, epidemiology registries provide useful data on disease incidence but no information is available on the number of relapsed FL patients. At the same time, new and effective drugs will be made available to the Hematologist/Oncologist community and thus, there will be an increase in the complexity of therapeutic algorithms.
Aims: The innovative modeling approach intends to offer a better understanding of the size of FL relapsed patient population in France. The objective of the model was to determine the yearly incidence of FL patients in first line and later lines of therapy between 2014 and 2018. Thus, the model provides relevant data to determine the patient population size that will be eligible for the new compounds and that could be implemented into a prospective economic model.
Methods: First, we used the "Registre Régional des Hémopathies Malignes de Basse-Normandie" (RRHMBN) to collect real world patient characteristics, such as patient subgroups and tumor staging. The RRHMBN is dedicated to Hematological malignancies and supported by official Health Authorities: "Institut National du Cancer"(INCA) and "Institut National de Veille Sanitaire" (INVS). Then, we applied these findings to the French National Network of Registers "FRANCIM" incidence and survival data. Thus, in 2014 the estimated number of patients with low risks (FLIPI 0-1, 36%) or high risk (FLIPI ≥2, 64%) is 985 and 1750 respectively. In 2018, it is estimated to reach 1151 and 2048 patients respectively. Afterwards, we applied progression free survival (PFS) results from medical literature for each standard of care treatment. The analysis showed that 36% of patients were classified FLIPI 0-1 and 60% of them had a stage I or II and they did not receive any treatment. 90% of patients with stages III/IV were treated by Rituximab alone (R) leading to a PFS equal to 26 months. Others patients stages III/IV and 90% of FLIPI≥2 were treated with Rituximab (R)−Cyclophosphamide (C)−Hydroxydaunorubicin (H)−Vincristine (O)−Prednisone (P) leading to a PFS equal to 42.2 months. For the remaining 10% of patients with FLIPI≥2, the Rituximab (R)−Cyclophosphamide (C))−Vincristine (O)−Prednisone (P) regimen led to a PFS equal to 34 months.
Results: The table below shows the yearly number of FL patients eligible for treatment in first and subsequent lines of therapy as produced by the model.

Table 1.

Category	Line of Therapy	2014	2016	2018
FL				
Stage I/II	1st line (L)	39	43	46
	≥ 2nd L	29	31	35
	Sub-total	68	74	81
Stage III/IV RCHOP	1st line (L)	1 755	1 898	2 052
	≥ 2nd L	1 086	1 174	1 271
	Sub-total	2 841	3 072	3 323
Stage III/IV RCVP	1st line (L)	350	379	410
	≥ 2nd L	238	258	278
	Sub-total	588	637	688
Total	1st line (L)	2 144	2 320	2 508
	≥ 2nd L	1 353	1 463	1 584
	Total FL population	3 497	3 783	4 092

Summary and Conclusions: Our modeling approach compensates for a lack of real world information on relapsed FL. We have shown that there is a significant number of relapsed patients who are in need of new treatments in France. The model produces estimates for the number of patients in later lines in need of novel therapies by 2018 and provides efficient prospective data that can be integrated into medico-economic model development. Furthermore, these data address a current unmet medical need for the development of new FL algorithms that are required for the upcoming therapies in the relapsed setting.

E1160

MANAGEMENT OF PRIMARY HEPATIC NON-HODGKIN'S LYMPHOMA AND CORRELATION WITH HCV INFECTION: EXCELLENT RESULTS WITH CONVENTIONAL CHEMOTHERAPY

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Background: Primary Hepatic (PHL) non-Hodgkin's Lymphoma is a rare entity, frequently associated with a poor prognosis. PHL was first described in 1965 by Ata el al and in 1986 Caccamo et al defined PHL as a localized lymphoma, limited to the liver without extrahepatic involvement. Small series of PHL have been reported, suggesting a non-fortuitous association with Hepatitis C Virus (HCV) infection. The prognosis is believed to be dismal, with early recurrence and short survival. To date, less than 150 cases have been published.
Aims: Eleven adult consecutive patients observed in our Division from 1990 to 2013 (median age: 58 years) fulfilled the diagnostic criteria for Primary Hepatic Lymphoma.
Methods: Our series of patients were derived from 1083 patients with non-

Hodgkin's Lymphoma observed in our institution in the same period (*i.e.* with a prevalence of 1.0% for PHL). We performed a study of the viral status and the result of cytotoxic treatment. The disease occurred in middle-aged patients (median age: 58 years). The main presenting complaint was right upper quadrant abdominal pain (4/11 patients). Tumor markers (Alfa-fetoprotein and CEA) were normal in 8 patients tested. Liver scans demonstrated either a solitary nodule or multiple lesions. Pathologic examination revealed diffuse large B cell lymphoma in seven patients, two cases of follicular lymphoma, one of small lymphocytic lymphoma and one case of T cell lymphoma. Eight patients (72%) were HCV-positive. Eight patients were treated with CHOP regimen (6 CHOP and 2 R-CHOP), two patients with R-FN, while a patient with a single focal lesion underwent to surgical treatment.
Results: The complete remission rate was 100% (11/11) after frontline therapy, and only one patient relapsed but underwent remission after additional chemotherapy courses; one of these patients, who had HCV-related cirrhosis, died because of hepato-renal syndrome, and another one died because of Acute Myeloid Leukemia.

Table 1.

NAME	CHEMO	ALIVE/ DEAD	DISEASE FREE SURVIVAL	OVERALL SURVIVAL FROM DIAGNOSIS
A.F.	R-CHOP X 6	ALIVE	86	91
C.M.	CEOP X 6	ALIVE	157	161
C.A.	CEOP X 6	ALIVE	200	204
M.A.	CHOP X 6	ALIVE	223	228
M.M.	R-CHOP X 6	ALIVE	120	123
P.F.	R-F X 6	ALIVE	39	44
P.R.	R-FN X 6	ALIVE	36	40
S.M.	CHOP X 6	DEAD	144	167
S.G.	CEOP X 6	ALIVE	188	192
T.L.	R-FN X 6	ALIVE	47	53
T.L.	R-FN X 6	ALIVE	114	119

Summary and Conclusions: Our study confirms the rarity of PHL. In our Division, the outcome of patients with PHL, who are treated with combination chemotherapy, seems excellent. The frequent association of PHL with HCV infection suggests a possible role of this virus in lymphomagenesis. HCV-infection does not appear to influence the outcome.

E1161

ORBITAL AND OCULAR ADNEXAL MALT LYMPHOMAS: A LONG-TERM OUTCOME

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Background: OAML(ocular adnexal mucosa-associated lymphoid tissue type lymphoma) is uncommon but its incidence is constantly increasing. There have been many attempts to identify prognostic factors for OAML. Recently, a new staging system and treatment modalities have been developed.
Aims: We studied the clinical features of OAML and evaluated the effectiveness of radiotherapy as a treatment modality. We also studied prognostic factors correlated with overall and progression-free survival in patients with OAML.
Methods: Ninety-four patients who were diagnosed with OAML at Yeungnam University Hospital between 1995 and 2014 were enrolled. Information on the patient age, tumor location, symptoms, and biochemistry profiles were collected. Computed tomography (CT), magnetic resonance imaging (MRI), positron emission tomography (PET), and bone marrow biopsies were performed in order to establish the stage. Most patients were treated with external beam irradiation (median dose 30 Gy). Treatment response was evaluated by using orbital CT or PET. The progression-free survival was analyzed in patient subgroups classified according to tumor bilaterality, the Ki 67 index, and the TNM-based stage.
Results: The median follow-up duration was 48 months (range 2–220 months). The median progression-free survival was 42 months (range 2–202 months). The median overall survival was 92.5 months (range 3–236 months). Seven patients experienced relapses, and 5 patients died. The 5-year progression-free survival and overall survival rates were 94.7% and 96.8%, respectively. The relapse rate was 7.4%. In the radiotherapy-only group, 6 of 86 patients had recurrences, but they achieved remission after additional treatment. Twelve of the 86 patients in the radiotherapy-only group had remnant lesions, but a remission rate of 92% was achieved even without additional treatment. Cervical lymph node metastasis was identified in 1 of 84 patients who underwent imaging, and bone marrow involvement was observed in 1 of 92 patients who underwent biopsy. Lactate dehydrogenase and β_2-microglobulin levels were not found to be correlated with relapse. Statistical analysis did not identify any correlation between progression-free survival and tumor bilaterality, the Ki 67 index, or the tumor stage.
Summary and Conclusions: Patients with OAML have a good prognosis. Because of the high survival rate and low relapse rate of OAML, it is difficult to identify prognostic factors that influence progression-free survival. Moreover, owing to the rarity of patients with advanced stages of OAML, additional studies are necessary to identify the economic benefits of performing thorough staging procedures. We concluded that radiotherapy is a very effective treatment modality for localized OAML, supporting the findings of previous studies.

E1162

RITUXIMAB WITH OR WITHOUT CHLORAMBUCIL FOR THE TREATMENT OF EXTRANODAL MARGINAL ZONE B-CELL LYMPHOMA: RESULTS OF A MONOCENTRIC STUDY.

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Background: Apart from localized gastric disease, there is no consensus on standard initial treatment of mucosa-associated lymphoid tissue lymphoma.
Aims: This study was launched to evaluate the use of Rituximab (R) alone or Rituximab plus Chlorambucil (RC) in patients not previously given systemic anticancer therapy.
Methods: All patients with histologically confirmed diagnosis of MALT lymphoma were selected from our data base. Only patients treated with R or RC were analyzed and evaluated. The scheme: rituximab 375 mg/sqm weekly for 4 doses, then monthly for 4 infusions alone or in combination with chlorambucil at the dosage of 0,1 mg/Kg/die for 45 days, then on days 1 to 15 monthly for 4 months.
Results: From January 2000 to February 2013, 136 patients with diagnosis of MALT lymphoma were diagnosed and treated in our Institution, 76 of them uniformly treated with R or RC. The median age at diagnosis was 68 years (range 32-85). In 20 patients the disease was localized in conjunctiva; 17 in the stomach; 14 in the lung; 13 in salivary glands; 3 intestine; 2 respectively in lacrimal gland, liver, skin; 1 in breast, cheek and tongue. Stage was IA in 63 patients (83%), IB in 1, IIA in 5 patients and IIB in 1, IIIA in 1 patient, IVA in 4 patients and IVB in 1. Bone marrow biopsy was negative in 59 patients, positive in 5 and not performed in 12 patients. The proliferative index was evaluated in 50 patients with Mib1 monoclonal antibody: 42 patients showed less than 30% of positivity and 8 had more than 30% of positivity. The diagnosis of MALT in 4 patients was associated with Sjogren Syndrome, in 4 patients with a positive HCV and in 1 with sclerodermia. According to treatment, 61 patients were treated with Rituximab plus Chlorambucil; 15 were treated with Rituximab alone.
At the end of treatment 68 patients (89%) obtained a complete remission and 7 (11%) a partial remission with an overall response rate of 100%. With a median observation period of 65 months (range 1-158) the overall survival was 88%. 9 patients died, 6 for disease progression (3 after a relapse) and 3 for causes not related to lymphoma. 12 patients (16%) relapsed, 8 of these patients were retreated with Rituximab and obtained a new complete remission. Both treatments were well tolerated without unexpected toxicities.
Summary and Conclusions: After a long follow-up the combination of Rituximab and Chlorambucil or Rituximab alone proved to be low toxic, feasible and effective therapy for MALT lymphomas.

Infectious diseases, supportive care

E1163

GRANULOCYTE TRANSFUSIONS IN THE TREATMENT OF SEVERE INFECTIONS DURING PROLONGED NEUTROPENIA: SINGLE CENTER EXPERIENCE IN YEARS 2005 - 2014

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Background: Use of granulocyte transfusions (GT) in patients with hematologic malignancy and prolonged neutropenia complicated with severe infection represents a strategy which positive and negative aspects are discussed.
Aims: To evaluate the efficacy and safety of GT applied over the last ten years period (2005-2014).
Methods: A single center retrospective analysis was performed. GT were collected after stimulation with 40mg i.v. Solu-Medrol from unrelated AB0 and Rh compatible donors. HLA compatibility was not evaluated and family donors were not used. Separation was performed at Cobe Spectra - Caridian BCT blood separator. GT were stored at room temperature and given to the patients on the day of collection after irradiation of the packs with 32 Gy using Gammacell 3000 Elan blood irradiator (Cs^{137}). Decisions to give GT in individual patients were made collectively after agreement minimally of 3 physicians (usually patients´ physician, head of the ward and head of Department). Decisions to stop GT treatment were taken according to clinical response to GT and degree of hematologic reconstitution. All patients were pre-medicated with Hydrocortison 100mg i.v. given immediately prior transfusion.
Results: GT were given to 41 patients (15 female and 26 male) at age from 22 to 69 years (median 45.5). There were 26 cases of acute leukemia, 7 patients with myelodysplastic syndrome, 5 patients with chronic lymphocytic leukemia and 3 patients with lymphoma. In most patients disease was active at the time of GT initiation (30; 73.2%), only 11 patients were in hematologic remission. 30 patients were after chemotherapy while 3 patients were after allogeneic hematopoietic stem cell transplantation. Duration of severe neutropenia (less than $0.5x10^9$/l) was from 4 to 80 days (median 19 days). Antibiotics were applied in all patients for 1 to 126 days (median 14 days) before initiation of GT. The main clinical indications were soft-tissue inflammations (26 cases) and pneumonias (16). 14 patients have suffered from proven invasive fungal infections (9 aspergilosis and 5 candidiasis) while coincident Gram-positive or Gram-negative sepsis was present in 9 and 16 cases, respectively, complicated in 12 patients with septic shock. Together 191 GT were given – in a single patient from 1 to 17 GT (median 3.5). GT contained from 0.5 to $3.0x10^{10}$ granulocytes per one transfusion unit (median $1.3x10^{10}$). No side-effects both in donors and patients were recorded. Decrease of fever, complete resolution of infection and clinical improvement after GT was observed in 36 (87.8%), 29 (72.5%) and 34 (82.9%) of patients, respectively. Clinical deterioration occurred in 6 (14.6%) patients (4 – 9.8% – of these were fatalities). In six patients immediately after GT treatment allogeneic stem cell transplantation was performed. We have not observed any significant clinical consequences of GT in these patients after transplantation, one patient transplanted with active disease died early after relapse of acute leukemia while all other patients achieved full donor chimerism with remission of hematologic disorder.
Summary and Conclusions: We can conclude, that in our hands GT harvested from unrelated donors after stimulation with steroids and transfused in selected patients with severe infections during prolonged neutropenia have a high efficacy and safety and enabled not only successful management of infections in most cases but also safe proceeding to allogeneic stem cell transplantation leading to cure in a significant proportion of our patients. Acknowledgement: Grant of IGA LF UP 01-2015.

E1164

INDUCTION PHASE INFECTIONS IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA AND IMPACT OF STEROID DOSE ON THE INCIDENCE OF INFECTIONS: A RANDOMIZED CONTROLLED STUDY

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Background: Infection is the most common cause of treatment related mortality in childhood acute lymphoblastic leukemia (ALL). Patients with ALL are prone to infections especially during induction phase of treatment.
Aims: Aim of the study is to determine incidence of common infections and infectious agents in pediatric patients with ALL during induction phase of treatment, and compare the infection frequency between the groups treated with 10 mg/kg or 20 mg/kg high dose methylprednisolone (MPZ).
Methods: Pediatric patients newly diagnosed with ALL between 2008 and 2014 were enrolled. Patients were treated according to modified St Jude Total XV Protocol, and they were randomized for high dose MPZ treatment, which was given for seven days before induction treatment at a dose of 10 mg/kg or 20 mg/kg orally. A retrospective data review, from electronical database and paper files, was performed. The incidences of diarrhea, pneumonia, neutropenic fever, typhlitis, fungal infections, use of antifungal agents, mucositis, vancomycin resistant enterococci colonization during induction phase were recorded.
Results: A total of 154 patients with newly diagnosed ALL were included in the study. Median age of patients at diagnosis was 5 (1-19) years. Neutropenic fever occurred in 87 (56.5%) of patients (Table 1), 30 of patients had neutropenic fever two or more times. Median duration of neutropenic fever was 2 (1-15) days. Microorganisms isolated in peripheral blood, central venous catheter (CVC), or urine samples in 48 (31.2%) of the patients. In peripheral blood cultures gram positive/negative ratio was 2.9, in CVC cultures this ratio was 3. CVC infection was detected in 42 (27.3%) of patients. Fungal infection was seen in 10 (6.5%) of patients. Infection related mortality occurred in 4 (2.6%) patients at induction, and one patient died due to tumor lysis syndrome at the induction phase. Of the patients, 141 were available for comparison of infection rates according to MPZ dose (78 received 10mg/kg, and 63 received 20 mg/kg MPZ). Only diarrhea frequency was statistically significantly more common in group treated with 20mg/kg dose of MPZ (*p*; 0,02).

Table 1. Infection rates in induction phase

Characteristics	n (%)
Neutropenic fever	87 (56.5%)
Catheter infection	42 (27.2%)
Diarrhea	33 (21.4%)
Mucositis	31 (20.1%)
Pneumonia	26 (16.9%)
Cellulitis, skin infections	14 (9%)
Fungal infection	10 (6.5%)
VRE colonization	13 (8.4%)
Typhilitis	5 (3.2%)

Summary and Conclusions: Our study revealed that 56.5% of patients had experienced neutropenic fever during induction phase. Catheter infections were among the most common reason of infections. Gram positive bacterial infections were seen three times more frequent than gram negatives in both peripheral blood stream and central venous catheters. In our study majority of induction deaths was secondary to infections (4/5). Additionally, the higher doses of MPZ had no impact on the rates of the various infections except for diarrhea and diarrhea was more common among the dose of 20 mg/kg group that might be related to oral application of the drug.

E1165

RELATIVE DOSE INTENSITY OF CHEMOTHERAPY AND PREVENTION OF FEBRILE NEUTROPENIA WITH BIOSIMILAR FILGRASTIM: A MULTICENTRIC OBSERVATIONAL STUDY OF 633 LYMPHOID MALIGNANCY PATIENTS (THE ZOHÉ STUDY)

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Background: Guidelines recommend granulocyte-colony stimulating factor (G-CSF) use to reduce the risk of neutropenic infections in patients receiving chemotherapy (CT) and to enable full-dose delivery. ZOHé is a French prospective non-interventional, longitudinal study describing biosimilar filgrastim (Zarzio®) use in routine practice, in patients receiving CT for solid tumor or lymphoid malignancies (LM).
Aims: To describe patients, Zarzio® treatment patterns, relative dose intensity (RDI) maintenance and outcomes in the LM cohort.
Methods: Patients ≥18 years old receiving treatment for LM were recruited. This analysis reports the impact in real life of EORTC guidelines on G-CSF prophylaxis for chemotherapy-induced febrile neutropenia (CIN/FN). Zarzio® treatment characteristics along with CT in terms of dose, day of initiation, duration and proportion of patients for which CT RDI was conserved (dose maintained in ≥85% of CT cycles) have been recorded. Data were collected at Zarzio® initiation time and 3 months after inclusion.
Results: 633 evaluable patients were recruited from June 2013 to April 2014. 32 patients (5%) received a CT regimen associated with a FN risk ≥20% justifying by itself the G-CSF prophylaxis. However, in patients receiving CT regimen with FN risk of <10%, 10-20% or unknown, the vast majority (586, 97.5 %) had at

least one risk factor for increased incidence of FN such as elderly patients (aged 65 and over) or advanced disease stage. Prophylaxis was initiated as either primary or secondary in 427 (67.5%) and 206 (32.5%) patients respectively. Zarzio® was started on average 6.5±2.2 days (median=6) after CT and given for 5.9±1.7 days (median=5). Dosing was 30MIU/day in 80%, 48MIU/day in 20% of the cases. 16.7% of patients developed at least one Grade 3 or 4 CIN episode of which 4.9% were febrile. CIN/FN-related hospitalizations were experienced by 5.7% of patients. 420 patients were evaluable for RDI (data collection was optional): the majority of patients 85.2% had a preserved RDI over 3 months follow-up (table). 37 non serious adverse events (AE) considered to be related to G-CSF by the investigator were recorded. One AE led to a modification of Zarzio® dose, 7 to Zarzio® treatment discontinuation.

Table 1. Relative dose intensity maintenance

Table: Relative dose intensity maintenance	Patients (n=420)		
	NHL (n=266)	HL (n=56)	Other chronic lymphoproliferative disorders (n=98)
Mean RDI (% ± SD)	94.1 ± 24.5	95.8 ± 25.8	92.4 ± 23.2
Median RDI (%, (Min-Max))	100 (0-266.7)	100 (16.7-200.3)	100 (0-138.4)
RDI conserved (n, (%))			
Yes	230 (86.5)	48 (85.7)	80 (81.6)
No	36 (13.5)	8 (14.3)	18 (18.4)

Summary and Conclusions: In this routine practice French cohort BS filgrastim (Zarzio®) appears effective for RDI maintenance in the vast majority of the patients. According to the EORTC guidelines, Zarzio® was initiated on primary prophylaxis treatment in 67.5% of patients and mostly at day 6 after CT.

E1166

BK VIRUS-HEMORRHAGIC CYSTITIS FOLLOWING ALLOGENEIC STEM CELL TRANSPLANTATION: CLINICAL CHARACTERISTICS AND UTILITY OF LEFLUNOMIDE TREATMENT

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Background: BK virus-hemorrhagic cystitis (BKV-HC) is a potential cause of morbidity and mortality in patients having undergone allogeneic stem cell transplantation (Allo-SCT).
Aims: We analyzed the clinical features of BKV-HC following Allo-SCT and discussed the utility of leflunomode therapy for BKV-HC.
Methods: From January 2005 to June 2014, among the 69 patients underwent Allo-SCT in our institution, the patients who experienced BKV-HC were investigated retrospectively. BK virus was confirmed by a qualitative PCR-based assay. Severity of HC was graded as: grade 0 (no hematuria), grade I (microscopic), grade 2 (macroscopic), grade 3 (macroscopic hematuria with presence of blood clots), and grade 4 (macroscopic hematuria clots and renal impairment due to urinary obstruction). Grade 3 and 4 were defined as high-grade HC. Complete response (CR) was defined as complete improvement in symptoms without hematuria; partial response (PR), downgrading of severity with persistent hematuria; no response (NR), unchanged or worsening urinary symptoms.
Results: HC was observed in 30 patients (43.5%), and among them, 18 patients (26.1%) were identified as BKV-HC. The median patients' age (12 males and 6 females) was 45 years (range, 13-63). The diagnosis of the patients were acute myeloid leukemia (AML, n=11), aplastic anemia (n=4), myelodysplastic syndrome (MDS, n=2), and non-Hodgkin lymphoma (n=1). The donor types were a HLA-matched sibling donor for 6 patients, HLA-matched unrelated donor for 9, and a haploidentical familial donor for 2. Nine patients (52.9%) received myeloablative conditioning regimen. Seven (38.9%) of patients received antithymoglobulin containing preparative regimen for *in vivo* T-cell depletion. The median onset and duration of BKV-HC was on day 21 (range, 7-97) after transplantation and 22 days (range, 6-107). There was no significant difference between the onset in patients with low-grade HC or with high-grade HC (21 days *vs* 21 days, p=0.633). Half of the patients had an acute graft-versus-host-disease (GVHD). Among them, seven (77.7%) patients developed an acute GVHD after onset of BKV-HC and, conversely, 2 (22.3%) patients developed a BKV-HC after the diagnosis of acute GVHD. Nine (50.0%) patients had concomitant cytomegalovirus viremia. Eleven patients (62.1%) had grade I-II HC and 7 patients (38.9%) had high-grade HC. Majority of the patients received supportive treatment, including intravenous hydration and/or blood transfusion. Urinary urethra catheter was inserted in 3 patients with grade 3 HC and 2 patients with grade 4 HC. After supportive care, treatment results showed that 9 patients (50.0%) achieved CR, 4 patients (22.2%) PR, and 5 patients (27.8%) NR. Out of the 7 patients who had high-grade HC, 1 had CR, 1 PR, and 5 NR, respectively. Among the 5 unresponders, one died of BKV-HC associated renal failure. The remaining 4 patients (3 AML and 1 high-risk MDS) were treated with leflunomide and tolerated well, with 3 patients having mild gastrointestinal symptoms, including anorexia and abdominal bloating. After leflunomide treatment, 2 (50%) patients achieved CR and 2 (50%) achieved PR. The median duration from the start of leflunomide therapy to response was 13 days (range, 8–17). There was no recurrence of hematuria in 2 patients achieving CR after discontinuation of leflunomide therapy.
Summary and Conclusions: BKV-HC was commonly observed in patients with HC following Allo-SCT. In high-grade BKV-HC patients who fail supportive care, leflunomide may be a feasible option without significant toxicity.

E1167

A COMPARISON OF ORAL LEVOFLOXACIN AND CEFEPIME IN PATIENTS WITH LOW-RISK FEBRILE NEUTROPENIA BY THE JAPAN FEBRILE NEUTROPENIA STUDY GROUP

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Background: Febrile neutropenia(FN) is a known severe adverse event in cancer patients undergoing chemotherapy. However, It is known that there is a group of patients with FN who falls into low risk for developing severe complications.
Aims: To determine whether low risk FN can be managed with oral antimicrobials, we conducted prospective study to evaluate oral use of levofloxacin (LVFX) .
Methods: We conducted a multicenter prospective study on cancer patients with low risk FN. Eleven institutions participated in the study which was approved by the institutional review board of each institution. Low risk was determined based on the Multinational Association of Supportive Care in Cancer (MASCC) score. After chemotherapy, only those patients with a neutrophil count of less than 500/μL, or less than 1,000/μL but expected to fall below 500/μL, and a recorded body temperature of over 38.0°C once or 37.5°C persistently for 1 hour were enrolled in the study. It consists of two components, *i.e.* either non-randomized treatment with LVFX or randomized treatment in which patients received either oral LVFX or intravenous cefepime (CFPM), a broad-spectrum cephalosporine. Primary endpoint is treatment success at 7th day; success is defined as defervescence and improvement in clinical symptoms and laboratory results. Secondary endpoints are safety profiles for 7-day study period and survival at 30th day.
Results: A total of 65 patients were enrolled. The results were analyzed as per protocol parameters. In the CFPM group (n=24), treatment success at 7th day was 66.7% (95%CI, 44.68-84.67), while In the LVFX group (n=41), it was 53.7% (95%CI, 37.42-69.34). The difference was not statistically significant (p=0.304). Serious adverse events were not observed in either group. All patients were alive at 30th day.
Summary and Conclusions: The study did not show a significant difference in effect or safety profiles between LVFX and CFPM in low risk FN, although the number of patients was too small. However, since all patients in the LVFX group survived at 30th day with no sequelae, oral use of LVFX could be considered a treatment option in low risk patients.

E1168

LONGITUDINAL RISK OF HERPES ZOSTER IN PATIENTS WITH NON-HODGKIN LYMPHOMA RECEIVING CHEMOTHERAPY: A NATIONWIDE POPULATION-BASED STUDY

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Background: The outcome of patients with non-Hodgkin lymphoma (NHL) was improved in recent decades, the treatment related adverse events on the quality of life may be emerging as an issue.
Aims: This study aims to evaluate the incidence rate and risk factors of herpes zoster in patients with NHL receiving anti-lymphoma treatment.
Methods: This study was conducted using the National Health Insurance Research Database (NHIRD) of Taiwan. The study cohort included patients with NHL between 2002 and 2008. Propensity score matching was performed to correct for sample selection bias. Logistic regression analyses were performed to obtain odds ratios (ORs) and 95% confidence intervals (95% CIs) for the risk evaluations.

Results: From 2002 to 2008, there were 3865 NHL patients with median age of 55 years who received CHOP/CEOP or R-CHOP/R-CEOP as front-line treatments; 2188 of these patients received CHOP/CEOP, and 1677 patients received R-CHOP/R-CEOP. The overall incidence rate of herpes zoster was 12.21% (472/3865); 11.79% (258/2188) of the patients received conventional chemotherapy and 12.76% (214/1677) of the patients received rituximab-containing chemotherapy. For the patients who received conventional chemotherapy, the risk factors included female gender, multiple courses of chemotherapy and autologous hematopoietic stem cell transplantation. For the patients who received rituximab-containing chemotherapy, the risk factors included female gender, diabetes mellitus, multiple courses of chemotherapy, autologous hematopoietic stem cell transplantation and higher accumulated rituximab dose. The analysis of the cumulative incidence rate revealed that the majority of the herpes zoster episodes occurred within the first two years after the diagnosis of NHL. After adjusting for the propensity score matching, rituximab-containing chemotherapy was not associated with a higher overall incidence rate of herpes zoster (p: 0.155). However, the addition of rituximab to conventional chemotherapy increased the short-term risk of herpes zoster with adjusted ORs of 1.38 (95% CI=1.05-1.81, p:0.021) and 1.37 (95% CI=1.08-1.73, p:0.010) during the 1-year and 2-year follow-up periods, respectively.
Summary and Conclusions: This nationwide population-based study evaluated the incidence rate and risk factors of herpes zoster in NHL patients who were receiving anti-lymphoma therapy. The incorporation of rituximab into conventional chemotherapy increased the short-term risk of herpes zoster.

E1169

MACROPHAGES FROM SEPSIS SURVIVING MICE PRESENT DIFFERENTIAL GENE EXPRESSION ASSOCIATED WITH AN IMMUNOTOLERANT PROFILE

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Background: Sepsis survivors are at a higher risk to present abnormalities of the immune system and die of secondary infections caused by low virulence pathogens. Such immunosuppressive state may favor tumorigenesis and experimental data obtained in murine models showed that sepsis led to expansion of regulatory T cells (Tregs) and macrophage polarization towards M2-like phenotype (characterized by high IL-10 production accompanied by low production of IL-12 and associated with suppression of T cell responses).
Aims: To report how sepsis affects the gene expression profile of macrophages.
Methods: C57Bl/6 mice were anesthetized and submitted to cecal and ligation puncture to induce severe sepsis. Naïve mice were used as controls. All animals received ertapenem 20 mg/kg ip. 6 h after surgery and bid for 3 days. Survivors (50%) were killed for bone marrow harvesting at day 15. Macrophages were differentiated using sarcoma L929 supernatant and adherent cells were collected after 7 days. M1 and M2-macrophage were used as controls and polarized through IFN-γ (50 ng/mL) plus LPS (10 ng/mL) and IL-10 (20 ng/mL), respectively. Naïve mice were inoculated with bone marrow-derived macrophage (BMDM) (10,000 cells) plus B16 melanoma (30,000 cells). Tumor growth and survival were assessed. RNA from isolated BMDM was isolated by columns and the cDNA was labeled and put in microarray slides. Data was analyzed using R (version 3.1.2, 2014-10-3) and Bioconductor (version 2.14). Differential gene expression was considered significant with a fold change of 20 for up and -2.0 for down-regulated genes and p <0.01. Pathway analyses were made through Nextbio. Data were deposited at GEO database (code GSE64498).
Results: Tumor growth was increased and overall survival was reduced in the group inoculated with BMDM from post-sepsis mice, compared with those who received BMDM obtained from naïve mice. In the microarray analysis, a set of 64 genes were up-regulated and 94 genes were found to be down-regulated. Genes related to innate immunity (*e.g.* Ccl5, Cxcl16, C3, Cfb, Ccr5 Tlr7 and Ccl8) and interferon alpha/beta signaling (*e.g.* Oasl1, Ifit3, Ifit2, Irf7 and Ifit1) were in general down-regulated. Also, several genes that encode chemotactic mediators were up-regulated (*e.g.* Ccl2, Ccl7, Ccl12) in BMDM from post-sepsis mice.
Summary and Conclusions: BMDM obtained from sepsis surviving mice present differential gene expression associated with an immunotolerant profile.

E1170

VIRAL RESPIRATORY TRACT INFECTIONS IN CHILDREN WITH MALIGNANCY: ISTANBUL PERSPECTIVE

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Background: Viral infections can cause a variety of clinical pictures in children on immunosuppression. RSV is the most commonly encountered viral respiratory pathogen in oncology patients, with parainfluenza virus (PIV) types 1, 3, 2, rhinovirus, influenza A and B following in order of decreasing frequency.
Aims: To evaluate the viral respiratory tract infections in children with malignancy
Methods: In this study, nasopharyngeal specimens were obtained from chidren in our Hematology-Oncology clinic who had such respiratory symptoms as fever, rhinorrhea, sneezing, cough or findings consistend with pneumonia on auscultation or imaging and also from patients who shared a room with them. Appropriate antiviral therapy was instituted after evaluation of the samples, namely, ribavirin po for 14 days for those positive for RSV and their roommates, and oseltamivir po for 5 days for those positive for influenza and their roommates.
Results: Between October 1st 2013 and February 15th 2015, a variety of viral respiratory pathogens were identified on specimens obtained on 32 occasions from 26 patients who were admitted due to a variety of malignancies, namely, acute lymphoblastic leukemia, acute myeloblastic leukemia and lymphomas. The alarming symptom was cough in 19 episodes (59.3%), fever in 4 episodes (12.5%), fever and cough in 9 episodes (%28.2). Pneumonia was diagnosed through pysical examination and imaging on 8 episodes (%24.2). 14 patients received ribavirin po, 6 patients received oseltamivir po and 15 of these patients also received intravenous immunoglobulin due to an IgG level below 500 mg/dL. Most of our patients had mixed infections where 2,3 or 4 pathogens were identified (RSV +PIV 2-3, hMPV +ADV, RSV +PIV4, RSV +rhinovirus, RSV +HBovCor229 +CorHKU, Corona63 +Corona43 +CoronaHKU +rhinovirus, ADV +rhinovirus, rhinovirus +Corona 63, RSV +rhinovirus +HBovCorona229 +ADV +hMPV, coronavirus +influenza A). 11 of the patients had rhinovirus, with 4 having rhinovirus only and 7 having rhinovirus and other viruses), 9 had coronavirus (3 had coronavirus 43, 1 had coronavirus 229, 2 had coronavirus 63, 2 had coronaHKU), 10 patients had RSV, with 4 having it in isolation and 6 having it as part of a mixed infection. 6 patients had parainfluenza viruses, with 4 having PIV only and 2 having a mixed infection. 2 patients each had human bocavirus, ADV and hMPV. 5 patients had influenza A (4 were H1N1 and 1 was H3N2), one patient had influenza B. Six of our patients got infected with different respiratory viruses at different times (Table). Eight patiens were given antivirals despite their lack of symptoms as prophylaxis because they shared a room with an infected patient. One patient who had familial hemophagocytic lymphohistiocytosis was younger than 6 months and therefore could not receive ribavirin. One patient with ALL was positive for CoronaHKU and H1N1, and received IVIG and oseltamivir and had to be transfered to the intensive care unit due to respiratory distress, hypotension and shock.

Table 1.

TABLE: Viral agent, clinic presentation and treatment

Patient (n,%)	Viral Agent	Syptom	Clinic presentation	Treatment
4 (12,5%)	RSV	Fever	URTI, pneuomonia	Ribavirin+IVIG (if IgG<500 mg/dl)
3 (9,3%)	INF A	Cough	URTI	Oseltavimir+IVIG(if IgG<500 mg/dl)
4 (12,5%)	RHINO	Cough	URTI	Supportive
1 (3,1%)	INF B	Cough	URTI	Supportive
4 (12,5%)	PARAINF V.	Cough, fever	URTI	Supportive
2 (6,2%)	METAPNEUMO V+ OTHERS	Cough	URTI	IVIG (if IgG<500 mg/dl)
4 (12,5%)	RSV+RHINO	Cough, fever	URTI	Ribavirin IVIG(if IgG<500 mg/dl
1 (3,1%)	H1N1+OTHER	Cough, fever	URTI, pneuomonia	Oseltavimir+IVIG(if IgG<500 mg/dl)
3 (9,3%)	RSV+OTHER	Cough	URTI, pneuomonia	Ribavirin+IVIG (if IgG<500 mg/dl)
3 (9,3%)	CORONA VIRUSES	Cough	URTI	Supportive
3 (9,3%)	RHINO+OTHER	Cough	URTI	Supportive

RSV:Respiratory syncytial virüs H1N1: İnfluenza A virüs Rhino:Rhino virüs İnf B:İnfluenza B virüs, Parainf: Para influenza virüs Metapneumo V+Others: Metapneumo virus, Adeno virüs, Corona virüs 229 İnfluenza A+Others: İnfluenza A, Corona HKU RSV+Others: RSV, Parainfluenza virüs 2,3,4, Human Boca Virus, Rhino virus Corona viruses: Corona 63, Corona HKU, Corona OC43 Rhino+Other: Rhinovirus, Corona virüs 63, URTI:Upper respiratory tract infection

Summary and Conclusions: Viral infections of the respiratory tract can kill immunocompromized children. Early diagnosis, close monitoring and treatment with antivirals and IVIG for those infected with influenza A and RSV, and meticulous supportive care for those infected with other viruses are needed to prevent mortality.

E1171

NEUTROPHIL CD64 INDEX AS A BIOMARKER OF EARLY SEPSIS IN MALIGNANT HEMATOLOGIC DISEASE

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Background: Malignant hematologic disease with sepsis has been character-

ized by high mortality and difficulties in diagnosis at early stage. A good biomarker may help us to improve the accuracy of diagnosis and to reduce the mortality rate.
Aims: The aim of this study was to investigate the usefulness of neutrophil CD64 expression in blood and procalcitonin (PCT) in the early diagnosis of sepsis in malignant hematologic disease.
Methods: Ninety-seven hematological malignancies were enrolled in the study. Sixty-three hematological malignancies were classified into three groups: no infection (n=35), local infection (n=20) and sepsis (n=43). Another 20 healthy people were included as a control group. Blood samples were collected within 24 hours after admission for neutrophilic CD64 index and neutrophilic CD163 index. Procalcitonin (PCT), high-sensitivity C-reactive protein (hs-CRP) were also determined in all groups.
Results: CD64 index and CD163 index did not differ between control group and no infection group (P>0.05); CD64 index were significantly increased in the groups with local infection and sepsis compared to the group without infection (P<0.0001); CD64 index were also significantly increased in the group with sepsis compared to the group with local infection (P<0.0001); The sensitivity, specificity, overall area under the receiver operating characteristic curve of CD64 index, CD163 index, PCT and hs-CRP for the diagnosis of early sepsis were determined. CD64 index had the highest sensitivity of 68.9%, specificity of 85% and overall area of 0.739 using a cut-off of > or =2.195. The cut-off value, highest sensitivity, specificity and overall area were 0.13ng/ml, 81.4%, 60% and 0.732 for PCT, 30.15mg/L, 79.1%, 60% and 0.716 for hs-CRP, and 448.895, 88.4%, 30% and 0.535 for CD163 index.
Summary and Conclusions: We conclude that neutrophil CD64 index can be incorporated with specific hematologic criteria as an additional marker for diagnosis of early sepsis in hematological malignancies.

E1172

FIVE CASES OF PNEUMOCYSTIS PNEUMONIA (PCP) INFECTION IN PATIENTS FOLLOWING TREATMENT WITH BENDAMUSTINE/RITUXIMAB: PROPHYLAXIS SHOULD BE CONSIDERED AS STANDARD PRACTICE

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Background: Pneumocystis pneumonia (PCP) is a life threatening infection associated with immunocompromised patients. It was initially described in HIV patients but is also well described following certain chemotherapy agents. It has a high morality rate, particularly in non-HIV patients (35 – 50%)[1]. PCP prophylaxis has been shown to effectively prevent PCP in at risk patients. Bendamustine is an alkylating chemotherapy agent, which is being increasingly used in clinical practice, particularly in treatment of lymphoproliferative disorders and myeloma. It is known to cause grade 3-4 lymphopenia in 74% of patients[2]. At present there is no standard recommendation for PCP prophylaxis with bendamustine treatment.
Aims: Identify all cases of PCP following bendamustine treatment in our unit and assess factors which increase the risk of infection.
Methods: Patients with a confirmed diagnosis of PCP who had received bendamustine were identified. Their case notes and investigations were reviewed.
Results: Seventy-two patients received bendamustine therapy between November 2009-February 2015. We identified five cases of PCP in our institution in patients undergoing treatment with bendamustine / rituximab (6.9%). Four cases of PCP occurred prior to routine introduction of PCP prophylaxis (August 2012) and 1 case occurred subsequently in a patient whose prophylaxis had been stopped. The patients infected consisted of 3 males and 2 females (average age of 69.6 years). Two patients received treatment for low grade Non Hodgkin Lymphoma (NHL), 2 for Chronic Lymphocytic Leukmaemia (CLL) and 1 patient received treatment for Waldenstrom's Macroglobulinemia (WM). None of our patients were on PCP prophylaxis at the time of infection. All infections were clinically and radiologically suggestive of PCP and 4 were confirmed using quantitative PCR on sputum samples. Four patients were undergoing treatment with bendamustine / rituximab at the time of infection (ranging from cycle 1-3 of treatment). One patient completed treatment twelve months earlier. All patients were lymphopenic (<0.5) at time of infection (average 0.18), 2 patients had a sustained lymphopenia (>2 weeks) prior to infection, and 4 had evidence of hypogammaglobulinemia prior to infection (average IgG 3.48, IgA 1.49, IgM 2.82).
Summary and Conclusions: PCP infection is common following bendamustine /rituximab treatment. It can occur during the first course of treatment and it occured up to twelve months after treatment in our patient cohort. Patients with severe lymphopenia (<0.5) and hypogammaglulinemia appear to be at particularily high risk of PCP infection. We recommend that all patients who receive bendamustine / rituximab treatment should be considered for PCP prophylaxis from the start of treatment and it then should be continued until adequate lymphocyte recovery occurs.

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E1173

SEVUPARIN DEMONSTRATES ANTI-ADHESIVE EFFECTS IN MALARIA PATIENTS SUGGESTING ITS POTENTIAL CLINICAL BENEFITS IN SEVERE MALARIA AND SICKLE CELL DISEASE

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Background: Microvascular obstruction is one of the main pathological events leading to the development of severe falciparum malaria and pain during vaso-occlusive crises (VOC) in sickle cell disease (SCD) patients. Excessive sequestration of *falciparum* infected red blood cells (IRBC) in malaria, and of sickle blood cells (SSRBC) in SCD are typical features of severe states of these diseases. The *falciparum* parasite and the SSRBC employ heparan sulfate (HS) during the process of adherence to the endothelium and to other blood cells causing obstructions of the blood flow. Inhibition of these abnormal cell and pathogen interactions with sevuparin working as a decoy receptor could restore a hampered blood flow and stop the parasite growth. Heparin and low molecular weight heparins (LMWHs) have demonstrated clinical benefits in the treatment of severe malaria (Munir M *et al.*, Heparin in the treatment of cerebral malaria. Paediatr Indones. 20, 47-50,1980) as well as of VOC in SCD (Qari MH *et al.*, Reduction of painful vaso-occlusive crisis of sickle cell anaemia by tinzaparin in a double-blind randomized trial. Thromb Haemostat 98: 392-396, 2007); however, the associated risk of bleeding limits their clinical use. Therefore, sevuparin was designed to maintain the anti-adhesive properties of heparins while minimizing the anti-thrombin-mediated anticoagulant activity. A 5-fold lower anticoagulant potency is found in sevuparin in comparison with LMWH. A double blind, randomized Phase I study in healthy volunteers has previously been completed.
Aims: A Phase I/II clinical study with sevuparin in patients with uncomplicated *falciparum* malaria was conducted with the aim to demonstrate sevuparins anti-adhesive effects.
Methods: The Phase I/II study consisted of an open labelled dose escalation phase followed by an open labelled, randomized phase. The study was designed to determine the tolerability and *ex vivo* efficacy of sevuparin when administered as an iv injection as adjunctive therapy to atovaquone/proguanil (Malanil®) in subjects affected with uncomplicated *Plasmodium falciparum* malaria, as compared to Malanil® alone (control). Subjects were treated with 4 daily intravenous (iv) injections of sevuparin during three consecutive days (12 doses).
Results: Sevuparin was found to be safe and well tolerated in patients with *falciparum* malaria when given by iv injections as adjunct therapy with Malarone®. Exploratory analysis revealed a potentially clinically meaningful anti-adhesive effect since 1) mature IRBC previously being sequestered, appeared in the circulating blood of sevuparin treated patients as compared to controls, one hour after sevuparin injection (p=0.031) and 2) the number of ring-stage IRBC was significantly decreased already one hour after the first injection of sevuparin while the parasites continued to expand in control patients not given sevuparin (p=0.022), suggests that the binding of malaria parasite (merozoite) to RBC for invasion was inhibited. These effects are consistent with previous *in vitro* results with sevuparin in both malaria and SCD related experiments. Sevuparin has been found to block IRBC and SSRBC binding to endothelium, and also to reverse already established blockade of the blood flow during malaria sequestration and during vaso-occlusion related to SCD in animals *in vivo*. In addition, sevuparin blocks the malaria merozoite invasion into RBC *in vitro*.
Summary and Conclusions: In the Phase I/II study in patients with uncomplicated *falciparum* malaria, sevuparin reverses the adhesion of blood cells and pathogens to endothelium and other blood cells. These anti-adhesive effects of sevuparin have the potential to normalize blood flow in indications like severe malaria and VOC in SCD, which could impact the overall outcome of these patients. Potentially sevuparin might have impact on other indications were reversing and preventing vascular obstruction can improve the disease status and progress.

E1174

EFFECTIVENESS OF ANKAFERD BLOOD STOPPER IN PROPHYLAXIS AND TREATMENT OF ORAL MUCOSITIS SEEN IN CHILDHOOD CANCERS AND CORRELATION WITH PLASMA CITRULLINE LEVELS

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Background: In last 3 decades, success rate has been markedly increased in childhood cancers; however, chemotherapy-related adverse events remain to be an important issue. Oral mucositis, one of the toxic effects of chemotherapy, is observed in 52-80% of the children receiving cancer therapy. Ankaferd Blood Stopper (ABS) is an herbal product that is used as a hemostatic agent. In previous studies, it was shown that ABS has anti-microbial, anti-inflammatory effects as well as positive effects on healing of tissue injury.

Aims: In our study, it was aimed to investigate effectiveness of ABS in prophylaxis and treatment of oral mucositis in patients receiving chemotherapy at childhood. In addition, plasma levels of citrulline, a biochemical marker for mucosal barrier injury, were measured and effectiveness of ABS therapy in mucositis was correlated by quantitative data in addition to clinical assessment.

Methods: This was a randomized, controlled and open study which included 27 patients aged 4-17 years receiving chemotherapy regimens with strong mucotoxic effect. The patients were asked to perform standard oral care (SOC) upon first day of chemotherapy for 10 days and oral mucosa was assessed daily upon completion of chemotherapy based on World Health Organization scale for oral mucositis. In addition, blood samples were drawn immediately before initiation of chemotherapy and at the period where mucositis became most intensive. Same patients receiving same chemotherapeutic agents in the second course of chemotherapy were asked to gurgle by using ABS four times daily in addition to SOC. Mucosa ratings were performed before second chemotherapy course and at the period where mucositis became most intensive, and blood samples were drawn to measure citrulline levels in second chemotherapy course.

Results: The study included 27 patients (mean age: 9.1±4.4 years; 15 boys [56%]). Stages of oral mucositis were found to be significantly lower in the second chemotherapy course given SOC plus ABS when compared to first chemotherapy course given SOC alone (p=0.007). Mean plasma citrulline level obtained before and after chemotherapy decreased from 44.08±11.20 to 23.99±12.16 nmol/mL in chemotherapy course given SOC alone (p<0.001) while it decreased from 38.67±11.46 to 26.78±11.99 nmol/mL (p<0.001). When extent of decrease in plasma citrulline level was assessed, it was greater in courses given SOC alone compared to those given SOC plus ABS (p=0.009).

Summary and Conclusions: ABS can be considered to be an approach with potential benefits, although its effectiveness hasn't been proven in the prophylaxis and treatment of oral mucositis. Based on our results, ABS exhibited beneficial effects in the prophylaxis and treatment of oral mucositis. However, multi-center experiences and further studies with larger sample size are needed for introduction of ABS into primary oral care and treatment protocols of oral mucositis.

E1175

SAFETY OF BIOSIMILAR FILGRASTIM IN PATIENTS WITH LYMPHOMA AND MYELOMA UNDERGOING NEUTROPENIA-INDUCING CHEMOTHERAPY: A SUBANALYSIS OF THE NEXT STUDY

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Background: Febrile neutropenia (FN) is a major risk factor for infection-related morbidity/mortality as well as a dose-limiting toxicity in patients (pts) undergoing chemotherapy (CT). Biosimilar filgrastim (Nivestim™, Hospira Inc.) is a granulocyte-colony stimulating factor (G-CSF) licensed for the treatment of neutropenia and FN induced by myelosuppressive CT.

Aims: NEXT (Nivestim™safety profile in patiEnts treated with cytotoXic CT in real-life clinical pracTice) assessed the safety of biosimilar filgrastim in pts undergoing CT for malignancies.

Methods: NEXT was a prospective, non-interventional, longitudinal, multicenter study conducted in France to evaluate the safety of biosimilar filgrastim. Recorded data included demographic and clinical characteristics; treatment-related data on efficacy and safety, such as adverse events (AEs) and FN. Pts were monitored for 1–6 CT cycles with three visits at inclusion, during treatment, and following CT. Here we present data for pts with lymphoma and myeloma.

Results: Of the total pts analyzed, 408 had lymphoma (mean age±standard deviation (SD): 63.3±16.3 years [62.0% male]) and 47 had myeloma (mean age±SD: 71.2±8.8 years [66.0% male]). At inclusion, the majority of pts had no prior FN (lymphoma: 89.2%; myeloma: 76.6%); 27.5% of lymphoma pts and 59.6% of myeloma pts had prior CT, while 26.7% and 38.3% of lymphoma and myeloma pts, had prior G-CSF therapy, respectively. Almost all pts received biosimilar filgrastim prophylactically (lymphoma: 98.8%; myeloma: 97.9%). In the group treated with curative intent, the median time to initiation of biosimilar filgrastim therapy was 14.0 days after the start of the last CT cycle for both lymphoma and myeloma pts; mean treatment duration±SD was 5.2±1.5 days and 3.0±0.0 days for lymphoma and myeloma pts, respectively. In this group, 40.0% of lymphoma pts and 100.0% of myeloma pts received a dose of 30 MIU; all pts received subcutaneous (SC) administration of biosimilar filgrastim. In the prophylactic group, the median time to initiation of biosimilar filgrastim was 6 days and 6.5 days after the last CT dose for lymphoma and myeloma pts, respectively; mean treatment duration±SD was 6.6±4.0 days and 5.9±4.5 days for lymphoma and myeloma pts, respectively. In this group, 72.5% of lymphoma pts and 78.3% of myeloma pts received a dose of 30 MIU; all pts received biosimilar filgrastim by SC administration. Anti-infective prophylaxis was reported in 42.9% of lymphoma pts and 67.4% of myeloma pts. In the prophylactic group, 7.1% (95% confidence interval [CI] 4.9, 10.1) of lymphoma pts and 10.9% (95% CI 4.3, 23.5) of myeloma pts experienced FN. More overall pts with lymphoma experienced an AE than those with myeloma (21.5% *vs* 8.5%). The most common AEs (>5.0% of pts) were bone/muscular disorders (lymphoma: 16.0%; myeloma: 4.3%) and muscle pain (lymphoma: 15.5%; myeloma: 4.3%). In this analysis, 7.1% of lymphoma pts and 10.9% of myeloma pts prescribed prophylactic treatment were hospitalized for FN and/or infection. The mean duration of hospitalization±SD for FN and/or infection was 11.4±17.4 days for lymphoma pts (data not available for myeloma pts), <3% of pts had a CT dose reduction (lymphoma: 2.8%; myeloma: 2.3%) and <12% of prophylactic pts (lymphoma: 5.9%; myeloma: 11.4%) had a delay in administration of CT due to FN and/or infection.

Summary and Conclusions: Biosimilar filgrastim was effective and well-tolerated in pts undergoing CT for hematological malignancies and is an alternative therapeutic option for pts with CT-induced neutropenia.

E1176

THE EFFICACY OF SERUM GALACTOMANNAN ANTIGEN TEST AS SURVEILLANCE TOOL OF IN-VASIVE PULMONARY ASPERGILLOSIS DURING ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION WITH PRIMARY ANTIFUNGAL PROPHYLAXIS

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Background: A serum galactomannan antigen (GM Ag) test has been used as routine surveillance tool of invasive pulmonary aspergillosis (IPA) for hematology patients who underwent allogeneic stem cell transplantation (alloSCT). In an era of effective primary antifungal prophylaxis (PAP), the routine surveillance of IPA with GM Ag test during alloSCT for hematologic malignancy need to be re-evaluated.

Aims: The purpose of this study was to evaluate the efficacy of GM Ag test as a routine surveillance tool for early detection of IPA in hematology patients receiving alloSCT and PAP, especially with micafungin.

Methods: From 2013 to 2014, medical records of 111 patients who received alloSCT for hematologic malignancy were analyzed. As an institutional protocol, all patients underwent routine surveillance with GM Ag tests twice a week during alloSCT, and received PAP with fluconazole or micafungin, if applicable. The diagnoses with IPA were classified according to the European Organization for Research on Treatment of Cancer/Mycoses Study Group (EORTC/MSG) criteria. GM Ag tests with one of respiratory symptom/sign or fever at the same time, were considered to have high suspicion of IPA, otherwise GM Ag tests without any symptom/sign/fever were considered to have low suspicion of IPA. Patient informed consent was waived because of the retrospective design of the study.

Results: For 111 patients, a total of 681 GM Ag tests were performed (median, 4 per patient [range 1-21]). The incidences of breakthrough IPA were as follows: 583(85.8%) no IPA, 72(10.6%) possible IPA, 21(3.1%) probable IPA, and 4(0.6%) proven IPA. The optimal cutoff index of GM Ag for diagnosis of probable/proven IPA was found to be 0.8. Probable/proven IPA were diagnosed in 6(1.0%) tests with GM Ag index <0.8, and in 19(36.5%) tests with GM Ag index ≥0.8 (P<0.001). Sensitivity and specificity were 76.0% and 95.0%, respectively. Of a total of 111 patients, PAPs with fluconazole and micafungin were performed in 79(64.9%) and 28(25.1%) patients, respectively. The cumulative incidences of probable/proven IPA at 3 months after completion of alloSCT were 1.5% in PAP with micafungin, and 4.0% in PAP with fluconazole (HR 0.18, 95% confidence interval 0.02-1.33, P=0.093, Log rank P=0.06). In patients receiving PAP with fluconazole, 423 GM Ag tests were performed. Among them, 204(48.2%) tests had low suspicion of IPA, and positive predictive value (PPV) was 33.3%. The performance of GM Ag tests improved when there is high suspicion of IPA, with PPV of 50.0%. In patients receiving micafungin prophylaxis, 136 GM Ag were tested. 57(41.9%) GM Ag tests had low suspicion on IPA. They all had GM Ag index <0.8, and no probable/proven IPA was diagnosed (NPV 100.0%, PPV not applicable). 79(58.1%) GM Ag tests had high suspicion of IPA, in which 72(91.1%) had GM Ag index <0.8 with NPV of 100%. Of the rest 7(8.9%) tests with GM Ag index ≥0.8, 2(2.5%) tests were proven to be probable/proven IPA with PPV of 28.6%, and 5 (71.4%) tests were false positive.

Table 1.

Groups	% (95% CI)
All GM Ag tests (N=681)	
Sensitiviy	76.0 (54.9 - 90.6)
Specificity	95.0 (93.0 - 96.5)
PAP with fluconazole (N=423)	
with low suspicion of IPA (N=204)	
PPV	33.3 (16.5-54.0)
NPV	99.5 (97.1-100.0)
with high suspicion of IPA (N=219)	
PPV	50.0 (18.7-81.3)
NPV	99.0 (96.3-99.9)
PAP with micafungin (N=136)	
with low suspicion of IPA (N=57)	
PPV	not applicable
NPV	100.0 (not applicable)
with high suspicion of IPA (N=79)	
PPV	28.6 (3.7-71.0)
NPV	100.0 (95.0-100.0)

Summary and Conclusions: In comparison to PAP with fluconazole, patients who received PAP with micafungin during alloSCT have lower incidence of IPA. Most of GM Ag tests in PAP with micafungin were negative or false positive, which renders routine surveillance of IPA by serum GM Ag tests less useful.

E1177

BIOSIMILAR FILGRASTIM IN PATIENTS UNDERGOING NEUTROPENIA-INDUCING CHEMOTHERAPY: OVERALL RESULTS FROM THE NEXT OBSERVATIONAL STUDY

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Background: Febrile neutropenia (FN) is a frequent and potentially serious complication of cytotoxic chemotherapy (CT). Biosimilar filgrastim (Nivestim™, Hospira Inc.) is a granulocyte-colony stimulating factor (G-CSF) approved in France for the treatment of neutropenia and FN resulting from myelosuppressive CT. Previous clinical trials have demonstrated similar efficacy and safety of biosimilar filgrastim to the reference product (Neupogen®, Amgen Inc).
Aims: NEXT (Nivestim™safety profile in patiEnts treated with cytotoXic CT in real-life clinical pracTice) evaluated the safety of biosimilar filgrastim in pts undergoing CT for malignancies.
Methods: NEXT was a prospective, non-interventional, longitudinal, multicenter study conducted in France to assess the safety of biosimilar filgrastim. Recorded data included demographic and clinical characteristics as well as treatment-related data on efficacy and safety, including adverse events (AEs) and FN. Pts were monitored for 1–6 CT cycles with three visits at inclusion, during treatment, and following CT. Here we present the overall study results.
Results: Of 2114 pts enrolled in the study, 2102 pts were included in the analysis (mean age±standard deviation [SD]: 63.5±12.7 years; 50.2% male). More patients had solid tumors (75.0%) than hematological malignancies (25.0%). At baseline, 92.5% of pts had no prior FN; 34.4% had previous CT; 20.2% had received previous G-CSF therapy. The majority (98.2%) of pts received prophylactic biosimilar filgrastim (primary prophylaxis: 91.0%; secondary prophylaxis: 7.3%). Of the pts receiving prophylactic biosimilar filgrastim, 79.9% received a dose of 30 MIU and therapy was administered subcutaneously in 99.4% of these pts. In the prophylactic group, median time to initiation of biosimilar filgrastim was 2 days after the last CT dose; mean treatment duration±SD was 6.0±3.8 days. Anti-infective prophylaxis was reported in 14.5% of these pts. The incidence of FN in this group was 4.9%, occurring a median time (range) of 14.0 (-13.0–155.0) days following the first CT cycle. Infections were reported in 3.1% of prophylactic pts, occurring a median time (range) of 23.5 (0.0–155.0) days following the first CT cycle. 4.9% of prophylactic patients were hospitalized for FN and/or infection; occurring 5.5 (0.0–64.0) median (range) days after initiating biosimilar filgrastim. 3.6% of pts were hospitalized for FN and/or infection after the first CT cycle, with ≤1% patients hospitalized for FN after each subsequent cycle. Reductions in CT dose due to FN and/or infection occurred in 4.7% of patients receiving prophylaxis. During the study 20.4% of pts had ≥1 AE; 12.7% of pts reported muscle and/or bone disorders and muscle pain (12.1%). The other most common AEs included gastrointestinal disorders (5.5%), nausea (3.0%), general disorders and administration site disorders (2.4%), diarrhea (2.3%) and headache (1.8%). Of the 205 physicians who completed the questionnaire concerning treatment and prevention of CT-induced FN, 73.1% routinely prescribed G-CSF therapy for prophylactic and curative reasons. The most common reasons for prescribing biosimilar filgrastim were cost savings and comparable efficacy and safety.
Summary and Conclusions: Biosimilar filgrastim (Nivestim™) was effective and well-tolerated in both the prophylactic and curative setting in patients undergoing chemotherapy for solid tumors and hematological malignancies. In real-world practice, most physicians prescribed G-CSF therapy for prophylactic or curative intentions in their patients receiving myelosuppressive chemotherapy.

E1178

ORGANIZING PNEUMONIA IN HEMATO-ONCOLOGY PATIENTS: A SOMEWHAT DISREGARDED DIFFERENTIAL DIAGNOSIS

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Background: Organizing Pneumonia (OP) is defined by clinical and imagiological aspects of pneumonia, with histological pattern of intra-alveolar buds of granulation tissue, consisting of intermixed myofibroblasts and connective tissue. OP develops in the context of an immune deregulation, a perpetuation of the inflammatory response in the presence of infection, autoimmune diseases or drug exposure. In hemato-oncology, OP has been extensively studied in the allogeneic transplant setting and, more recently, it has also been described in lymphoproliferative diseases, myelodysplastic syndromes and chronic myeloproliferative diseases.
Aims: Analyze clinical characterisitics and outcomes of diagnosed OP in hemato-oncology patients, outside the allogeneic transplant setting.
Methods: Retrospective revision of biopsy-proven OP cases diagnosed in patients with hematological diseases, in a Portuguese hospital centre, between 2005 and 2014.
Results: Ten cases with biopsy-proven OP were obtained. The median age was 62 years (33-75) and 7 cases (70%) were male. The hemato-oncological diagnoses were: Lymphoma (n=7; 70%); B-cell Acute Lymphoblastic Leukemia (n=1; 10%); Myelodysplasia (n=2; 20%); Acute Myeloid Leukemia (n=2; 20%). In two (20%), the OP diagnosis was made simultaneously with onset of hematological disease, seven (70%) during chemotherapy and one (10%) during the first 100 days after autologous transplantation. Prior to the OP diagnosis, none was submitted to bone marrow allogeneic transplantation or pulmonary radiotherapy. Clinically, nine (90%) patients had fever and nine (90%) had respiratory insufficiency. Of the latter, three (30%) patients needed non-invasive ventilation and two (20%) invasive ventilation. The thoracic CT scan reported findings varying between nodules (n=4; 40%), consolidations (n=3; 30%), interstitial infiltrates (n=3; 30%), ground-glass opacities (n=3; 30%) and pleural effusions (n=3; 30%), with findings overlapping in 9 (90%) cases. The perpetuation of an unfavorable clinical course despite antimicrobial/antifungal therapy or the lack of typical CT findings led to the decision of lung biopsy. Percutaneous transthoracic CT-guided lung biopsy was performed in eight (80%) patients and thoracotomy in other two (20%), with no procedure related complications. In terms of OP therapy, corticosteroids were the choice in nine (90%) patients (n=5 isolated; n=4 in association with azithromycin; n=1 no treatment), with clinical and imagiological improvement in seven cases. Pulmonary aspergillosis was a non-confirmed differential diagnosis in 5 (50%). Of the 4 OP exacerbations, 2 occurred with hematological disease progression. Of the 6 (60%) deaths, 4 occurred with hematological disease evolution, 2 with OP exacerbation in association with sepsis/multiorganic dysfunction. The Overall Survival and the Exacerbation Free Survival were 12 months and 4 months, respectively.
Summary and Conclusions: OP should be considered a differential diagnosis of pulmonary lesions in hemato-oncological diseases. The lack of specificity of the clinical and imagiological presentation, urges the need of considering the lung biopsy in highly suspicious lesions. The correct diagnosis is essential to begin the treatment with corticosteroids, which showed to be capable of reasonable clinical and imagiological disease control in our series.

E1179

THE EFFICACY OF POLYVINYLPYRROLIDONE – ZINC GLUCONATE AND TAURINE GEL (GEL X) IN PROPHYLAXIS AND TREATMENT OF ORAL MUCOSITIS IN CHILDREN TREATED WIH CHEMOTHERAPY

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Background: Polyvynilpyrrolidone-zn gluconate and taurine (GelX) is an oral lubricating gel used in the management of oral mucositis (OM). OM is an important medical and nursing problem secondary to use of chemotherapy, associated with oral pain and reduced oral intake, difficult to manage, especially in young children, with important impact for quality of life and nutritional status.

GelX forms an adherent barrier and cover the oral mucosa lesions, thus protecting the sensitive nerves endings and lubricating the oral tissue.
Aims: To evaluate the efficacy of Gel X as prophylactic or curative treatment for children treated with chemotherapy for hematologic malignant diseases.
Methods: We conducted a single-centre prospective and observational study in children with acute leukemias receiving chemotherapy according to the BFM protocols. The parents signed the informed consent. The study was non-sponsored. In the prophylactic arm, the oral cavity nursing with GelX started on the first day of chemotherapy and continued until the recovery from neutropenic phase; for the curative arm, the oral cavity nursing started on the first day of chemotherapy with other oral rinses and was switched to GelX after OM development and continued until OM recovery. The oral rinses were recommended to be used at least 3 times a day according to the product specifications. The OM was assessed daily by the physicians using the WHO grading 0-4 (0=absent, 1= soreness and/or erythema, 2= erythema, ulcers and patient can swallow solid food, 3= ulcers with extensive erythema and patient cannot swallow solid food, 4=mucositis to the extent that alimentation is not possible). We performed also a patient assessment for pain (general, mouth and throat) VAS scale 1-5 (1=no pain, 2=mild pain, 3=moderate pain, 4=severe pain, 5=very severe pain) and saliva VAS scale 1-5 for a) swallowing (1=normal, 2=cannot swallow certain solid foods, 3=can only swallow soft foods,4=can only swallow liquid foods,5=cannot swallow), b) saliva amount (1=normal amount, 2=mid loss of saliva, 3=moderate loss of saliva, 4=severe loss of saliva, 4=severe loss of saliva, 5= no saliva) and c) consistency of saliva (1=normal, 2=slightly thick, 3=moderately thick, 4=extremely thick, 5=saliva that dries in mouth or lips). The tolerability of oral rinses was evaluated daily by the patients or parents using the Visual Analog Scale (VAS) scoring 1-5: 1= tolerable without any problems, 2=satisfactory, 3= indifferent, 4= unsatisfactory, 5=intolerable. The OM pain reduction and food intake improvement were assessed by the patients/parents using the VAS scoring 1-5 (1=excellent, 2=good, 3=slight effect, 4=almost no effect, 5=no effect at all). Basic statistics univariate analysis were performed using statistical software with the Fisher's exact test. The "P" values <0.05 were considered as statistically significant differences.
Results: A total of 15 patients were enrolled in this analysis, 8 in the prophylactic arm (group A) and 7 in the curative arm (group B). Characteristics of the groups were shown in Table 1. The OM maximum grade was higher in the B group than in the A group, p=0,018. The patient/parent assessment of mouth pain (p=0,01) and of saliva consistency (p=0,033) were significantly lower in the prophylactic group. We also observed a significant difference regarding the OM pain reduction (p=0,001), oral intake improvement (p=0,001) and use of systemic analgesics (p=0,005). There were no difference in the median value of tolerability of GelX rinses between the group A and B.

Table 1.

	prophylactic arm (group A)	curative arm (group B)	P=
No.of patients	8	7	-
Age (years) median, (range)	5,5 (2-10)	4, (0,75-5)	-
Sex: M/F	3/5	1/6	0,287
Diagnosis: ALL/AML	5/3	6/1	0,287
OM maximum grade, (range)	1, (1-3)	4, (3-5)	***0,018***
Patient assessment median, (range)			
- General pain	1, (1-2)	2, (2-3)	0,123
- Mouth pain	2, (1-3)	4, (3-5)	***0,001***
- Throat pain	1, (1-2)	3, (1-4)	0,369
- Swallowing	1, (1-4)	3, (1-4)	0,304
- Amount of saliva	1, (1-2)	2, (1-5)	0,091
- Consistency	1, (1-4)	2, (1-5)	***0,033***
Tolerability of oral rinses, median, (range)	1, (1-2)	2, (1-4)	0,369
OM pain reduction, median days	2, (0-4)	7, (4-8)	***0,001***
Oral intake improvement, median days	2,(0-3)	8, (5-10)	***0,001***
Systemic analgesic treatment with analgesics	2/8	7/7	***0,005***

Summary and Conclusions: There are limited data regarding the use in children as prophylactic or curative measures. In our study, the oral spray was administered as prophylaxis or as curative treatment. Our results showed better results in the OM development and quality of life in the prophylaxis arm. These results, however, should be considered as informative and larger cohorts of patients needs to be analyzed.

E1180

INFECTION AND COLONIZATION BY CARBAPENEM RESISTANT KLEBSIELLA PNEUMONIAE IN HAEMATOLOGY PATIENTS

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Background: Klebsiella Pneumoniae is a frequent nosocomial pathogen inhabiting the digestive tract. In the era of antibiotic resistance and multi-drug resistant bacteria, the emergence and spread of carbapenem-resistant Klebsiella pneumoniae (CRKP),has become a major health concern for hospitalized patients. Hematological patients are particularly prone to infection by CRKP due to a combination of factors: prolonged neutropenia, recurrent use of chemotherapy and broad spectrum antibiotics, severe mucositis, immunosuppression, catheters.
Aims: Description an outbreak of CRKP colonization between May and September 2014 in our centre despite strict hygiene.
Methods: Colonization (carrier state) was defined as the presence of at least one rectal swab positive for CRKP. Eradication of colonization was confirmed when three consecutive rectal swabs are negative after decontamination therapy (after 3 days, 7 days and 1 month). Infection was defined as the presence of the microorganism from blood cultures or other body site (urinary tract in particular) with clinical signs. Carbapenemase detection was made by Hodge test and disk diffusion, confirmed by specific PCR.
Results: 11 patients were identified as carriers CRKP over a period of 4 months. *(Patients characteristics are described in table 1)*. Colonization was followed by a microbiologically documented infection in 5 patients (1 septic shock, 1 bacteraemia concomitant UTI and followed by septic shock, 1 bacteraemia, catheter and soft tissue infection, 2 UTIs recurrent). As risk factors, four of these patients had severe neutropenia and only one patient had a normal WBC count, but suffered from gastrointestinal GVHD. All infected patients were initially treated with high doses carbapenem therapy administered by prolonged infusion (3 hours) combined with gentamicin and/or fosfomicine and/or tigecycline. In two episodes of bacteremia we used second-line treatment with ceftazidime/avibactam, with good safety profile and efficacy (negative cultures at 72 hours). The infection-related mortality rate was 40% (2/5).
Decontamination therapy consisted in oral gentamicin (80 mg/6h for 14 days), if three days after rectal swab continued positive, treatment was started with oral neomicine and estreptomicin (40/80 mg, 1 tablet/8h) or treatment was repeated with gentamicin. 10 patients received decontamination therapy (1 patient developed septic shock and died before receiving treatment) being successful in 5 of them (50%). It was necessary to repeat courses with an average of two lines (1-4) and a median duration of 2 month (15days-6meses).

Table 1.

PATIENT	AGE GENDER	DIAGNOSIS	TREATMENT	RESOLUTION OF COLONIZATION	DECONTAMINATION THERAPY LINES	INFECTION	RESOLUTION OF INFECTION	OUTCOME
1	67/F	MPDs	IS for GVHD	No	3	UTI	Yes	Death [b]
2	54/M	AML	Chemotherapy	No	4	Bacteraemia Catheter Soft tissue	Yes Yes Yes	Alive
3	17/M	AML	Allo-SCT	No	4	UTI recurrent	Yes	Alive
4	33/F	AML	IS for GVHD	No	1	UTI Bacteraemia Septic shock	Yes Yes No	Death [a]
5	65/M	AML	2ºAllo-SCT	-	-	Septic shock	No	Death [a]
6	39/M	AML	Chemotherapy	Yes	4	-	-	Alive
7	24/M	HD	Chemotherapy	Yes	2	-	-	Death [b]
8	80/M	MDS	5-AZA	Yes	2	-	-	Alive
9	68/M	MPDs	HU	Yes	1	-	-	Alive
10	49/F	AML	2ºAllo-SCT	Yes	1	-	-	Death [b]
11	59/M	CLL	Chemotherapy	No	2	-	-	Alive

[a] Death from CRKP infections. [b] Death from other non-CRKP causes

Summary and Conclusions: 1. In our center 5/11 colonized patients developed infection. Our experience with ceftazidime/avibactam was favourable because it managed to eradicate bloodstream infection. 2. We have done a good control of the outbreak in only four months. Special health measures such as surveillance with weekly rectal swabs, bed isolation and strict hygiene measures have been very important in controlling the spread of the epidemic. 3. Hematology patients need several courses of decontamination therapy to succeed.

E1181

INFECTIONS IN PATIENTS WITH MYELODYSPLASTIC SYNDROME/ ACUTE MYELOID LEUKEMIA TREATED WITH AZACITIDINE: REPORT FROM A SINGLE CENTER

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Background: Infections are common and potentially fatal events affecting patients with myelodysplastic syndromes (MDS). Predisposing factors that are likely to be associated with increased risk of infections in MDS patients are neutropenia and/or neutrophil functional impairment; B-, T- and NK-cell defects; secondary iron overload related to red blood cell transfusions; comorbidities; treatment toxicity; previous severe infections. Few data are available on the incidence and pathogens involved in infectious events, most of these originating from retrospective studies or clinical trials with primary end points other than infection.
Aims: An Italian single Center real-life experience assessing the incidence, risk factors and impact of infections on outcome of patients with MDS treated with hypomethylating agents is herein reported.

Methods: From March 2008 to October 2013, 50 patients, aged 40 years and older (median age: 69, 40-84 years) with diagnosis of MDS (WHO2008 categories: 22.4% RA/RCMD, 32.6% RAEB-1, 28.5% RAEB-2, 12.2% CMML, 4% AML), were treated with 5-azacitidine (75 mg/m^2/die for 7 days every 4 weeks), both in on-label and off-label drug use setting. Forty-four percent of patients had intermediate-2 or high International Prognostic Scoring System, 68% were neutropenic and 12% had high MDS-Comorbidity Index. Prophylactic antibiotics were administered to 12 patients (24%), prophylactic antifungal to 17 patients (34%) and granulocyte colony-stimulating factor was administered to 24 patients (48%).
Results: Median number of cycles received by a single patient was 5 (range 1-21); 48% received more than 6 cycles of therapy. 30.4% of the entire cohort was considered responsive to treatment (14.4% hematologic improvement, 8% partial response, 8% complete response, according to IWG2006 criteria); 24% of patients achieved a stable disease. Out of 50 patients, 25 (50%) developed 25 infectious events (1 for each patient), during 325 treatment cycles (7.7%); 14/25 (56%) events required hospitalization. Only one patient died from an infectious complication. Twenty-two of 25 infectious events (84%) were bacterial, mostly pneumonia; 3 (12%) were fungal (invasive aspergillosis) and 1 (4%) was viral (H1N1). Infectious events did not significantly affect overall survival (27 *vs* 18 months, p=0.606), progression free survival (6.0 *vs* 6.1 months, p=0.48) or overall response to therapy (13 *vs* 17.4%, p=0.693). However, no complete responses were documented in the cohort of patients who suffered from infectious episodes. In a univariate analysis, age, sex, low neutrophil count, high comorbidity index, antibiotic prophylaxis and use of G-CSF were not found to be associated with infections. Only high IPSS and the presence of pancytopenia, seemed to be correlated with an increased risk for infections.
Summary and Conclusions: Infectious events, specifically bacterial infections, are one of the most frequent complications during therapy with azacitidine in patients with MDS. These data suggest that there are not predisposing risk factors for infection in patients except those connected with disease severity (high IPSS and pancytopenia). Routine antibiotics, antifungal prophylaxis and/or use of G-CSF appear not to reduce the incidence of infectious events. Moreover, bearing in mind the risk of bacterial and fungal resistance associated with extended use of anti-infective drugs, they should be used with caution in selected subsets of MDS patients.

E1182

EVALUATION OF SERUM GALACTOMANNAN ASSAY FOR THE DIAGNOSIS OF INVASIVE ASPERGILLOSIS IN CHILDREN WITH HEMATOLOGICAL MALIGNANCIES

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Background: Fungal infection is a major concern during treatment of hematological malignancies. Establishing diagnosis is a challenge and often frustating for the treating physicians. Rapid and diagnostic test is valuable for timed intervention. Galactomannan assay is a non invasive test, however it's efficacy needs to be validated in our population.Only few studies in Indian population, hence to establish a clinically relevant cut off value this prospective study is done.
Aims: Diagnostic efficacy of galactomannan assay (GA) for invasive aspergillosis (IA) is variable. the cut off value is debated.
Methods: Children ≤14-years with hematological malignancies and fever were enrolled prospectively. Blood sample for GMA was drawn on day of admission; levels were measured with *Platellia Aspergillus* enzyme immunoassay. Diagnostic criteria were adapted from EORTC-MSG. GMA was evaluated at various cut-offs, with proven, probable and possible episodes being considered as disease group.
Results: 100 febrile episodes in 78 patients were included. Mean-age was 6.1 years. Majority (75%) episodes were in patients with ALL, followed by AML (17%). CT-scan-lung was performed in 23 episodes. BAL, transbronchial-lung-biopsy and functional-endoscopic-sinus-surgery were performed in 2-episodes, each. Post-mortem investigations included autopsy (1) and organ biopsies (6). Diagnosis of IA was proven and probable in one case each. A possible diagnosis was made in 23 episodes; remaining 75 were categorized as "No IA". Other fungal infections diagnosed included mucormycosis (3), candidiasis (1) and fusariosis (1). Best results were obtained with a cut-off value of 1.0, with sensitivity, specificity, positive and negative predictive value of 60%, 93%, 75 and 87, respectively. With GMA >1.0 as cut-off, the probability of a positive test to be true or false positive was 0.71 (95% CI: 0.48-0.88) and 0.28 (95% CI: 0.12-0.52), respectively. For a negative test, the probability of true negative was 0.87 (95% CI: 0.78-0.93) and false negative was 0.13 (95% CI: 0.16-0.22). The sensitivity dropped to 40%, at cut-off value of 1.5 and specificity was 38%, at a cut-off of 0.5. Significant correlation of a higher GMA was observed with pulmonary nodules (p=0.037), duration of Amphotericin >10-days (p=0.043) and mortality (p=0.001).
Summary and Conclusions: Confirming the diagnosis of aspergillosis is a challenge; this renders assessment of efficacy of GMA difficult. At a cut-off value of 1.0, the sensitivity and specificity were 60% and 93%, respectively.

E1183

ANIDULAFUNGIN THERAPY FOR HIGH RISK OF INVASIVE FUNGAL INFECTION HEMATOLOGIC PATIENTS: ITS ROLE IN REAL-LIFE SINGLE CENTER EXPERIENCE.

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Background: Anidulafungin in an echinocandin licensed for the treatment of invasive candidiasis in adult non-neutropenic patients. This drug is neither metabolized by the liver, nor has renal elimination. Liver dysfunction is common after allogeneic stem cell transplantation and intensive chemotherapy. In our hospital patients who fulfill criteria of high risk for Invasive fungal infection (IFI) routinely receive antifungal prophylaxis with voriconazole. It has become routine clinical practice to substitute voriconazole for anidulafungin in cases with hepatic involvement of GVHD and/or liver toxicity manifested as elevated liver function tests (LFTs).
Aims: The aim of this study is to analyze the safety, toxicity and feasibility of off label anidulafugin as prophylaxis and treatment of high risk of IFI hematologic patients in a real life scenario.
Methods: We have retrospectively studied all episodes of adult high risk of IFI hematological patients treated with anidulafungin in our hospital from March 2010 to October 2014. IFI was defined according to EORTC guidelines.
Results: Fifty patients were included, 38 were treated once, 9 twice and 3 thrice, thus 65 different therapy episodes were finally analized. Median age was 50 years (IQR 39.2-61.2), with 63.1 % (n=41) cases being male. Most (81.6%) had undergone allogeneic stem cell transplantation (Allo-SCT). The most common baseline diagnoses were acute leukemia in 31 (47.7%) and non Hodgkin´s lymphoma in 12 (8.3%). Fifty-five cases (85%) had no prior history of IFI. The remaining ten cases previously had proven (n=3), probable (n=5) or possible (n=2) IFI and therefore received secondary anti-fungal prophylaxis. Anidulafungin was administered as prophylaxis in 48 episodes (73.9%), in 3 episodes for proven candidiasis (4.6%) and in 14 (21.5%) as empirical treatment of IFI. Elevated LFTs was the most common cause to commence on anidulafungin (n=46, 71.8%), in 19 of them (29.7%) voriconazole had to be discontinued. Other reasons to start anidulafungin were chronic graft versus host disease (n=6), voriconazole intolerance (n=4), hallucinations due to voriconazole (n=3), candidiasis (n=3), and others (n=3). Median number of days on therapy was 11 (IQR 5-19.5). Treatment with anidulafungin was well tolerated in most of our patients (93.8%). The drug was withdrawn in three cases: one due to acute kidney failure, one due to drug intolerance during infusion and one due to progressive liver failure. Among the 48 cases receiving anidulafungin as prophylaxis there were 36 with abnormal LFTs and 6 with liver GVHD. In this very high risk of IFI cohort there were 3 proven (2 cases of mucormicosis and one *C.guillermondii* candidemia), 2 probable and one possible IFI, during the duration of prophylaxis or in the week following the discontinuation of anidulafungin. In addition, 4 more proven IFIs were diagnosed in the following 100 days: one disseminated histoplasmosis (day 11), one C.krusei candidemia (day 18), one pulmonary invasive aspergillosis (day 10) and one esophageal candidiasis (day 36). All patients but the last one, had received other anti-fungal agent after anidulafungin was discontinued. Nearly half of the patients receiving anidulafungin as prophylaxis (45.8%) died during the 100 days follow up period, 2 of them (9.6%) due to IFI.
Summary and Conclusions: Anidulafungin is a safe and feasible alternative to azole therapy, especially in patients with elevated LFTs or liver involvement by GVHD. Further studies are needed to establish the role of anidulafungin in this patient population.

E1184

LOW-RISK MYELODYSPLASTIC PATIENTS SUPPORTED WITH ERYTHROPOIETIN PLUS LIPOSOMIAL IRON SHOWS A REDUCED NUMBER OF FEBRILE EPISODES THAN PATIENTS WITH INTRAVENOUS IRON SUPPORT.

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Background: Intravenous iron support simultaneous to erythropoietin administration improve response to erythropoietin in myelodysplastic patients. Oral liposomal iron, bypassing normal intestinal mechanism of absorption, shows similar effects, better than oral iron sulfate, usually poorly absorbed. Ther'are many evidences that iron, useful for bacterial growth, might increase risk of infection.
Aims: Aim of this study is to verify incidence of number of febrile episodes in low-risk myelodysplastic patients without support or supported with erythropoietin (epo) only or with epo plus oral liposomal iron, oral iron sulfate or iv sodium ferrigluconate.
Methods: This study is a retrospective, multicentric study.Between july 2008 and december 2014, 107 patients affected by low-risk refractory anemia were

studied.Median follow-up was 24 months (R12-60).20 patients had no support, 27 epo support, 30 epo+liposomial iron (14 mg 2 tablets orally/day for 3 months), 15 epo+iron sulfate (525 mg2 tablets orally/day for 3 months), 15 epo+iv sodium ferrigluconate (62.5 mg iv in NS 100 ml in 1 h/day for 5 day/month).All patients supported with epo received calcium levofolinate 7.5 mg/day orally +Vitamin B12: 400 mg/day orally.Epo support was performed with originator or biosimilar epo alpha or epo zed 40000 IU sc/week or with epo beta 30000 IU sc/week.Statistical analysis was performed by Chi Squre test and Fisher exact test.

Results: In group with no support median hemoglobin level was 9.5 g/dl (R8-11). Median neutrophils count was 1000/mcl (R300-2500). Median packed red blood cells unit (PRBCU) transfused was 0.2/month (R0-0.5). Median number of febrile episodes/year was 1.5 (R0-2). In group supported with epo only median hemoglobin level after 3 month treatment was 11 g/dl (R7.5-12). Median neutrophils count was 800/mcl (R400-1200). Median packed red blood cells unit (PRBCU) transfused was 0.4/month (R0-0.7). Median number of febrile episodes/year was 2 (R0-2).In group supported with epo+iron sulfate median hemoglobin level after 3 month treatment was 10.5 g/dl (R8-12). Median neutrophils count was 750/mcl (R300-1100). Median packed red blood cells unit (PRBCU) transfused was 0.3/month (R0-0.6). Median number of febrile episodes/year was 3 (R0-3). In group supported with i.v. sodium ferrigluconate median hemoglobin level after 3 month treatment was 12 g/dl (R9-13). Median neutrophils count was 700/mcl (R250-1000). Median packed red blood cells unit (PRBCU) transfused was 1.5/month (R1-3). Median number of febrile episodes/year was 6 (R0-9). In group supported with liposomial iron median hemoglobin level after 3 month treatment was 12.8 g/dl (R10-13). Median neutrophils count was 280/mcl (R150-1300). Median packed red blood cells unit (PRBCU) transfused was 0.2/month (R0-1). Median number of febrile episodes/year was 1 (R0-2).

Summary and Conclusions: Number of febrile episodes is low in each treatment group. Febrile episodes seem not related to basal neutrophil count or hemoglobin level reached after 3 month treatment. Number of febrile episodes is higher in group with higher transfusion need and in group treated with i.v. sodium ferrigluconate (p 0.02). Probably liposomial iron support provides a reduced amount of non- transferrin bound iron that might block bacterial growth. These data need confirmation on a larger cohort of patients.

E1185

RISK FACTORS FOR FEBRILE NEUTROPENIA AND BLOODSTREAM INFECTIONS IN RECIPIENTS OF HEMATOPOIETIC STEM CELL TRANSPLANTS

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Background: Number and indications for Hematopoietic Stem Cell Transplantations (HSCTs) in adults continue to grow worldwide. Febrile neutropenia (FN) remains one of the most common complications in the HSCT patients. Bloodstream bacterial infections (BSI) stay common causes of FN among neutropenic patients. Choice of initial strategy of antibacterial treatment in FN patients is based mainly on clinical and epidemiological risk factors, because of the low frequency of culture isolation and reduced clinical manifestations of infection.

Aims: The aim of the study was to determine the risk factors for febrile neutropenia or microbiologically proven bloodstream infection in adult patients receiving HSCT.

Methods: 242 patients undergoing allogeneic or autologous HSCT at the Belarus National Centre for Hematology and Bone Marrow Transplantation from January 2013 to January 2015 were monitored and their clinical data was reviewed. Age of the patients included in this study was 18-65 years, 42% of them were male, 58% - female. The primary outcome was the episode of FN(fulfilled criteria of Freifeld *et al.*, 2011), the secondary outcome was microbiologically proven bacterial bloodstream infection (BSI). Isolation of pathogens was performed by standard means with BacT/ALERT Standard Aerobic/Anaerobic bottles and BacT/ALERT 3D automated microbial detection system, identification and antibiotic resistance was studied with VITEK 2 system and disc-diffusion methods.

Categorical variables were analyzed with χ2 test and Fisher's exact test, and continuous variables were analyzed with the Mann-Whitney U test and Odds Ratio. A multivariate analysis with logistic regression was conducted for the categorical variables with P-value ≤0,2 in previously performed univariate analysis. Significant P-value considered to be <0,05.

Results: There were 87 patients with episodes of FN, the incidence of FN in HCST recipients was 36%. Among them 39 patients had microbiologically proven BSI, *i.e.* 16% of all HSCT recipients or 45% of those who had FN. Most of the cases of BSI were caused *by E. coli, Kl. pneumoniae, P. aeruginosa, A. baumannii, Streptococcus spp.* Among independent statistically significant risk factors for both FN and BSI were: profound neutropenia (OR 2,34, 95% CI 1,19-13,24, p=0,012 for FN; OR 2,44, 95% CI 1,96-9,54, p=0,005 for BSI); neutropenia duration >14 days (OR 1,37, 95% CI 1,08-12,93, p=0,049 for FN; OR 1,68, 95% CI 1,14-8,73, p=0,045 for BSI) and active main disease on start of HSCT procedure (OR 3,41; CI 2,32-8,63, p=0,01 for FN; OR 1,28, CI 1,04-3,81, p=0,049 for BSI). Prior to HSCT patients colonization with ESBL-positive *Enterobacteriaceae spp.* and prior ICU hospitalization had a trend towards the statistical significance as a risk factors of BSI, what may be proved by using larger number of patients in the future studies (OR 1,64, 95% CI 0,89-4,36, p=0,64 for colonization; OR 2,31, 95% CI 1,27-6,41, p=0,72 for ICU hospitalization).

Summary and Conclusions: The above named risk factors and most common pathogens should be taken into account when choosing a clinical approach to empiric antibacterial treatment and prophylaxis in adult HSCT patients.

E1186

ANALYSIS OF 75 CASES OF ACUTE LEUKEMIA WITH INVASIVE FUNGAL DISEASE

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Background: Acute leukemia patients are susceptible to invasive fungal disease, proper diagnosis and treatment is important for reducing morbidity and mortality.

Aims: To analyze the clinical characteristics of invasive fungal infections in patients with acute leukemia (AL), explore the early diagnosis and prevention strategy.

Methods: Acute leukemia patients with invasive fungal disease (IFD) treated in cancer center of the First Hospital of Jilin University from 2014 January -12 months were analyzed retrospectively according to the "criteria for the diagnosis and treatment principles of hematological malignant patients with invasive fungal disease (The Fourth Edition of China)", the outcome of the disease and the curative effect were analyzed simultaneously.

Results: In 211 cases of AL patients, 75 cases (35.5%) include 49 AMLs and 26 ALLs occurred IFD, the male to female ratio was 49:26, the median age was 48 years old (8□83 years old). 73 cases were lungs IFD, 1 case was liver and spleen IFD, 1 case was intestinal IFD. Among of the 75 cases, there were 1 case with proven IFD (blood culture positive for Candida), 8 cases with probable IFD, 23 cases with possible IFD and 43 cases with uncertain IFD. Host factors included neutropenia (70 cases, 93.3%), the application of hormone for more than 3 weeks (10 cases, 13.3%), a history of previous fungal infection (4 cases, 5.3%), allogeneic transplantation (1 cases, 1.3%), prior application of broad-spectrum antibiotics was confirmed in 69 patients (92%). 31 cases (41.3%) showed specific CT signs, wherein the air crescent sign in 1 case, pulmonary cavity in 1 case, liver and spleen buphthalmos sign in 1 cases, and the rest cases showed compact, clear boundary lesions, including 5 cases with halo sign. 30 cases (40%) of patients showed non-specific CT signs, such as small nodules, multiple patchy shadow, ground glass shadow, emphysema or bronchiectasis. Positive G test was found in 12 patients and positive GM test was in 10 cases, of which both test positive was in 9 cases. All 23 patients with possible IFD were identified as fungal infections according to the subsequent treatment response and disease evolution, 23 cases (53.5%) was eventually identified as fungal infection in 43 uncertain IFD patients, so in total 75 patients, 55 patients were eventually identified as fungal infection, accounting for 26.6% of all patients with 211 leukemia patients. All 75 patients received antifungal treatment, including 9 cases of target therapies, 46 cases of diagnosis driven therapies, 20 cases of empiric therapies. 7 cases (77.8%) were effective in target therapies, 36 cases (78.3%) was eventually identified as fungal infection in 46 diagnosis driven therapies patients, in which 31 case (86.1%) were effective. 10 cases (50%) was confirmed with fungal infection in 20 empiric treatment patients, and were all effective. The overall effective rate was 89.1% (48/55).

Summary and Conclusions: IFD is one of the main causes of infection in acute leukemia patients. High resolution CT and G test, GM test were important in the diagnosis of IFD. Early diagnosis and treatment is the key to improve the efficacy of IFD. The application of "criteria for the diagnosis and treatment principles of hematological malignant patients with invasive fungal disease (The Fourth Edition of China)" can be a very good guide for the clinical diagnosis and treatment.

E1187

ACINETOBACTER BAUMANNII SURVEILLANCE MEASURES IN HAEMATOLOGICAL MALIGNANCIES PATIENTS

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Background: Acinetobacter baumannii is a gram-negative, strictly aerobic,nonfermentative coccobacillus that causes infections in immunocompromised and chronically ill patients. Recently, it has emerged as a major cause of health care-associated infections (HCAIs) in addition to its capacity to cause community-acquired infections. It is associated with multidrug resistance and it is considered to be among the most difficult anti-microbial-resistant bacilli to control and treat. Given to its antimicrobial resistance, treatment options are severely limited, and to the best of our knowdlwdge there aren't any controlled trials to guide therapeutic choices. In severely ill patients multidrug-resistant *Acinetobacter* infections are associated with an extremely high morbility and mortality rate. It is therefore necessary the developement of measures that can prevent or early identify Acinetobacter infection.

Aims: Despite the high proportion of infection related death, particularly sustained by multidrug-resistant bacteria among wich A. Baumanii, there are only few reports on Acinetobacter infection and measures to prevent and treat the bacillus spread and infection in patients affected by haematological malignancies. Moreover, these reports are case reports or describe small population of patients. Here we describe our experience on Acinetobacter measures of early detection.
Methods: Between January 2014 and December 2014 pharyngeal swabs were performed on 139 haematological patients on the date of admission in the hematology Section of Catania University and none was positive for Acinetobacter. We also performed 32 serial pharyngeal swabs on hospitalized patients and when cases of Acinetobacter bacteraemia were detected (2 cases) as empirical test to early detect contamination and treat positive symptomatic cases.
Results: Seven swabs were positive for A. Baumanii, all in heavily treated and advanced stage patients (3 AML patients, 1 MM, 2 CLL, 1 LNH) and, of these, 3 patients died for concomitant complications, 2 patients developed Acinetobacter baumannii bacteremia that caused septic shock and death, and 2 were successfully treated with inhaled colistin for the eradication of bacterium from the respiratory tract, preventing bacteriemia. This method was not described before and allows an early detection of the Bacillus. Moreover, it could be used not only for cohorting patients but also as a signature marker for subsequent clinical infections. In addiction, early treatment with aerosolized Colistin for the eradication of MDRAB colonization in the respiratory tract resulted as salvage therapy for the 2 patients described that are still alive after eradication.
Summary and Conclusions: In conclusion A. Baumanii colonization are difficult to manage in immunocompromised patients but an appropriate swabs surveillance, application of strict infection control measures and use of aerosolized Colistin therapy could be of help.

E1188

DOES NEUTROPENIA AFFECTS HERPES VIRUS REACTIVATION IN CRITICALLY ILL PATIENTS WITH HEMATOLOGICAL MALIGNANCIES AND PNEUMONIA?
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Background: Chemotherapy applied in hematological malignancies management leads to blood cells count decreasing which in turn leads to a deterioration in the condition of the patient. Leucocyte count diminishment involves not only tumor cells but neutrophils as well. It can cause ICU admission. Neutrophils are essential for immune regulation and opportunistic infection prevention. Nevertheless life-threatening complications like nosocomial pneumonia and or respiratory failure can occur within any granulocyte count during chemotherapy. Herpes viruses tend to reactivate in immunodeficient person. Acute herpes virus infection can cause lung injury.
Aims: The aim of the study was to estimate frequency of herpes viruses in critically ill patients with hematological malignancies and pneumonia during immunodeficiency
Methods: 38 critically ill patients with hematological malignancies suffered from pneumonia were enrolled in the study: 18 with acute leukemia, 8 with ly… 2… le… si… D… P… **R**… h… o… B… (2… si… d… E… vi… p… ir… fi… fr… w…

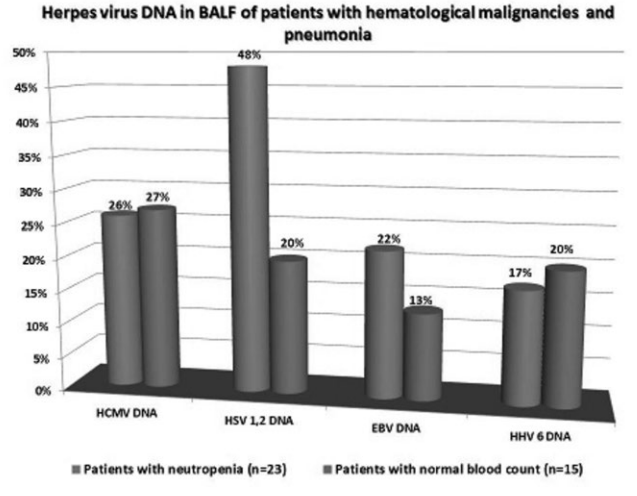

Figure 1

Summary and Conclusions: Obtained data show twice higher frequency of herpes virus DNA detection in BALF of immunodeficient hematological patients. Viral load is also higher if neutropenia occurs. This proves, granulocyte count reduction can be associated with viral lung injury complications.

E1189

NOCARDIA INFECTION IN PATIENTS UNDERGOING ALLOGENEIC STEM CELL TRANSPLANTATION
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Background: Patients undergoing allogeneic stem cell transplantation are at increased risk of opportunistic infections. *Nocardia*, a gram positive bacterial agent, is an uncommon cause of infection. The diagnosis of Nocardiosis can be quite challenging because of technical difficulties (difficulty in obtaining adequate samples and growing cultures). The disease usually presents in a subacute manner in immunocompromised patients but its course is often life threatening.
Aims: The objective of this study was to record the incidence of nocardiosis in patients who received stem cell transplants in our hospital and to describe their clinical characteristics.
Methods: Seventy patients who had undergone allogeneic stem cell transplantation between 2009 and 2014 were retrospectively evaluated. Reason for transplant, graft *vs* host disease (GvHD), site of infection, *pneumocystis jiroveci* prophylaxis, treatment regimens and outcome were recorded.
Results: In the above period of six years, 40 out of 70 patients developed graft *vs* host disease (acute GvHD:24 cases, chronic GvHD:23 cases) that required corticosteroid treatment. None of the remaining 30 patients developed nocardiosis. Of the 40 patients with GvHD, 35 patients received co-trimoxazole as prophylaxis for *pneumocystis jiroveci* and five patients alternative prophylaxis such as pentamidine and atovaquone. Again in the 35 patients who received co-trimoxazole, no nocardiosis occurred, while three out of five patients (60%) on alternative prophylaxis developed infection due to *nocardia*: osteomyelitis in a man with acute lymphoblastic leukemia, disseminated disease (involving the lung, brain, liver, spleen, kidneys and possibly the duodenum) in a man with myelofibrosis and pulmonary nocardiosis in a woman with chronic myeloid leukemia. The woman eventually died of nocardiosis. The two men with nocardiosis were successfully treated with adequate antibiotic combinations (one of them is still under co-trimoxazole).
Summary and Conclusions: Though uncommon, nocardiosis should be kept in mind when it comes to patients undergoing allogeneic stem cell transplantation. Graft *vs* host disease and its treatment seriously affect the host's immune status and could increase patient susceptibility to the disease in this subgroup of patients. Interestingly in our center all patients who were diagnosed with nocardiosis were not receiving co-trimoxazole as prophylaxis for pneumocystis jiroveci while none of the patients under co-trimoxazole prophylaxis ever developed any infection caused by *Nocardia spp*.

E1190

INFECTIOUS COMPLICATIONS IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES/ACUTE MYELOID LEUKEMIA TREATED WITH 5-AZACITIDINE
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Background: Azacitidine (AZA) is the standard of care for high-risk myelodysplastic syndromes (MDS). Its mechanism of action includes demethylation, cytotoxicity and the induction of apoptosis. Thus, cytopenias are a major problem during the first cycles of treatment, when hematological improvement has not yet been achieved. The risk of infection in this period of treatment has been demonstrated to be substantial. Indeed, in the AZA-001 trial, infections occurred in about 50% of AZA-treated patients. The incidence and features of these infectious complications has been addressed mainly in controlled clinical trials, with highly selected patients, making it controversial to extrapolate the results to "real life" patients.
Aims: We aimed to compare the incidence of infectious complications during AZA treatment in a cohort of "real life" patients with the published data.
Methods: We retrospectively evaluated the incidence of infectious complications during AZA treatment in a cohort of patients with high risk MDS and Acute Myeloid Leukemia (AML).
Results: Fifty one patients started AZA treatment between 31/07/2006 and 24/03/2014. Median age at diagnosis was 69 years (49-83 yrs, 34 males, 17 females). Median age at the start of treatment was 71 years (49-83). There were 44 MDS and 7 AML. MDS classification according to the WHO 2008 was: Refractory cytopenia with multilineage dysplasia (n=10); Refractory anemia with excess blasts 1 (n=12); Refractory anemia with excess blasts 2 (n=17); Chronic Myelomonocytic Leukemia (n=4); Refractory anemia with ring sideroblasts (n=1). AZA was started at a median of 4.99 months from diagnosis in a standard dose of 75 mg/m2, on a 5-2-2 schedule. A median of 5 (1-34) cycles of AZA were delivered to each patient. In a total of 353 cycles, 31 (8.8%) infectious episodes occurred in 12 patients (23.5%). The median time to first infection was 1.9 (0.2-41.9) months. Six patients (11.8%) had a single episode of infection after a median of 3.2 months from the start of AZA (0.4-41.9 months).

In the 6 patients who had two or more infectious episodes, the second one occurred after a median of 4.7 (3.2-25.2) months from the start of AZA. The median neutrophil count at the time of infection was 0.35 (0-16)/μL. In 17 of 31 (54.8%) infections, grade 3-4 neutropenia was present. In these 31 infectious episodes, a positive microbiologic test was available in 11 cases (35.4%). Eighteen bacteria and one fungus were isolated (table). Twenty-three hospitalizations were required in 11 (91.7%) of the 12 patients who had infections, with a median duration of 17 (2-59) days. The most frequent infection sites were the lungs (32.2%) and the skin (19.4%). Infections were the attributable cause of death in 7 hospitalized patients: five of them died due to pneumonia and 2 to fever of unknown origin. Eighteen patients with MDS (38.3%) progressed to AML under AZA, after a median of 7.5 (2.5-23.0) months. With a median follow-up of 16.3 (2.1-223.7) months, 15 patients were alive, resulting into a median overall survival of 20,5 (10,1-30,8) months.

Table 1.

Product	Microbiologic result
Blood	*Staphylococcus epidermidis* (n=2) Oxacilin-resistant *Staphylococcus haemolyticus* *Staphylococcus hominis* *Staphylococcus aureus* (n=2) *Pseudomonas aeruginosa* (n=2) *Escherichia coli (n=2)* *Enterobacter cloacae* *Enterococcus faecium* *Acinetobacter baumannii*
Bronchoalveolar lavage	*Staphylococcus aureus*
Sputum	*Aspergillus flavus*
Skin	Methicillin resistant *Staphylococcus aureus*
Urine	*Escherichia coli* *Klebsiella pneumoniae*
Faeces	*Clostridium ramosum*

Summary and Conclusions: We found a relatively high probability of infectious complications following treatment of MDS/AML with AZA, especially pneumonia, wich was also the main cause of death. However, in our cohort, the incidence of infections (23.5%) was inferior of that related in the published data. We concluded that in a setting of not highly selected patients, like our cohort, infections during AZA treatment are not more incident than those described in the literature.

E1191

FACTORS AFFECTING THE OUTCOME OF FEBRILE NEUTROPENIA IN PATIENTS WITH HEMATOLOGIC MALIGNANCIES

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Background: Febrile neutropenia(FN) is one of the most seroius complications in patients (pts) with hematological malignancies.The prompt initiation of empirical antiboitics within 1 hour of fever has lead to reduction of mortality and improvement of survival .Several risk assesment guidlines have been adopted to identify pts as low and high risk of complications.We conducted this single institution study to determine specific risk factors that may affect the outcome in pts with FN.
Aims: To identify specific factors that may affect the response to treatment in pts with febrile neutropenia.
Methods: During the peroid of 1st of April 2014 untill the end January 2015, pts with hematological malignancies who presented to clinical oncology department and developed FN during management were enrolled in this prospective analysis. At the onset of fever, pts underwent complete physical examination, in addition to blood culture, urine and stool culture. Computer tomography of the chest and paranasal sinuses and serial galactomannan (GM) test were requested in cases with uncontrolled fever or suspected invasive fungal infection (IFI).Polymerase chain reaction analysis of bacteria and fungi from the blood and bronchoalveolar lavage were performed in selected cases.The data were analyzed using chi-square test.
Results: One hundred and thirty five (135) pts were identified and analyzed. The mean age was 38.5 years (range 14-76). 51% had acute myeloid leukemia, 36% acute lymphoblastic leukemia (ALL), non hodgkin's lymphoma, chronic lymphocytic leukemia, and multiple myeloma were diagnosed in 7%, 4%, and 2% respectively .According to Multinational Association for Supportive Care in Cancer (MASCC) index, 80 pts (60%) were categorised as high MASCC score (<21), while 55 pts (40%) had low MASCC score (>21). Blood culture was negative in 114 pts (84%), while it was positive in 21 pts(16%). Gram negative bacteria constituted 60% of cases, while gram positive were 40 %.Serial GM test was positive in 24 pts only (17%),88 pts (65%) did not receive antifungal agents. Fluconazole was used as antifungal prophylaxis in the majority of pts (n=95)(71%), of those who did not receive fluconazole (n=40), only 15 pts (37.5%) developed IFI and were classified according to 2008 European organization for research and treatment of cancer/invasive fungal infections cooperative group and the national institute of allergy and infectious diseases mycoses study group (EORTC/MSG) Consensus; 12 pts had probable fungal infections,2 pts had possible fungal infections and 1 patient had definitive fungal infection. The choice between first line antibiotics (ceftazidime, maxipime, imipenem) or antifungal (amphotericin, voriconazole) had no impact on the recurrence of FN attacks (P<0.08 and p<0.23 respectively).In terms control of fever on 1st line antibiotics, there was statistically significant difference in favour of low risk MASCC score (p<0.001),change of antibiotics due to uncontrolled fever was required in 62 pts (45%),there was a significant difference between defervescence and low/high MASCC score (P<0.001). With respect to first antifungal used, a significant correlation was observed between low/high MASCC score and control on first antifungal therapy (P<0.006). In the present study ,16 pts(12%) only were diabetics, there was no significant correlation between diabetes and uncontrolled fever, prolonged neutropenia .Prolonged FN (>7days) was observed in 40 % of pts ,the use of corticosteroids, and non administration of granulocyte colony stimulating factor (G-CSF) were the predominant risk factors (P<0.001 ,P<0.002 respectively). In terms of mortality, only pts with high MASCC score(n=11) (8%) had the worst outcome compared to low score(n=1) (P<0.0001).
Summary and Conclusions: There are multiple factors that may affect the outcome of pts with FN and it should be taken in considerations during mangment of FN such as MASCC score, previous use of corticosteroid and G-CSF adminstration during FN.

E1192

VARIOUS PATTERNS OF HEPATITIS B VIRUS REACTIVATION IN B CELL LYMPHOMA

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Background: It has been reported that reactivation of hepatitis B virus (HBV) could be fatal in patients with malignancy following systemic immunosuppressive chemotherapy, especially combined with rituximab. The prophylactic nucleoside analog therapy is recommended in the current guidelines to treat HBV reactivation in which the incidence of HBV reactivation was proved to be reduced. On the while, how HBV reactivation exhibits clinical course in patients with lymphoma treated with rituximab-containing chemotherapy has to be elucidated.
Aims: To investigate various patterns of HBV reactivation in lymphoma patients receiving Rituximab-contaionig chemotherapy.
Methods: We retrospectively analyzed 541 patients with B cell lymphoma who were treated with rituximab-containing regimens between 2006 and 2013 at Kansai Medical University Hospital.

Table 1. Characteristics of 18 patients with HBV infection in B-cell lymphoma.

Age	Sex	Diagnosis	Chemotherapy	HBsAg	HBcAb	HBsAb	HBV DNA (log copies/ml)	HBV prophylaxis	Reactivation	HBV treatment after reactivation	IgG(mg/dl)	Time to event(days)
64	M	DLBCL	R-CHOP	+	+	+	2.1	entecavir	-	-	-	-
67	F	DLBCL	R-CHOP	+	-	+	6.1	entecavir	-	-	-	-
61	M	DLBCL	R-CHOP	+	-	+	7.7	entecavir	-	-	-	-
70	M	DLBCL	R-CHOP	+	-	+	2.1	entecavir	-	-	-	-
69	M	DLBCL	R-CHOP	+	-	+	5.2	entecavir	-	-	-	-
81	M	DLBCL	R-CHOP	+	-	+	2.1	entecavir	-	-	-	-
62	M	MCL	R-hyperCVAD/HDMTX-AraC	+	-	+	6.2	entecavir	-	-	-	-
58	F	FL	R-CHOP	+	-	+	2.1	entecavir	-	-	-	-
68	M	FL	R-CHOP	+	-	+	2.4	entecavir	-	-	-	-
60	M	FL	R-CHOP	+	-	+	2.6	lamivudine	breakthough	entecavir	-	194
74	F	DLBCL	R-CHOP	+	-	+	5.3	lamivudine	breakthough	entecavir	-	509
70	M	DLBCL	R-CHOP,R-ESHAP,R-CHASE,R-CSD	+	-	+	undetected	-	+	entecavir	292	807
70	F	DLBCL	R-CHOP,ASCT	+	-	+	5.0	entecavir	secondary reactivation	entecavir	304	607
64	M	DLBCL	R-CHOP	+	-	+	undetected	-	+	entecavir	988	684
66	M	DLBCL	R-CHOP,R-DeVIC,R-ESHAP,ASCT	-	+	+	3.0	-	+	entecavir	299	633
69	M	DLBCL	R-CHOP	-	+	+	5.5	-	+	entecavir	604	23
84	M	DLBCL	R-CHOP,R-DeVIC	-	+	+	2.2	-	+	entecavir	565	268
80	M	DLBCL	R-CHOP	-	-	+	3.2	-	+	entecavir	786	182
81	M	DLBCL	R-CHOP	-	-	+	2.1	-	+	entecavir	723	59

Abbreviations: DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma; MCL, mantle cell lymphoma; HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; HBsAb, hepatitis B surface antibody; HBcAb, hepatitis B core antibody; ASCT, autologous stem cell transplantation; M, male; F, female; Time to event, days to reactivation/breakthrough

Results: A total of 541 patients were studied. Most common subtype of lymphoma was diffuse large B-cell lymphoma. Among 541 patients, 14 patients (2.5%) were hepatitis B antigen (HBs Ag) positive carrier. Among these 14 carriers, 12 patients whose HBV-DNA level was positive at diagnosis were treated with nucleoside analog. Two patients whose HBV-DNA were undetected at diagnosis, without prophylactic nucleoside analog, converted to reactivation after the chemotherapy. Two patients who received prophylactic nucleoside analog therapy developed breakthrough hepatitis long after diagnosis (median: 351 days). One-hundred and seven patients (19.7%) had occult HBV infection (HBs Ag negative, HBc Ab positive or HBs Ab positive). Among occult patients, 5 patients converted to reactivation (de novo hepatitis) during the chemotherapy and received nucleoside analog. All of these de novo hepatitis patients were presented with hypogammaglobulinemia (median: 511 mg/dl) at reactivation. Of all reactivated patients, 2 patients underwent autologous stem cell trans-

plantation (ASCT). One was a carrier received prophylactic nucleoside analog, and whose HBV-DNA became undetected, but she developed secondary reactivation long after ASCT. The other was de novo hepatitis, he converted to late-onset reactivation after ASCT. These two patients were also presented with hypogammaglobulinemia (median: 301 mg/dl) at reactivation.
Summary and Conclusions: We achieved improvement on management against HBV reactivation through the guideline to prevail on us to use prophylactic nucleoside analog therapy. Here we addressed that HBV reactivation could exhibit various patterns as we develop to treat lymphoma with new immunosuppressive agents as well as hematopoietic cell transplantation. Therefore, we have to pay attention to each pattern of HBV reactivations in treating them beyond the guidelines.

LB2080

REACTIVATION OF HEPATITIS B INFECTION IN HAEMATOLOGY PATIENTS RECEIVING RITUXIMAB THERAPY: EVIDENCE OF SUB-CLINICAL ALT FLARES IN HBSAG-/HBCAB+ PATIENTS

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Background: Reactivation of hepatitis B virus (HBV) following Rituximab therapy is a well-recognized phenomenon. In patients with pre-existing serological markers of chronic (surface antigen positive [HBsAg+]) HBV infection, the rate of Rituximab-associated HBV reactivation is approximately 80-100% in the absence of nucleotide analogue (NA) prophylaxis. In patients with serological markers of past infection (HBsAg-negative [HBsAg-]/core antibody positive [HBcAb+]), this rate varies between 0% and 45%. European guidelines therefore recommend that all patients are HBV serotyped (with HBsAg and HBcAb) prior to treatment with Rituximab. Patients with active hepatitis B disease should not receive therapy with Rituximab, and those with positive HBV serology but no active disease should be referred to a hepatologist for specialist monitoring and therapy, including measuring HBV DNA levels and starting prophylactic NA therapy.
Aims: To determine: i) the proportion of patients appropriately screened prior to Rituximab and if HBsAg-/HBcAb+, the proportion tested for HBV DNA levels: ii) the rate of ALT flares in the HBsAg-/HBcAb+ patients
Methods: Between January - October 2014, Haematology patients in a tertiary level centre who received Rituximab were identified from the electronic prescribing database. Pathology records were used to determine demographic characteristics, evidence of HBV serotyping, HBV DNA levels and whether HBsAg-/HBcAb+ patients received NA therapy. Patients with a high ALT at any time following Rituximab were categorised into groups of ALT >50 or >150 IU/L.
Results: Of the 230 patients administered Rituximab, 82% had malignant diagnoses and 18% non-malignant diagnoses. 166 patients (72%) underwent HBV serotyping. Of these, 19 (11%) were HBsAg-/HBcAb+ and 2 were chronic HBsAg+/HBcAb+ carriers. Of the HBsAg-/HBcAb+ patients, seven (33%) were tested for HBV DNA at least once and none were positive. Both HBsAg+/HBcAb+ patients had detectable HBV DNA. Four (21%) of the HBcAb+ and both the HBsAg+ patients were started on NA therapy. The proportion of patients with an ALT >50 IU/L following Rituximab therapy was higher in the HBcAb+ group compared to the HBcAb- group (47% *vs* 25% p = 0.04). There was a trend to a greater number of patients with an ALT >150 IU/L in the HBcAb+ group compared to the HBcAb- group, but this was non-significant (21% vs. 13% p = 0.07). Of the 64 patients who did not undergo any HBV serotyping, 30 patients (18%) had an ALT of >150 and one developed an ALT of >1000 IU/L.
Summary and Conclusions: Although there were no patients with HBV DNA-confirmed reactivations in the HBsAg-/HBcAb+ patients in this study, the higher proportion of patients with an ALT >50 IU/L in the HbsAg-/HBcAb+ group suggests that there were sub-clinical ALT flares in this group, and suggests that the frequency of HBV DNA monitoring impacts on the measured reactivation rate in previous studies. The study also highlights that increased awareness amongst Haematology clinicians is needed to improve rates of HBV serotyping prior to and monitoring for reactivation following Rituximab administration to avoid preventable HBV-related complications.
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LB2081

USING PET/CT TO ASSESS FDG NODAL UPTAKE AND IMPACT OF VIRAEMIA IN HIV-POSITIVE PATIENTS: A COMPARISON OF REACTIVE LYMPHADENOPATHTY, MULTICENTRIC CASTLEMAN DISEASE AND HIV-RELATED LYMPHOMA

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Background: PET/CT is a widely used imaging modality in the investigation, diagnosis, staging and assessment of treatment response in patients with lymphoma and has an increasing role in investigating HIV-positive patients with pyrexia of unknown origin (PUO). Its use in differentiating malignancy from infection in the evaluation of intracranial lesions in the context of HIV, for example, is well-recognized. HIV-related immunosuppression predisposes patients to a number of additional phenomena however, including opportunistic infections, HIV-related reactive lymphadenopathy, immune reconstitution and the lymphoproliferative disorder Multicentric Castleman disease (MCD). These can lead to false-positive interpretations of malignancy, particularly in untreated patients with high viral loads (VL) and therefore special consideration is required when evaluating PET/CT in patients with HIV. There remains considerable debate about which factors are most important in predicting the aetiology of lymphadenopathy using PET/CT in the HIV setting.
Aims: To compare PET/CT findings in HIV-positive patients with lymphoma, reactive lymphadenopathy and MCD to identify differentiating factors. We aimed to look specifically at the effect of VL on nodal and splenic characteristics and used radiological parameters to assess this.
Methods: Patients with HIV who presented with PUO and who subsequently had imaging with18F-FDG PET-CT were identified. Patients were categorised into 4 groups after final diagnosis: i) reactive lymphadenopathy ii) MCD iii) HIV-associated lymphoma and iv) confirmed infectious or other miscellaneous condition; the latter group were excluded from analysis in this study. Electronic records were used to record baseline demographic characteristics and CD4 count, plasma HIV load and ART status. All lymphoma and MCD diagnoses were confirmed on biopsy and all patients with reactive lymphadenopathy had extensive inpatient investigations which excluded an infectious or other aetiology. PET/CT imaging was reviewed by a radiologist and SUV_{max}, distribution of lymphadenopathy and liver-spleen ratio calculated.
Results: 80 patients were identified, of which 39 had HIV-related reactive lymphadenopathy, 26 had lymphoma and 15 had MCD. The mean age was 42 years and 74% were male. There were higher median SUV_{max} values among patients with lymphoma compared to reactive lymphadenopathy and MCD (12.0 vs. 5.6, p=0.0021, 12.0 vs. 5.7, p=0.0041, respectively). There was a trend to higher median SUV_{max} values in the reactive lymphadenopathy group with a detectable VL compared to the undetectable group, but this difference was not significant (5.9 *vs* 4.0) and there was no difference between the MCD and reactive lymphadenopathy groups. Reversal of the liver:spleen avidity ratio was observed more commonly among those with detectable viral loads but did not aid discrimination between diagnoses.
Summary and Conclusions: This is the largest PET/CT series of HIV-positive patients with fever and lymphadenopathy. Significantly higher SUV_{max} values were observed among lymphoma cases and despite MCD being a lymphoproliferative disorder, showed similar median SUV_{max} values to reactive lymphadenopathy. There was no effect of high or low viraemia on SUV_{max} in patients with both MCD or lymphoma which may be helpful clinically when serial PET/CT imaging is available. Finally, this study confirms that both histological and radiological evidence of HIV-related reactive nodal changes were seen even in patients with complete serum virological suppression and this should therefore be borne in mind when investigating HIV-positive patients for lymphoma.
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LB2090

ANTIMICROBIAL-RESISTANT GRAM-NEGATIVE BACTERIA IN BLOODSTREAM INFECTIONS IN PATIENTS WITH PEDIATRIC HEMATOLOGY & ONCOLOGY AND PEDIATRIC BONE MARROW TRANSPLANTATION UNIT

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Background: Patients with cancer are at high risk for infections caused by antibiotic resistant gram-negative organism.
Aims: To survey blood cultures taken from the patients hospitalized in pediatric hematology & oncology clinic and pediatric bone marrow transplantation unit and inspect the antibiogram of antimicrobial-resistant gram-negative organisms.
Methods: The blood cultures taken from children hospitalized in pediatric hematology & oncology and pediatric bone marrow transplantation clinics from April 2011 to April 2014 during fever and/or clinic infection existence were evaluated retrospectively. The gram-negative organisms grew in the blood cultures and their antibiograms were inspected. Antibiotic-resistance was classified as multidrug resistant (MDR), extensively drug resistant (XDR) and pandrug resistant (PDR).
Results: Pathogen organisms had grown in 632 (12%) of 5287 blood cultures. Among all of the organisms, 86.5% gram positive and 13.5% (n=85) gram negative organisms were identified. Forty-three (50%) of gram negative organisms were antimicrobial-resistant. The anti-microbial resistant gram-negative organisms identified in the blood cultures were *Klebsiella pneumoniae* (n=24; 11 with ESBL resistant, 11 with MDR/XDR; and 2 with PDR), *Acinetobacter baumannii* (9; 8 with MDR/XDR; and 1 with MDR), *Pseudomonas aeruginosa* (n=5; 4 with MDR/XDR and 1 with MDR), Escherichia coli (n=5 with ESBL resistant).
Summary and Conclusions: In pediatric hematology-oncology patients, bloodstream infections with antibiotic-resistant gram negative pathogens has recently increased. They are associated with increased morbidity and mortality. It is clear that the introduction of novel antibiotics will lead to improvements in the treatment of MDR, XDR and PDR.

E1193

CAR MEDIATES DIFFERENTIATION AND MIGRATION OF ERYTHROPOIETIC PROGENITOR CELLS AND IS SPECIFICALLY DOWN-REGULATED IN MDS

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Background: Myelodysplastic syndromes (MDS) are myeloid neoplasms characterized by peripheral cytopenia, an accumulation of dysplastic cells in the bone marrow (BM) and a high risk of transformation to acute myeloid leukemia. Transfusion-dependent anemia develops in most patients. In the refractory anemias the accumulation of dysplastic erythroid BM progenitor cells (EryPC) which paradoxically accompanies peripheral anemia, is a typical finding.
Aims: The biochemical basis of the maturation defect of erythroid cells and their abnormal accumulation in the BM are still unknown. Therefore, we were interested to examine abnormally regulated and functionally relevant genes expressed in MDS EryPC.
Methods: In our study, we have established a screening program involving mRNA expression profiling studies of EryPC in patients with low-risk MDS and control BM samples obtained from patients with other BM neoplasms as well as patients with unexplained cytopenia, normal BM or reactive/deficiency cytopenias. EryPC were defined as $CD45^{low}/CD105^{+}$ cells and purified from BM mononuclear cells by multicolor flow cytometry (MFC) and cell sorting. mRNA expression profiles were analyzed by Affymetrix array technology and confirmed for a panel of selected mRNA species by qPCR.
Results: In mRNA- and MFC-validation experiments, we found that the major Coxsackie-Adenovirus Receptor (CAR) is specifically down-regulated in $CD45^{low}/CD105^{+}$ EryPC in MDS patients when compared to EryPC in normal BM or other control cohorts. In line with this observation, the immature erythroblastic cell lines HEL, K562 and KU812 stained negative for CAR. Lentiviral transduction of the full-length *CAR* gene into these cells resulted in a significantly increased expression of various erythroid differentiation antigens, including CD36, CD71 and Glycophorin-A as determined by flow cytometry and qPCR. Furthermore, *CAR* transduction resulted in an increased migration of HEL cells against a serum protein-gradient in a transwell assay. Transfection with truncated variants of *CAR* did not result in an increased expression of erythroid antigens or an increased directed migration.
Summary and Conclusions: In conclusion, our data show that CAR is a functionally relevant molecule that promotes the expression of early erythroid differentiation antigens on myeloid progenitor cells and their migration against blood serum proteins. In patients with MDS, CAR is downregulated on EryPC, which may have implications both in terms of the pathogenesis of the disease and the application of this novel marker in diagnostic MFC algorithms. With regard to functional consequences, we hypothesize that CAR-deficiency is pathogenetically relevant as it may not only contribute to the maturation-defect of EryPC in MDS but also to the related accumulation of erythroid cells in the BM that is accompanying the peripheral anemia in these patients.

E1194

RUNX1 MUTATION AND LOW RUNX1 TRANSACTIVATING ACTIVITY PREDICT HIGHER RISK OF AML TRNASFORMATION AND INFERIOR LEUKEMIA-FREE SURVIVAL IN CHRONIC MYELOMONOCYTIC LEUKEMIA

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Background: We have previously described a high frequency of *RUNX1* mutations in chronic myelomonocytic leukemia (CMML), the prognostic impact of *RUNX1* mutation and its biological activity on disease progression remain to be investigated.
Aims: We aimed to correlate *RUNX1* mutation status and its biological activity with clinical features in patients with CMML as well as to determine the role of RUNX1 mutants in secondary acute myeloid leukemia (sAML) transformation.
Methods: Bone marrow (BM) samples from 105 CMML patients at diagnosis were examined for *RUNX1* mutations by PCR-based analysis covering entire coding sequences from exons 3 to 8 of *RUNX1b* gene. Additional 25 genes related to the myeloid neoplasms were also analyzed. The RUNX1 mutant activity on target *C-FMS* gene induction was determined by luciferase reporter assay. The relative activity of each RUNX1b mutant was derived by measurement of luciferase intensity of each transfectant and normalized with wt-RUNX1b. Pyrosequencing was used to measure allele burden of mutated genes.
Results: Thirty-six of 105 patients (34.3%) with CMML had *RUNX1* mutations, 33 of them had co-existing mutations, including 13 *SRSF2*, 10 *ASXL1*, 9 *TET2*, 8 *EZH2*, 6 *NRAS*, 5 *CEBPα*, 3 *CBL*, 3 *SETPB1*, 3 *U2AF1*, 3 *SF3B1*, 2 *DNMT3A*, 2 *FLT3*-TKD, and one each of *IDH2*, *PTPN11*, *MLL*-PTD, and *CSF3R*. The RUNX1 mutants were grouped into high and low activity according to their relativet ransactivating activities (cut-off was 0.52), 30% of 103 CMML patients had low RUNX1 activities. Both *RUNX1* mutation and low biological activity were significantly associated with lower platelet count, higher WBC count and monocytosis, and higher BM blasts. Patients with low activity were younger and had more CMML2 subtype. There was no correlation of hemoglobin level, cytogenetics or IPSS-R with *RUNX1* mutation status or mutant activity. Forty-five patients progressed to sAML, 32 had matched paired CMML/sAML samples available for clonal evolution analysis; 17 were positive for *RUNX1* mutations at both phases. The allele burden of *RUNX1* mutations remained stable except 3 in which a new *RUNX1* mutant clone emerged while the previous one declined at sAML phase and accompanied the co-emergence of *NRAS* mutation and *FLT3*-TKD in one each of them. In addition, *RUNX1* mutant clones evolved in another two patients and with the acquisition of *IDH2 with FLT3-ITD* and *SRSF2* mutations, respectively in each of them during sAML progression. We also found that one lost *TET2* mutation and the other lost *JAK2*V617F during disease progression. *RUNX1* mutation was associated with an increased risk of sAML transformation compared with non-mutated patients (58% *vs* 33%, *P*=0.021) and shorter leukemia-free survival (LFS) (23.3±3.1 months *vs* 47.8±17.7 months, *P*=0.042) but with no impact on overall survival (12.5±3.2 months *vs* 13.2±1.5 months, *P*=0.147). CMML patients with low RUNX1 activities had a higher risk of sAML transformation (64.5% *vs* 31.9%, *P*=0.004) and inferior LFS (19.7±4.6 months *vs* 47.8 months, *P*=0.010) compared with high activity group. By including additional gene mutations for outcome analysis, both *RUNX1* mutation status and biological activity were the most important prognostic predictors.
Summary and Conclusions: Our results showed that *RUNX1* mutation and low RUNX1 transactivating activity predicted higher risk of sAML progression and shorter LFS in CMML patients. (Grant support: NHRI- EX103-10003NI, OMRPG3C0021, MMH-E-101009, MOST103-2321-B-182-015, and MOHW103-TD-B-111-09).

E1195

ABNORMAL MONOCYTE POPULATIONS IN THE PERIPHERAL BLOOD OF PATIENTS WITH LOWER RISK MYELODYSPLASTIC SYNDROMES

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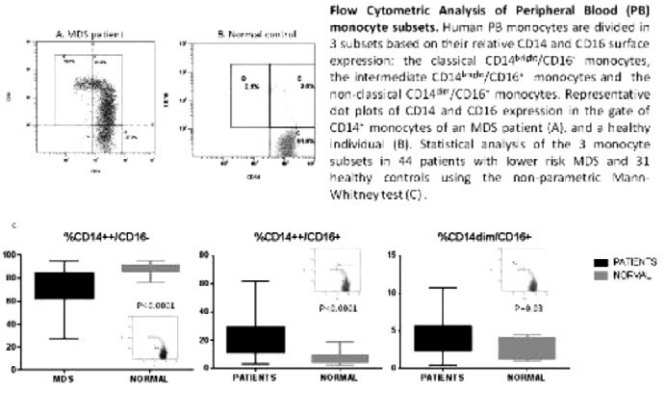
Background: Immune deregulation is implicated in the pathogenesis of Myelodysplatic Syndromes (MDS). Monocytes are an important part of the innate immune system; circulating human monocytes are phenotypically and functionally heterogenous. They typically express the CD14 antigen and are divided in the $CD16^{-}$ (classical) monocytes and the $CD16^{+}$ monocytes which can be further subdivided in the $CD14^{bright}/CD16^{+}$ (intermediate) monocytes and the $CD14^{dim}/CD16^{+}$ (non-classical) monocytes. Classical monocytes seem to be involved in tissue repair while intermediate monocytes are mainly pro-inflammatory. The role of the monocyte cell subsets in the pathogenesis/pathophysiology of MDS has not been extensively studied.
Aims: The aim of this study was to investigate for quantitative abnormalities in peripheral blood (PB) monocyte subsets of patients with MDS that might have an impact on the pathogenesis/pathophysiology of the disease.
Methods: We have studied 44 patients with Very Low/Low risk MDS according to the R-IPSS score and 31 age and sex matched healthy individuals. Four-colour Flow Cytometry was used to assess the three monocyte subsets based on their relative CD14 and CD16 surface expression. Furthermore, the Mean Fluorescence Intensity (MFI) of the CCR2 and the CX_3CR1 chemokine receptors, which are characteristically expressed by monocytes, was determined in each monocyte subpopulation.
Results: Analysis was performed within the CD14+ cell population. A significant increase in the proportion of the $CD16^{+}$ monocytes was observed in MDS patients (26.4±15.4%) compared to normal controls (10.2±4.9%) (P<0.0001) which was associated with a significant decrease of the $CD16^{-}$ monocytes in patients (73.6±15.3%) compared to controls (89.8±4.9%) (P<0.0001). Among the $CD16^{+}$ monocytes, the most prominent increase was observed in the intermediate $CD14^{bright}/CD16^{+}$ monocytes which were increased both as cell proportions (22.1±13.8%) and absolute cell numbers (133.5±118.5/μl) in patients compared to healthy controls (7.8±4.2% and 50.1±36.3/μl, respectively) (P<0.0001 and P=0.0012, respectively). Furthermore, the percentages of the $CD14^{bright}/CD16^{+}/CCR2^{+}$ and the $CD14^{bright}/CD16^{+}/CX_3CR1^{+}$ cells, were increased in MDS patients (19.2±14.2% and 16.2±13.3%, respectively) compared to normal controls (6.9±3.2% and 7.4±4.8%, respectively) (P=0.0008 and P=0.0273, respectively). The $CD14^{bright}/CD16^{+}$ monocytes of MDS patients consisted of a higher proportion of $CCR2^{+}$ (91.8±6.4%) and a lower proportion

of CX_3CR1^+ (87.1±15.6%) cells compared to healthy individuals (87.7±6.7% and 97.3±3.6%, respectively) (P=0.04 and P=0.001, respectively). Interestingly, the MFI of CCR2 was significantly increased in the $CD14^{bright}/CD16^+$ monocytes of MDS patients (12.9±3.2) compared to normal controls (7.2±2.4) (P<0.0001) (Figure 1).



Flow Cytometric Analysis of Peripheral Blood (PB) monocyte subsets. Human PB monocytes are divided in 3 subsets based on their relative CD14 and CD16 surface expression: the classical $CD14^{bright}/CD16^-$ monocytes, the intermediate $CD14^{bright}/CD16^+$ monocytes and the non-classical $CD14^{dim}/CD16^+$ monocytes. Representative dot plots of CD14 and CD16 expression in the gate of $CD14^+$ monocytes of an MDS patient (A), and a healthy individual (B). Statistical analysis of the 3 monocyte subsets in 44 patients with lower risk MDS and 31 healthy controls using the non-parametric Mann-Whitney test (C).

Figure 1.

Summary and Conclusions: We have showed for first time that patients with lower risk MDS display significantly increased numbers of circulating $CD14^{bright}/CD16^+$ monocytes which are also phenotypically altered. The possible involvement of this abnormal cell population in the excessive pro-inflammatory cytokine production and ineffective hematopoiesis of lower risk MDS is currently under investigation.

E1196

GLUTATHIONE S-TRANSFERASE P1 (GSTP1) PROMOTER HYPERMETHYLATION IN MDS/AML: ASSOCIATION WITH SPECIFIC CHROMOSOME ABBERATIONS

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Background: Gene silencing by epigenetic alterations is a common event in carcinogenesis. The GSTP1 gene is a well-known housekeeping gene involved in carcinogen detoxification. Its expression has been linked to cancer incidence and drug resistance. It has been reported to be the target of somatic CpG island promoter hypermethylation in various types of human neoplasia, such as prostate, breast and liver cancer. Recently, there is an ongoing preclinical and clinical platform of drug discovery based on the activity of GSTP1 inhibitors in stimulating multilineage differentiation of hematopoietic progenitors.
Aims: The aim of the study was to investigate the possible contribution of epigenetic inactivation of the GSTP1 gene in MDS and AML *via* DNA promoter hypermethylation. We also assessed possible associations of GSTP1 methylation pattern with specific chromosomal aberrations and the presence of $A^{313}G$ GSTP1 germline inactivating polymorphism.
Methods: Our case-control study enrolled 116 patients [(62 MDS, 29 *de novo* AML and 25 AML cases preceded by MDS (MDS-AML)] and 25 healthy donors. Cytogenetic analysis of bone marrow samples was successfully performed for all patients at diagnosis. Total genomic DNA was extracted from patients' and controls' samples using the QIAamp DNA-extraction midi kit (Qiagen, Hilden, Germany). GSTP1 promoter methylation status was studied by methylation-specific PCR (MSP) using the CpG WIZ® GSTpi Amplification Kit (Chemicon, Canada, USA). The $A^{313}G$ GSTP1 genotyping was performed by Real-Time PCR on the Biorad CFX96 (Biorad, California, USA) using GoTaq® qPCR Master Mix (Promega, USA).
Results: Our results showed that GSTP1 gene promoter was hypermethylated in 39 out of 116 patients (33.6%). All control samples were found to be completely unmethylated. Hypermethylation of GSTP1 promoter was found in 21 out of 62 MDS (33.9%), 6 out of 29 AML (20.7%) and 12 out of 25 MDS-AML cases (48.0%). A significantly increased frequency was observed in MDS (p<0.001) and MDS-AML patients (p<0.001). The GSTP1 promoter hypermethylation was not found to be associated with the $A^{313}G$ GSTP1 polymorphism. Stratification of patients according to their cytogenetic findings revealed a significantly increased frequency of methylated cases in the group of patients showing a normal karyotype, as compared to those with an abnormal karyotype (60.0% *vs* 38.9%, respectively). Among patients with an abnormal karyotype, the most common chromosome aberrations were trisomy 8 (+8), -5/del(5q) and -7/del(7q) followed by del(20q) and loss of Y chromosome (-Y). The higher frequencies of GSTP1 hypermethylation were found in cases with -7/del(7q) (45.0%) and -5/del(5q) (33.5%) (p<0.001).
Summary and Conclusions: The results indicate an important role of the GSTP1 epigenetic silencing in MDS/AML pathogenesis, extending our knowledge on epigenetic alterations of the GSTP1 gene in hematological malignancies. The remarkably high frequency of GSTP1 promoter hypermethylation in patients with chromosome 7 abnormalities is consistent with the use of DNA demethylating agents in treatment of MDS with loss of chromosome 7. Further, the findings might suggest that GSTP1 hypermethylation could be a potential epigenetic biomarker for MDS response to treatment, mainly in cases under treatment with GSTP1 inhibitors.

E1197

CIRCULATING MICRORNAS IN PLASMA OF PATIENTS WITH MYELODYSPLASTIC SYNDROMES

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Background: Myelodysplastic syndromes (MDS) represent a wide range of hematopoietic stem cell disorders characterized by ineffective hematopoiesis, peripheral blood cytopenias, and a tendency to evolve into acute myeloid leukemia. MicroRNAs (miRNAs) are short endogenous non-coding RNA molecules that play an essential role in the regulation of gene expression at post-transcriptional levels. Existence of miRNAs has been recognized not only in the intracellular environment but it has recently been shown that these molecules are also present in a wide variety of body fluids, including blood plasma. Cell free miRNAs (referred to as circulating miRNAs) have been repeatedly proved to function as new promising semi-invasive diagnostic markers of various types of cancer but only little information is known about their deregulation in MDS.
Aims: In this study, we focus on alteration of miRNA levels circulating in plasma of MDS patients with the emphasis on differences between low and high risk disease.
Methods: Peripheral blood plasma was collected from 40 patients with untreated primary MDS and from 10 healthy, age-matched donors with no medical history. Written informed consent was obtained from all tested subjects in accordance with the Institutional Review Board. Total RNA was extracted from plasma using Qiagen miRNeasy mini kit. Agilent Human miRNA Microarrays were used to assess the amount of 2,006 human miRNAs in blood plasma from 13 MDS patients and 6 healthy donors. The microarray results were confirmed in the complete set of samples by RT-qPCR.
Results: Statistical analyses identified significant differences in a plasma profile of circulating miRNAs between different study groups. In MDS, 19 down-regulated and 25 up-regulated miRNAs were detected compared to healthy controls. Some hematopoiesis and/or oncology-associated miRNAs were found in the list of deregulated molecules, *e.g.* down-regulation of miR-92a-3p, miR-142-3p/5p, miR-320a/b/d/e, and miR-451a, and up-regulation of miR-150-5p, miR-188-5p, and miR-548q. Low-risk and high-risk groups of patients significantly differed in levels of several miRNAs, namely in reduction of miR-185-5p and miR-4306 and elevation of miR-548q, miR-623, miR-4707-5p, miR-4721, and miR-4739 in high-risk patients.
Summary and Conclusions: Expression profiling of plasma miRNAs in patients with MDS revealed several miRNAs with altered levels gradually changing within the disease progression, suggesting that circulating miRNAs may be new candidate molecular markers for monitoring of the disease.
This work was supported by the grant NT13847-4 from the Ministry of Health of the Czech Republic.

E1198

***IN VITRO* CULTURE OF BM CELLS OF MDS PATIENTS AND CYTC POLYMORPHISMS CAN PREDICT THE *IN VIVO* HEMATOLOGICAL RESPONSE OBSERVED DURING IRON CHELATION THERAPY WITH DEFERASIROX**

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Background: Several studies showed that iron chelation therapy (ICT) can induce hematological improvement and transfusion independence in a significant percentage of MDS patients. However, we do not have clinical or biological parameters to identify patients with high probability of such hematological response.
Aims: The aim of the study was to identify either a molecular marker and/or an *in vitro* assay to predict *in vivo* hematological improvement to deferasirox treatment.
Methods: 22 MDS patients from 9 Italian Centers were enrolled in the study.

Five were RA, 4 RARS, 8 RCMD, 4 RAEB I, 1 CMML. In 6 of them ICT induced RBC transfusion independence during the first 6 months of therapy, one experienced hematological improvement but he stopped therapy after few months for progression.
BM samples were collected from 22 patients before deferasirox treatment and during follow up. BM cells were incubated with deferasirox 50 micromolar for 12 hrs and tested for colony formation in semisolid culture medium. In addition, different mitochondrial genes, including COX1, COX2 and CytB were sequenced in all the patients enrolled.
Results: In 8 out of 22 patients (3 RA, 2 RARS, 2 RCMD, 1 CMML) the *in vitro* incubation with deferasirox resulted in a significant increase of colonies (BFU-E, CFU-GM and CFU-GEMM). (mean value of BFU-E: 9±4 before incubation and 19±11 after incubation). Interestingly, 6 of these 8 patients who showed an "*in vitro*" response experienced transfusion independence after *in vivo* treatment with deferasirox. The responders continued to maintain the "*in vitro* response " throughout subsequent analysis during the follow-up. The other two 22 "*in vitro* responder" could not be evaluated for *in vivo* response.
By contrast patients who did not respond *in vitro* to deferasirox did not reduce the transfusion requirement. In parallel we analyzed mtDNA in BM MNC cells and we found a strict association between two polymorphisms of CytB (14766 and 15326) and the hematological response to deferasirox therapy.
Summary and Conclusions: The hematological improvement during deferasirox therapy in MDS patients can be predicted by colony assay after *in vitro* incubation with deferasirox. Mitocondrial gene polymorphisms seems to be associated with hematological response. Several other patients are under evaluation and may confer further solidity to this correlation. At the same time, new studies may shed new light on the mechanism of deferasirox activity.

E1199

MUTATIONAL PROFILE IN MYELODYSPLASTIC SYNDROMES BY HIGH-DEPTH NEXT GENERATION SEQUENCING AND CLINICAL RELEVANCE
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Background: Myelodysplastic syndromes (MDS) are a heterogeneous group of clinical entities. Cytogenetic abnormalities are an important hallmark of prognosis, but they are only present in 30-50% of patients. New technologies, such as next generation sequencing (NGS), have found somatic mutations in a considerable number of patients.
Aims: To optimize the technique of high-depth NGS in our center, to compare our preliminary results with the main previously published outcomes and to define their clinical significance in SMD patients.
Methods: We analyzed the mutational profile and copy number variation (CNV) of 86 MDS patients, using the *Ion Torrent Proton (Life Technologies)* system for NGS. We selected a library of 35 genes related to myeloid pathologies. An optimal depth of coverage, median about 2000, and uniform sequence coverage (92%) were reached, contributing to a high sensitivity. Additionally, clinical characteristics were considered: sex, age, WHO classification, cytogenetic risk, IPSS-R, and outcome.
Results: Eighty six MDS patients, median age: 76 (range: 44-90), were selected. The diagnosis, according to WHO classification, were: RA (4), RARS (9), RCMD (32), RAEB1 (13), RAEB2 (11), MDS associated with isolated del5q (2), CMML (12), and MDS/AML 20-30% blasts (3). Very low, low, intermediate, high and very high risk categories according to IPSS-R were found in 23, 26, 11, 9, and 15 patients, respectively. The median follow-up time was 12 month (range: 1-50). A total of 163 somatic mutations were found in 76 out of 86 patients (88%). The more frequent mutations were: TET2 (21%), SF3B1 (19%), ASXL1 (15%), TP53 (13%), DNMT3A (13%), JAK2 (13%), RUNX1 (9%), EZH2 (8%), ZRSR2 (7%), IDH1 (7%), CBL (6%), MLL (6%), IDH2 (6%), ETV6 (6%) and the remainder had frequencies of ≤5%. The results are very similar to those than have been previously published. CNV analyses showed alterations, gains or losses of copies, in 65 out of 86 patients (76%). HRAS, involved in regulating cell division, the erythroid transcription factor GATA1 and the transcriptional regulator ATRX presented gains in an appreciable proportion of patients, 28%, 23% and 20%, respectively. In contrast, genes, such as KIT and TET2, presented losses of copies in 16% and 15% of patients. Considering the clinical characteristics, SF3B1 mutations were associated to WHO classification and IPSS-R; and TP53 mutations were associated to cytogenetic risk and to IPPS-R. In univariate analysis, cytogenetic risk, IPSS, IPSS-R, WHO classification and presence of TP53 mutations were associated with shorter survival. Patients with TP53 mutations had a significantly poorer overall survival than those without the mutation (median 7 m *versus* 38 m; *P=0.001*). In multivariate analysis, only TP53 remained the independent prognostic factor for overall survival (HR=5.2; 95% CI: 2.0-13.5; P=0.001).
Summary and Conclusions: Taking into account mutations and CNV, and using methods of high-depth NGS, more than 95% of MDS patients present genetic abnormalities. TP53 mutations, that were closely associated to high risk cytogenetic and poor prognosis categories of IPSS-R, remained the independent prognostic factor for overall survival. In conclusion, high-depth NGS seems to be a promising tool to improve our understanding of the genetic alterations in SMD in order to consider them in future algorithms for diagnosis and prognosis.

E1200

TS, MTHFR ANDxRCC1 GENETIC VARIANTS AFFECT THE CLINICAL COURSE OF MDS PATIENTS IRRESPECTIVELY OF IPSS RISK
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Background: Myelodysplastic syndromes (MDS) are hematologic neoplasms characterized by ineffective hematopoiesis and an increased risk of transformation into acute myeloid leukemia. The pathogenesis and the outcome of MDS is thought to evolve from accumulation and selection of specific genetic or epigenetic events. We hypothesized that genetic variants in DNA repair, synthesis or methylation genes may affect the clinical course of MDS patients.
Aims: To investigate the prognostic value of genetic polymorphisms in BER system, DNA synthesis and folate-metabolizing genes on survival of MDS patients.
Methods: We genotyped 113 MDS patients, 54 with IPSS low/int-1 receiving only best supportive care (BSC) (low risk group) and 59 with IPSS int-2/high treated with azacitidine (high risk group), for the following polymorphisms: xRCC1 194 and 399, xRCC3 241, TS5'-UTR (2R/3R and G/C) and 3'-UTR (6bp+/6bp-), MTHFR 677 and 1298, APE1 148. Genomic DNA was analyzed by High Resolution Melting assay and restriction digests of PCR products. Overall survival (OS) was calculated using the Kaplan-Meier estimate probabilities, and differences between survival curves were analyzed by the log-rank test. The association between genetic variants and survival was assessed using stepwise logistic regression model.
Results: For all target genes, the distribution of genotypes was consistent with the Hardy-Weinberg equilibrium. The median period of observation of patients was 27 months for low risk group and 13 months for high risk group. In the low risk group,xRCC1 399 GG [Hazard ratio (HR)=4.65; p=0.05], TS3'-UTR -6/-6 (HR=7.07; p=0.02), TS5'-UTR 2R/3G, 3C/3G, 3G/3G (HR=11.44; p=0.02) and MTHFR 677 TT (HR=67.12; p=0.0001) variant alleles were associated with a statistically significant adverse clinical outcome, if compared to the reference groups. Interestingly, when we subsequently analyzed the association between the genetic variants and OS in the high risk group, we found that 3 out of the 4 variants associated with an adverse outcome in the low risk group were still significantly associated to lower survival even in the high risk group [XRCC1 399 GG (HR=5.71 p=0.002),MTHFR 677 TT (HR=8.58 p=0.0001), TS3'-UTR +6/+6 (HR=0.097 p=0.004)]. Finally, we performed an exploratory analysis to investigate the combined effect of the unfavorable genotypes on survival. In the low risk group, the 3-year OS was 33% for those patients with ≥2 variant alleles, as compared to 62.5%, and 100%, respectively, for those with 2 or 0/1 variant alleles. The predictive role of the combination of the adverse genotypes on survival was confirmed also in the high risk group, suggesting that patients with a higher number of genetic polymorphisms had a shorter survival. Interestingly, treatment with azacitidine conferred a survival advantage to patients with unfavorable genetic variants, resulting in the absence of a survival difference between low-risk patients treated with BSC only and high-risk patients treated with azacitidine.
Summary and Conclusions: These data suggest, for the first time, that genetic variants in TS, MTHFR andxRCC1 genes could independently modulate OS of both low and high risk MDS patients, representing a possible new prognostic marker able to provide guidance for clinical management of MDS patients.

E1201

SIRPB1: BIOMARKER OF RESPONSE TO 5-AZACITIDINE TREATMENT IN MDS AND AML PATIENTS
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Background: Myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML) are a group of diseases of the elderly that initiates in a hematopoietic stem cell and are characterized by clonal hematopoiesis and uncertain prognosis, mostly due to cytogenetic background. In both diseases, 5-Azacitidine (5-Aza) has been successful, inducing prolonged survival and delayed AML evolution.

Aims: To identify the genes mostly predictive of treatment response we use high-throughput genomic analysis (SNP arrays and/or NGS-RNA-seq and/or NGS-WES and/or GEP) in azacitidine-sensitive and resistant MDS/AML patients. Furthermore, we sought to correlate the specific alterations with clinical outcome.
Methods: The high-density CytoScan HD Array (Affymetrix, Inc) includes 2.67 million markers for copy number analysis, with 750,000 SNP probes, and 1.9 million non polymorphic (based on Copy Number Variants, CNV) probes for comprehensive whole-genome coverage. The results obtained by SNP Array, were analyzed by Chromosome Analysis Suite (ChAS) v1.2 (Affymetrix Inc.): using this software it is easy to distinguish between aberrations and artifacts, moreover it let to discover which genes are involved in the disease and their type of lesion (*e.g.* loss, gain, loss of heterozigosity, allelic imbalance, high copy gain, homozygous copy loss). We used also another important software, which is Nexus Copy Number™ v7.5 (BioDiscovery): it present the same features of ChAS, but it also allows to compare two or more group of patients with a statistical relevance.
Results: We treated 246 adult patients with MDS or AML: 214 patiens were AML and 32 were MDS with a median age of 59 and 70 years, respectively. Forty-five pts were treated with 5-Aza (32 MDS/13 AML), while 201 AML were treated with conventional chemotherapy. Forty-five MDS/AML patients were treated with at least one complete cycle of 5-Aza (75 mg/sqm/daily). SNP arrays was done in 22/45 (49%), 13 pts were defined "insensitive/resistant". Macroscopic CNAs affecting a complete chromosome or its arms were detected in 5 of 22 pts (23%), while classical cytogenetic was able to detect only two cases of trisomy 8 (9%), suggesting superiority of SNPs array for CNAs identifications. Chromosomic aberrations disease-related are more statistically frequent on patients "insensitive" *versus* patiens "sensitive" (64% *vs* 35%) ($p \leq 0.01$). Moreover we found that from the median of chromosomic alterations lenghts (in kbp) the group of "insensitive" MDS/AML patients to 5-Aza therapy present more losses than "sensitive" ones. By Nexus Copy Number software, we identify 137 genes highly differentially gain (SIRPB1 and KIT with $p \leq 0.05$) or loss (SIRPB1, LCE1C, BCAS1, EXD3 with $p \leq 0.05$) or LOH between "insensitive" *versus* "sensitive" to 5-Aza ($p \leq 0.05$). Among these genes, we focused on SIRPB1 (cytoband 20p13, 56Kbps), since it was loss on 14/22 (64%) "insensitive" pts (p=0,023) and gain on 7/22 (32%) "sensitive" ones (p=0,0324), respectively. SIRPB1 common deletion region length is 27 kbps and the common amplified region length is 30 kbps. By NGS-WES we analyzed 35/214 (16%) AML samples at diagnosis. We found mutations in SF3B1, NPM1, CBL, RUNX1, BCOR, KIT, GATA2, IDH2, KDM6A, KIAA1324L, PRIM2, RRN3, APOBR and again in SIRPB1 an heterozygous missense variant (rs45545343; p. H/D).
Summary and Conclusions: We conclude that SIRPB1 is a promising marker of response in patients with myelodysplastic syndromes and acute myeloid leukemia treated with 5-Azaciditine.
Acknowledgement: Celgene, ELN, AIL, AIRC, progetto Regione-Università 2010-12 (L. Bolondi), FP7 NGS-PTL project.

E1202

RPS14 EXPRESSION IN MYELODYSPLASTIC SYNDROME WITHOUT 5Q DELETION

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Background: Reduced RPS14 expression in myelodysplastic syndrome (MDS) with 5q deletion and the better prognostic of these patients are well demonstrated, however the role of lower expressed RPS14 in MDS without 5q deletion remains unknown.
Aims: We studied RPS14 expression in MDS patients without 5q deletion and the role it plays in their prognosis and survival.
Methods: This study included bone marrow samples of 87 patients, 58 MDS patients (15 with 5q deletion and 43 without 5q deletion), 4 myelomonocytic leukemia, 12 chronic myeloproliferative disorders, 10 acute myeloid leukemia patients and 3 healthy donors; between years 2000 and 2014. We used the ABI PRISM® 7900HT (Applied Biosystems) detection system to quantify RPS14 expression. Statistical analysis was performed using the Statistical Package of Social Sciences (SPSS 15.0). Survival was estimated by the Kaplan-Meier method and compared with the log-rank test.
Results: Myelodysplastic syndrome and chronic myelomonocytic leukemia patients showed RPS14 lower expression than acute myeloid leukemia, chronic myeloproliferative disorders patients and healthy donors (p=0,08). MDS patients with 5q deletion showed lower RPS14 expression when compared to MDS patients without 5q deletion. The 56% (24/43) of MDS without 5q deletion shown low RPS14 expression. The mean follow-up of the patients was 51 months (0,1-172). There was no significant difference for overall survival rate between RPS14 lower and normal expression patients with MDS without 5q deletion. Patients in Intermediate-1 group with RPS14 lower expression, had longer overall survival than Intermediate-1 patients with RPS14 high expression (81% *vs* 50%).p=0.08 (Figure 1).
Summary and Conclusions: Lower expression of RPS14 in MDS without 5q deletion patients is associated with prolonged survival in Intermediate-1 prognosis group.

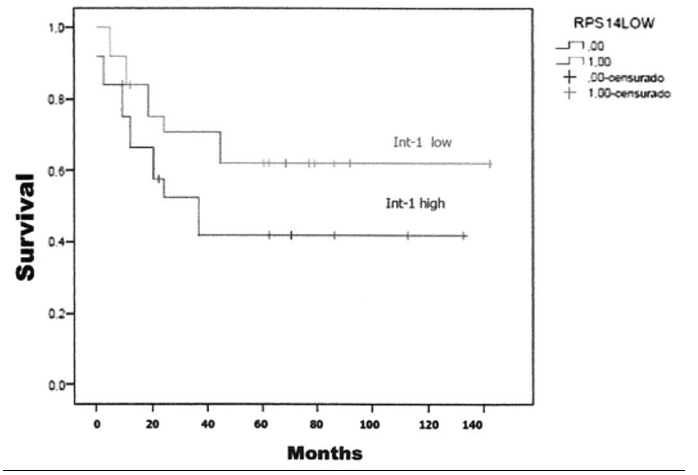

Figure 1.

E1203

AUTOPHAGY LEVEL WAS ABNORMAL IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES

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Background: Myelodysplastic syndromes (MDS) are clonal hematopoistic stem/preginitor cells disorders with cytopenia, myelodysplasia, ineffective hematopoisis, and high risk transform to acute myeloid leukemia (AML). The majority of patients are hepercelluar bone marrow while decreased peripheral blood cells.
Aims: To explore the change of autophagy and its significance in the myelodysplastic syndrome (MDS) patients by detecting the expression level of Beclin 1, mTOR, LC3 in bone marrow mononuclear cells (BMNC).
Methods: Thirty-eight patients with MDS and 26 non-malignant anemia patients were enrolled in this study. The iron content of BMNC was detected by iron staining. The level of reactive oxygen species (ROS) was detected by flow cytometry. The quantity of autophagic vacuoles were detected by transmission electron microscopy (TEM) and monodansylcadaverine (MDC) staining. The LC3 protein positive cells were counted by Immunofluorescence assays. The expression of Beclin 1, LC3A, mTOR mRNA were measured by real time PCR. The expression of Beclin 1 and LC3 proteins were detected by Western blotting.
Results: (1) The iron particles appeared more in MDS patients when viewed under a microscope and the intracellular iron content of MDS patients was also higher than that of controls. (2) The ROS level of MDS patients was significantly higher than that of controls. (3) The autophgic vacuoles of double membrane that surrounds lysosomes appeared in MDS patients. (4) The percentage of MDC positive cells was significantly higher in MDS patients than that of controls. (5) The percentage of LC3 protein cells was also increased in MDS patients (6.13%±1.03% *vs* 1.5%±0.58%, P<0.05. (6) The expression of Beclin 1, LC3A mRNA in low-risk and intermediate-1 MDS were higher compared with controls (3.61±3.02 *vs* 1.55±1.03 and 6.56±3.97 *vs* 1.21±0.95 respectively, both P<0.05. The expression of mTOR mRNA was down-regulated in low-risk and intermediate-1 MDS compared with controls (0.39±0.37 *vs* 1.50±1.03□P<0.05. There were no significant difference in expression of Beclin 1 and mTOR mRNA among the intermediate-2 and high-risk MDS and the controls. (7) The expression of Beclin 1 protein and LC3B were higher in MDS patients than that of controls.
Summary and Conclusions: The enhanced autophagy in low-risk and intermediate-1MDS might be considered as a cell protective mechanism. The relatively defective autophagy in intermediate-2 and high-risk MDS might contribute to its progress to AML.

E1204

CLINICAL RELEVANCE OF IL-6 AND TNF-ALFA IN THE CHARACTERIZATION OF LEUKEMIC STEM CELLS IN MDS PATIENTS

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Background: Myelodysplasic Syndrome (MDS) is considered a clonal hematopoietic stem cell (HSC) disease, whereby the existence of changes in these type of cells, besides being based on the aetiology of the disease can also affect the progression to AML. Recently, a small subset of these cells was identified with unique ability to auto renovate and differentiate into different lineages of cancer cells that comprise the tumour, the cancer stem cells (CSC). These cells have been identified in many types of cancers, in particular cancer of the brain, breast, prostate, colon and pancreas, as well as in multiple myeloma and leukaemia (leukemic stem cells, LSC). Moreover, the development of MDS is accompanied by immune changes and production of antibodies and cytokines, such as IL-6 and TNF-α. However, the evaluation of these cytokines in the LSC and HSC has not been examined in patients with MDS.
Aims: To assess the relevance of IL-6 and TNF-α in HSC and LSC characterization, in MDS patients, in order to identify new prognostic and predictive markers as well as new therapeutic targets.
Methods: Analysis of clinical and analytical data of 102 patients with *de novo* MDS. The distinction between HSC and LSC was performed by flow cytometry using a panel of monoclonal antibodies conjugated with fluorescent probes in the following combinations: CD34/CD117/CD123/GIP and CD34/CD11/CD123/IL-6/TNF-α. All data were analysed using statistical tests (p<0,05).
Results: The sample consisted of 102 MDS patients with median age of 74 years (22-89) and with a ratio Male/Female 0.8. The MDS subtypes according to the WHO are: refractory cytopenia with multilineage dysplasia (RCMD) (n=52), refractory cytopenia with unilineage dysplasia (RCUD) (n=12), refractory anemia with excess blasts (RAEB) -1 (n=8), RAEB-2 (n=8), refractory anemia with ringed sideroblasts (RARS) (n=6), 5q- syndrome (n=4) and chronic myelomonocytic leukemia (CMML) (n=12), with IPSS: low (n=37), intermediate-1 (n=39), intermediate-2 (n=10) and high (n=1). Eleven patients progressed to AML: 7 patients with RAEB-2, 2 patients with RCMD, 1 patient with RAEB-1 and another with CMML. In our study we noticed an increase statistically significant in CD34+ cells in MDS patients in more advanced stages (RAEB-2), as well as in cells that co-express CD117, which can be related to the evolution of these patients to AML. Furthermore, the increase in CD34+/CD117+ cells observed in patients with RCMD may identify those who have a higher risk of leukemic transformation. Analysis of stem cell also allowed the identification of two groups of cells, one phenotypically 'normal' (HSC) and other with neoplastic characteristics (LSC) that differentially express the inflammatory cytokines IL-6 and TNF-α in the several MDS subtypes studied. The expression of IL-6 was observed in the HSC in RAEB-2 and in the LSC in refractory thrombocytopenia subtype. TNF-α was also observed in the HSC in CMML and in the LSC in RAEB-2 subtype. The presence of both cytokines in patients with RAEB-2 (p <0.05) is marked, although IL6 predominates in HSC and TNF-α in LSC. On the other hand, the presence of stem cells with the phenotype CD34+/ CD117+/IL-6+ may influence the progression to AML (p <0.05), and is also associated with a decreased overall survival (p <0.05).
Summary and Conclusions: These results suggest the importance of IL-6 and TNF-α in distinguishing two groups of hematopoietic stem cells in MDS patients, as they could influence the development and progression of the disease, as well as patient survival.

E1205

CONCORDANCE BETWEEN CYTOLOGY AND FLOW CYTOMETRY IN THE DIAGNOSIS OF CHRONIC MYELOMONOCYTIC LEUKEMIA

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Background: The diagnosis of chronic myelomonocytic leukemia (CMML) relies on cytology and cytogenetics, and both play an important role in the prognosis of this entity. Recently, other complementary tests such as flow cytometry (FCM) have been incorporated in clinical practice and become especially relevant in the diagnosis of doubtful cases.
Aims: The aim of this study was to analyze the discrepancies of the percentage of monocytes and the sum of promonocytes plus blasts (PM+BL) between cytology and FCM in CMML
Methods: In this study we carried out a retrospective analysis of the discrepancies between the percentage of monocytes and the sum of promonocytes plus blasts (PM+BL) at diagnosis, in cytological and FC bone marrow aspirate reports of 17 CMML cases diagnosed at our institution between 2010 and 2014. The diagnosis was stabilized according to the 2008 WHO classification. In order to identify myeloid precursors, promonocytes, monocytes, dysplastic changes in the monocytic population, and granulocytic maturation patterns by FCM, a panel with the following antibodies in 2 tubes was used: CD16/CD13/CD34/CD33/CD117/CD11b/CD45/HLA-DR and CD36/CD64/CD34/CD33/CD14/CD11b/CD45/HLA-DR. The acquisition of cells was performed in a flow cytometer BD FACSCanto II™ (BD Biosciences) and data were analyzed using InfinicytTM program (Cytognos). Statistical analysis was performed using SPSS version 21.0. The interclass correlation coefficient (ICC) was used to assess agreement between the 2 diagnostic methods and was interpreted as follows: excellent ICC >0.9; very good ICC 0.7-0.9; good ICC 0.5-0.70; moderate ICC 0.3-0.50, poor ICC <0.30
Results: Table 1 summarizes patient's characteristics, as well as the percentages of monocytes and BL+PM assessed by cytology and FCM. It is worth noting that most cases (15/17) were classified as CMML-1, and in the only 2 patients with CMML-2, the percentage of BL+PM detected by FCM was below 10%. FCM detected dysplastic alterations in all cases, being the most prevalent: hipogranulation in mature neutrophils, abnormal maturation patterns in neutrophils, increased percentage of monocytes, and altered expression of monocytic markers such as CD13 and CD33. We failed to find statistically significant differences in the percentage of BL+PM detected by cytology or FCM respectively (4 *vs* 2.8 P=0.2) neither in the percentage of monocytes (9 *vs* 10.7; P=0.2). In addition, the concordance between the percentages of BL+PM (ICC=0.453) and monocytes (ICC=0.488) by cytology and FCM was moderate.

Table 1. Patients characteristics and percentages of monocytes and BL+PM assessed by cytology and flow cytometry.

Patient	Gender	Age	2008 WHO	FAB	Cariotype	% BL+PM cytology	% BL+PM FCM	% monocytes cytology	% monocytes FCM	Outcome	Follow up (months)
1	Male	72	CMML-1	MD	Normal	2	2.88	9	6.7	Alive, progression	47
2	Male	55	CMML-1	MD	Normal	9	1	5	13.1	Alive, stable disease	25
3	Male	81	CMML-1	MP	Normal	3	2.09	6	6.52	Alive, progression	43
4	Female	91	CMML-1	MD	Normal	8	4.7	5	13.01	Dead	29
5	Female	75	CMML-1	MD	Normal	0	1.23	11	20.03	Alive, stable disease	45
6	Male	76	CMML-2	MP	Normal	17	9.58	9	10.36	Alive, progression	20
7	Male	73	CMML-1	MD	45X, -Y	0	3.34	7	10.01	Dead	27
8	Female	81	CMML-1	MD	Normal	2	4.66	10	12.63	Alive, stable disease	16
9	Female	81	CMML-1	MD	Normal	0	4.71	14	11.49	Alive, stable disease	32
10	Male	68	CMML-1	MD	Normal	0	0.34	25	10.7	Alive, stable disease	29
11	Female	78	CMML-1	MD	47XX, +8	7	3.6	11	11.2	Alive, stable disease	46
12	Male	85	CMML-1	MD	Normal	8	0	3	6	Alive, stable disease	12
13	Male	79	CMML-1	MP	Normal	4	2.62	10	11.35	Dead	14
14	Female	82	CMML-1	MD	Normal	2	3.71	7	6.43	Alive, stable disease	9
15	Male	73	CMML-2	MP	Normal	15	1.28	10	10.01	Alive, stable disease	7
16	Male	58	CMML-1	MD	Normal	4	2.56	9	7.76	Alive, stable disease	6
17	Male	81	CMML-1	MD	Normal	9	8.35	17	16.99	Alive, stable disease	3

Summary and Conclusions: FCM supported the diagnosis of CMML in all our cases, and therefore should be implemented specially in unclear cases by cytology. We could not find adequate concordance between cytology and FCM in the determination of the percentage of BL+PM, and monocytes. Cytology should still be considered the gold standard for the classification of CMML-1 and CMML-2 according to the 2008 WHO criteria.

E1206

MOLECULAR PROFILE OF TRANSFORMED AND NON TRANSFORMED INDIAN PATIENTS WITH MYELODYSPLASTIC SYNDROMES

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Background: Myelodysplastic syndromes are characterized by bone marrow failure and a risk of progression to acute leukemia. So far, blast counts and cytogenetic abnormalities are known as major determinants of the risk for transformation. Molecular pathogenesis and the molecular basis for its progression to AML remain largely undefined. Although some case reports are available but no molecular study from India is available on the transformed MDS cases. Probably this is the only study with largest transformed MDS cases from Indian subcontinent.
Aims: This study was an attempt to look for the molecular changes that may be associated with transformation in MDS patients by comparing the molecular profile of transformed and non transformed cases.
Methods: Both DNA and RNA were extracted from mononuclear cells from patients Blood/ BM. Conventional cytogenetics was used for the detection of karyotypic abnormalities. Nested PCR was used for the screening of RAS gene mutations and RT-PCR was used to detect FLT3-ITD mutation and hTERT expression respectively. Telomerase activity was measured by PCR-ELISA-TRAP Assay. Methylation specific PCR was used to detect the methylation status of TSGs (p15, SOCS-1, FHIT & Calcitonin).
Results: A total of 100 MDS patients were studied, progression was observed in twenty one patients and no progression was found in 79 cases. Of the transformed cases, two patients progressed from RA to AML, 10 patients from RAEB2 to AML, 5 patients with RAEB-1 to AML, 1 patient with RAEB-2 to ALL, 2 patients with RA to RCMD and 1 patient with RAEB2 with del 5q to MF. The median time of progression was 15 months (Range 1-16 months). Of these progressed patients 6 were females and 15 were males. Median age of the transformed patients was 48 years (range : 9-77 years) whereas for non transformed 48 years (range :11-44 years).In non transformed patients favorable cytogentics was more evident than transformed patients (p <0.03).FLT3 mutations was not found in any patient or suppressor genes. The frequency of RAS mutations were significantly higher in transformed patient as compared to non transformed cases. Telomerase activity was significantly increased in transformed patients (p<0.009). Of all the four tumor suppressor genes studied, frequency of hypermethylation was significantly increased for the three genes in transformed patients *i.e.*, p15 (p<0.02), SOCS 1 (p<0.02), FHIT gene (p<0.02). After multivariate analysis of all the factors, $p15^{INK4b}$ gene methylation was found as an independent predictor for progression of disease (HR 5.15, 95% CI, 1.64-16.1 p=0.005) (Figure 1).

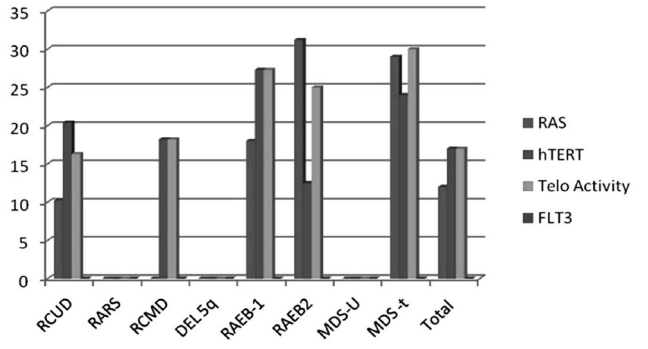

Figure 1. Frequency (%) of mutations within different WHO subtypes of MDS.

Summary and Conclusions: Molecular profiling of the Myelodysplastic Syndromes may give the information about the transformation and prognosis of the patients. Gene methylation may be one of the important factors in the process of transformation.

Myelodysplastic syndromes - Clinical

E1207

MANAGEMENT OF MYELODYSPLASTIC SYNDROMES WITH ERYTHROPOIESIS STIMULATING AGENTS : EVALUATION OF ERYTHROPOIETIC ASPECTS AND ANALYSIS OF RESPONSE

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Background: Erythropoiesis stimulating agents (ESAs) are the frontline treatment in lowriskanemic MDS patients and an employment of this therapy in the earlier stage of the disease can delay the need for RBC transfusion, hypothetically by slowing the disease course. It is matter of debate whether the clinical response is a result of proliferation and maturation of the dysplastic clone or stimulation of residual normal erythropoiesis by ESAs.
Aims: Macrocytosis is one of the cytological hallmarks of dyserithropoiesis in MDS : an analysis of the erythropoietic response to ESAs therapy in a cohort of anemic non trasfusion-dependent MDS patients, enrolled in a retrospective register, RECAMDS, subgroup of Italian MDS register, was performed.
Methods: 137 patients, treated with standard-dose ESAs, have been retrospectively analyzed (Table 1). Data analysis was performed, according to IWG 2006 criteria, at the baseline, after three and six months of continuous treatment, with a subanalysis of the patients according to WHO and R-IPSS risk stratification. ESAs treatment was started at mean Hb concentration of 9.5 g/dl, mean serum EPO concentration : 41mU/L, after a mean time from diagnosis of six months (r.1-118).
Results: Overall response rate (ORR) was 83% (114/137) and no difference among WHO and IPSS subgroups was found :76% achieved response after three months of treatment, while other 7% after six months. 2 patients with stable disease (non responders IWG), in which treatment was continued, achieved response after 9 months. In the macrocytic-responders group 87% exhibits again macrocytosis after 3 months, while 13% become normocytic. In the normocytic-responders group 92% exhibits again normocytosis, while 4/52 (8%) become macrocytic : in these 4 patients after three months there was a contemporary worsening in neutropenia and thrombocytopenia, with transfusion-dependence, regarded as first signs of progression of disease. Non responders were 23/137 (17%) : in the macrocytic-non responders group 89% exhibit again macrocytosis after 3 months, while 11% become normocytic; in the normocytic group 80% exhibits again macrocytosis, while 20% become normocytic : of these 74% become transfusion-dependent at 6 months (median time to transfusion 12 months : range 1-23).

Tablel 1.

MDS PATIENTS	137
M	66 (48%)
F	71 (52%)
WHO	
AR	44 (32%)
DEL 5q	9 (6%)
RAEB-1	5 (4%)
RAEB-2	2 (2%)
RARS	20 (14%)
RCMD	57 (41%)
R-IPSS	
VERY LOW	46 (34%)
LOW	79 (58%)
INTERMEDIATE	10 (7%)
HIGH	2 (1%)
ERYTHROPOIESIS	
BASELINE HB (mean, g/dL)	9.5 g/dL (r. 7.1-11.3)
BASELINE SERUM EPO (mean, mU/mL)	41 mU/mL (r.3-30)
OVERALL RESPONSE RATIO	
RESPONDERS	114/137 (83%)
RESPONDERS AT 3 MONTHS	105/137 (76%)
RESPONDERS AT 6 MONTHS	9/137 (7%)
RESPONDERS AT 9 MONTHS (NON RESPONDERS IN IWG 2006)	2/137 (2%)
NON RESPONDERS	23/137 (17%)

Summary and Conclusions: These preliminary data can suggest that, in the majority of MDS patients responsive to ESA treatment, the increase of hemoglobin concentration occurs mainly stimulating erythroid production in MDS clones; in the minority of patients probably it happens recruiting residual polyclonal erythropoiesis. It is interesting to note that stimulating effects of ESAs last even when the expression of dysplasia progresses.

E1208

IMPACT OF TREATMENT WITH RECOMBINANT HUMAN ERYTHROPOIETIN ALPHA ON CARDIAC REMODELING IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES: RESULTS FROM A PROSPECTIVE STUDY

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Background: Myelodysplastic syndromes (MDS) are typical diseases of the elderly. The most frequent symptom is anemia. Both age and comorbidities have a relevant negative impact on the clinical outcome. In the case of a concomitant cardiac disease, the presence of anemia and its related iron overload, frequently observed in transfusion-dependent MDS pts, synergistically act with a harmful effect on the probability of survival. It is not a case that cardiac disease is the more common single comorbidity observed in the majority of MDS cohorts. Erythropoiesis stimulating agents (ESAs) have been demonstrated to reduce the transfusion requirements in pts affected by MDS with low/Intermediate-1 IPSS. Anemia appears to be strongly associated with cardiovascular morbidity and mortality.
Aims: Aim of our study is to investigate of early treatment with rHuEPO alpha may reverse cardiac remodeling, improve Ejection Fraction (EF) and reduce cardiovascular morbidity in "lower risk" MDS pts.
Methods: From January 2013 to January 2015, 40 MDS pts were included in a prospective observational multicentric study; 15 females and 25 males, of mean age 78 (range 61-92). According to the WHO 2008 classification, morphological MDS diagnosis were as follows: 17% Refractory Anemia (RA), 55% Refractory Cytopenia with Multilineage Dysplasia, 8% Refractory Anemia with Ringed Sideroblasts, 2% del 5q syndrome, 17% Refractory Anemia with excess of blasts-1. All pts had PSS low or int-1 risk and epo serum levels <500 mU/L.ESAs treatment was allowed when hemoglobin (Hb) was lower than 10.5 g/dL. All pts were analyzed with echocardiography before starting ESAs and every 6 months during the treatment. All echocardiography measurements were carried out according to the recommendations of the American Society of Echocardiography by un external investigator. Left ventricular mass (LVM) was calculated according to the Devereux formula and indexed to height. Left ventricular hypertrophy (LVH) was defined by a LVM index >47 in women and >50 in men. We also analyzed comorbidity by CIRS and cardiovascular risk factors. All pts were treated with rHuEPO alpha 40000 U weekly.
Results: At baseline all patients were transfusion-free and with mean Hb concentration of 9.66 g/dl. The rate of erythroid response to rHuEPO according to IWG criteria 2006 was 54%. No thrombotic events was eported. One or more comorbidity of any grade of severity was seen in 90% of pts at diagnosis. The more common comorbidity was cardiac (62,5%). Cardiovascular disorders were more frequent among older subjects (52% in >75y *vs* 30% in <75y), and among males. The median EF at baseline was upper than 55% and half pts had normal LVM. In 90% of pts with erythroid response EF was stable, in 10% improved and in 0% decreased while LVM was reduced in 35% of pts. The EF improvement and decrease of LVM was more evident in pts with cardiovascular comorbidities. None pts among "responders group" had cardiac comorbidity worsening ooor hospitalization. Anemia improvement and FE increase were associated with an imanananprovement of quality of life (QOL). The median observation time was 10 months (range 1-24). During the study period, 4 patients (10%) have died, respectively: 2 sepsis, 1 hearth failure, 1 leukemia evolution.
Summary and Conclusions: rHuEPO alpha treatment is effective and safe also in pts with cardiac comorbidities. In responders "lower risk" MDS pts, early treatment has been shown to improve FE and reverse cardiac remodeling with conseguence a reduction of cardiovascular risk/ hospitalization and improvement qment men uality of life. In pts with cardiac comorbidities the benefit of early treatment was more evident. The aim of our ongoing prospective study is to demonstrate the relationship between Hb levels, FE and LVM, cardiovascular events and mortality. This correlation is more evident in patients with cardiac comorbidities. Further larger studies are warranted to confirm these data and to evaluate if rHuEPO treatment could be worthwhile in term of cost-efficacy in the very old pts.

E1209

ACUTE MYELOID LEUKEMIA PROGRESSION IN ARGENTINEAN PATIENTS WITH MYELODYSPLASTIC SYNDROMES.

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Background: At least, one third of patients with Myelodysplastic Syndromes (MDS) develop Acute Myeloid Leukemia (AML) during the follow-up depending on diverse prognostic factors and their respective group of risk. Once the leukemic evolution occurs, the outcome of patients is very poor with short survival.
Aims: To analyze prognostic variables and scoring systems regarding leukemic evolution in Argentinean population. Also, to test each category of risk trying to access differences in terms of overall survival in patients with or without leukemic evolution.
Methods: This is a multicentric retrospective study of 966 MDS patients (532 patients belonging to the Argentinean MDS Registry sponsored by de Argentinean Society of Hematology and the remaining to a previous multicentric study) diagnosed from 1981 to 2014. Patients were classified following FAB and WHO criteria and 59 patients presented with secondary MDS. The median age was 66.7 (14-95) years with 74.6% above 60 years, a male/female (546/420) ratio of 1.3. During the follow-up (median: 18.3 months), 424 (43.9%) died and 211 (25%) evolved to AML. BM transplanted patients were censored till the moment of the procedure.
Results: Age (limit of 60 years), percentage of bone marrow blast (0-2, 3-4, 5-9, ≥10%), hemoglobin level (<8, 8-<10, ≥10 g/dL), platelets (<50, 50-100, >100x 10^{12} /L) and neutrophil count (limit of 800 /μL), cytogenetic group of risk (according to the IPSS-R), and red blood cell transfusion requirements were significant predictive variables for leukemic evolution (Kaplan-Meier and Long-Rank test, p<0.05). FAB and WHO classifications and scoring systems (IPSS, IPSSR) also allowed us to differentiate groups of risk for evolution to AML.
The majority of prognostic categories showed statistical differences when we tried to ascertain if patient that evolve to AML depict differences in term of survival when compared with patients that do not evolve (Table 1). However, higher blast counts, worst cytogenetic findings and, correspondingly, higher IPSS and IPSSR groups of risk showed similar survival regardless their leukemic progression. Among patients who died during the follow-up, those patients that previously evolved to AML showed lower age (p<0.001), hemoglobin level (p=0.022), neutrophil (p<0.001) and platelet count (p=0.015), and higher blast percentages (p<0.001)

Table 1.

	With Evolution to AML		Without Evolution to AML		
	Pts (N)	Survival (50%, months)	Pts (N)	Survival (50%, months)	*p value*
Age					
<60	77	23	165	120	*<0.001*
≥60	129	15	584	64	*<0.001*
Gender					
Male	131	15	411	50	*<0.001*
Female	77	18	339	121	*<0.001*
Hemoglobin (g/dL)					
<8	64	14	145	32	*<0.001*
8-<10	74	13	213	64	*<0.001*
>10	51	26	356	98	*<0.001*
Platelet count (x10^9/L)					
<50	63	13	112	27	*=0.009*
50-99	23	11	58	25	*=0.006*
≥100	106	18	549	94	*<0.001*
Neutrophil count (/μL)					
<800	58	14	88	70	*<0.001*
≥800	136	17	630	75	*<0.001*
Cytogenetic Risk					
Very Good	5	**44**	21	**NR**	*=0.328*
Good	93	17	481	80	*<0.001*
Intermediate	35	20	96	37	*=0.001*
Poor	18	**18**	25	**25**	*=0.748*
Very Poor	23	7	28	**13**	*=0.238*
Blast (%)					
0-2	47	16	481	82	*<0.001*
3-4	22	28	87	80	*<0.001*
5-9	57	17	94	22	*=0.077*
≥10	76	**13**	65	**18**	*=0.119*
IPSS					
Low	8	18	269	121	*<0.001*
Int-1	65	23	227	64	*<0.001*
Int-2	40	**18**	64	**19**	*=0.396*
High	36	**12**	22	**13**	*=0.754*
IPSS R					
Very Low	6	17	161	136	<0.001
Low	30	30	232	75	<0.001
Int	25	19	62	63	=0.022
High	41	14	42	21	=0.013
Very High	24	**12**	35	**13**	**=0.904**

Summary and Conclusions: Clinical parameters, age, classifications and the applied scoring systems were useful tools to evaluate prognosis in our series that showed a similar rate of leukemic progression to previous reports. Interestingly, the survivals in patients with higher blast percentages, worst cytogenetic findings and, correspondingly, higher IPSS and IPSSR groups of risk were similar between patients with or without evolution to AML.

E1210

PULMONARY INFECTIONS IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES RECEIVING AZACYTIDINE TREATMENT

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Background: Azacytidine (AZA) is a demethylating agent widely used in the treatment of patients with high-risk Myelodysplastic Syndromes (MDS). Pulmonary infections represent the most frequent complication in these patients, however, their incidence, etiology and impact in the overall outcome during AZA treatment are still unclear. A major problem in the characterization of infections in MDS patients receiving AZA treatment is represented by the low level of clinical and microbiological documentation being most of patients managed on an outpatient basis.
Aims: Aim of this study was to evaluate the incidence and clinical role of pulmonary infections in a cohort of MDS patients receiving AZA treatment in our Institute, where the availability of a dedicated hematological emergency ward made their access and their infective diagnostic work-up homogeneous in the presence of fever or other infective clinical signs.
Methods: We retrospectively evaluated 86 MDS patients (M/F 52/34, median age 70 years, range 41-82) treated with AZA at our Institution from 04/2009 to 01/2015. All patients received AZA cycles at standard dosage (75 mg/m^2 for 7 days every 28 days) as outpatients. Microbiological work-up included blood cultures, culture from other sites, galactomannan assay from serum and from sputum/bronchoalveolar lavage.
Results: The total number of AZA cycles was 988, with a median of 8 cycles per patient (range 1-56 cycles). There were 41 episodes of lung infection documented by chest CT in 32 patients (37.2% of patients and 4.1% of AZA cycles). Based on the above diagnostic work-up, pulmonary infiltrates were considered of fungal origin in 9 cases (21.9%), associated to bacteremia in 3 cases (7.3%) and of unknown origin in the remaining 29 cases (70.7%). As to the time of occurrence of lung infections, 12 episodes were documented in the first 2 cycles of AZA treatment (14% of 86 patients), 13 after the cycles 3-5 (13.3% of 71 evaluable patients), and the remaining 16 episodes beyond the fifth cycle of AZA treatment (29.6% of 54 evaluable patients) (Figure 1). Overall, a pulmonary fungal disease was documented in 6 of 165 (3.6%) cycles 1-2, in 1 of 196 (0.5%) cycles 3-5 and in 2 of 627 (0.3%) cycles since cycle 6 (p=0.001). Out of 32 patients who developed a pulmonary infection 25 (78%) interrupted the AZA treatment within 3 months from the infectious episode due to deterioration of clinical conditions, hematologic disease progression and/or death, including 8 of 9 (89%) patients who developed a pulmonary fungal disease.

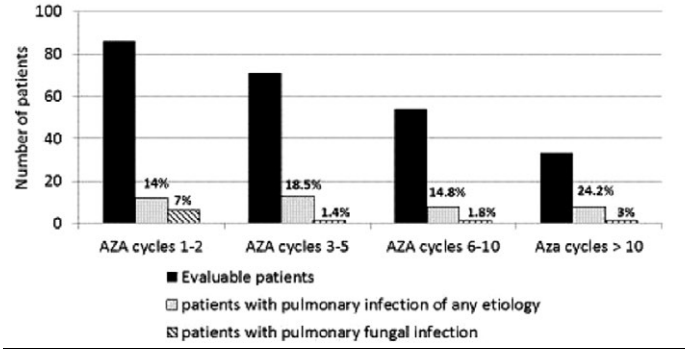

Figure 1. Incidence of pulmonary infections according to AZA cycles.

Summary and Conclusions: In conclusion, pulmonary infections are a common complication in MDS patients receiving AZA treatment, and are often associated to an interruption of AZA therapy. Pulmonary fungal infections are more frequently observed early during the first cycles of treatment. It should be defined if the poor outcome of patients who develop pulmonary infections during AZA therapy is an epiphenomenon of an immunologic deterioration associated to the hematologic disease progression or is independently related to the complication. If confirmed in other experiences, the results of our study raise the issue of the need of an antibacterial and/or antifungal prophylaxis particularly during the first months of AZA therapy.

E1211

HIDDEN MDS: A PROSPECTIVE STUDY TO CONFIRM OR EXCLUDE MDS IN PATIENTS WITH ANEMIA OF UNCERTAIN ETIOLOGY

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Background: Anemia is one of the most frequent abnormalities detected in hemograms and it can be the first sign of MDS, especially in the elderly patients. Diagnosis of MDS when anemia is the only abnormality can be complicated, so the diagnosis is delayed until the degree of anemia becomes severe (transfusion dependency) and/or other cytopenias/blasts appears.
Aims: To analyze the impact on the diagnosis of MDS among anemic patients, by systematically and prospectively reviewing all hemograms performed in 3 centers in Spain.
Methods: We performed a prospective study within 69 days (Dec 2012-March 2013). Ethic committee approved the protocol as an observational study. All hemograms performed in the laboratories (137,453 hemograms) were reviewed. Those with Hb level <12g/dL and/or MCV >100 fL were selected (2,702 patients). Surgery and oncology departments patients or with a known disease causing anemia were excluded. In the second step, a complete peripheral blood analysis was performed (reticulocyte count, peripheral blood smear, Vitamin B12, folic acid, ferritin, renal, thyroid and hepatic function). The results were reviewed with the clinical history and if no cause of anemia/macrocytosis was found, the patient was referred to the haematologist (n=221).
Results: From 137,453 hemograms, we selected 211 candidates to MDS screening. 133 final diagnoses have been made (see Table 1), included 12 MDS. 23 patients have currently been investigated. In 117 patients, we could not complete the study due to comorbidity/age >90 years (N=99) or death (N=18).

Table 1.

	Salamanca	Hospital de la Ribera	German Trias i Pujol	TOTAL
Days	69	63	37	169
Nº of revised hemograms	60.655	53.863	22.935	137.453
Revised patients	442	2.125	135	2.702
Patients candidates	182	101	7	211
Total diagnostics	93	36	4	133
In study	22	1	0	0
Hematological malignacies detected	5 MDS 2 MGUS 2 TTP 1 AML 1 cMPN Ph(-) 1 AA	7 MDS 1 MGUS		12 MDS 3 MGUS 2 TTP 1 AML 1 cMPN Ph (-) 1 AA
Iron deficiency	57	0	1	58
Hypothyroidism	4	3	0	7
Megaloblastic	15	24	0	39
Others	5	1	3	9
Not included (age/comorbidities)	89	9	1	99
Death	18	0	0	18

Summary and Conclusions: This prospective approach is a reasonable screening procedure in the hematological laboratories and will probably allow us to diagnose the more relevant causes of anemia including MDS, early, avoiding delay in providing the patients with the necessary intervention.

E1212

EFFECTIVENESS OF AZACITIDINE COMPARED TO CONVENTIONAL CARE REGIMENS FOR THE TREATMENT OF HIGH-RISK MYELODYSPLASIA IN THE REAL-WORLD: RESULTS FROM THE DUTCH POPULATION-BASED PHAROS MDS REGISTRY

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Background: In 2009, azacitidine (AZA) was registered in Europe for the treatment of transplant-ineligible patients with high-risk myelodysplastic syndromes (HR-MDS). Population-based studies assessing its effectiveness compared with that of conventional care regimens (CCRs) in daily practice are lacking.
Aims: In this population-based study, we assessed the effectiveness of AZA on overall survival (OS) in HR-MDS compared with that of two CCRs.
Methods: We identified 515 transplant-ineligible, WHO-defined MDS patients diagnosed between 2008-2011 from the Dutch PHAROS MDS registry, which encompasses ~40% of the Dutch population (31 centers) and is notified by the nationwide Netherlands Cancer Registry. The PHAROS MDS registry includes data on patient features at baseline, classification, prognostication, treatment, response to treatment and survival. All cases were confirmed by bone marrow

examination. Overall, 151 (29%) patients were lower-risk and 113 (22%) higher-risk by IPSS. In the remaining 251 (49%) patients, an IPSS score could not be computed mainly due to unperformed cytogenetics. CCRs were defined as best supportive care (BSC) only, including treatment with growth factors, and intensive chemotherapy (IC). OS was measured from time to HR-MDS diagnosis, which was estimated by the Kaplan-Meier method and compared by the log-rank test. Unstratified Cox proportional hazard models were used to estimate the hazard ratio (HR). Hematological response was assessed according to the revised IWG-MDS criteria (Cheson 2006). Grade 3-4 adverse events (AEs) were graded by CTCAE v3.

Results: Of all 113 HR-MDS patients, 65 received AZA, 32 BSC only and 16 IC. As expected from a real-world population, patients who received BSC only were older and had poorer ECOG performance status (ie, ≥2) than patients who received AZA or IC. Their median age (range) was 77 (52-88), 74 (55-83) and 66 (52-75) years, and 25%, 3% and 0% had an ECOG score ≥2, respectively. After a median follow-up of 14.7 months, the median OS (95% CI) was 17.6 months (15.1 to 24.7) in the AZA group *versus* 9.1 months (3.5 to 14.3) in the BSC only group (HR 0.60, *p*=.027; Figure 1A) and 19.0 months (4.8 to 25.3) in the IC group (HR 0.73, *p*=.321; Figure 1B). One-year survival (95% CI) was 72% (59% to 81%) in the AZA group *versus* 38% (21% to 54%, *p*=.005) and 75% (46% to 90%, *p*=.935) in the BSC only and IC group, respectively. AZA and IC were given for a median (range) of 7 (1-26) and 2 (1-3) cycles, respectively, and BSC only for a median of 3.9 (0.0-26.4) months. Responses of CR, PR and mCR were seen in 12%, 3% and 15% in the AZA group, 38%, 0% and 31% in the IC group and 0%, 0% and 0% in the BSC only group, respectively. Further, hematological improvements were seen in 42%, 38% and 0% in the AZA, IC and BSC only group, respectively. Grade 3-4 anemia, thrombocytopenia and neutropenia, respectively, were seen during treatment in 48%, 52%, and 71% with AZA, 44%, 88% and 69% with IC and 44%, 40%, and 19% with BSC only.

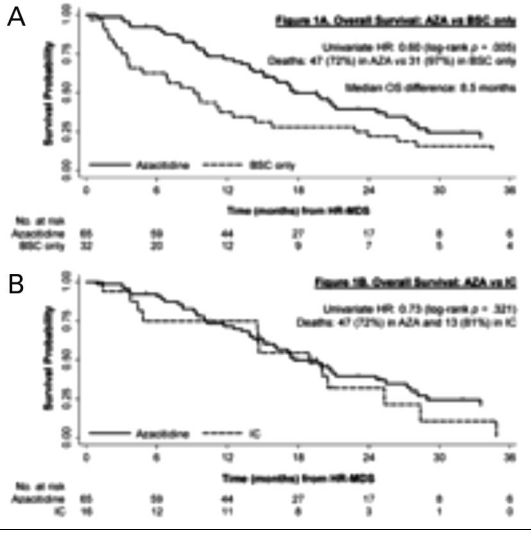

Figure 1.

Summary and Conclusions: In our population-based study, AZA prolonged median OS compared with BSC only, but not with IC. Overall response rates and grade 3-4 hematological AEs were similar with findings from the AZA-001 trial (Fenaux 2009). However, our real-world patient population experienced poorer survival than reported in the AZA-001 trial. Population-based studies provide data complimentary to findings from clinical trials and are important to assess uptake and outcome of new interventions in daily practice and may be part of guideline development. Our results, however, should be interpreted with caution due to the modest patient numbers and the fact that population-based studies are uncontrolled and confounded by indication.

E1213

CLINICAL CHARACTERISTICS, TREATMENT AND OUTCOMES OF PATIENTS WITH THERAPY-RELATED MYELODYSPLASTIC SYNDROMES AND ACUTE MYELOID LEUKAEMIA (T-MDS/AML)

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Background: Therapy-related myeloid neoplasms (t-MN) include therapy-related acute myeloid leukaemia (AML), therapy-related myelodysplastic syndromes (MDS) and therapy-related myelodysplastic syndrome/myeloproliferative neoplasms MDS/MPN) occurring as a late complication of cytotoxic chemotherapy or radiation. t-MN is becoming increasingly common and known to be associated with poor outcomes.

Aims: To identify incidence, characteristics, treatment and outcomes of t-MN patients in our centre.

Methods: We investigated the clinical characteristics, treatment and outcomes of patients with t-MN diagnosed in a single centre between 2003 and 2014 and compared with 198 *de novo* MDS patients for whom we have comprehensive data.

Results: Of 446 patients with AML (152) or MDS (294) diagnosed between 2003 and 2014, we identified 39 patients with features of t-MN (8.7%); 35 patients with t-MDS (11.9%) and 4 with t-AML (2.6%). Median age at presentation and gender distribution were similar to *de novo* MDS with 25.6% of patients under 65 years. The degree of cytopenias at presentation was greater in t-MN group but the percentage of marrow blasts at diagnosis was comparable between two groups. Twenty two patients (56.4%) had a prior haematological malignancy, 10(25.6%) had solid tumours and 3 (7.7%) had cytotoxic therapy for autoimmune disorders. Thirty four patients (87%) received chemotherapy, 12(31%) received radiotherapy and 7(18%) received combined chemotherapy and radiation as primary therapy. The time from therapy to development of t-MN was shortest for patients with haematological disorders (45.5 months) compared to solid tumours (84.5 months) and autoimmune disorders (109 months). Patients who developed t-MN after acute leukaemia had had topoisomerase II inhibitors with other cytotoxic agents and were found to have the shortest latency (16.5 months) and overall poor survival. A higher proportion of patients with higher risk cytogenetics were observed in t-MN group compared to *de novo* MDS. Forty one percent of patients had a normal karyotype and were observed to have better survival (31 *vs* 13 months). Improved survival was associated with very low, low and intermediate risk IPSS-R. Twenty two patients received best supportive care, 12 received Azacitidine and 5 received intensive chemotherapy, of which 2 had allogeneic marrow transplantation. For patients treated with Azacitidine, a median of 4 courses were given with 25% achieving partial response, haematological improvement or stable disease. Median overall survival for all patients was 14 months (0-122) compared to 30 months (0-156) in *de novo* MDS. Survival declined markedly after two years and 5-year survival was only 13.8%. Improved survival was associated with blast count <5%, prior radiotherapy only, lower risk IPSS-R and a normal karyotype. Patients who received Azacitidine had similar survival to those who were treated with intensive chemotherapy (10.5 *vs* 14 months) (Table 1).

Table 1. Characteristics, survival and treatment of patients with t-MN.

Category	Number (%)	Latency (months)	Survival (months)
Primary disorder			
Haematological disorder	**22(56.4%)**	**45.5 (3-132)**	**14 (0-74)**
▪ Acute leukaemia	6	16.5 (3-46)	14 (5-57)
▪ Mycloma	6	45 (24-110)	20 (1-74)
▪ Lymphoproliferative disorders	9	69 (22-132)	14 (0-59)
▪ MPN	1	---	---
Solid Tumour	**10 (25.6%)**	**84.5 (12-366)**	**37 (0-122)**
▪ Breast	3	60 (23-152)	8 (5-96)
▪ Prostate	3	44 (12-130)	46 (4-68)
▪ Others	4	163 (66-366)	37 (0-122)
Autoimmune disease[1]	**3 (7.7%)**	**109 (28-131)**	**24 (18-29)**
More than one disorder[2]	**4 (10.3%)**	**44 (24-103)**	**8.5 (5-13)**
Primary therapy			
Chemotherapy alone	27 (69.2%)	54 (0-163)	14 (0-122)
▪ Alkylating agents	22	71 (23-163)	12.5 (0-96)
▪ Topoisomerase II inhibitors	17	54 (3-152)	14 (1-96)
▪ Others	25	---	---
ASCT	3	83 (32-110)	30 (5-74)
Radiotherapy alone	5 (12.8%)	71.5 (12-366)	42 (0-68)
Combined chemotherapy & radiotherapy	7 (17.9%)	84 (23-153)	7 (5-96)
Diagnostic parameters			
Blast count in bone marrow			
▪ <5%	24 (61.5%)	---	21.5 (0-122)
▪ >5%	15 (38.5%)	---	8 (1-96)
Cytogenetics (available in 34 patients)			
Normal	**14 (41.2%)**	**67 (3-366)**	**31 (4-122)**
Abnormal	**20 (58.8%)**	**45 (12-152)**	**13 (0-96)**
▪ Complex>3 abnormalities	8	---	---
▪ Monosomy 7	2	---	---
▪ Translocation of chromosome 3	1	---	---
▪ MLL rearrangement	2	---	---
▪ Trisomy 8	1	---	---
▪ Any other single or double abnormalities	6	---	---
IPSS-R (available in 32 patients)			
Lower risk	**16 (50%)**	**61 (3-366)**	**31 (0-122)**
▪ Very low	5	66 (13-132)	29 (5-122)
▪ Low	7	68 (3-366)	44 (30-59)
▪ Intermediate	4	40.5 (22-74)	7.5 (0-36)
Higher risk	**16 (50%)**	**49 (0-152)**	**10.5 (1-96)**
▪ High	8	44.5 (14-131)	13.5 (4-96)
▪ Very high	8	77 (0-152)	7 (1-14)
Treatment for t-MN			
Supportive care only	22 (56.4%)	---	28 (0-122)
Azacitidine	12 (30.8%)	---	10.5 (5-57)
Intensive chemotherapy	5 (12.8%)	---	14 (5-96)
Allogeneic stem cell transplant	2 (5.1%)	---	---

MPN- Myeloproliferative neoplasm; 1- Autoimmune haemolytic anaemia, Rheumatoid arthritis, Fibrosing alveolitis (received either Methotrexate or Azathioprine); 2- Inflammatory bowel disease/Prostate cancer, AML/Prostate cancer, Ovarian cancer/Breast cancer, Myeloma/Urothelial cancer; ASCT- Autologous stem cell transplant; t-MN- Therapy-related myeloid neoplasms

Summary and Conclusions: Patients with t-MN showed more adverse features of MDS and cytogenetics hence have lower survival. A longer latency period and better survival is observed in patients who received prior radiotherapy only. Patients with low blast count at presentation, normal karyotype and radiotherapy only as primary treatment had a survival advantage. The use of IPSS-R can identify patients with good outcome and can be useful in risk-adapted approach to management. Azacitidine is effective in selected patients not fit for intensive therapies; however no survival advantage was noted in our small cohort.

E1214

CLINICAL FEATURES, TREATMENT DECISIONS AND TREATMENT OUTCOMES OF PATIENTS WITH CHRONIC MYELOMONOCYTIC LEUKEMIA (CMML) IN THE REAL-WORLD: RESULTS FROM THE DUTCH POPULATION-BASED PHAROS MDS REGISTRY

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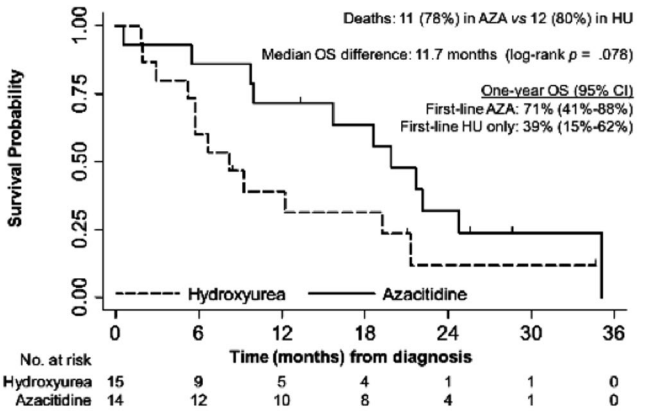
Background: CMML is a very rare hematological malignancy characterized by features of both myelodysplastic syndromes and myeloproliferative neoplasms. Clinical practice guidelines for the management of CMML are ill-defined due to the scarcity of CMML-specific phase 3 clinical trials. Clinical characterization and clinical decision-making can be supported by information gained from population-based studies.
Aims: To present clinical features, treatment decisions and treatment outcomes of newly diagnosed CMML patients in daily practice in the Netherlands.
Methods: We identified 85 CMML patients (median age 74 years, age range 54-94 years, 63% males) diagnosed between 2008-2011 from the Dutch population-based PHAROS MDS registry, which encompasses ~40% of the Dutch population (31 centers) and is notified by the nationwide Netherlands Cancer Registry. For all cases, the diagnosis was confirmed by bone marrow examination and classified according to WHO 2008 classification. The PHAROS MDS registry includes data on patient characteristics at baseline, classification, prognostication, treatment, response to treatment and survival. Overall survival (OS) was measured from time of diagnosis until death or latest follow-up.
Results: Of all patients, 72% were diagnosed in patients over 70 years of age. The vast majority of patients had an ECOG performance score ≤1 (92%) and ≥1 co-morbidity at diagnosis (84%). Overall, 84% patients had CMML-1 and 16% CMML-2. Cytogenetic assessments were performed in 52 (62%) patients, of which 73%, 12% and 15% had good, intermediate or high-risk cytogenetics based on the definition used in the CPSS (Such 2013), respectively. Cytogenetic assessment decreased with older age from 83% in 18-59-year-olds to 44% in over 80-year-olds (*p* for trend=.027). Data on *ASXL1* mutational status were not available for patients diagnosed during the study period of 2008-2011. A wait-and-see strategy was applied in 32 (38%) patients, of which 11 (34%) ultimately started with therapy after a median of 8.9 months after diagnosis. Of 64 patients who received first-line therapy, 19 received hydroxyurea (HU) only, 19 transfusions only, 14 AZA, 11 EPO and 1 lenalidomide. Only 14 (22%) of 64 patients received second-line therapy: 9 received AZA, 2 HU, 2 transfusions only and 1 proceeded to an allograft. With respect to treatment outcome, we assessed the outcome of first-line AZA (*n*=14, median age 72.5 years; 93% ECOG score ≤1) *vs* first-line HU without cross-over to AZA (*n*=15, median age 72 years; 87% ECOG score ≤1). Their characteristics are shown in Figure 1. Median OS (95% CI) was 19.9 (9.8-24.8) *vs* 8.2 (2.9-19.3) months in the AZA and HU treated group, respectively (log-rank *p*=.078; Figure 1). AZA was given for a median (range) of 5.5 (1-30) cycles and HU for a median of 5.1 (0.3-35.8) months. Hematological improvements per revised IWG-MDS criteria (Cheson 2006) were observed in 29% and 0% in the AZA and HU treated group, respectively.
Summary and Conclusions: In our population-based study, risk-stratification by means of cytogenetic assessment decreased with older age. Without cytogenetics, accurate risk-stratification cannot be made, which possibly could lead to inappropriate, risk-adapted management. Although limited by small patient numbers, there was a trend towards higher median OS in patients receiving AZA as opposed to those receiving HU only. In the absence of CMML-specific phase 3 trials, population-based studies are important for providing data on clinical characteristics and treatment outcomes of CMML patients.



Variable	AZA (*n* = 14)	HU (*n* = 15)	*P*	Variable	AZA (*n* = 14)	HU (*n* = 15)	*P*
Male sex	57%	53%	1.0	**CPSS cytogenetics**			
CMML-2	36%	27%	.70	Low	43%	53%	.016
Leukocytes, x 10^9/L				Intermediate	0%	13%	
Median	31.7	45.7	.14	High	43%	0%	
Range	3-99	8-196		Not performed	14%	33%	
Elevated LDH	62%	64%	1.0				

Figure 1.

E1215

A NEW PROGNOSTIC INDEX TO PREDICT SHORT-TERM PROGNOSIS IN MDS PATIENTS TREATED WITH AZACITIDINE; COMBINATION OF P53 EXPRESSION AND CYTOGENETICS

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Background: Azacitidine (Aza) is a currently used demethylating agent for myelodysplastic syndrome (MDS). Since Aza epigenetically modulates various genes, there may be a new prognostic factor to predict outcome of MDS patients treated with Aza. p53 is a tumor suppressor protein encoded by the *TP53* gene. Although it was reported that p53 expression was associated with poor outcome in del(5q) myelodysplastic syndrome (MDS) patients treated with lenalidomide, its value is controversial in MDS patients treated with Aza.
Aims: In this study, we intended to identify prognostic factors including p53 expression of bone marrow in the era of Aza for MDS.
Methods: We retrospectively analyzed 60 consecutive MDS patients treated with Aza. To assess p53 protein expression, immunohistochemical analyses of bone marrow clot sections were performed. Patients were categorized as p53-positive when the percentage of p53-positive cells was 5% or more. The prevalence of p53 expression was analyzed as a prognostic factor.
Results: Median age was 73 years old (range 48-90). In 29 patients, p53 was positive. Numbers of patients according to the World Health Organization (WHO) (2008) classification were 4 refractory cytopenia with unlineage dysplasia, 15 refractory cytopenia with multilineage dysplasia, 16 refractory anemia with excess blasts with type 1, 14 refractory anemia with excess blasts with type 2 and 11 acute myeloid leukemia, and those according to WHO classification-based prognostic scoring system (WPSS) were 2 very low, 10 low, 9 intermediate, 24 high and 15 very high. There was no significant difference of WPSS distribution between p53-negative and -positive patients. As to cytogenetic abnormalities, it was normal in 29 patients, complex or monosomy7 (poor cytogenetics) in 14 and other abnormalities in 17. There was more patients with poor cytogenetics in p53-positive cohort than those in p53-negetive cohort (P=0.03). More patients in p53-negative cohort responded to azacitidine treatment than those in p53-positive cohort according to International Working Group response criteria revised in 2006 (42% *vs* 17%; P=0.04). Overall survival (OS) was significantly lower in p53-positive patients compared with p53-negetive patients (85% *vs* 59% at 12 months after the start of Aza administration; P=0.006). Multivariate analysis demonstrated that p53-positive was a significant prognostic factor for OS along with poor cytogenetics [p53-positive: Hazard ratio (HR) 1.26, 95%CI 1.04-1.53; P=0.02, poor cytogenetics: HR 3.72, 95%CI 1.52-9.11; P=0.004]. We propose a new prognostic index to predict short-term prognosis of MDS patients in the era of Aza; High: p53-positive and poor cytogenetics, Intermediate: p53-positive or poor cytogenetics, and Low: p53-negetive and absence of poor cytogenetics. OS was significantly different among

three groups according to this new prognostic index (Low 92%, Intermediate 65% and High 27% at 12 months; P<0.0001) (Figure 1).

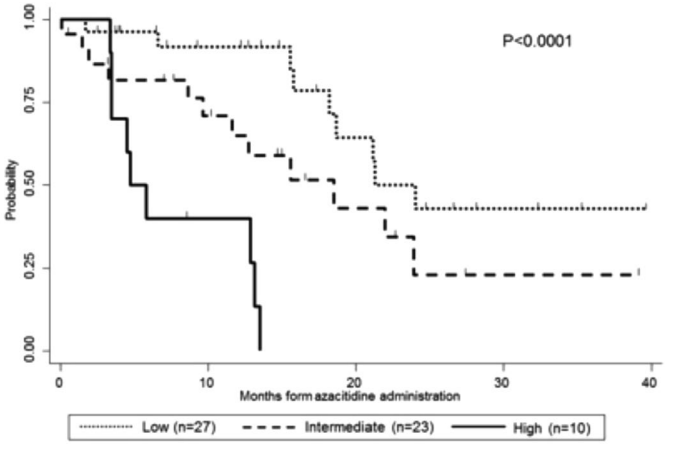

Figure 1.

Summary and Conclusions: p53 expression of bone marrow was a significant prognostic factor in MDS patients treated with Aza. In combination with cytogenetic abnormalities, it is possible to predict short-term prognosis.

E1216

DIAGNOSIS, PROGNOSTICATION AND TREATMENT OF MYELODYSPLASTIC SYNDROMES (MDS) IN DAILY PRACTICE: RESULTS FROM THE DUTCH POPULATION-BASED PHAROS MDS REGISTRY

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Background: The Dutch Population-based HAematological Registry for Observational Studies (PHAROS) in MDS is established to document clinical features, management and outcomes of newly diagnosed MDS patients in the Netherlands. The PHAROS registry documents data in addition to the minimal dataset collected by the nationwide Netherlands Cancer Registry (NCR). The purpose of the PHAROS MDS registry is to provide insight into the delivery of care to MDS patients in order to improve the quality of diagnosis and management of MDS patients in the Netherlands.
Aims: To assess clinical features, classification, prognostication and management of newly diagnosed MDS patients in daily practice in the Netherlands.
Methods: We identified 536 WHO-defined MDS patients (median age 75 years, age range 23-94 years, 60% males) diagnosed between 2008-2011 from the PHAROS MDS registry, which covers ~40% of the Dutch population (31 centers) and is notified by the NCR. All cases were confirmed by bone marrow (BM) examination. The PHAROS MDS registry includes data on patient characteristics at baseline, classification, prognostication, treatment, response to treatment and survival.
Results: Of all patients, the vast majority had an ECOG performance score ≤1 (91%) and ≥2 co-morbidities (57%). Further, 21% had received therapy for a prior malignancy and 5% were transfusion dependent. In BM aspirates, the percentage of erythroid, myeloid and megakaryocytic dysplasia (ie, <10% or ≥10%) was reported in 35%, 45% and 30% of the records, respectively. Assessment of ringsideroblasts was done in 70% of all BM aspirates. The degree of BM fibrosis and CD34-positivity were assessed in 82% and 74% of all BM biopsies, respectively. In 17% of all MDS cases, a MDS subtype could not be assigned. Cytogenetic assessments were performed in 61% of the patients, and decreased sharply with older age from 78% in 18-59-year-olds to 43% in over 80-year-olds (*p* for trend <.001). As a result, risk stratification could not be performed in a substantial number of patients as cytogenetic data are essential to assess prognosis. In patients with data available to assess prognosis by IPSS-R (*n*=268), 20%, 25%, 22%, 17% and 16% had very low, low, intermediate, high and very high risk MDS at diagnosis. Overall survival by IPSS-R risk group is shown in Figure 1A. A total of 64 (74%) of 86 patients aged <65 years received first-line therapy: 22% received an allograft, 8% lenalidomide, 6% azacitidine (AZA), 14% growth factors, 13% intensive chemotherapy and 38% supportive care only (Figure 1B). Among 345 (77%) of 450 patients aged ≥65 years, the corresponding percentages were: 0%, 1%, 17%, 34%, 2% and 46 (Figure 1B). Data on subsequent treatment are shown in Figure 1B as well. Of all 102 AZA-treated patients, 36% received AZA outside the EMA-approved indication.

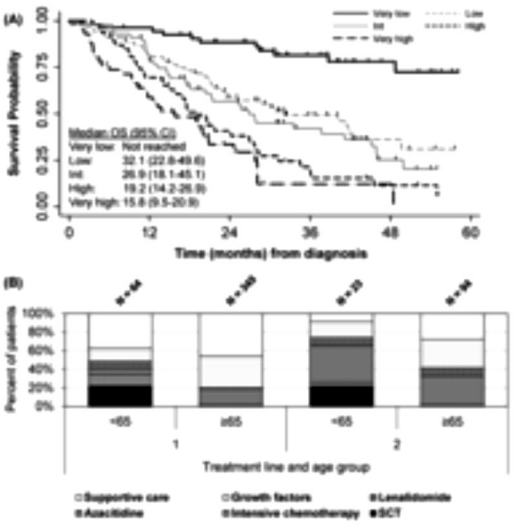

Figure 1.

Summary and Conclusions: We show that most patients from daily practice do not undergo a full and proper diagnostic work-up, such as assessment of dysplasia and cytogenetics, as recommended by previous and current European guidelines for the diagnosis of MDS (Malcovati 2013). Although treatment options in MDS are limited, especially for the elderly, prognosis should at least be assessed in order to accurately inform patients about their life expectancy and to provide information on appropriate risk- and age-adapted treatment options. Population-based registries, such as PHAROS, can be useful instruments to evaluate guideline adherence. Also, they provide information complementary to findings from clinical trials as the latter usually addresses a small minority of the general patient population.

E1217

THE UTILITY OF GRANULOCYTE MATURATION PATTERNS ASSESSED BY FLOW CYTOMETRY IN THE DIAGNOSIS OF MYELODYSPLASTIC SYNDROME

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Background: The routine role of flow cytometry (FC) in the diagnosis of myelodysplastic syndrome (MDS) is currently limited to the quantitative measurement of bone marrow (BM) CD34+ blast percentage. However, morphologic diagnosis can be challenging, particularly when dysplastic changes in the marrow are subtle. The expression of specific antigens at various stages of normal granulopoiesis is tightly controlled but dysgranulopoiesis may be associated with variant antigen expression which can be identified by FC.
Aims: 1) To assess the utility of variant granulocyte maturation antigen patterns (GMAP) in supporting MDS diagnosis. 2) To develop an immunophenotyping scoring system to rank dysgranulopoiesis.
Methods: The BD FACsCanto II analyser™ and BD FacsDiva software™ were used for acquisition and interpretation. A total of 60 BM aspirate samples from adult patients were analysed; 31 controls (normal n=18, previous lymphoma n=5, staging lymphoma n=1, paraproteinaemia n=3, myeloma n=3, systemic lupus erythematosus n=1) and 26 confirmed MDS cases based on the standard WHO diagnostic criteria. Three patients with subtle dysplastic BM, insufficient for diagnosis, were also included. The International Prognostic Scoring System for MDS (IPSS-R) risk stratify patients with MDS; low risk n=16 (IPSS-R >1.5-3.0), intermediate risk n=5 (IPSS-R >3-4.5) and high risk n=5 (IPSS-R >4.5-6). Antibodies CD10, CD11b, CD13, CD16, CD34, and CD45 were added to aspirates. Parameters assessed were total CD34+ blast percentage, CD11b/CD16 GMAP, CD13/CD16 GMAP, and four previously published scoring parameters[1,2] which include myeloblast%, B progenitor%, myeloblast *vs* lymphocyte CD45 ratio and granulocyte: lymphocyte side scatter peak channel ratio. Each feature carries 1 point and a total score ≥2 is suggestive of changes often seen in MDS.
Results: The majority of control subjects showed standard GMAP (Figure 1); 84% showed a uniform 'biphasic' pattern with CD13/CD16 and 81% showed a uniform 'orthogonal' pattern with CD11b/CD16. Eighty-five percent of MDS patients correlated with morphology by deviating from the standard 'biphasic' GMAP using CD13/CD16 whereas 77% deviated from the standard 'orthogonal' GMAP using CD11b/CD16. Of patients with low risk MDS, 63% had deviated

GMAP with CD11b/CD16 and 69% had deviated GMAP with CD13/CD16. Compared to control BM, all GMAP deviations in MDS patients presented as a non-uniform pattern or as a reduction of CD11b and/or CD16 expression. A GMAP score <2 was calculated in 90% of controls. A score ≥2 was seen in 85% of total MDS patients. A score ≥2 was seen in 75% of patients with low risk IPSS-R. Interestingly, of 3 morphologically sub-diagnostic patients, 2 had scores ≥2 and two had deviated GMAP using CD11b/CD16 and CD13/CD16. Patients with low risk IPSS-R had a median GMAP score of 2, those with intermediate risk IPSS-R had a median score of 4 whereas high risk IPSS-R patients had a median score of 5.

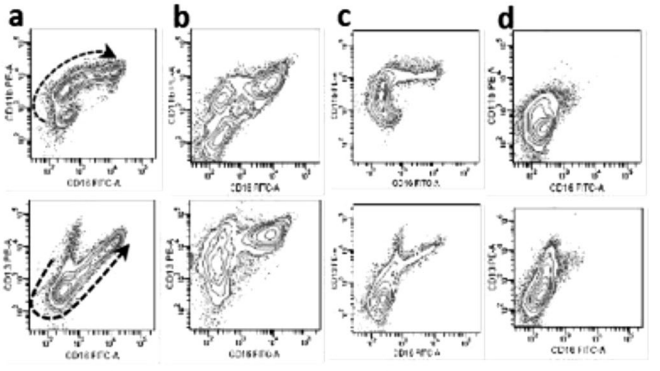

Diagram 1 Contour plots of GMAP using CD11b/CD16 (top panel) and CD13/CD16 (bottom panel); (a) shows the characteristic uniform 'orthogonal' and 'biphasic' patterns seen in the majority of control BM. The arrows show the direction of granulocyte maturation; (b) shows non-uniform GMAP in a patient who had sub-diagnostic morphological dysplasia which was insufficient for diagnosis; (c) shows a reduction of CD16 expression in a MDS patient with low risk IPSS-R; (d) shows an almost complete loss of CD11b and CD16 expression in a MDS patient with intermediate risk IPSS-R.

Figure 1.

Summary and Conclusions: We have shown that GMAP has diagnostic utility in MDS and that median GMAP scores may rank to IPSS-R score. Validation of the GMAP and GMAP score using a larger MDS patient cohort, and refinement by inclusion of FC assessment of dysmegakaryopoiesis is warranted.

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E1218

PATIENTS WITH THERAPY-RELATED CHRONIC MYELOMONOCYTIC LEUKEMIA (TR-CMML) HAVE SHORTER MEDIAN OVERALL SURVIVAL THAN *DE NOVO* CMML (DN-CMML): MAYO CLINIC EXPERIENCE

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Background: CMML is a malignant hematologic neoplasm characterized by peripheral blood monocytosis and bone marrow dysplasia. The World Health Organization (WHO) classifies therapy-related myeloid neoplasms (tr-MN) as another category. It is known that tr-MNs tend to have a worse prognosis than *de novo* MN (dn-MN). Tr-CMML is an under-described entity with scarce literature in comparison to dn-CMML.
Aims: To study the differences in clinical outcomes between tr-CMML and dn-CMML patients.
Methods: A retrospective single institution study chart review of cases with CMML at Mayo Clinic Rochester between 1994- 2011 was performed. DN-CMML, tr-CMML and leukemic transformation (LT) were defined according to WHO classification (Swerdlow *et al.* 2008). Prior exposure to chemotherapy, radiotherapy or both were defined as tr-CMML while lack of both as dn-CMML. Cytogenetic risk was defined according to Such E. *et al.* (Hematologica 2011). Appropriate IRB approval was obtained in accordance with the Helsinki declaration. Comparison between group medians was done using Wilcoxon test, to comparison values were done contingency and Onaway analyses (Means/Anova/Pooled t), while survival estimates were calculated using Kaplan-Meier curves JMP V10.
Results: Out of 265 CMML patients, 30 (11%) had tr-CMML. Median age was 72 years, with 19 (63%) patients being males. Labs at diagnosis included median white blood cell (WBC) of $16.8x10^9$, hemoglobin (Hg) 10.5 g/dL, platelet (PLT) $65x10^9$, monocytes (Mon) $3.8x10^9$, bone marrow (BM) blasts 4% (range 0-18), peripheral blood blasts (PB) 0% (0-7), Lactate dehydrogenase (LDH) 241 U/L. CMML-1 was found in 28/30 patients (93%) and CMML-2 in 2 patients (7%). Splenomegaly was present in 7 (23%) patients and absent in 21(70%). Two pts had splenectomy (7%). Cytogenetic were low 21 pts (72%), intermediate 2 (7%), and high in 6 (20%). LT was seen in 5 (17%) patients. When evaluating prior therapy, 17(57%) patients were treated with chemotherapy (CT), 6 (20%) patients were treated with radiotherapy (RT) and 7 (23%) CT+RT. Out of 265 patients, 235(89%) had dn-CMML. Median age was 71, with 158 (67%) were males. Labs at diagnosis were median WBC $12x10^9$, Hg 10.4 g/dL, PLT $95x10^9$(p=0.046), Mon $2.1x10^9$, BM blasts% 4 (range 0-19), PB 0% (0-19), LDH 222 U/L. CMML-1 was found in 210 pts (89%) and CMML-2 in 25 pts (11%). Splenomegaly was present in 57 (24%) and absent in 166 (71%). Twelve pts had splenectomy (5%). Cytogenetic were low 163 pts (71%), intermediate 31(13%), high 37(16%). LT was found in 26(11%) patients. Median overall survival was 30 months in dn-CMML *vs* 11 months in tr-CMML group (p=0.02). Upon comparing LT between these groups, time to LT was 10 *vs* 11 months, respectively (p=0.6). Median overall survival was in CT+ group 9 months, in RT+ group 4.4 months and in CT/RT+ group 13 months (p=0.7).
Summary and Conclusions: Tr-CMML comprises a small portion of all CMML cases (11%). Upon comparison, only lower platelets were found to be statistically significant compared to dn-CMML. Median overall survival in dn-CMML group was longer than tr-CMML group but LT seemed to be similar in terms of incidence and time to occurrence. Prior therapy (CT *vs* RT) in tr-CMML patients did not effect median overall survival. Additional larger studies are needed to confirm our results.

E1219

PROGNOSTIC FACTORS FOR TREATMENT FREE SURVIVAL IN PATIENTS WITH MYELODYSPLASTIC SYNDROME WHO STOPPED HYPOMETHYLATING AGNETS WITHOUT PROGRESSION

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Background: Hypomethylating agents (HMA) constitute an essential tool in the treatment of myelodysplastic syndrome (MDS). The use of these drugs as treatment for MDS was shown prolonged survival in MDS. However, there were no definitive prognostic factors to predict survival or treatment outcomes after using HMA in MDS. And guideline about HMA treatment recommend continuous therapy because patients can be shown rapid progression if they stop the treatment. However, in real clinical practice, many patients are impossible to continue treatment of HMA because they have more problems such as comorbidities, old age, poor economics, etc than patients have those who enrolled to clinical trials.
Aims: The purposes of this study were to estimate porgnostic factors for survival in patients with MDS who were treated with HMA, and especially, to document prognostic factors for HMA treatment free survival (TFS) in patients who were stopped HMA without disease progression because of other causes such as toxicities, cost, etc.
Methods: The medical records of this study were collected from nationwide registry data which was performed in AML/MDS working party in South Korea. Data from patients at fourteen university hospitals in South Korea between Jan 2001 to Oct 2013 were collected retrospectively. All included patients had been diagnosed to MDS and they were treated with HMA such as azacitidine or decitabine as front line therapy and they were treated continuously at least four cycles. The evaluation of response were estimated by IWG response cri-

teria. Early response was defined to be shown response after two cycles of HMA treatment. The TFS was defined duration from the end date of HMA therapy to the date of disease progression, relapse, or death from any causes.
Results: The median age of the 217 patients was 68 years (range, 24-92 years) and the male to female ratio was 1.8:1.0. 144 patients were treated with azacitidine and 71 patients with decitabine. 109 patients stopped HMA therapy because of disease progression and the others stopped HMA therapy without progression because of other causes. The 5 years overall survival rates (OS) and TFS of patients who were more than 65 years old, have blast counts more than 5% in bone marrow, have poor cytogenetics, have higher risk of IPSS(International Prognostic Scoring System), WPSS (WHO Classification-Based Prognostic Scoring System) and IPSS-R (Revised International Prognostic Scoring System) were inferior than those of patients less than 65 years old, blast less than 5% in bone marrow, good cytogenetics, lower risk of IPSS, WPSS, and IPSS-R (Table 1). Especially, the 5 years OS and TFS of patients who stopped HMA therapy because of other cause without disease progression were also inferior in bone marrow blast more than 5%, higher risk of IPSS, WPSS and IPSS-R than those of patients who bone marrow blast less than 5%, lower risk of IPSS, WPSS, and IPSS-R. However, patients who have early good response including hematologic improvement, and more than stable disease according to IWG response criteria, and early improvement of platelet counts were also shown trends to be superior OS and TFS, but not shown significant differnences (early good response *vs* not good response ; 70.2% *vs* 45.6%, p=0.228 (OS), 54.0% *vs* 46.0%, p=0.172 (TFS), and early platelet response *vs* not shown ; 70.2% *vs* 43.3%, p=0.179 (OS), 54.0% *vs* 43.4%, p=0.124 (TFS)).

Table 1. Factors affecting OS and TFS (treatment free survival).

Value	5yrs OS (%)	*p*-value	5yrs TFS (%)	*p*-value
Age, years				
< 65	46.0	0.055	37.4	0.022
≥ 65	36.1		23.9	
BM blast, %				
< 5	55.4	< 0.001	45.8	< 0.001
≥ 5	16.6		0.0	
Cytogenetics				
Good	36.0	0.001	23.6	0.013
Intermediate	32.2		0.0	
Poor	19.4		18.8	
IPSS risk, n (%)				
Lower risk	48.7	< 0.001	39.5	< 0.001
Higher risk	21.6		10.3	
WPSS risk, n (%)				
Lower risk	59.4	< 0.001	49.0	< 0.001
Higher risk	21.4		0.0	
IPSS-R risk, n (%)				
Lower risk	48.0	< 0.001	36.4	0.005
Higher risk	19.6		11.9	

Summary and Conclusions: In our study, good prognostic factors for OS and TFS were younger age (<65 years), blast counts in bone marrow (<5%), good cytogenetics, lower risk of IPSS, WPSS, and IPSS-R. And in case of patients who stopped HMA therapy without disease progression because of other causes, they might be shown prolonged survival in less than 65 years old, blast less than 5% in bone marrow, lower risk of IPSS, WPSS, and IPSS-R. However, further studies are needed to determine more impressive clinical factors to predict survival in patients with MDS treated with HMA in clinical real practice.

E1220

HYPOMETHYLATING AGENTS ARE EFFECTIVE IN SHRINKING SPLENOMEGALY IN PATIENTS WITH CHRONIC MYELOMONOCYTIC LEUKEMIA (CMML): MAYO CLINIC EXPERIENCE

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Background: CMML is a malignant hematologic neoplasm characterized by peripheral blood monocytosis and bone marrow dysplasia. Cytoreductive therapy (Hydroxyurea) and hypomethylating agents (HMA) (Decitabine (DAC) and Azacitidine (AZA)) are the main therapy options. Splenomegaly is commonly found in CMML patients. As of today, no consensus criteria for response in CMML exist, and most clinicians use MDS International Working Group (IWG) criteria (Cheson *et al.* 2006). Such criteria do not account for spleen response. In addition, data is scarce on HMA effect on splenomegaly.
Aims: To study the efficacy of HMA on splenic size in CMML patients.
Methods: A retrospective single institution study chart review of cases with CMML at Mayo Clinic Rochester between 1994- 2011 was performed. Spleen size was documented only via clinical examination. Due to lack of criteria on splenomegaly response, we used "modified" splenic response criteria of international working group-myeloproliferative neoplasms research and treatment (IWG-MRT) and European LeukemiaNet (ELN) consensus report for myelofibrosis (Tefferi A. *et al.* 2013). In our study, CR was resolution of splenomegaly and PR was shrinkage over 50%. Patients with splenectomy were excluded. Appropriate IRB approval was obtained in accordance with the Helsinki declaration. Comparison between group medians was done using Wilcoxon test, while survival estimates were calculated using Kaplan-Meier curves JMP V10.
Results: Out of 269 CMML patients, 36 (13.4%) were treated with HMA. Median age was 70 years, with 21(58%) patients being males. Labs at treatment included median white blood cell (WBC) was $14.4x10^9$, Hemoglobin (Hg) 10.2 g/dL, platelet (PLT) $67x10^9$, monocytes $3.36x10^9$. CMML-1 was found in 26 patients (72%) and CMML-2 in 10 patients (28%). When evaluated patients with treated HMA therapy, 29(81%) patients were treated with DAC and 7(19%) patients were treated with AZA. Median number of therapy cycles was 4.5. Out of 36 patients, 11(31%) had splenomegaly documented at time of start of HMA and at follow-up. Median age was 66, with 7(64%) were males. Labs at treatment were median WBC $39.3x10^9$, Hg 10 g/dL, PLT $67x10^9$, monocytes $3.37x10^9$. CMML-1 was found in 8 patients (73%) and CMML-2 in 3 patients (27%). Nine (82%) patients had DAC and 2 (18%) AZA. Median number of therapy cycles was 4. When evaluated pretreatment splenic size below left costal margin with physical examination, median splen size was 6 cm (range 1-18 cm) while during treatment best median spleen size was 2 cm (range 0-13cm). Among these patients splenic complete and partial response (CR 3/PR 2) was 45% with rest having stable spleen response (SR). Median cycles of therapy for CR/PR group was 5(range 4-6) *versus* 3 (range 1-6) in SR group. Median survival was 10 months in splenic response positive (CR/PR) whereas 4.8 months in SR group (p=0.11).
Summary and Conclusions: HMA is frequently used for CMML patients. Significant improvement was found on splenic size after HMA treatment for many splenomegalic CMML patients. In addition splenic responders correlated with longer length of therapy. Overall Survival in splenic positive response group was longer than negative response group, but this did not reach statistical significance, likely due to small number of sample. Additional larger studies are needed to confirm our results.

E1221

HEMATOLOGIC MALIGNANCIES, MOSTLY MYELODYSPLASTIC SYNDROMES AMONG 1740 INFLAMMATORY BOWEL DISEASE PATIENTS: LONG TERM FOLLOW-UP DATA FROM A TERTIARY CENTER

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Background: It is not an uncommon situation that patients (pts) with inflammatory bowel disease (IBD) are referred to hematology for suspected hematologic malignancies (HM). There is concern that IBD and medications especially thiopurines and anti TNF agents used in the treatment may be associated with increased risk of various HM.
Aims: We aimed to describe IBD pts who developed HM.
Methods: Retrospective review of medical records from all 1740 pts treated for IBD at a single gastroenterology clinic from 1999 to 2014 was performed. Pts with HM were further evaluated for the following parameters in the Table 1 and mean corpuscular volume (MCV) before azathioprine (Aza) use, MCV at the time of HM diagnosis and duration of Aza treatment. Informed consent was obtained from all pts.
Results: In total 6 pts (3/3, M/F) were identified with HM (5 MDS and 1 AML). Characteristics of the pts are summarized in Table 1. Median ages at diagnosis of IBD and HM were 38.5 (18-55) and 48.5 (29-59) respectively. All of these 6 pts except one had been exposed to Aza and 4 were treated also with anti TNF. All 5 patients experienced leukopenia during Aza therapy. Median time of Aza treatment duration was 10 (6-18) months. Median time between IBD and HM was 6.5 years. At the time of IBD diagnosis MCV changed between 84-101 fl. Only 1 patient had a MCV >100 fl before Aza. At the time of HM diagnosis 4 pts had a MCV of >100 fl. Most of the pts (4/6) did not have active IBD at time of HM diagnosis. Iron deficiency anemia with/without chronic disease anemia was seen in 5 of 6 pts. Median hemoglobin was 11.7 (6.9-12.5) g/dl.

Table 1. Patients characteristics. AML, acute myeloid leukemia; Aza, azathioprine; MDS, myelodysplastic syndrome; GIBD, gastrointestinal Behcet disease; MTX, methotrexate; SSZ, salazopyrin; TNF, tumor necrosis factor.

Patient number	Sex	IBD	Age at diagnosis of IBD	Aza treatment	Other agents	Type of HM	Age at diagnosis of HM	Time between IBD and HM (years)	Follow-up time (years)	Life status at end
1	M	CD	32	yes	Anti TNF, MTX	MDS	47	15	15	alive
2	F	CD	18	yes	Anti TNF, SSZ	MDS	29	11	11	alive
3	M	CD	45	yes	Anti TNF	MDS	50	5	5	alive
4	F	GIBD	25	yes	Steroids, anti TNF	MDS	30	5	8	alive
5	F	UC	55	yes	SSZ	AML	59	4	5	alive
6	M	CD	52	no	SSZ	MDS	52	0.15	0.5	alive

Summary and Conclusions: Overall risk of myeloid malignancies mostly MDS may be increased in pts with IBD either related to the disease itself or as a consequence of its treatment. Pts with leukopenia during Aza and MCV >100 fl (after exclusion of other causes) may be prone to develop HM and deserve increased awareness. Concomitant iron deficiency may affect values especially MCV and its increase.

E1222

IMPACT OF AZACITIDINE ON RED BLOOD CELL ALLOINMUNIZATION IN MYELODISPLASTIC SYNDROME

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Background: Reed blood cell (RBC) transfusion remains an essential component for the management of patients with myelodisplastic syndrome (MDS). Alloimmunization to RBC antigens is an important complication associated to multiple transfusion. The incidence of alloimmunization in MDS ranges from 12 to 56%. Hipomethylating agents such as Azacitidine (AZA) and Decitabine are standard care for the treatment of patients with MDS, however its impact in RBC alloimmunization has not been evaluated.
Aims: The objective of this study was to examine whether AZA may influence over the incidence of RBC alloimmunization in MDS.
Methods: Clinical and transfusion data regarding all consecutive patients with MDS and secondary acute myeloid leukemia from MDS who received >6 RBC concentrates between January 2009 and December 2014 were recorded. Data included: diagnosis according WHO 2008 classification, RBC support, treatment (AZA *versus* supportive care), alloantibody detection and identification, and the time between first transfusion and evidence of alloantibodies. We compare the data with that of MDS patients who received >6 RBC units between 1995 and 2008, previous to AZA approbation by EMEA.
Results: A total of 59 patients (34 males/25 females) with a median age of 75 years (53-89) received ≥6 RBC units between 2009-2014. Twenty-seven patients received AZA (75 mg/m2/day, 7 days without administration on the weekend) when a median of 6 cycles (range 1-14). Thirty-two patients received best supportive therapy (BSC) (RBC transfusions and erythropoietin when baseline level is low). The median follow-up from first transfusion was 13 months for AZA group and 12 months for BSC group. We identified 6 cases (18.8%) of alloimmunization in the BSC group and no cases in the AZA group (p=0.02). The WHO 2008 subtype and alloantibodies specificities are showed in Table 1. During a median follow-up of 23 months, the incidence of RBC alloimmunization in 94 patients who received their first transfusion before 2009 was 14.9% (14 patients) without differences with the 2009-2014 group (6 patients: 10.2%) (p=0.39). We observed significant differences in number of RBC units transfused between AZA group (median 24 units; range 6-212) and BSC group (median 10.5 units; range 6-198) (p <0,005). The median RBC units transfused per month was higher in AZA group (3.5; range 0.09-14) than in BCS group (0.91; 0.17-21) (p=0.002). A higher number of RBC units were transfused to patients in 1995-2008 group (median 36; range 6-307) compared to 2009-2014 group (median 17; range 6-206) (p<0.001). However, they were no significant differences in the median RBC units per month between groups. The alloantibody free survival from first transfusion was significantly superior in patients who received AZA (Figure 1) (Log-rank p=0,046).

Table 1. The WHO 2008 subtype and alloantibodies.

	RCUD	RARS	RCMD	RAEB 1	RAEB 2	AML	MDS/MP	5q-
No. Patients	5	9	18	2	10	7	6	2
Alloantibodies	2	0	2	0	0	0	1	1

RCUD: Anti-C, Anti-D; RCMS: Anti-E, Anti-Kell; MDS/MP: Anti-Kell; 5q-: Anti-Kell.

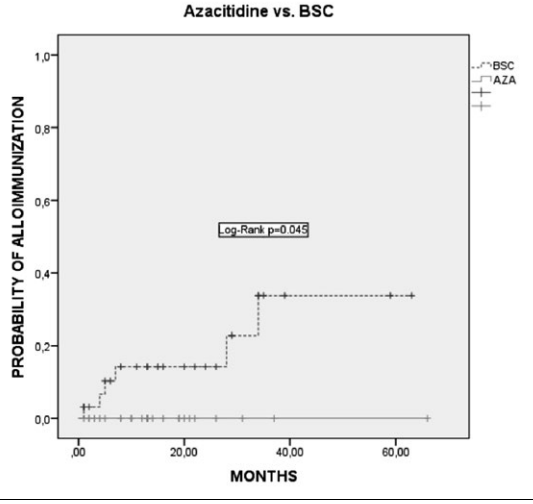

Figure 1. Time to alloimmunization from first transfusion.

Summary and Conclusions: Patients with MDS who received treatment with AZA form RBC antibodies at a lower rate than patients who received supportive care. However, these results were not influenced by the period in which the transfusion was performed. We suggest that the immunosuppression due to AZA therapy could develop an immunological tolerance or no response to incompatible transfusions.

E1223

IRON CHELATION THERAPY IN MYELODYSPLASTIC SYNDROME (MDS) IN ROUTINE CLINICAL SETTING: AN INTERIM ANALYSIS OF THE NON-INTERVENTIONAL STUDY EXSEPT

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Background: MDS is a diverse group of hematopoietic stem cell disorders with anemia as one of its major clinical features often leading to transfusion dependency. A substantial proportion of MDS patients (pts) experiences iron overload (IOL) due to chronic red blood cell transfusion. Although the impact of IOL on morbidity and mortality in patients with MDS remains a matter of debate, there is some evidence that iron chelation might be beneficial in this patient group. Deferasirox (DFX) is an oral iron chelator, which has been shown to be effective in removing excess iron from the body.
Aims: The study was planned to evaluate the feasibility, safety and effectivity of the iron chelator DFX in a setting outside of clinical trials.
Methods: In the present non-interventional study EXSEPT, started in Aug 2010 and closed recruitment in Dec 2014 with a 2-year-follow-up-period still ongoing, we have performed a subgroup analysis with interim data on 199 patients with MDS and iron overload (data cut-off in Nov 2013). A total of 294 pts with IOL due to different transfusion requiring diseases were enrolled in this prospective, multicenter study. Efficacy was measured by change in serum ferritin (SF) during chelation with DFX. All pts were treated with DFX according to the guidelines for iron chelation therapy in pts with MDS. Within the first year, visits were planned every 3 months and twice in the second year. Pts with at least 1 follow-up visit were eligible for analysis and last observation was carried forward defined as last visit. Dose groups of 5 mg steps were analyzed according to the prescribed dose given.
Results: 190 pts with MDS and IOL were analyzed with 57% being male pts. Median age was 74 years (range 14-93). The IPSS risk categories were available in 62% and were as follows: low in 19.5%, intermediate-1 in 26.8%, intermediate-2 in 9.5% and high in 4.7% of the pts, respectively. Mean baseline SF level was 2090 ng/mL (range 423 to 5840 ng/mL) and showed a moderate mean reduction of 237 ng/mL during course of DFX treatment until last visit. The reduction in iron burden differed within the initially planned dose groups with no effective reduction in the dose groups <10 mg/kg (+207 ng/mL; mean baseline 1759 ng/mL; n=27) and 10-≤15 mg/kg (-28 ng/mL; mean baseline 1750 ng/mL; n=16) compared to an effective reduction in the planned dose groups 15-≤20 mg/kg (- 625 ng/mL; mean baseline 2236 ng/mL; n=14) and 20-≤25 mg/kg (-348 ng/mL; mean baseline 2094 ng/mL; n=46). Mean creatinine levels increased slightly from baseline serum creatinine level of 0.90 mg/dL to 1.06 mg/dL at last visit. Adverse events were documented in 152 pts (80%). DFX was generally well tolerated, with the most common adverse events being gastrointestinal disturbances (15%), decrease in renal creatinine clearance (8%) and blood creatinine increase (7%).
Summary and Conclusions: We performed this analysis to evaluate the safety and efficacy of DFX in a routine setting outside clinical trials. The results demonstrated efficacious iron chelation using adequate doses of DFX in elderly pts with MDS. Changes in SF were moderate possibly reflecting the early stage of the study with limited data on pts with visits >6 months. Moreover, treatment was possibly sub-optimal in pts with doses below 10 mg/kg/days, but no data on the reason for the chosen dose were available. DFX was generally well tolerated, with the most common adverse events being gastrointestinal disturbances. This study with all its limitations due to the non-interventional nature supports the results of previously conducted trials.

E1224

HEMOCHROMATOSIS GENE MUTATIONS MAY AFFECT THE SURVIVAL OF PATIENTS WITH MYELODYSPLASTIC SYNDROME

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Background: Myelodysplatic syndrome patients are at risk of developing an iron-replete state and hemochromatosis due to frequent transfusion dependency and characteristics of the disease itself.

Aims: The recent availability of potent oral iron chelators is renewing an interest in the assessment of the possible impact of *HFE* genetics in MDS**.**
Methods: 36 newly diagnosed patients with MDS were studied for parameters of iron metabolism in addition to C282Y and H63D mutations of the *HFE* gene.
Results: Mutations were present in 11 out of 36 patients (31%), which was not different from our general population and were equally distributed among MDS subtypes. Mutated patients had higher ferritin levels (p=0.039) and lower TIBC (p=0.018). Ferritin was found to be higher for the untransfused mutated patients (p=0.017), but not for transfusion-dependent patients in whom ferritin levels correlated significantly with the number of blood units received (p=0.04). There was no difference in the number of blood units received between the mutated and wild type patients. A new observation made was that the mutated patients had a lower overall survival (OS) in addition to a poorer leukemia free survival (LFS) (p=0.004 and p=0.003, respectively) (Figure 1).

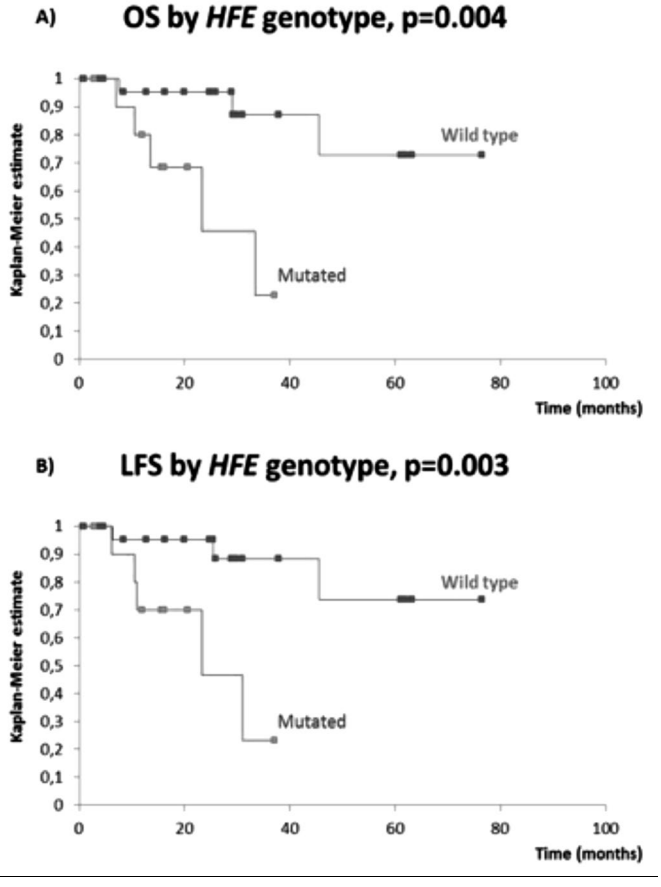

Figure 1.

Summary and Conclusions: The *HFE* gene mutations are not more frequent in MDS patients. Iron overload in mutated patients was higher. The effect of mutations on survival could be mediated by changes in iron metabolism. The *HFE* gentopye may predict MDS prognosis and there is a need for further studies. It remains a challenging question if *HFE* mutated MDS patients should be considered for potent iron chelation therapy.

E1225

P53 EXPRESSION IN PATIENTS WITH MYELODYSPLASTIC SYNDROME AT DIAGNOSIS AND FOLLOWING TREATMENT WITH AZACITIDINE

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Background: The Myelodysplastic Syndromes (MDS) are a heterogeneous group of malignant stem cell disorders characterised by ineffective and dysplastic haematopoiesis resulting in a hypercellular and dysplastic bone marrow with a risk in some patients of transformation to acute myeloid leukaemia (AML). Previous studies have demonstrated consistent correlation between immunohistochemical staining for the tumour suppressor gene p53 (*TP53*) and the presence of *TP53* mutation identified by molecular methods including deep sequencing. P53 expression has also been shown to have value as an independent prognostic indicator in some haematological malignancies, including Del(5q) MDS.

Aims: Our study aimed to assess the prognostic impact of p53 expression in bone marrow biopsies at diagnosis in MDS patients (n=47). We also set out to assess the effect, if any, of treatment with the hypomethylating agent Azacitidine on p53 expression.
Methods: Formalin-fixed paraffin embedded 4µm sections of bone marrow trephines were stained with Dako p53 mouse monoclonal antibody (DO-007) using standard protocol. Three hundred haematopoietic cells were manually counted. Positive expression is defined as strong nuclear staining in at least 1% of the haematopoietic cells examined
Results: p53 expression (strong positivity in >1% of cells examined) was seen in a significant number of patients (22%) at diagnosis of MDS (n=10). Using a cut-off point of 5% of cells, strong positivity was identified in 14% (n=6). P53 expression was correlated with MDS WHO category and IPSS-R score. Strong p53 staining in >5% of cells was associated with a significantly lower survival rate. Not surprisingly, patients with known cytogenetic abnormalities involving the short arm of chromosome 17 had particularly strong expression of p53. Comparison of p53 expression in marrow biopsies prior to treatment and following 4 cycles of treatment in 25 patients treated with Azacitidine showed varying patterns, with significant decrease in expression following treatment in 11 patients (44%). Patients with higher levels of expression had more substantial decrease in expression post treatment. No association was observed between changes in p53 expression and survival among Azacitidine treatment group but this may be due to a small sample size (Figure 1).

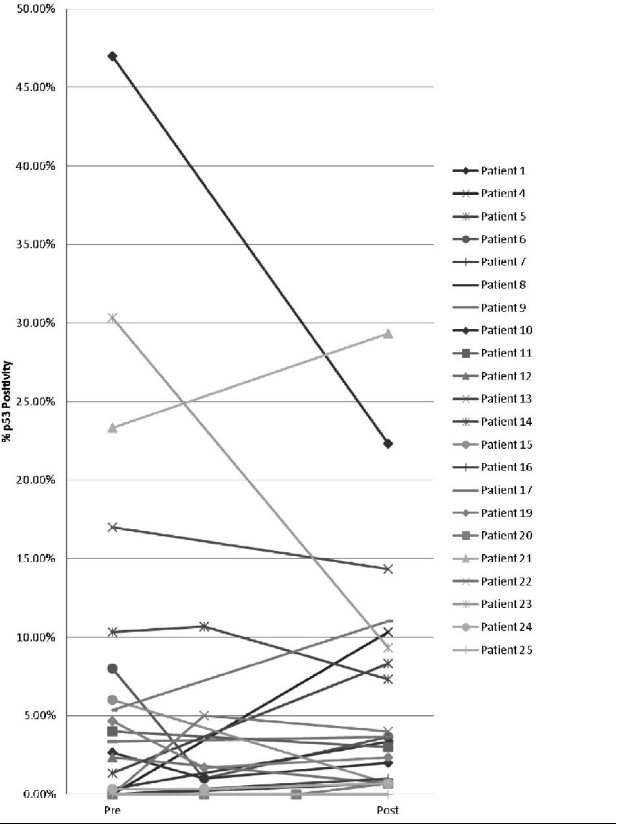

Figure 1. Difference in P53 expression pre and post treatment with Azacitidine.

Summary and Conclusions: Immunohistochemical analysis of p53 expression in marrow trephine biopsies shows significant promise for future use as a readily available prognostic indicator with a short turn around time for patients with MDS. Further research is warranted to assess the on the impact of Azacitidine and other therapeutic agents on p53 expression, and to establish whether immunohistochemical analysis of p53 expression might help predict either disease responsiveness or resistance.

E1226

SUBGROUP ANALYSES OF A PHASE 3 STUDY IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES FAILING HMA TREATMENT: IDENTIFICATION OF A HOMOGENEOUS POPULATION WHO BENEFIT FROM RIGOSERTIB THERAPY

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Background: Myelodysplastic syndrome (MDS) is heterogeneous, with varying categories and prognosis as defined by the Revised International Prognostic Scoring System (IPSS-R; Greenberg 2012). Patients with higher IPSS-R scores have worse clinical outcomes; those with Very High Risk (VHR) IPSS-R have the worst prognosis, with most dying of bone marrow failure complications. ONTIME was the first randomized study in patients with MDS failing hypomethylating agents (HMAs), with overall survival (OS) as the primary endpoint. ONTIME enrolled RAEB-1, RAEB-2, RAEB-t and CMML patients previously treated with HMAs, with marrow blast count at entry of 5-30%. Thus, it included a heterogeneous population of MDS patients. When the study was designed, little information was available regarding the prognosis for patients with MDS failing HMAs. Subsequently, Prebet and others showed a short survival expectancy (<6 months) for these patients (Prebet, JCO 2011; Jabbour, Cancer 2010). ONTIME demonstrated a 2.3-month benefit in median OS (mOS) in the ITT population that was not statistically significant (8.2 mo rigosertib *vs* 5.9 mo BSC; p=0.33; HR=0.87; n=299). The study was well-balanced, permitting post-hoc analyses of OS in defined subgroups of patients, including those with the worst prognosis, based on the (i) types of failures of HMA therapy, (ii) duration of exposure to HMA therapy, (iii) prognostic risk categories per the IPSS-R, and (iv) cytogenetic aberrations.
Aims: We sought to identify MDS subtypes who may benefit from rigosertib for a future clinical trial by defining a prospective patient population based on data from ONTIME and biological rationale.
Methods: Distribution of OS in each risk category and each arm was estimated by Kaplan-Meier method and log-rank test was used for treatment effect. Hazard ratio was estimated by Cox regression.
Results: We analyzed OS in patients classified as Primary 5-Azacytidine (AZA) Failures (patients who failed to respond to or progressed during AZA treatment) or as Secondary AZA Failure (patients who responded and then progressed), following Prebet 2011. The mOS was longer with rigosertib than with BSC among the Primary AZA Failure patients (8.6 months *vs* 4.6 months; p=0.032; HR=0.65) but not among patients classified as Secondary AZA Failure (5.5 months for rigosertib, 6.8 months for BSC; p=0.87; HR=1.05). In both treatment arms, the IPSS-R results correlated with OS in this study. Among patients who had VHR IPSS-R, the mOS was significantly longer with rigosertib than with BSC (7.6 *vs* 3.2 months; p=0.0050; HR=0.56); in contrast to all other IPSS-R risk categories (see Table 1). All of the patients with monosomy 7 and 66% of those with trisomy 8 were also VHR IPSS-R. The analysis of karyotypic aberrations found that mOS was significantly longer with rigosertib than with BSC for patients with the monosomy 7 (5.6 mo rigosertib *vs* 2.8 mo BSC; p=0.0033; HR=0.24) or trisomy 8 (9.5 mo rigosertib *vs* 4.5 mo BSC; p=0.035; HR=0.34) mutations.

Table 1. Overall survival by IPSS-R risk category (intention-to-treat).

IPSS-R Risk Category	N	Median (months)	N	Median (months)	Log-rank p-value	Hazard ratio (Rigosertib/BSC) (95% CI)
Overall	199	8.2	100	5.9	0.33	0.87 (0.67-1.14)
Intermediate	14	9.1	14	12.6	0.48	1.39 (0.56-3.47)
High	67	9.7	26	9.7	0.93	1.03 (0.61-1.74)
Very High	**93**	**7.6**	**41**	**3.2**	**0.0050**	**0.56 (0.37-0.84)**
Unknown	24	8.2	19	6.3	0.79	0.90 (0.44-1.82)

Summary and Conclusions: We conducted in-depth analysis of ONTIME, and found that patients with the worst prognosis at entry, and thus with the greatest unmet medical need, appeared to benefit most from rigosertib treatment; namely, those with Primary AZA Failure, VHR- IPSS-R, and monosomy 7. The duration of prior HMA treatment inversely correlated with survival benefit. Based on these results, a new randomized prospective controlled study in this high-risk MDS patient population comparing rigosertib to physician's choice will be conducted to confirm these important observations.

E1227

PROGNOSTIC AND PREDICTIVE VALUE OF IPSS-R IN ASSESSING OVERALL SURVIVAL (OS) IN A PHASE III STUDY OF RIGOSERTIB IN SECOND-LINE HIGHER-RISK (HR) MDS PATIENTS

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Background: ONTIME was a randomized (2:1) controlled study of rigosertib (RIG) *vs* best supportive care (BSC) in 299 pts with HR-MDS who had relapsed after, failed to respond to, or progressed during treatment with hypomethylating agents (HMAs). The study showed a non- significant trend favoring RIG in the analysis of overall survival (OS). The Revised International Prognostic Scoring System (IPSS-R) is a refinement of an earlier widely used index for risk assessment and prognosis for MDS (Greenberg 2012). Here we present the results of analyses in the subgroup of patients with very high risk (VHR) per the IPSS-R.
Aims: To examine the utility of the IPSS-R and the correlation between baseline characteristics and OS in a Phase III study of 2nd-line HR -MDS pts with IPSS-R VHR.
Methods: A log-rank test was run on each baseline disease characteristic from the 93 RIG and 41 BSC pts with IPSS-R VHR in the ONTIME study.
Results: Promising improvement (p<.01) with RIG on median OS *vs* BSC was shown in several subgroups defined by baseline characteristics (see Table 1). Overall, adverse events (AEs) were reported in 100% of RIG pts and 95% of BSC pts with IPSS-R VHR. AEs ≥Grade 3 in ≥10% of pts were: anaemia (RIG 24%, BSC 11%), thrombocytopenia (21%, 11%), febrile neutropenia (17%, 11%), neutropenia (15%, 13%), pneumonia (12%, 13%).

Table 1. Median (months) OS by baseline disease characteristics in Pts with IPSS-R VHR.

Characteristic	RIG N	RIG OS	BSC N	BSC OS	Log-rank p-value	Hazard ratio (RIG/BSC) (95% CI)
All patients with IPSS-R VHR	93	7.6	41	3.2	0.0050	0.56 (0.37-0.84)
Primary HMA failure*	55	8.1	21	2.6	0.0055	0.48 (0.28-0.81)
FAB classification of RAEB-t	23	5.8	9	3.4	0.0031	0.26 (0.10-0.68)
ECOG performance status 0 or1	79	8.9	30	3.6	0.0006	0.44 (0.28-0.71)
Bone marrow blast 20-30%	24	5.9	9	3.4	0.0020	0.25 (0.10-0.64)
Hemoglobin < 9 g/dL	63	6.9	21	2.3	<0.0001	0.30 (0.17-0.54)
Platelet count = 40 $\times 10^9$/L	37	10.1	13	4.4	0.0009	0.27 (0.12-0.62)
Neutrophil count = 0.8 $\times 10^9$/L	24	8.5	12	2.7	0.0038	0.29 (0.12-0.70)
FAB classification of CMML	1	9.2	5	4.7	<0.0001	--

*Failed to respond to or progressed during HMA treatment (Prebet 2011)

Summary and Conclusions: IPSS-R is a useful prognostic tool for 2nd-line MDS pts. After HMA failure, MDS pts with IPSS-R VHR in certain subgroups identified by baseline disease characteristics showed an OS advantage when treated with RIG compared to BSC. Such characteristics should be considered in the design of future 2nd-line studies in MDS patients with IPSS-R VHR.

E1228

INCREASED IMMUNE ACTIVATION AND IMPAIRED CELL SUPPRESSION ON MYELODYSPLASTICS SYNDROMES PROGRESSION

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Background: The Myelodysplastics Syndromes (MDS) are characterized by clonal proliferation of bone marrow cells with inefficient production. Its pathogenesis is still unknown. Some patients present autoimmune phenomena and abnormal immune function. Furthermore, some immune alterations remain unexplained according to the disease progression
Aims: This study aims determine cell-mediate suppression by Treg in MDS patients in 2 periods: zero and 12months
Methods: Peripheral blood of 38 patients was obtained initially (T0) and after 12 months (T12). Tregulatory (Treg) were sorted by flow citometry and cocultured for 7 days with T effector cells (Teff) in different concentration (Teff isolated with stimulation by phytohemagglutinin(Teff+PHA), Teff:Treg:1:1 and Teff:Treg:8:1) to evaluate cell-mediate suppression by Treg and quantify interleucines production (IL2, IL4, IL6, IL10, IL17, TNFa and IFNg) in culture supernatant by CBA. Relative gene expression of IRF-1(exons 2 and 4/5) was detected by qRT-PCR in peripheral Treg cells and peripheral blood and gene expression of Foxp3 was detected only in peripheral Treg cells
Results: Treg number (T0=5,6x10^5±2,8 *vs* T12=4,8x10^5±2,60; p=0.3) and Teff percentage (T0=16,8%±9,5 *vs* T12=13,1%±6,3; p=0.06) did not differ after 12 months, albeit there was a trend toward a decrease of Teff. Co cultures of Treg:Teff 1:1 showed an increased Teff proliferation in T12 (41,9%±3,1) compared to T0 (28,2%±4,7; p=0.02). The cytokine production was significantly

increased in culture supernatant (pg/mL) after 12 months: isolated Teff+PHA (IL2:p=0,048;IL4:p=0,001;IL6:p<0,001;IL10:p=0,001;TNF:p=0,024;INF:p<0,001 ;IL17:p<0,001), coculturesTeff:Treg:1:1 (IL2:p=0,007;IL4:p=0,002;IL6:p<0,001; IL10:p<0,001;TNF:p=0,002,INF:p<0,001;IL17:p<0,001) and Teff:Treg:8:1 (IL2:p=0,85;IL4:p=0,128;IL6:p=0,001;IL10:p<0,001; TNF:p=0,08; INF:p=0,002; IL17:p<0,001). Relative genetic expression of IRF-1 exon 4/5 was increased in whole blood (T0=0,02±0,14 *vs* T12=0,99±0,2;p=0,001) and Treg (T0=0,07±0,07 *vs* T12=0,14±0,08; p<0.001) after 12 months. However, genetic expression of exon 2 was only increased in whole blood (T0=0,002±0,01 *vs* T12=0,01±0,01; p<0.001), while the genetic expression of Foxp3 in Treg did not present statistical differences (T0=0,06±0,06 *vs* T12=0,07±0,12; p=0.57). Interestingly, a moderate positive correlation between the genetic expression of Foxp3 and IRF-1 exon 4/5 (R=0,659; p=0.002)

Summary and Conclusions: The increase of Teff proliferation and cytokine production suggests an impairment of Treg suppression ability and might be related to dysfunctions in inhibition by contact and inhibitory cytokine production. The initial reduced expression of IRF-1 in MDS patients evolved with an important increase during the disease progression. This gene is a powerful tumor suppressor and its low expression may be associated to disease progression. Moreover, IRF-1 is involved in several immune functions and high expression was described in MDS patients with autoimmune manifestations, thus the observed higher expression of IRF-1 might be associated to increased cytokine production, like IFN. In mice, IRF-1 inhibits Foxp3 expression in Treg cells. However, our results showed a positive correlation of IRF-1 exon 4/5 and Foxp3. Thus it is possible that the high IRF-1 expression was disproportionally induced by Foxp3 in MDS patients along disease progression

E1229

IMMUNE EXACERBATION ON MYELODYSPLASTICS SYNDROMES

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Background: The Myelodysplastics Syndromes (MDS) are characterized by clonal proliferation of bone marrow cells with inefficient production. However, the pathogenesis is still unknown. Some patients present autoimmune phenomena and abnormal immune function

Aims: This study aims to evaluate T cell function in MDS patients. Associated to autoimmune manifestations and prognostic scores will be detailed and compared to controls

Methods: The diagnosis of MDS was made according to WHO classification including the analysis of peripheral blood/marrow cell morphology and karyotype, and patients were stratified for prognosis according to IPSS, IPSS-R, WPSS. Peripheral blood from 38 MDS patients, 28 HC(healthy controls) and 17 AID (autoimmune disease composed by lupus and rheumatoid arthritis) were obtained. Tregulatory (Treg) were sorted by flow citometry and cocultured for 7 days with T effector cells (Teff) in different concentration (Teff isolated with stimulation by phytohemagglutinin, Teff:Treg:1:1 and Teff:Treg:8:1) to evaluate cell-mediate suppression by Treg and quantify interleukines production (IL2, IL4, IL6, IL10, IL17, TNFa and IFNg) in culture supernatant by CBA. Relative gene expression of IRF-1 was detected by qRT-PCR in peripheral Treg cells and peripheral blood, and Foxp3 was detected only in peripheral Treg cells. Antinuclear antibodies (ANA) were determined by indirect immunofluorescence. Total complement (CH100) and C2 serum levels were determined by radial immunohemolysis

Results: Treg number (x10^5cells) was not different in the MDS patients subgroups determined according to prognosis score: IPSS (high=4,9±1,1 *vs* low=5,6±1,6; p=0,5), WPSS (high=5,8±2,2 *vs* low=5,2±0,4; p=0,29), and IPSS-R (high=5,8±2,5 *vs* low=5,6±0,6; p=0,6). Hemoglobin serum levels was lower in high risk (7,9mg/dL±1,2) compared to low risk patients (9,9mg/dL±0,2; p=0.025), according to IPSS score. There was no difference in Treg number (x10^5cells) according to the presence of normal (5±0,8) or abnormal karyotype (6,5±1,2; p=0.12). Treg number (x10^5cells) was significantly higher in MDS (5±2,8) than in HC (3,1±2,0; p=0.007) and AID (2,8±2,4; p=0.008). Peripheral Teff percentage was similar in HC (20±8,3), MDS (13,3±9,0; p=0,27) and AID (14±6,8; p=0,98). Suppression of proliferation Teff mediated by Treg cell was decreased in the AID group compared to HC (p=0,03) and MDS (p<0.003). Overall cytokine production detected in culture supernatant was decreased in MDS, except for IL17, which was higher in AID (98,7pg/mL±155) compared to HC (16,4pg/mL±49,3) and MDS (9,1pg/mL±95,7; p<0.001). The relative gene expression of IRF-1 exon 2 (0,01±0,015 *vs* 0,001±0,01; p<0.02) and 4/5 (0,10±0,11 *vs* 0,02±0,14; p<0.01) in total peripheral blood were lower in MDS patients compared to IAD. Additionally, relative gene expression of IRF-1 exon 4/5 in Treg cell was increased in AID (0,11±0,11; p=0,005) compared to MDS(0,07±0,07) and HC (0,02±0,07; p=0,003). The relative gene expression of Foxp3 in Treg cell was similar in the three groups (MDS=0,06±0,06 *vs* AID=0,04±0,1 *vs* HC=0,04±0,04; p=0,96). MDS (46,2U/mL±2,1) patients presented lower CH100 than IAD (98,2U/mL±1,5; p=0,014). The frequency of ANA-positive individuals was higher in MDS than in HC (p=0.029)

Summary and Conclusions: Our results showed that suppression of Teff cell by Treg cell in MDS is similar to the controll, however the peripheral number of Treg cell is higher than in HC and AID, suggesting that function might be increased in MDS patients. One can hypothesize that it occurs secondarily, in an attempt of controlling the disease immune hyper activation. Interestingly, cytokine production was globally impaired in MDS, revealing a possible exhaustion of immune system, emphasized by complement consumption

E1230

LONG-LASTING HEMATOLOGIC RESPONSE TO AZACITIDINE IN MYELODYSPLASTIC SYNDROMES: UPDATE OF CLINICAL RESULTS FROM A SINGLE INSTITUTION

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Background: Although Azacitidine (AZA) has proven effective in myelodysplastic syndromes (MDS), the duration of haematologic response is usually limited (median: 13.6 months) *(Fenaux 2009)*. The French Group *(Itzykson 2011*) identified some clinical and haematologic parameters (poor ECOG performance status (p.s.), IPSS intermediate and poor risk cytogenetics, circulating blasts, high transfusion need) independently associated with a a poorer outcome, and these 4 criteria were integrated in a 3-risk-group prognostic score.

Aims: These data prompted us to retrospectively analyse our MDS pts treated with AZA who showed a favourable long-lasting response to AZA (*i.e.*: duration of response ≥20 months), in order to enucleate the clinical and hematologic features of long-responder pts.

Methods: The type of response was defined according to IWG criteria *(Cheson 2006)*: Complete Remission (CR), Partial Remission (PR), Marrow CR (mCR), Hematologic Improvement (HI). The response duration was measured from the date of achievement of a first response until the date of disease progression or death. Overall Survival (OS) was measured from the start of AZA treatment. Moreover, we quantified the degree of phosphoinositide-phospholipase C (PI-PLC) beta1 methylation and gene expression before and during AZA administration.

Results: From September 2004 in our Institution, 70 pts (50 males, median age 70, range 37-87 yrs) were treated with AZA. 40/70 pts (57.1%) showed a favourable response. 6 pts underwent allogeneic stem cell transplantation. 11/70 pts (15.7%, 27.5% of the responder pts) (4 males, median age: 68, range 60-84 yrs) showed a response duration ≥20 months. At AZA onset, WHO diagnosis was: RCMD-RS: 1 pt; RAEB-1: 4 pts; RAEB-2: 5 pts; MDS with Fibrosis: 1 pt. MDS was therapy-related in 3 pts. ECOG p.s. was <2 in all cases. IPSS risk was: Low: 1 pt; INT-1: 3 pts; INT-2: 6 pts; High: 1 pt. WPSS risk was: Low: 1 pt; INT: 2 pts; High: 5 pts; Very High: 3 pts; IPSS-R risk was: Low: 2 pts; INT: 3 pts; High: 4 pts; Very High: 2 pts. IPSS cytogenetic risk was: Good: 5 pts; INT: 4 pts; Poor: 2 pts. Transfusion need was high (≥4 RBC units/8 weeks) in 4 pts. No patient showed circulating blasts. Following Itzykson's AZA prognostic scoring system, the risk was: Low: 3 pts; INT: 8 pts. The pts received a median of 24 (8-59) cycles of AZA (3 lower-risk pts discontinued therapy after 8th cycle). The median duration of treatment was 35 (8-68) months. The best response achieved was: CR in 6 pts; marrow CR+HI: 1 pt; HI: 4 pts. First Response and Best Response were observed after a median of 1 (1-5) and 8 (1-12) cycles, respectively. An abnormal karyotype persisted in 3 pts. The median duration of response was 40 (21-75) months. In 8 pts, after the 1st 8 cyles, the intervals between cycles were prolonged on average up to 8 weeks, and in 5 pts erythropoietin was added during the maintenance therapy. Grade >2 non hematologic toxicity was observed in 2 pts. In 8 pts the disease evolved into AML (4 pts), or into a more advanced MDS (2 pts), or into a chronic myeloproliferative neoplasm (2 pts). 6 pts are still alive, and 2 pts are still maintaining the response after 49 and 71 months, respectively. Median OS from the start of AZA was 50 (25-125) months. All the pts showed an increase in PI-PLCbeta1 expression, that was maintained along with the hematologic response.

Summary and Conclusions: Our data show that a limited but significant fraction of MDS pts show a long-lasting hematologic and molecular response to AZA.

E1231

THE EFFICACY OF THE 5-AZACITIDINE IN THE THERAPY-RELATED MYELOID NEOPLASIA: A RETROSPECTIVE MULTICENTRIC EXPERIENCE

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Background: Therapy-related myelodysplastic neoplasia (t-MDS/AML) is an increasingly complication in patients (pts) treated with radiotherapy or

chemotherapy for previous hematologic or solid tumors. Conventionally, these pts are considered having a bad prognosis and allogeneic stem cell transplantation is the gold standard therapy. However, this approach is often not exploitable due to patient ineligibility or lack of stem cells donor. In these cases, if pts had a medullar blast count below 30%, 5-azacitidine (5-AZA) may be a suitable alternative. Very few data in this setting are available.
Aims: To evaluate the effectiveness of 5-AZA in this group of pts in the clinical practice. To assess the influence of cytogenetic risk into the outcome.
Methods: A retrospective analysis of t-MDS/AML cases into a large cohort of pts treated with 5-AZA in 10 centers in Lombardia region was performed. The cytogenetic risk was evaluated in according to the recently developed cytogenetic system for Myelodysplastic Syndromes (Schanz JCO 2012) and used as parameter in the prognostic score IPSS-R. Considering the small sample, the "very low", "low" and "intermediate" risk patients were evaluated together and their survival was compared to the survival of the "high" or "very high" cytogenetic risk pts. Response to therapy was considered evaluable if pts had reached, at the time of observation, at least 6 cycles of 5-AZA. The Kaplan Meier method, followed by the Logrank test was applied to evaluate the survival, starting from the beginning of treatment, and in relationship to the cytogenetic stratification.
Results: From a population of 187 pts affected by a myeloid malignancy with a medullar blast count <30% and treated with 5-AZA, data about 29 cases of t-MDS/AML were carried out. Of these 16 were female and 13 were male. Median age was 70 (range 40-87). Bone marrow blasts count was ≥20% in 2 cases, ≥10% in 10 cases and <5% in 3 cases. In 13 cases Red Blood Cells transfusion dependence was present. In the other 16 cases median hemoglobin level was 9.4 g/dl (range 8.6-12). Only 2 pts had platelet count (PLT) <10.000/mm^3, in other 10 case PLT were <50.000/mm^3. In 7 cases neutrophil count was less than 800/mm^3. The median number of 5-AZA courses administered was 5 (range 1-20). A response to the treatment (including the cases of "stable disease" maintenance) was achieved in 15 cases on 21 evaluable. The median survival was 12.1 months (Figure 1a). The median survival of the responders was significantly higher than the non responders one (23.8 months *versus* 4.3 months; p=0.023, Figure 1b).
The cytogenetic analysis, at the starting therapy time, was available in 27 cases. Considering the cytogenetic risk, the pts were divided as shown in Table 1.

Table 1.

Cytogenetic risk	Very Low	Low	Intermediate	High	Very High
Pts (n°)	1	8	2	7	9

The survival of "very low", "low" or "intermediate" cytogenetic risk pts did not differ significantly from the survival of "high" or "very high" risk ones (p=0.1890; Figure 1c).

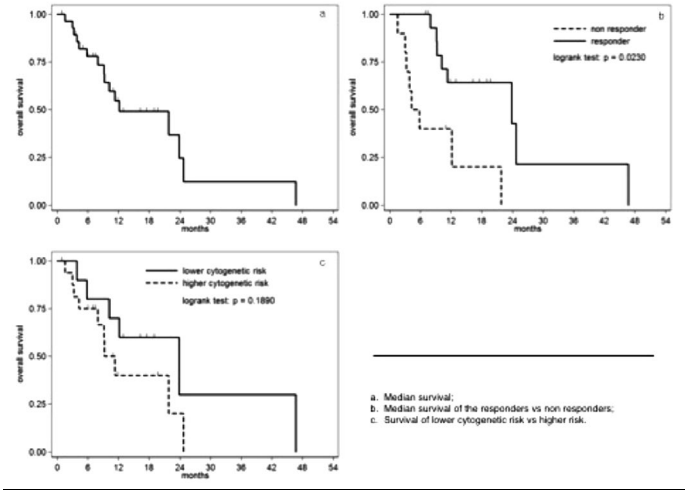

Figure 1.

Summary and Conclusions: Treatment in t-MDS/AML patients with 5-azacitidine seems to be effective to induce a response in these patients (71.4% of evaluables). Responding pts had a significative benefit in overall survival. The presence of a "high" or "very high" cytogenetic risk according to the IPSS-R stratification did not influenced the survival.

Myeloma and other monoclonal gammopathies - Biology

E1232

AN ENDOGENOUS RETROVIRUS EXAPTATION EVENT SIGNIFICANTLY CONTRIBUTED TO THE GENERATION OF SOLUBLE RANKL MRNA: IMPLICATIONS ON THE TRANSCRIPTIONAL PROFILE OF THE TRANSCRIPT VARIANT

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Background: Transposable elements (TEs) are known to promote genomic plasticity by affecting both the expression patterns and the splicing motifs of genes. Endogenous retroviruses *(*ERVs) represent remnants of ancient retroviral insertions into the host genome DNA that throughout the course of Vertebrate evolution became fixed in the germ line. Human ER*Vs* lack the ability to retrotranspose however their long terminal repeats (LTRs) contain transcription regulatory elements that, in the sense orientation, are functional and could be adopted for use by nearby host genes. *TNFSF11*, the gene encoding for RANKL, generates by alternative splicing two validated mRNA variants. *TNFSF11* variant 1 (NM_003701.3) contains five exons and encodes for the membrane-bound form of RANKL. Proteolytic cleavage of this transmembrane form allows for the generation of a soluble form of the protein. *TNFSF11* variant 2 (NM_033012.3) contains a set of alternate 5′ exons compared to variant 1, lacks the exon encoding for the intracellular and transmembrane domains of membrane-bound RANKL and can be directly translated into the soluble form of RANKL protein. Originally identified in SCC-4 and T3M-1 Cl.2 squamous cell carcinoma cell lines, it has been deduced that variant 2 is predominantly expressed in malignant cell types. Interestingly, it has recently been suggested that transcription of this variant is mediated via a distinct proximal promoter sequence.
Aims: Highlight, via *in silico* analysis, that exon 1A of *TNFSF11* variant 2 (soluble RANKL mRNA, *sRANKL*) was evolutionary provided by an exapted ERV-classI sequence. Show that transcription regulatory sequences of the putative *sRANKL* proximal promoter were also provided by this genetic element as well as by a nearby, upstream, TEs.
Methods: *TNFSF11* and the genomic region spanning 5000 nts upstream were downloaded from the NCBI Gene database and scanned for the presence of TEs by RepeatMasker. *TNFSF11* locus syntenic alignments of numerous mammals were downloaded from the UCSC Genome Browser Database. The syntenic DNA segments extracted were scanned by RepeatMasker. EMBOSS Cpgplot was used to identify putative CpG islands located in the genomic region spanning 5000 nucleotides upstream *sRANKL* transcriptional start site (TSS). PROMO Version 3.0.2 software was used to evaluate transcription factor (TF) binding affinity of the region spanning 250 nucleotides upstream *sRANKL* TSS; assumed to include precious regulatory elements of the variant's putative proximal promoter. Only human factors and human binding sites were concidered, applying a stringent maximum matrix dissimilarity rate of 7.
Results: *TNFSF11* exon 1A locates in a region reported from RepeatMasker to match a sense orientation LTR78 element, belonging to the ERV-classI family. Thorough computational analysis, considering fragments of interrupted repeats as joined by RepeatMasker, combined with in-depth phylogenetic analysis strongly support that the genomic region ranging from 1638 nts upstream to 3155 nts downstream *sRANKL* TSS was evolutionary provided by a "TE cluster"; namely a region where older TEs, integrated into the host genome, were interrupted by the insertion of younger ones. The genomic region spanning 4000 nts upstream *sRANKL* TSS contains CpG-rich genomic content. A CpG island was identified in this region, ranging from 2977 to 2775 nts upstream *sRANKL* TSS. Of note, the DNA segment including the CpG island was evolutionary provided by a LTR12C genetic element, locating short upstream the "TE cluster". The region spanning 250 nts upstream *sRANKL* TSS includes many high-affinity TF binding sites. A putative binding site for *CREB1*, a potent stimulator of *TNFSF11* variant 1 expression, locates 41 nts upstream *sRANKL* TSS. In addition, potential binding sites for factors inducing gene transcription during T cell-dependent immune response (for example *STAT4* and *NFATC2*) as well as for factors regulating apoptosis and tumor-suppression (*TP53, IRF1*) were identified (Figure 1).
Summary and Conclusions: Exaptation of an ERV-classI repeat allowed for a novel *TNFSF11* transcriptional pattern to occur. TEs, especially ERVs, are known to represent common targets of DNA methylation. Transcription of these genetic elements increases in the hypomethylated tumor microenvironment.

In this context, the CpG-rich content of the region spanning 4000 nts upstream *sRANKL* TSS, including the presence of a CpG island, could explicate the observed preferential expression of *sRANKL* in malignant cells.

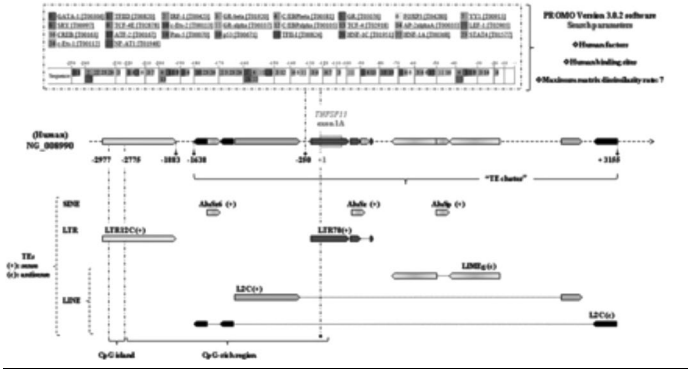

Figure 1.

E1233

IMPACT OF NOVEL THERAPIES ON MULTIPLE MYELOMA SURVIVAL-CURRENT AND FUTURE OUTCOMES

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Background: Multiple myeloma (MM) survival improved dramatically with the introduction of new therapies in the mid-late 2000s. These therapies essentially transformed MM from an acute condition to a potentially manageable chronic disease. The impact of further advances in MM therapy is uncertain, but can be projected by analyzing historical data and expected future clinical trial outcomes.
Aims: The aim of the study is to estimate the current and future patient outcomes in MM, specifically overall survival, using a novel approach.
Methods: For the historical perspective in the US (1990-2008), we calculated overall survival for patients diagnosed in a given year (cohort OS) by analyzing data from Surveillance, Epidemiology & End Results (SEER). For the predictive analysis, we calculated cohort OS using a patient flow model by integrating data from clinical trials, cancer registries and demographics as well as assumptions from key opinion leaders. We simulated progression of each cohort based on treatment rates and time spent in each line of therapy. The resulting OS curves were calibrated to 19 years of historical data.
Results: The median cohort OS in the US remained relatively static from 1990-2001 at ~30 months, but increased by ~43% to 43 months in 2008. The median cohort OS is expected to continue rising to ~72 months in 2022, which represents ~67% improvement from 2008 and ~140% improvement from 2001 (Figure 1). The life expectancy of patients diagnosed with MM in 2022 would thus be ~75 years, but will still be shorter than the current ~86 year life expectancy of the general population at the median diagnosis age at ~69 years. In European geographies, we expect to see similar trends, albeit with a slight delay that reflects the access restrictions and adoption patterns of novel therapies by treating physicians.

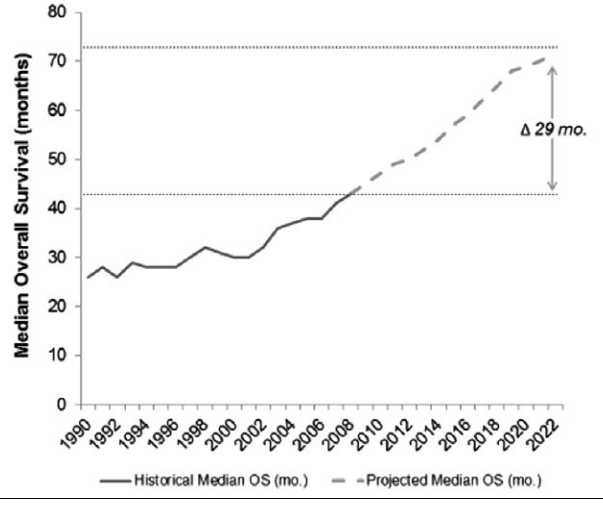

Figure 1.

Summary and Conclusions: This analysis highlights the dramatic improvements in MM survival since 2000 and the expected improvement in the future, demonstrating the transformative impact and value of novel therapies. Over the next few years, with better scientific understanding of MM and the use of novel drug regimens, we expect to see additional improvement of ~67% in OS for MM patients. Nevertheless, there is opportunity to further bridge the life expectancy gap between the general population and MM patients.

E1234

LOOKING FOR HYPOXIA FINGERPRINT IN MALIGNANT PLASMA CELL APPLIED TO NORMOXIC ENVIRONMENT.

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Background: Background: Recently, it was described that myeloma cells are chronically exposed to hypoxic environment in the bone marrow. Although the stabilization and nuclear localization of HIF-1 alpha protein in malignant plasma cells, was confirmed in many studies, the hypoxia transcriptional signature was not revealed thus far in MM patient. In fact, in order to determine such signature it has to be demonstrated how the long term sorting process of CD138 positive cells, conducted in the presence of oxygen, influences the expression of genes triggered in hypoxic environment.
Aims: Aim: To determine the stability of the hypoxia induced transcriptional expression profile of malignant plasma cells applied to oxygen environment, what mimics the condition during the sorting process of CD138 positive cells. Our goal is to *provide the bases* for a proper determination of hypoxia signature in multiple myeloma patients, taking into the consideration the influence of diagnostic procedure (CD138 cell sorting) on hypoxia-induced gene expression changes in malignant plasma cells.
Methods: Methods: Malignant plasma cells were cultured in hypoxic environment for 16h. The proper hypoxic stimulation was confirmed by the presence of HIF-1 alpha transcription factor in hypoxic cells by western blot and up-regulation of established hypoxia target genes by RT-PCR. Among the analyzed gene (ADM, BNIP3, VEGFA, PFKFB4, AK3L) we chose *PFKFB4*, as an excellent surrogate *marker* of *hypoxia in malignant plasma cells. Next,* we determined the stability of the hypoxia induced transcriptional expression changes of PFKFB4 in malignant plasma cells applied to oxygen environment, mimicking the conditions during the sorting process of CD138 positive cells. Upon indicated time points we analyzed the influence of normoxic environment on hypoxia-induced stabilization of HIF-1 alpha (Western Blot) and on expression of hypoxia-induced PFKFB4 (RT-PCR). The analysis was performed in the room temperature and in 4^0C.
Results: Results: In our present study we identified *PFKFB4*, as an excellent surrogate *marker* of *hypoxia in malignant plasma cells. We demonstrated very low expression of* PFKFB4 in normoxic condition in all analyzed cell lines, opposite to other established HIF-1 alpha target genes, which expression differs between different cell lines. Importantly, *PFKFB4* was strongly upregulated by hypoxia in all tested multiple myeloma cell lines. Next, we determined the stability of the hypoxia induced HIF-1 alpha accumulation and hypoxia-induced changes of PFKFB4 mRNA expression in malignant plasma cells applied to oxygen environment, what mimics the condition during the sorting process of CD138 positive cells. Our study revealed that HIF-1 alpha transcription factor is stable for at least 6h in hypoxic cells applied to normoxic environment, provided they are kept in 4^0C. Nevertheless, however, in room temperature (RT), the HIF-1 alpha transcription factor is not detected anymore upon 5 minutes in normoxic environment. As for the hypoxia-induced transcriptional response, we determined that PFKFB4 mRNA expression changes in malignant plasma cells are significantly influenced by normoxic environment (RT), after the estimated time required for CD138 sample preparation (from the bone marrow aspiration to the step of RNA isolation of CD138 sorted plasma cells).
Summary and Conclusions: Summary and Conclusions: Our research demonstrated that hypoxia-induced changes in the gene expression are not stable in normoxic environment during the time required for the bone marrow (BM) aspirate preparation. Thus, based on this study, we can conclude that the delay in CD138 plasma cells analysis strongly influences the gene expression of surrogate hypoxia markers in malignant plasma cells and only very strict laboratory practice allows the detection of hypoxia-induced transcriptional signature in malignant plasma cells. Our study clearly points the approach, which should be applied for BM aspirates to detect the hypoxia-induced gene expression profile.

E1235

IN HOUSE NEXT GENERATION SEQUENCING METHOD OF INMUNOGLOBULIN GENES FOR MINIMAL RESIDUAL DISSEASE QUANTIFICATION IN MULTIPLE MYELOMA

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Background: Multiple myeloma (MM) is a B-cell malignancy characterized by the bone marrow (BM) accumulation of a clonal population of plasma cells, which secretes a monoclonal IgH protein. The first step to the cure of MM is achieving a clinical complete response (CR). However, a majority of patients relapse in part due to the persistence of low levels of pathological plasma cells after treatment (Minimal residual disease (MRD)), sometimes more 10^8 pathologic plasma cells. In addition, residual cells have a heterogeneous clonal architecture and clonal evolution. Accordingly, the technology employed to assess MRD should not also be able to identify pathological clones, but also be able to assess clonal evolution.
Next generation sequencing (NGS) allows the identification of clonogenic B or T cells with high sensitivity and specificity and is suitable for detection of MRD as shown recently in patients with chronic lymphocytic leukemia (CLL).
Aims: To present a new method for quantification of immunoglobulin (Ig) gene clonal rearrangements from a polyclonal background by next generation sequencing (NGS). To test the suitability of the method for minimal residual disease (MRD) detection in Multiple Myeloma (MM) patients compared with Flow Cytometry (FC) data.
Methods: We analyzed DNA from bone marrow samples of 50 patients (50 diagnostic samples and 76 follow-up samples). The method employs the primers developed by BIOMED-2 for IGH (VDJ, DH-JH) and IGK (VJ and Kdel), and a set of specific mathematical and bioinformatics tools.
First, we identified the clonal rearrangement(s) (clonotype) in the diagnostic sample by fragment analysis and NGS. Second, we searched the NGS sequences from follow-up samples to detect the presence of the clonotype identified in the diagnostic sample.
PCR fragments shorter and longer than 250pb were sequenced with Proton and PGM sequencers respectively (Ion Torrent) following manufacturer instructions (Life Technologies) with slight modifications.
Sensitivity was calculated with serial dilutions of samples of known MRD values with polyclonal samples.
In follow-up samples we analyzed at least 1 µg of DNA to achieve a sensitivity of 10^{-5} EMR values were calculated using the DIFF algorithm and the equation: $EMR=[Lc*(D/K)]/Lt^2$
Where: *Lc* sample clonal reads, *Lt* sample total reads, *D* ng of gDNA used for PCR amplification, and *k* ng of gDNA per diploid cell.
Results: The sensitivity of the method is 10^{-5} for 150.000 cells. Reproducibility is >90%. The *linear correlation coefficient* between NGS and FC data for diagnosis and follow-up samples was R=59 and R=0.51, respectively. The Pearson *correlation* coefficient was 0.765, p<0.0001 for diagnostic and 0.716, p<0.0001 for follow-up samples. The Ig variable region usage identified by NGS was in accordance with the described in the literature for MM.
Summary and Conclusions: NGS sequencing of Ig genes with the Ion Torrent methodology is an effective technology to identify and quantify pathological clonal cells in MM. It is a methodological and economical alternative to CF and other methods for MRD follow-up.

E1236

MET INHIBITION AS A NEW THERAPEUTIC OPTION IN MULTIPLE MYELOMA PATIENTS WITH MET OVEREXPRESSION

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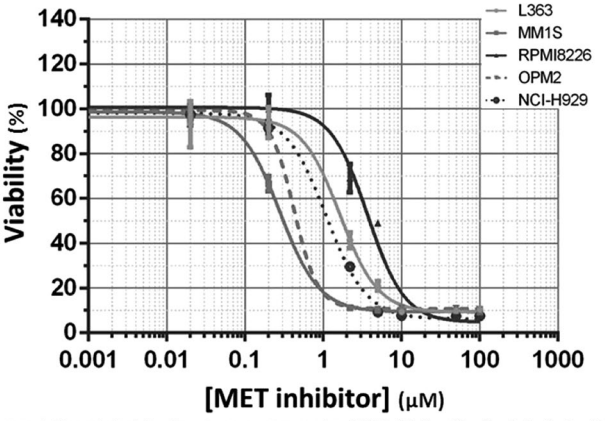
Background: Through the last years, the importance of HGF/MET signaling related to Multiple Myeloma (MM) pathogenesis has been described. Because of this, the inhibition of this signaling pathway could constitute a new therapeutic target. Nowadays, there are lots of drugs whose mechanism of action is inhibiting this signaling pathway.
Aims:-Analyze the mechanism of action that one MET inhibitor has on MM patient's plasma cells and MM cell lines. - Discover any possible synergism combination among MET inhibitor and other antimyeloma drugs (Dexamathasone, Lenalidomide, Bortezomib and Melphalan). - Evaluate the effect of MET inhibitor on MM clonogenic cells.
Methods: Bone marrow plasma cells from: 44 MM patients at diagnosis, 8 patients without bone marrow infiltration and 6 MM cell lines, were studied. *MET* and *L1-MET* expression was carried out on cDNA from plasma cells purified from all the samples. Levels of expression were quantified on the three types of samples by SYBR assay; GUSB was used as control gene and MM cell line U266 as control sample. Methylcellulose assays were carried out to study the effect of MET inhibitor on MM clonogenic plasma cells. MET mutations were studied by Next Generation Sequencing on MM plasma cells. Dose response curves for cell viability of MM plasma cells and cell lines treated with MET inhibitor, antimyeloma drugs or combination of both was stablished by MTT assay. Combinations were carried out with IC30, IC50 and IC70 values for each drug alone and plasma cell to study synergistic combinations.
Results: MM patients showed *MET* overexpression regarding to control bone marrows that don't have plasma cell infiltration (*p-value*=.0012). MM cell lines MM1S and OPM2, were the most sensitive to MET inhibitor treatment (IC50 of 0.06 and 0.26 µM, respectively). These cell lines only showed *MET* expression. *L1-MET* and *MET* transcripts were exhibited by RPMI, U266 and NCI-H929 MM cell lines and have higher IC50 values (3.6, 1.31, 1.09 µM, respectively). This relation between *L1-MET*, *MET* levels expression and IC50 values were also showed in MM patient's plasma cells. We didn't observe any mutation on *MET* gene, so aberrant MET activation in MM is not due to activated mutations on this gene but for *MET* overexpression. Besides that, MET inhibitor had cytotoxic effect on clonogenic plasma cells around IC50 values; we didn't observe any colony at higher concentrations of 0.02 µM MET inhibitor. MM cell lines resistant to MET inhibitor presented synergism among the MET inhibitor and anti-myeloma drugs (Dexamathasone, Lenalidomide, Bortezomib and Melphalan), having high dose reduction index (DRI) (from 3.08 to 62.7) (Figure 1).



Representative cytotoxicity dose response curves for MET inhibitor. The X-axis indicates the dosage (µM) and the Y-axis indicates the percentage of cell viability (%). Colors represent different MM cell lines grouped by *MET* and *L1-MET* level expression.

Figure 1. Cytotoxicity of MET inhibitor.

Summary and Conclusions: MET inhibition could be a new therapeutic option in multiple myeloma. MET inhibition is synergistic with proteasome inhibitors and immunomodultor drugs, as well as it kills clonogenic myeloma cells. *L1-MET* transcript may be a biomarker in MM treated with MET inhibitors.

E1237

INITIAL EVALUATION OF NOVEL DUAL PIM/PI3K AND TRIPLE PIM/PI3K/MTOR INHIBITORS IN MULTIPLE MYELOMA

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Background: Multiple myeloma (MM) is characterised by clonal expansion of malignant plasma cells in the bone marrow (BM). Despite significant advances in treatment it remains incurable. This is largely due to the supportive role the BM environment plays in migration, survival, proliferation and drug resistance. BM microenvironmental signalling along with other factors such as treatment with proteasome inhibitors (PI) can contribute to activation of the PI3K/AKT survival pathway. Multiple small-molecule inhibitors have been developed to target PI3K/AKT or mTOR kinases, but the efficacy of these drugs is likely to be compromised by the stimulation of compensatory signalling pathways. The redundancy of signalling pathways provides back-up mechanisms allowing escape from targeted inhibition. One such compensatory pathway is that driven by PIM kinases, which produce parallel oncogenic signals to AKT and mTOR and share several downstream molecular targets. As with PI3K/AKT, the BM microenvironment plays a major role in PIM activation. PIM1 and particularly PIM2 are known to be highly expressed in MM and play important roles in regulating MYC-driven transcription, apoptosis, cytokine signalling, cell proliferation and protein translation. Combinations of separate PI3K and PIM inhibitors have shown evidence of synergy in MM cell lines and animal models and a PIM kinase inhibitor has recently shown activity in relapsed/refractory MM.
Aims: We wished to evaluate the activity of a novel family of kinase inhibitors capable of inhibiting not only PIM kinases but also PI3K/AKT (dual inhibitors) and PI3K/AKT/mTOR (triple inhibitors).
Methods: We evaluated the in-vitro activities of a single pan-PIM (pPIMi), dual PIM/PI3K (IBL-202) and triple PIM/PI3K/mTOR (IBL-301) inhibitor in a number of MM cell lines alongside the pan-PI3K inhibitor GDC-0941 and the pan-PIM inhibitor AZD1208. IBL-202 and IBL-301 are low nanomolar pan-PIM/PI3K and pan-PIM/PI3K/mTOR inhibitors respectively. These dual and triple inhibitors show excellent kinase selectivity profile against a panel of 456 kinases. Cell viability was assessed using the Cell-Titre Glo assay and apoptosis was deter-

mined using Annexin-V/PI staining. To examine the impact of the microenvironment on the efficacy of these compounds MM cells were co-cultured with HS-5 stromal cells or in a hypoxic glove box (1% O_2).
Results: Inhibiting PIM kinases and the PI3K pathway simultaneously using IBL-202 was significantly more potent than a pan-PI3K inhibitor, GDC-0941 alone or pan PIM kinase inhibitors pPIMi or AZD1208. This was observed in all MM cell lines tested and in addition for the compound IBL-301, a triple inhibitor of PIM kinases, PI3K and mTOR. IC50 values were in the 0.5μM and 0.05μM range for IBL-202 and IBL-301, respectively. In comparison pPIMi and GDC-0941 scored an IC50 value between 5 and 10μM while the IC50 for AZD1208 was >20μM (Figure 1). In addition IBL-202 and IBL-301 caused a reduction in pAkt and known PIM targets, pBad and pS6. Bortezomib caused a marked increase in levels of PIM2 in MM cell lines. A combined treatment of IBL-202 or IBL-301 with bortezomib resulted in a synergistic effect in these cell lines (CI<1). In an effort to mimic the tumour microenvironment MM cell lines were co-cultured with the stromal cell line HS-5s. We observed strong induction of PIM2 in MM cells following co-culture. Co-culture protected MM cell lines against bortezomib-induced cell death, while promoting the apoptotic effect of both IBL-202 and IBL-301 with an increase in Annexin V positive cells from 15% to 40%. This suggests that microenvironmental stimulation could potentially induce synthetic lethality in the presence of these inhibitors. The tumour microenvironment in MM patients is hypoxic compared to healthy controls. Hypoxic conditions led to an increase in levels of PIM1 which is reported to regulate the surface expression of the CXCR4 receptor. The CXCR4 receptor is important for MM cell survival, migration and microenvironment interaction. We have observed a decrease in levels of CXCR4 following treatment with IBL-202 in a dose dependent manner. The dual and triple inhibitors are optimized with respect to their *in vitro* ADME properties and have excellent oral bioavailability. In-vivo IBL-301 has been well tolerated, with no signs of toxicity even 20 times above the efficacious dose in a transgenic ($KRAS^{V12}$NSCLC) mouse model. Testing of IBL-202 in a relevant MM mouse model is planned in the near future.

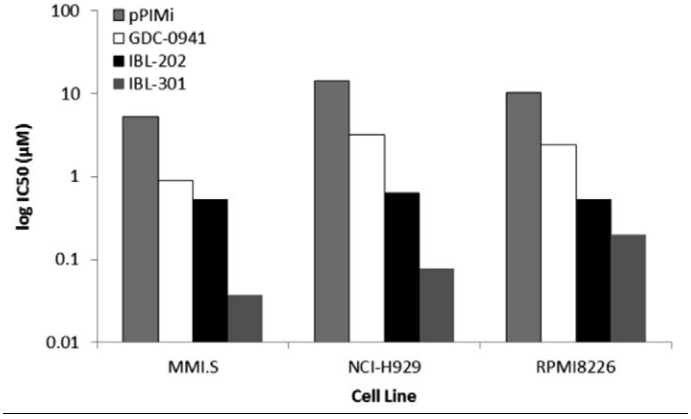

Figure 1.

Summary and Conclusions: IBL-201 and IBL-301 show promising activity in MM cellular models with increased potency compared to inhibitors targeting PIM or PI3K alone and warrant further evaluation in this disease.

E1238

PILOT ASSESSMENT OF PROXIMITY EXTENTION IMMUNOASSAY IN PATIENTS WITH MONOCLONAL GAMMOPATHIES: PARALLEL EVALUATION OF 92 CANCER-RELATED PARAMETERS IN BONE MARROW AND IN SERA

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Background: The assessment of prognostic factors in cancer is vital for correct stratification and targeted therapy. In multiple myeloma, both tumor cells and bone marrow microenvironment play an important role in pathogenesis, and take part on the development and the extent of myeloma bone disease (MBD).
Aims: The aim of our project was to address the utility of Proximity Extention ImmunoAssay technique (PEA) in patients with monoclonal gammopathies instead of standard ELISA techniques in the assessment of tumor microenvironment parameters.
Methods: We assessed 92 cancer-related parameters from the Oncology I ^{96x96} protein biomarker panel (Olink, Uppsala, Sweden). The parameters are known to be altered in various cancer types and are related to angiogenesis, cell-cell signaling, growth control and inflammation. Both sera and bone marrow samples were acquired from 30 patients with monoclonal gammopathies-25 patients with multiple myeloma (MM) and 5 individuals with monoclonal gammopathy of undetermined significance (MGUS) taken at the time of diagnosis.PEA is a sensitive and specific alternative of ELISA methods. For each sample there are two different antibody probes labeled with A- and B-oligonucleotides. Proximity of the oligonucleotide chains to the binding site leads to PCR amplification of the signal with specific primers. For statistical estimation we used Mann-Whitney U-test, Kruskal-Wallis test and Spearman correlation analysis at $p<0.05$ and Principal Component Analysis (GenEx, SSPS).
Results: In 3 patients the data did not pass the quality control. In the rest 27 individuals we analysed parallelly bone marrow and sera analytes. In 58 analytes the levels in bone marrow and serum were significantly different, with up to 38 fold difference (over 5 log scale), the rest 34 analytes had similar levels in both bone marrow and sera. Out of the whole panel, 9 analytes were significally different when comparing individuals with or without the presence of renal impairment. We found 19 proteins that significantly differed between MGUS and MM in both serum and bone marrow. There were several perspective candidate biomarkers correlating with disease stage, extent of myeloma bone disease, M-protein type and therapeutic outcome, such as tartrate resistant alkalinephosphatase-5 (TRAP-5), matrix metalloproteinase 1 (MMP-1), tumor necrosis factor (TNF), transforming growth factor alpha (TGF-α), Fas antigen ligand (FasL), Fms-related tyrosine kinase 3 ligand (Flt3-L) or epithelial cell-adhesion molecule (Ep-CAM).
Summary and Conclusions: Proximity ImmunoAssay is a very sensitive technique enabling parallel assessment of several analytes. Unlike ELISA, it has many fold larger dynamic range, and is suitable for analyte levels beyond the calibration curve. Out of the Oncology panel, 9 parameters were influenced by renal impairment. Despite limited number of patients, we could trace several potential candidates for the assessment of MM pathogenesis as well as for the extent of myeloma bone disease.
Supported by the grant NT14393.

E1239

THE USE OF MAGE C1 AND FLOW CYTOMETRY TO DETERMINE AND MONITOR THE MALIGNANT CELL TYPE IN MULTIPLE MYELOMA

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Background: Multiple Myeloma (MM) is a haematological malignancy, characterised by the accumulation of neoplastic plasma cells in the bone marrow (BM). Unfortunately, the full disease mechanism and exact malignant cell phenotype of this cancer remains unresolved. This affects effective MM treatment, as it limits the development of target-specific drugs and importantly prevents optimal detection of residual primary disease-causing cells. There is a definite need to identify a common molecular marker that is expressed in the majority of the diseased patients and directly associated to disease pathogenesis, thereby allowing monitoring of the malignant disease-causing cell directly. Cancer testis antigen MAGE C1 has been extensively studied in MM, is putatively involved in the pathogenesis of the cancer and is expressed in >90% of symptomatic MM patients at diagnosis, indicating a potential link to the malignant cell phenotype and use as a minimal residual (MRD) marker.
Aims: The aim of this study was to use aberrant MAGE C1 expression to identify the malignant cell phenotype in MM using flow cytometry and then investigate the use of this marker to monitor MRD in patients undergoing therapy.
Methods: BM and peripheral blood (PB) was collected from fifteen untreated symptomatic MM patients at diagnosis, with PB also being collected every three months over a two year period. Additional BM and PB samples were collected from five healthy donors for comparison. Mononuclear cells were isolated using density-gradient centrifugation, stabilized in 80% ethanol and analysed via flow cytometry following incubation with relevant B-cell development cell-surface markers and the nuclear-staining antibody MAGE C1.
Results: MAGE C1 expression was observed consistently in the early stem cells ($CD34^+$) and early pro-B to pre-B cells ($CD34^{-/+}/CD19^+$), as well as the proliferating plasma cells in the MM BM and PB. The expression of MAGE C1 in the $CD34^+$ cells suggests that these cells are the disease progenitors that sustain the downstream accumulation of abnormal plasma cells. The reactivation of MAGE C1 expression in a sub group of these plasma cells may be responsible for the proliferation, leading to a second disease mechanism that accelerates plasma cell mass expansion. The expression of MAGE C1 in the PB indicates that the malignant phenotype is also found in circulation and thus could easily be used for MRD monitoring. Importantly, the changes in MAGE C1 expression correlated to the decrease/increase in serum M protein, beta-2-microglobulin and creatinine levels over the two year treatment period, connecting this cancer marker to disease pathogenesis and indicating a direct link to active disease. It was further observed that current treatments are targeting different cell populations not necessarily eradicating all the malignant cell types.
Summary and Conclusions: MAGE C1 was successfully used to determine the malignant cell phenotype as well as to monitor MRD with specific B cell markers via flow cytometry. MRD monitoring using PB is easily accessilbe to

all MM patients compared to stressful BM biopsies. Furthermore, analysis of PB using this assay is similar than current flow cytometry methods giving a clear indication of the malignant cells found in circulation with better sensitivity to residual disease. MAGE C1 expression in PB can provide clinicians with access to the percentage of malignant cells in circulation prior to ASCTs as well as other MM treatments in a time efficient manner in turn improving the outcome of patients.

E1240

PERSONAL HISTORY OF INFECTIONS ASSOCIATED WITH AN INCREASED RISK OF MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE (MGUS) AND MULTIPLE MYELOMA: A POPULATION-BASED NESTED CASE-CONTROL STUDY

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Background: Infections are a major contributor to the global cancer burden and are a leading cause of morbidity and mortality in individuals with monoclonal gammopathy of undetermined significance (MGUS) and multiple myeloma (MM). Both MGUS and MM are associated with immune dysregulation which may not only increase the propensity to infection but may also act as a trigger for the development of MGUS and/or progression to MM or associated lymphoproliferative disorders.
Aims: To investigate the association between prior common community-acquired infections and subsequent risk of MGUS and MM using the UK Clinical Practice Research Datalink (CPRD).
Methods: Conditional logistic regression models were used to estimate odds ratios (OR) and associated 95% Confidence Intervals (CI) excluding the 12 month period prior to MGUS/MM diagnosis/control selection. Findings were adjusted for a number of potential confounders including age at diagnosis, comorbidities, autoimmune disease, prior cancer, and lifestyle variables.
Results: In total, 4,654 MGUS and 3,801 MM patients matched to 23,101 and 18,991 controls respectively were identified. A personal history of infections was associated with an increased risk of both MGUS and MM, with the strongest associations observed for MGUS (MGUS: OR 1.32, 95% CI 1.23-1.41; MM: OR 1.22, 95% CI 1.13-1.32). A number of infections, in particular those affecting the respiratory tract were more commonly diagnosed in individuals who later developed MGUS/MM. A dose response relationship was also evident, with individuals more prone to infections more likely to later develop MGUS/MM.
Summary and Conclusions: The findings of this study suggest infections may play an important role in the etiology of the plasma cell disorders and/or be markers of an underlying immune disturbance. Further exploration of the mechanisms involved may aid our understanding of the pathogenesis of MGUS and MM.

E1241

INFLUENCE OF ABC TRANSPORTERS' GENETIC PROFILES IN THE DEVELOPMENT OF MONOCLONAL GAMMOPATHIES

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Background: Genetic variability inxenobiotic transport related proteins can influence drugs' efficacy and could contribute to the susceptibility to hematological conditions, such as Monoclonal Gammopathy of Unknown Significance (MGUS) and Multiple Myeloma (MM). ABC (ATP-binding cassette) transporters superfamily includes membrane proteins which function is to transport substrates through intra- and extracellular membranes. MDR1 and MRP1 are membrane transporter proteins that, besides the above described functions, may play a role in development, progression and therapeutic outcome in several hematological pathologies.
Aims: In this context, we investigated the relevance of polymorphisms in *MDR1* (C3435T) and *MRP1* (G1666A) genes as risk factors for the development of monoclonal gammopathies, such as MGUS and MM, as well as their possibility of prognostic risk factors.
Methods: This study included 145 patients diagnosed with monoclonal gammopathies (GM) (68 MGUS, 77 MM) and 121 healthy controls (CTL). The genetic profiles of *MDR1* (C3435T) and *MRP1* (G1666A) were assessed by RFLP-PCR. The strength of association between polymorphisms and disease's development risk were estimated by odds ratio (OR) with 95% confidence interval (CI 95%). The influence of these polymorphisms in the patients overall survival (OS) was calculated by Kaplan Meier method.
Results: Our results show that allele C from *MDR1* is the most prevalent in CTL (52.1%) and in MGUS (52.2%), while allele T is predominant in MM (52.0%). Moreover, both alleles have the same prevalence in CTL (50.0%), and genotype CT is the most common in all groups (CTL=46.3%; GM=62.8%; MM=70.1%; MGUS=54.4%). As to *MRP1* gene, we observed that allele A is the most frequent in all groups (CTL=72.6%; GM=67.9%; MM=67.5%; MGUS=68.4%) and we also perceived that the most common genotype is AG (CTL=54.8%; GM=51.7%; MM=46.8%; MGUS=57.4%). After calculating the OR for individual genotypes we realized that CC genotype from *MDR1* is a protector factor for the development of MM (OR=0.37; p=0.0093; CI95% 0.17-0.79) and CT genotype represents a risk factor for the development of GM in general (OR=1.9; p=0.0092; CI95% 1.2-3.2) and for the development of MM (OR=2.7; p=0.0012; CI95% 1.5-4.9). The Kaplan Meier analysis revealed that MM patients with the AA genotype in *MRP1* gene have a decreased overall survival in about 18 months (AA-20.1±2.4 months; AG or GG-38.1±3.9 months; p=0.035).
Summary and Conclusions: This work suggests that *MDR1* and *MRP1* genotypes can be predictive of the predisposition to the development and progression of monoclonal gammopathies. However, their role in therapy response remains unknown.

E1242

ANALYSIS OF RHOU AND RHOV EXPRESSION IN MULTIPLE MYELOMA REVEALS A POSSIBLE CORRELATION WITH BONE MARROW DEPENDENCE

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Background: Rho GTPases, in their active GTP-bound state, interact with effector proteins controlling many biological processes: cytoskeletal regulation, membrane trafficking, cell adhesion and polarization, transcriptional activity, apoptosis and proliferation. The RhoUV subfamily consists of two atypical Rho proteins: RhoU and RhoV that due to their spontaneous activation are normally expressed at very low levels in most cell types and tissues. In Multiple Myeloma (MM), a tumor microenvironmental niche, which protects MM cells from chemotherapy and proapoptotic noxae, is created by the adhesion between primary malignant plasma cells and stromal cells. RhoU and RhoV GTPases are able to alter cell-cell/cell-extracellular matrix adhesion, cell motility and actin dynamics. Also, RhoU levels are known to be upregulated upon IL-6 stimulus, which is a pivotal growth factor for malignant plasma cells. On these basis, we hypothesize that changes in the expression of these GTPases and therefore in their function could lead to dynamic alterations of MM-associated niche.
Aims: Analyze RhoU and RhoV expression and actin cytoskeleton dynamics in normal B cells *versus* MM malignant plasma cells. Evaluate if changes in the expression of these GTPases have an impact in adhesion, migration/motility and structural features of MM cells. Study the role of RhoU and RhoV in malignant cell-stromal cell adhesion and in the creation of protective bone marrow niches.
Methods: mRNA expression was analyzed by qRT-PCR in MM cell lines, MM cells from patients and normal B cells from healthy donors. Malignant plasma cells were isolated from bone marrow or peripheral blood of patients using "EasySep CD138 positive selection kit". Normal B cells were purified using "EasySep human B cell isolation kit". Fluorescence microscopy was performed using Zeiss LSM 700 microscope. Staining was done with Phalloidin 594 (Abcam), rabbit anti-RhoU or RhoV (Abcam), goat anti-rabbit Alexa fluor 488 (Life Technologies) and DAPI (Vector).
Results: MM cell lines showed a significantly higher expression of RhoU and RhoV as compared to normal B cells. While RhoV displayed the highest expression in stroma/IL-6-dependent cell line (INA-6), RhoU was expressed at high levels in INA-6 but also in U-266, in this latter probably because of its autocrine IL-6 production. Remarkably, RhoU and RhoV expression was actively modulated when MM cell lines were co-cultured with stromal cells. MM patients also showed a significantly higher expression of both proteins when compared to normal B cells. Interestingly, in the stage of plasma cell leukaemia (PCL) RhoV mRNA levels were high but RhoU levels seemed to be even lower than in normal B cells. Immunofluorescence showed that RhoU and RhoV colocalize with actin in normal B cells while in INA-6 these were located both in the cytoplasm and nucleus. Upon co-culture of INA-6 with stromal cells, RhoU accumulated in the nucleolus. In freshly purified plasma cells from MM patients' bone marrow, RhoU and RhoV were found both in the cytoplasm and nucleus being RhoU particularly abundant in the nucleolus. On PCL patients there was a very low signal for both proteins and RhoV seemed to be present only in the cytoplasm.
Summary and Conclusions: RhoU and RhoV are widely over-expressed in MM. Their expression seems to correlate with bone marrow dependence, being that it decreases in more aggressive, bone marrow-independent stages where malignant plasma cells are no longer dependent on the stromal signals. RhoU and RhoV could be important in MM pathogenesis and suitable targets to disrupt the MM plasma cell adhesion to protective bone marrow niches.

E1243

GRANULOCYTE-LIKE MYELOID DERIVED SUPPRESSOR CELLS (G-MDSC) ARE INCREASED IN MULTIPLE MYELOMA DUE TO IMMUNOLOGICAL DYSREGULATION OF MESENCHYMAL STEM CELLS (MSC)

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Background: Granulocytic-Myeloid-derived suppressor cells (G-MDSC), a heterogeneous population of myeloid cells with peculiar immunosuppressive properties against T-cells due to increased levels of Arginase-1 (Arg-1), are increased in Multiple Myeloma (MM), as recently described by our and other groups. MM plasma-cells depend on the bone marrow microenvironment for the growth and survival; important in this context is the role of mesenchymal stem cells (MSCs), stromal adult stem cells with immunomodulatory properties.
Aims: Investigating the role of MSCs from MGUS and MM patients on expansion and activation of G-MDSCs.
Methods: G-MDSC (CD11b+CD33+CD14-HLADR-) were evaluated by flow cytometry and functional test, including the expression of the enzyme arginase (ARG-1) and ability to suppress lymphocytes activation when co-cultured with T-lymphocytes obtained from normal donors. Briefly, human peripheral blood mononucleated cells (PBMCs) isolated from healthy volunteer donors were cultured alone, with HD (n=6), MGUS (n=5) or MM MSCs (n=6) (1:100 ratio). After one week, PBMCs were collected. G-MDSCs were then isolated using anti-CD66b magnetic microbeads. The phenotype of G-MDSC was confirmed by cytofluorimetric analysis. Their immunosuppressive capacity was analyzed, evaluating T-cell anergy when co-cultured with autologous CFSE-labeled T cells stimulated by phytohaemagglutinin (PHA).
Results: G-MDSC percentage in MM was greater than healthy controls (61.2±1.6% *versus* 51.7±1.4%, p<0.001). We found a T-cell anergy dose- and time-dependent driven by MM-G-MDSC, due to overexpression of ARG-1 (p=0.0012). Only MM MSCs-educated G-MDSC exhibited suppressor effect with a reduction of T cell proliferation of about 34±9.6% (p<0.01) compared to G-MDSC control (isolated from PBMCs cultured in medium alone). Notably, neither MDSCs control nor myeloid cells co-cultured with HD or MGUS MSCs showed suppressor activity on T cell proliferation. Using real time PCR, we analyzed the expression of immune modulatory factors (Arg-1, NOS2, COX2, TNFα, TGFβ, IL6, IL10, IL1β) by MSCs after 48 h of co-culture with PBMCs. MM MSCs showed an increase of COX2, IL6, IL10, IL1β, TGFβ and NOS2 expression (p<0.05) compared to HD MSCs, suggesting that multiple mechanisms are involved in MDSCs induction by MM MSCs. The same immune modulatory factors were investigated in MDSCs before incubation with T cells. MM MSCs-educated MDSCs expressed higher levels of arginase1, NOS2 and IL6 (p<0.05) compared to MDSCs control.
Summary and Conclusions: MSCs from MM but not MGUS patients are able to activate G-MDSCs with potential implication in immune escape that favours plasma-cells growth, survival and resistance to drugs.

E1244

MICRORNA PROFILE IN BONE MARROW FIBROBLASTS FROM PATIENTS WITH MULTIPLE MYELOMA

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Background: Multiple Myeloma (MM) is a plasma cell dyscrasia with specific localization in the bone marrow that is almost invariably preceded by a Monoclonal Gammopathy of Undetermined Significance (MGUS). Several studies have clearly proved that a permissive microenvironment is required for MM to emerge. Recently, we demonstrated that bone marrow fibroblasts (cancer associated fibroblasts, CAFs) from 1st diagnosed MM patients show a relevant activated phenotype that acts as a promoter for MM initiation and progression as well as angiogenesis (1). The microRNAs (miRNA, miR) are a class of highly conserved short non-coding RNA molecules that regulate gene expression at the post-transcriptional level. They are involved in regulation of several biological processes, including cell proliferation, differentiation, apoptosis, morphogenesis and metabolism. Specific miRNA expression signatures can distinguish cancer from benign tissues and may provide the basis for developing new diagnostic and therapeutic strategies. Despite numerous studies focused on the miRNAs profile in MM cells, their analysis on CAFs has not been investigated yet.
Aims: We wondered whether miRNAs could play an important role in the functional conversion of MGUS CAFs into the activated phenotype of the 1st diagnosed MM CAFs.
Methods: CAFs were purified from bone marrow aspirates of 15 patients with MGUS and 15 patients with 1st diagnosed MM using appropriate microbeads, and cultured *in vitro* until the second passage. 100ng of RNA enriched in miRNA fraction were labeled and hybridized onto commercially array slides. Differentially expressed miRNAs were analyzed with Rank Product algorithm. The resulting *p*-values were corrected for multiple testing using the False Discovery Rate (FDR) method by Benjamini and Hochberg. Results were validated using quantitative real-time PCR.
Results: CAFs from 1st diagnosed MM patients show a different miRNA profile compared to those from MGUS patients. Heighten differentially expressed miRNAs were identified: 8 were up-regulated, and 10 down-regulated.
Among the up-regulated miRNA, 5 were significantly up-regulated (miR-125b-5p, miR-5100, miR-199a-5p, miR-214-3p and miR-27b-3p) and they were engaged in tumor growth, differentiation, proliferation and drug resistance. Among the down-regulated miRNA, 5 were significantly down regulated (miR-4281, miR-4530, miR-4430, miR-6089 and miR-6087), and they were involved in post-transcriptional regulation of gene expression for differentiation and metastatic progression. In particular, miR-199a-5p and miR-214-3p play a major role in fibroblast activation by targeting Caveolin1, a key player in TGFβ signaling, and miR-125b-5p is involved in tumor drug resistance (Figure 1).

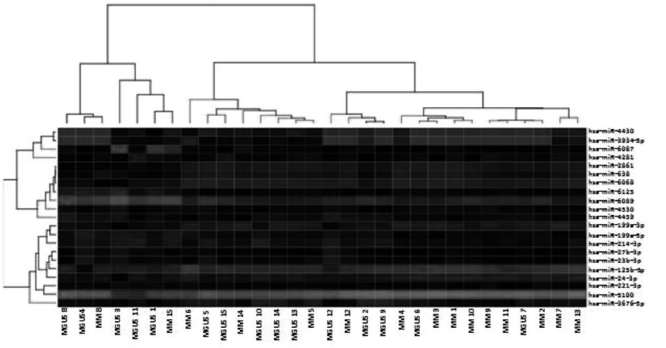

Figure 1.

Summary and Conclusions: These findings indicate that miRNA profile differs between 1st diagnosed MM and MGUS patients and that the activated phenotype of 1st diagnosed CAFs might be related to the expression of specific miRNAs. Several studies are ongoing to identify a correlation between the differentially expressed miRNAs and the activated phenotype of CAFs and their role in the cross-talk between MM plasma cells and CAFs.

Reference

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E1245

TUMOR NECROSIS FACTOR-RELATED APOPTOSIS-INDUCING LIGAND AND DEATH RECEPTORS: RELEVANCE IN THE PATHOGENESIS OF MONOCLONAL GAMMOPATHIES

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Background: Tumor Necrosis Factor-related Apoptosis-inducing Ligand (TRAIL) was identified in 1995 and, since then, growing interest has emerged in oncology due to its reported ability to selectively trigger cancer cell death. Pro-apoptotic TRAIL signaling is mediated through DR4 (also TRAIL-R1) and DR5 (also TRAIL-R2) receptors. Despite intensive investigations very little is known regarding expression of TRAIL and pro-apoptotic TRAIL receptors in plasma cells (PCs) of patients (pts) with monoclonal gammopathies (MG).
Aims: The aim of this investigation is to contribute to clarify the involvement of TRAIL and death TRAIL receptors in the development of MG, in the progression of MGUS to MM and in the prognosis of MM.
Methods: Between April 2010 and July 2013, we evaluated bone marrow PCs from 125 pts with MG, 59 symptomatic multiple myeloma (MM), 66 monoclonal gammopathy of uncertain significance (MGUS) and 11 healthy controls (Ctr). PCs were analysed by flow cytometry and the 2 populations were identified by gating CD138+/CD19- and CD138+/CD19+. We evaluated the expression of TRAIL, TRAIL-R1 and -R2 with monoclonal antibodies in both populations by flow cytometry. The results are expressed as percentage of PCs expressing these proteins and expression levels in mean intensity of fluorescence (MIF).
Results: In our population, median age was 70 (39-86) years, 52% were male. We found that the mean percentage of CD138+/CD19+ PCs expressing TRAIL and TRAIL-R1 in MM (70,8% and 73%, respectively) is significantly higher than in MGUS (54,8% and 46,1%, respectively) pts (p=0,0001) and in MGUS compared to Ctr (46,2% and 13,2%; p=0,043 and p=0,0001, respectively); TRAIL-

R2 was also higher in MGUS *vs* Ctr (56,7% *vs* 17%, p=0,0001). TRAIL expression level in PCs with the same phenotype was higher in MM (46,6±3,4 MIF) than in MGUS (35,3±1,4 MIF) pts (p=0,026), as well as the level of expression of TRAIL-R1 and -R2 in MM (32,7±1,1 MIF and 44,8±2,2 MIF, respectively) and in MGUS (37,1±1,7 MIF and 60,9±1,9 MIF, respectively) pts compared to Ctr (27,5±0,65 MIF and 24,8±0,7 MIF, respectively; p=0,0001). We also evaluated the expression of TRAIL and TRAIL pro-apoptotic receptors in CD138+/CD19- PCs in MGUS and in MM pts. In MM, we found increased expression levels of TRAIL and TRAIL-R2 in CD138+/CD19+ (46,6±3,4 MIF and 44,8±2,2 MIF, respectively) compared to CD138+/CD19- (29,2±1,8 MIF and 34,2±2 MIF, respectively) PCs (p=0,0001). Similar data were observed in MGUS pts only for TRAIL-R2 (60,9±1,9 MIF in CD138+/CD19+ *vs* 54,8±2,4 MIF in CD138+/CD19- PCs; p=0,001). In our investigation, we also searched for prognostic markers in MM pts and we found a significant better survival for pts with a percentage of CD138+/CD19- PCs expressing TRAIL superior to 13,5% (p=0,008).

Summary and Conclusions: Our study showed that TRAIL and TRAIL pro-apoptotic receptors, R1 and R2, are highly expressed in CD138+/CD19+ PCs of MG pts compared to Ctr with increased levels in MM *versus* MGUS, suggesting a significant role of these proteins in the development and progression of MG. We also demonstrated a prognostic impact of TRAIL expression in MM pts, with an improved prognosis associated with a higher percentage of CD138+/CD19- PCs expressing TRAIL.

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E1246

THE NOVEL MOLECULE STK405759 HAS SELECTIVE CYTOTOXICITY AGAINST MULTIPLE MYELOMA *IN VITRO* AND *IN VIVO*

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Background: Despite advances in treatment, including high-dose chemotherapy and novel agents such as proteasome inhibitors and immunomodulators, multiple myeloma (MM) remains incurable due to development of drug resistance in the bone marrow microenvironment.

Aims: To evaluate the use of STK405759, a synthetic small molecule, as a potentially novel drug for MM treatment in preclinical models.

Methods: Cytotoxic activity of STK405759 was evaluated byxTT assay. Apoptosis and cell cycle were checked by flow cytometry. Protein expression was assessed by Western blot. Histochemistry was used to evaluate apoptosis in tumors of mousexenograft model.

Results: Our data show that STK405759 has a potent cytotoxic activity against a wider variety of MM cell lines and patient-derived MM cells, regardless of their sensitivity to conventional therapy or novel agents. In contrast, the viability of normal peripheral blood mononuclear cells derived from healthy donors and MM patients was not inhibited. Importantly, STK405759 induced cell death of RPMIS cells co-cultured with HS-5 BMSCs. At low concentrations (nanomolar), STK405759 triggered G2 cell cycle arrest and induced apoptotic cell death associated with the cleavage of caspase-8 and poly (ADP-ribose) polymerase, as well as down-regulation of the Mcl-1 anti-apoptotic protein. These biological responses were associated with decreased AKT and CREB protein expression in MM cell lines. Combination studies of STK405759 with bortezomib, lenalidomide or dexamethasone showed significant synergistic cytotoxicity in MM cells. *In vivo* studies revealed decreased MM tumor burden and prolonged survival of STK405759-treated mice as compared with controls. STK405759 induced apoptosis of tumors cells from treated mice.

Summary and Conclusions: Our results from *in vitro* and *in vivo* studies showed the anti-myeloma activity of STK405759 and provided the rationale for further evaluation of STK405759 as monotherapy and combination therapy for treating patients with MM.

E1247

CYTOGENETIC ABNORMALITIES OF UNDETERMINED CLINICAL SINIGICANCE CORRELATE WITH POOR PROGNOSIC FACTORS IN MULTIPLE MYELOMA

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Background: The prognosis of multiple myeloma (MM) depends on the underlying cytogenetic subtype. Recurrent cytogenetic changes including hyperdiploidy of odd-numbered chromosomes, -13/13q- and balanced translocations involving *IGH* have already been incorporated into risk stratification strategies and are used in standard clinical practice. Nevertheless, MM often exhibits a heterogenous and complex karyotype, and many other chromosomal abnormalities of uncertain clinical significance are observed.

Aims: We performed a comprehensive analysis for the relationship between cytogenetic abnormalities of undetermined prognostic significance and known prognostic factors in MM.

Methods: Conventional G-banding was performed in 333 newly diagnosed MM. Karyotypes were described using the most recent version of the International System for Human Cytogenetic Nomenclature. Translocations with breakpoints at p10, p11, q10, or q11 were all regarded as whole arm (centromere to centromere) translocations. Poor prognostic indicators as follows: hemoglobin (Hb) level <10 g/dL, calcium level >12 mg/dL, IgG level >7 g/dL or IgA level >5 g/dL, b2-microglobulin level >5.5 mg/L, and albumin level <3.5 g/dL. Creatinine level >2 mg/dL, IgA type, lambda type, elevated free light chain level >100 mg/dL, abnormal kappa/lambda ratio of >4.0 or <0.5, and elevated LDH level were also included.

Results: Among the 333 MM patients, 212 showed a normal or good/intermediate karyotype (195 with normal karyotype; 17 with abnormal karyotype associated with good or intermediate prognosis); these served as the control group in this study. The remaining 121 had other cytogenetic abnormalities (with or without hyperdiploidy, or translocations associated with good or intermediate prognosis). Chromosomes 1p (12.0%), 1q (18.9%), 6q (7.2%), 8q (6.9%), 11q (5.4%), 12p (5.1%), 14q (9.3%) and 19q (5.1%).showed frequent abnormalities. Common breakpoints were p10-12, p13, p22 in 1p, q10-12, q21, q25, q32 in 1q; q21, q25 in 6q; q10, q22, q24 in 8q; q10, q13 in 11q; p10-12 in 12p; q24, q32in 14q and q10, q13.3 in 19q (Figure 1). Certain chromosomal abnormalities were associated with known prognostic factors (p-value <0.05): 8q-IgA type; 1q-IgG level of >5 g/dL or IgA level of>3 g/dL; 14q-kappa or lambda type chains >100 mg/dL; 1p, 1q, 6q, and 19q-Hb <10.0 g/dL; 1p and 12q-albumin <3.5 g/dL; 14q and 19q -creatinine >2.0 mg/dL; 1p, 1q, 6q, or 19q-b2-microglobulin >5.5 mg/L; 1p, 8q, 11q, and 14q-calcium >12 mg/dL; 1p, 3p, 12p, or 14q-elevated LDH level.

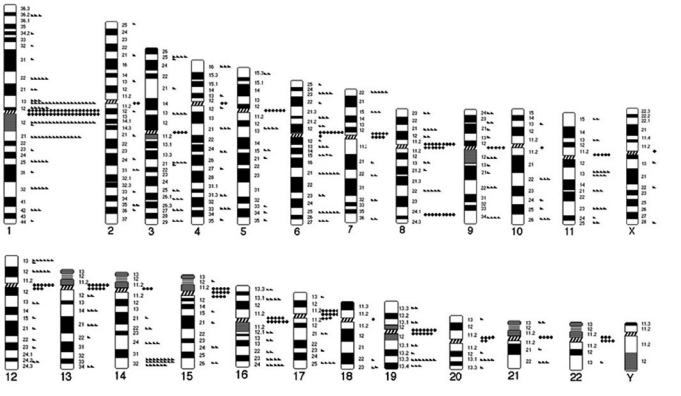

Figure 1.

Summary and Conclusions: We analyzed the distribution and clinical significance of the less commonly occurring cytogenetic abnormalities for the first time. Abnormalities in chromosomes 1p, 1q, 3p, 6q, 8q, 11q, 12q, 14q, and 19q were associated with clinical factors associated with a poor prognosis. Not all chromosomes showing frequent aberrations correlated with prognostic factors, indicating that it is not the presence of a cytogenetic abnormality in itself, but the particular chromosome(s) involved in the abnormality that is the key factor that determines the initial clinical characteristics of MM

E1248

PLASMA CELLS NEGATIVE SELECTION TECHNIQUE IN SAMPLES OF PLASMA CELL DYSCRASIA PATIENTS.

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Background: A 15-20% of plasma cells (PCs) is the main prerequisite for diagnosis of genetic abnormalities by FISH techniques in bone marrow interphase plasma cell dyscrasia patients. Plasma cells (PCs) enrichment from bone marrow samples patients are frequently performed by the traditional method of immunomagnetic separation (magnetic activated cell sorting, MACS) using anti-CD138 MicroBeads. The main inconveniences of the MACS method are: its high cost, it is unsuccessful if the percentage of PCs in the initial sample is less than 5% and the PCs positive selection affect to the quality of the cells. RosetteSep Human Multiple Myeloma Enrichment Cocktail The RosetteSep

enrichment cocktail cross-links unwanted cells in human bone marrow to multiple red blood cells (RBCs) using Tetrameric Antibody Complexes (TAC), forming immunorosettes. This increases the density of the unwanted cells, in such a way that they pellet along with the free RBCs when centrifuged over Ficoll-Paque PLUS. Desired cells are never labeled with antibodies and are easily collected as a highly enriched population at the interface between the plasma and Ficoll-Paque PLUS. With this above negative selection cells technique, it obtain a great quantity and high quality cells.
Aims: The aim of this paper is to find the optimal algorithm, results and advantages in cost and pure and quality cell populations of the RosetteSep separation technique for samples of 50 bone marrow plasma cell dyscrasia patients with various percentages of neoplastic cells for applying FISH methods in these diseases.
Methods: Specimens: We analysed 76 heparinized bone marrow aspirates with known PCs dyscrasias for FISH diagnosis of genetic abnormalities. The percentage of monoclonal PCs varied between 0.48 and 73.5% (mean 14.39%) according to flow cytometry analysis on the previous study before PCs selection. The cytology analysis was used when the percentage was between 3 to 4%, to determine the PCs infiltration, when there was an infiltration ≥7% or presence of cell nests the sample was processed. Cell separation: RosetteSep technique was used for separation of PCs in all specimens. Briefly, we added RosetteSep Multiple Myeloma Enrichment Cocktail at 50 µl/ml of bone marrow aspirate sample, incubated 20 minutes at room temperature and diluted it with an equal volume of Ficoll-Paque PLUS. After centrifuging for 20 minutes at 1200xg at room temperature we removed and washed the enriched cells from the density medium (plasma interface). For all cases, enrichment was accomplished. Flow cytometry analysis: It was proved the percentage of PCs before and after the separation. Enriched sample was stained with CD38-FITC and CD45-V450 and acquired using a BD FACSCanto II. Percentage of PCs among non-erythroid cells was obtained by Infinicyt software (Cytognos). FISH analysis: FISH analysis was carried out following standard procedures on interphase cells. The number of interphase cells analyzed were 200.
Results: 76 bone marrow samples were analyzed from patients with a range age between 38-89 years old (mean 67 years old). 19 sample patients with ≤5% of preselected PCS by flow cytometry (0.48%>4.9%) showed hemodilution (1 cases). Cytology analysis were carried out in spite of do the PCs selection in the rest of the bone marrow samples with ≤5% PCs (6 cases); the PCs selection was realized when the samples showed a ≥7% of PCs and/or nest *cells* by cytology. All samples were successfully separated. The median of PCs after enrichment was 60.5% (range 45.93- 86.75%). We obtained results in all of 77 FISH samples analyzed.
Summary and Conclusions: The introduction of RosetteSep contributed markedly in increasing the effectiveness of plasma cell separation from bone marrow samples, mainly in samples with low plasma cell content where the MACS method is not unsuccessful (<5%), its negative selection strategy enabled us to obtain sufficient amounts of highly purified and quality PCs required for subsequent diagnosis techniques proposed. On the other hand, the above technique has a low cost as opposed to MACS. The increase in the PCs concentration permits better FISH results incrementing the genetic abnormality detections which have a high prognosis value in these pathologies.

Myeloma and other monoclonal gammopathies - Clinical

E1249

CT IS SUPERIOR TOx-RAY IN DIAGNOSING OSTEOLYTIC LESIONS IN THE SPINE AND PELVIS: A PROSPECTIVE STUDY OF NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS

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Background: The presence of osteolytic lesions may define if a patient has multiple myeloma needing treatment or smouldering myeloma, and once developed the skeletal damage tends to be irreversible. Thus, a sensitive examination of the skeleton at diagnosis is of great importance for the decision to start anti-myeloma treatment. Conventionalx-ray has been the method of choice in many centres, despite increasing evidence that other imaging modalities may be more sensitive, especially in the spine and pelvic areas.
Aims: In a prospective clinical trial we have compared low-dose Computed Tomography (CT) head to head with conventionalx-rays for the demonstration of osteolysis in the spine and pelvis in newly diagnosed multiple myeloma patients. Simultaneously the value of adding 18-flourdeoxyglukose (^{18}FDG) Positron Emission Tomography (PET) and Single Photon Emission computed tomography (SPECT) to CT was evaluated.
Methods: 35 previously untreated multiple myeloma patients (21 males, 14 females; mean age 64 (49-81)) were included in a prospective single centre phase-II study evaluating the safety and efficacy of a five drug combination of doxorubicin, cyclophosphamide, bortezomib, dexamethasone and lenalidomide. Informed consent was obtained. At baseline the bone status of the patients was evaluated by conventionalx-ray, CT, ^{18}FDG-PET-CT and SPECT-CT. The presence of osteolysis in the pelvis and spine was determined byx-ray and CT, and focal activity was studied using PET-CT and SPECT-CT. The imaging was evaluated by a group of dedicated experts.x-rays were evaluated independently from CT.
Results: CT was significantly more sensitive thanx-rays for detection of osteolytic lesions in both pelvis ($p<0.01$, n:32) and spine ($p<0.05$, n:32). Osteolysis in the pelvis was diagnosed in 69% (24/35) of the patients by CT and only in 28% (9/32) of the patients byx-ray. Two patients were diagnosed with osteolysis in the pelvis usingx-ray, which was not found at CT. In the first of these, PET was also positive, and after retrospective comparison to a follow-up CT-scan osteolysis was also recognized on the study CT. The second, had in addition to a negative CT, also negative PET and SPECT, and after retrospective comparison to follow-upx-rays, the studyx-ray was considered as false positive due to misinterpreting of intestinal air. In the spine osteolysis or malignant fractures were diagnosed in 69% (24/35) of the patients by CT and in 38% (12/32) byx-rays. In the spine none of the patients with osteolysis byx-rays had a negative CT. Additionally, CT revealed three cases with imminent affection of the spinal cord and one with extra medullary disease. In both spine and pelvis PET positive foci were found in 9% (1/11) of the negative CT-scans, while PET was negative in 50% (12/24) of the positive CT-scans of pelvis, and in 48% (11/23) of the positive CT-scans of spine (no corresponding PET of spine to one of the 24 positive CT-scans). SPECT was occasionally positive, in positive CT-scans (spine:14/23, pelvis:8/21) but never in CT-negative, indicating that SPECT positivity may reflect enhanced bone remodelling in the respective areas.
Summary and Conclusions: CT of the pelvis and spine diagnose significantly more patients with osteolysis thanx-rays of the respective anatomical area, and should be the preferred modality for the diagnosis of bone involvement in multiple myeloma. The additional value of adding the more expensive and time-consuming ^{18}FDG-PET or SPECT to CT was in this study low.

E1250

SEVERE INFECTION IN ELDERLY PATIENTS TREATED WITH RELAPSED OR REFRACTORY MULTIPLE MYELOMA: ANALYSIS OF THE INTERGROUPE FRANCOPHONE DU MYÉLOME (IFM) 2009 01 PROTOCOL

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Background: The combination of bendamustine, bortezomib and dexamethasone (BVD) demonstrated a high efficacy and a good tolerability in elderly patients with relapsed and/or refractory multiple myeloma (RRMM). However, severe infection was a major toxicity.
Aims: We reviewed clinical aspects and risk factors of severe infection occurring in the IFM 2009 01 protocol.
Methods: The IFM 2009 01 protocol evaluated the BVD combination as 2nd-line treatment in elderly patients (>65 years) with RRMM (Rodon P. *et al.*, Haematologica 2015;100(2):e56-9). Twelve cycles were scheduled: 6 monthly cycles followed by 6 cycles every 2 months. Patients did not receive any prophylactic antibiotics. All adverse events were collected and graded prospectively according to the National Cancer Institute Common Terminology Criteria for Adverse Events (version 3.0). We report here an analysis of the episodes of severe infection (grade 3 or more).
Results: Seventy-three patients were included. The median age was 75.8 years (range 66-86). The median number of cycles administered was 7. The overall response rate was 69.8%. The median progression-free survival was 10.8 months, and the median overall survival was 23 months. Twenty episodes of grade 3 or more infection occurred in 17 patients (23.2%): lung and respiratory tract infection 13 episodes, bloodstream infection 6 episodes and pyelonephritis 1 episode, respectively. A recurrence of lung infection was observed in 3 patients. Fourteen episodes were diagnosed in the early phase of therapy (within the 4 first BVD cycles). Only 1 severe infection underwent during a chemotherapy-induced neutropenic phase (absolute neutrophil count <1000/mm^3). Fifteen episodes occurred among 34 patients older than 75 years *versus* 5 episodes in 39 younger patients (p=0.0028). Sepsis was responsible of death in 5 of these 17 patients (29.4%), all occurring in the early phase of the BVD therapy.
Summary and Conclusions: Infection was a major severe adverse event in elderly patients treated for RRMM in the IFM 2009 01 protocol, occurring in approximately one quarter of the patients. Its incidence was significantly higher in patients older than 75 years. Severe episodes of infection mainly occurred in the early phase of treatment. The mortality rate was high. These findings suggest that a systematic prophylactic use of antibiotics may be needed in this population of patients.

E1251

IMMUNOGLOBULIN HEAVY/LIGHT CHAIN IMMUNOASSAYS FOR RESPONSE EVALUATION IN MULTIPLE MYELOMA: COMPARISON WITH IMMUNOFIXATION, SERUM FREE LIGHT CHAIN, AND MULTICOLOR FLOW- CYTOMETRY

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Background: Multiple myeloma (MM) patients are monitored to assess treatment response. Serum immunoglobulin (Ig) heavy/light chain immunoassays are new methods, which allow quantification of Ig light chain types (IgGκ, IgGλ, IgAκ, IgAλ).
Aims: We compared Ig heavy/light chain assays with standard methods for response assessment, and evaluated advantages and limitations of this test. We also studied whether the test predicted prognosis.
Methods: 368 samples were obtained from 128 patients with IgG and IgA type MM at Kameda Medical Center and Kanazawa University Hospital (median age: 70 years, range: 44-89; median follow-up: 29 months, range: 0.9-189.6). The proportions of patients in ISS stages 1, 2, and 3 were 24%, 37%, and 39%, respectively. Paraprotein type was IgG in 84 patients (59 IgGκ, 25 IgGλ) and IgA in 44 (23 IgAκ, 21 IgAλ). Samples were taken at various times after treatment and analyzed retrospectively by serum Ig heavy/light chain immunoassays. Heavy/light chain ratio (HLCR) was calculated with Ig'κ (G or A) as the numerator and compared to normal ranges (IgGκ: 3.84-12.07 g/L; IgGλ: 1.91-6.74 g/L; IgGκ/IgGλ: 1.12-3.21; IgAκ: 0.57-2.08 g/L; IgAλ: 0.44-2.04 g/L; IgAκ/IgAλ: 0.78-1.94). The results were compared to serum protein electrophoresis (SPEP), immunofixation electrophoresis (IFE), and serum free light chain immunoassay (FLC). HLC-pair suppression was defined as uninvolved immunoglobulin of the same isotype 50% below the lower limit of normal (IgGκ, IgGλ, IgAκ, IgAλ). Residual neoplastic plasma cells (MM-PCs) in bone marrow were assessed by multicolor flow cytometry (MFC) in 158 samples from 66 patients at very good partial response (VGPR), complete response (CR), and stringent CR (sCR). MFC negativity was defined as <10^{-4} MM-PCs. Overall survival (OS) and progression-free survival (PFS) were estimated by the Kaplan-Meier method, and survival was compared using the log rank test.
Results: MM responses were assessed according to international response criteria. Patients' samples at various responses were: Before therapy, n=60 (16%); sCR, n=81 (22%); CR, n=30 (8%); VGPR, n=136 (37%); and PR, n=61 (17%). Normal HLCR was obtained at sCR, CR, VGPR and PR in 70 (86%), 29 (97%), 76 (55%) and 9 (15%) cases, respectively. Among PR samples with normal HLCR, many SPEPs showed small M-spike with broad polyclonal peak. In the sera from patients who achieved a CR or better, abnormal HLCR and FLC ratio were seen at rates of 9.7% and 22.3%, respectively, and no sera had abnormal value in both assays simultaneously. Among the patients who achieved a VGPR or better, patients with a normal HLCR had fewer MM-PCs than those with an abnormal HLCR (median 1x10$^{-3.42}$ *vs* 1x10$^{-3.04}$, respectively, P=0.01). Shorter OS were shown in patients with highly abnormal HLCR; >100 or <0.01 at diagnosis than in those without (36.3 months *vs* not reached, P=0.038). Longer OS were observed in patients who achieved HLCR normalization at best response than in those who did not (not reached *vs* 119.7 months, P=0.0002). Presence of HLC pair suppression at best response was associated with poorer OS, but that at diagnosis was not (Figures 1,2 and 3).

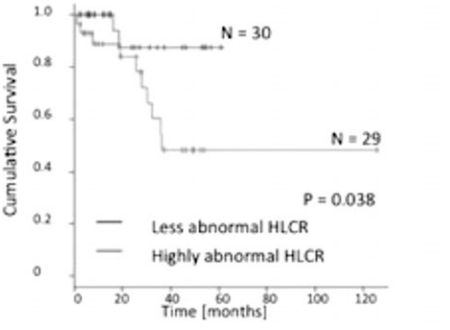

Figure 1. Highly abnormal HLCR at diagnosis is associated with shorter OS.

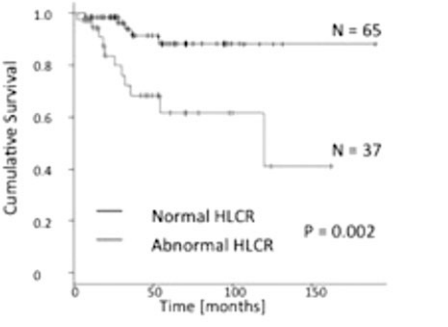

Figure 2. Abnormal HLCR at best response is associated with shorter OS.

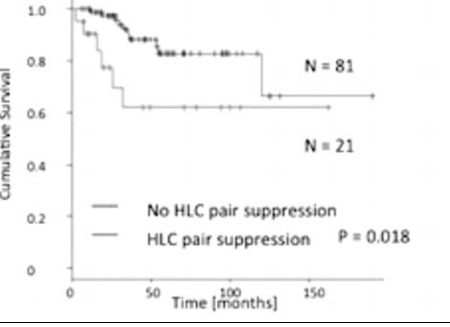

Figure 3. HLC pair suppression at best response is associated with shorter OS.

Summary and Conclusions: This study suggests the usefulness of HLC assays for the assessment of MM response. Highly abnormal HLCR at diagnosis was a poor prognostic factor. Abnormal HLCR and HLC-pair suppression at best response were associated with poorer survival. Response assessment by SPEP alone seems inaccurate, especially in patients with PR or VGPR with uninvolved Ig recovery. HLC assay enables more accurate estimation of involved Ig quantification, and should be used for response assessment in the near future.

E1252

DYNAMIC CONTRAST-ENHANCED MAGNETIC RESONANCE IMAGING PARAMETERS CORRELATE WITH ANGIOPOIETIN-1/ANGIOPOIETIN-2 RATIO AND OTHER HIGH-RISK FEATURES IN PATIENTS WITH MULTIPLE MYELOMA

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Background: Dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) studies the kinetics of the distribution of paramagnetic contrast in the microvessels and in the interstitial space of the studied tissue. It has been previously reported that DCE-MRI parameters correlate with increased angiogenesis and event-free survival of patients with multiple myeloma (MM) in the conventional chemotherapy era.
Aims: The aim of this study was to evaluate the value of DCE-MRI in the era of novel anti-myeloma agents and explore possible correlation with angiogenic cytokines that have prognostic value in MM, such as the ratio of angiopoietin-1/angiopoietin-2 (Angp-1/Angp-2).
Methods: We studied 46 MM, previously untreated, patients (24M/22F, median age 67 years, range 33-88 years) at diagnosis (37 with symptomatic MM and 9 with smoldering MM-SMM) and 5 patients with MGUS. All patients underwent MRI of the lumbar spine and DCE-MRI was performed. The following perfusion parameters that were firstly described by our group (Moulopoulos *et al.*, Ann Oncol 2003;14:152-8) were evaluated: Wash-in (WIN), washout (WOUT), time-to-peak (TTPK), time-to-maximum slope (TMSP) and WIN/TMSP (WTSP). All these parameters can be obtained from the time-intensity curves and the first derivative function [f(t)=d(DSE)/dt] of the EMG equation. The following serum indices of angiogenesis were also measured on the day of MRI: VEGF, Angiogenin (Ang), Angp-1 and Angp-2, using an ELISA methodology (R&D Systems, Minneapolis, MN, USA).
Results: MRI evaluation of the spine revealed that 11 (23.9%) patients had focal, 12 (26.1%) diffuse, 16 (34.8%) normal, and 7 (15.2%) had a variegated pattern of marrow involvement. Symptomatic MM patients had increased WIN values [median 18.7 sec^{-1}, range (2-111 sec^{-1})] compared to SMM [5.7 (1.3-30), p<0.05) and MGUS patients [1.7 (1.1-5), p=0.001]. TTPK values were decreased and WTSP values were increased in both symptomatic MM [47 sec (18-76 sec) and 0.47 sec (0.04-9.73 sec), respectively] and SMM [60.4 (36-98) and 0.35 (0.02-1.01) respectively] MM patients compared to MGUS patients [77 (67-120) and 0.04 (0.02-0.1), p<0.05 for all comparisons]. Only symptomatic patients had decreased TMSP values [32 sec (11-56 sec)] compared to MGUS patients [46 (41-71), p=0.004]. Patients with normal MRI pattern had increased WIN, TMSP and WTSP values and decreased WOUT and TTPK values compared to other patients (p<0.05 for all comparisons). The Angp-1/ Angp-2 ratio was reduced in symptomatic MM patients [13.5 (1.8-39.7)] compared to SMM patients (22.1 (9.9-32.3); p=0.017] and MGUS patients [64.2 (26.7-89.5); p<0.001]. TTPK values correlated with serum Angp-2 (r=-0.330, p=0.018) and Angp-1/Angp-2 ratio (r=0.361, p=0.009), as well as serum M-protein (r=-0.331, p=0.02). WIN values correlated with M-protein (r=0.432, p=0.002), albumin (r=-0.376, p=0.008), β2-microglobulin (r=0.335, p=0.021), hemoglobin (r=-0.309, p=0.028) and serum creatinine (r=0.371, p=0.007). The median follow up of symptomatic myeloma patients was 31 months and the median overall survival was 67 months. All symptomatic patients were treated with novel agent-based regimens. Thirteen patients progressed after first line therapy. There was a trend for shorter time to progression in patients with low TTPK values (<upper quartile, median TTP 44 months) compared to patients with high TTPK values (median TTPK not reached, p=0.09).
Summary and Conclusions: DCE-MRI parameters, such as the TTPK, correlate with increased angiogenic capacity, as reflected by the low ratio of Angp-1/Angp-2, while others, such as the WIN, correlate with high risk features of MM. Higher number of patients and longer follow-up is needed to reveal if DCE-MRI can be of prognostic significance in the era of novel anti-MM agents.

E1253

SERUM LEVELS OF VON WILLEBRAND FACTOR (VWF) BUT NOT OF ADAMTS-13 PREDICT FOR EARLY DEATH IN PATIENTS WITH AL AMYLOIDOSIS, INDEPENDENTLY OF CARDIAC BIOMARKERS

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Background: The deposition amyloid fibrils composed of immunoglobulin light chains in tissue microcirculation causes organ dysfunction in systemic light chain (AL) amyloidosis. Light chains or of oligomers of light chains may also have a direct toxic effect on cells such a myocardial cells or endothelia l cells. vWF is mainly produced, stored and secreted by endothelial cells (ECs), is critical for thrombus formation but the secretion of vWF by ECs is also triggered by several conditions and may reflect a state of "stimulation" of the endothelium. ADAMTS-13 is a protease, primarily synthesized and released from hepatic stellate and endothelial cells, which cleaves the cell bound large ultrapolymeric vWF strings. Both vWF antigen (vWFAg) and ADAMTS-13 antigen levels have been implicated as prognostic markers associated with cardiovascular events, potentially reflecting endothelia dysfunction. Cardiac dysfunction is the main determinant of prognosis in AL amyloidosis, but the role of endothelium has not been studied.
Aims: To evaluate the prognostic role of vWF and ADAMTS-13 in patients with AL amyloidosis.
Methods: Both vWFAg and ADAMTS-13 levels were measured in stored serum, using commercially available assays, in 81 consecutive patients with newly diagnosed AL amyloidosis who were treated in the Department of Clinical Therapeutics (University of Athens, Greece) and in 30 age-matched healthy controls.
Results: Median age was 68 years (range 42-82 years) and the median number of involved organs was 2; heart was involved in 62% and kidneys in 74%. Median NTproBNP level was 2,318 pg/mL (range 33-75,000 pg/mL); 36% had NTproBNP ≥4,000 pg/mL and 28%, 38% and 34% of patients had Mayo stage -1, -2 and -3, respectively. Primary therapy based on bortezomib was given in 52%, on lenalidomide in 44%, while 4% received MDex. Median survival was 47 months; 3- and 6-month mortality was 12% and 20%, respectively. The median serum level of vWFag in patients with AL amyloidosis was 181 (range 20-557) U/dL, significantly higher than in healthy controls (median: 84 U/dL, range 48-124; p <0.001). Median ADAMTS-13 levels were similar between AL patients and controls (median 1044 *vs* 1170 ng/ml, p=NS). There was no correlation of vWF and ADAMTS-13 levels, and no association of vWFag levels with renal, cardiac, nerve or liver involvement or with the levels of NTproBNP, hsTnT or Mayo stage, eGFR, serum albumin levels or proteinuria or the levels of involved free light chains. ROC curve analysie identified that levels of vWFag within the top quartile (≥230 U/dL) were associated with a very poor outcome (median survival 4 months *vs* 47 months, p=0.001). There was no association of ADAMTS-13 levels with any outcome (early death or overall survival). In multivariate analysis vWFag levels ≥230 U/dL were independently associated with a high risk of death at 6 months (HR: 15, 95% CI 2.6-84, p=0.002) together with NTproBNP ≥4,000 pg/mL (HR: 16.8, 95% CI 3- 94, p=0.001) and for overall survival (HR: 2.64, 95% CI 1.2-5.8, p=0.01), along with NTproBNP levels ≥4,000 pg/ml (HR: 4.17, 95% CI 1.98- 8.8, p<0.001). Among patients with both vWF≥230 IU/L and NTproBNP>4000 pg/ml, 75% died within 3 months from initiation of therapy.

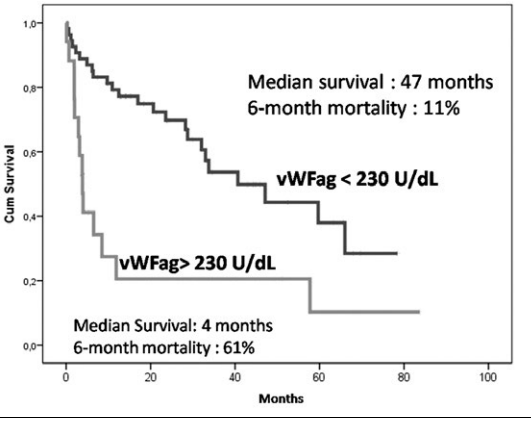

Figure 1.

Summary and Conclusions: Serum vWFag levels in patients with AL amyloidosis are elevated, without a compensatory increase of ADAMTS-13, are not correlated with other features of the disease and are associated with a high risk of early death independently of cardiac biomarkers.

E1254

CHEMOTHERAPY-INDUCED NEUROPATHY AMONG MULTIPLE MYELOMA PATIENTS AND THE INFLUENCE OF CHEMOTHERAPEUTIC AGENTS: RESULTS FROM THE POPULATION-BASED PROFILES STUDY.

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Background: Chemotherapy-induced peripheral neuropathy (CIPN) is a common adverse event of antimyeloma treatment, which may affect patients' health-related quality of life (HRQOL). Because there is no proven therapy for CIPN, it is important to closely monitor the development of this side effect in order to modify chemotherapy dose on time.
Aims: To evaluate the prevalence and severity of CIPN and the influence of antimyeloma treatment with neurotoxic chemotherapy on the development of CIPN among a population-based sample of multiple myeloma (MM) patients.
Methods: All MM patients diagnosed between 2000 and 2014 as identified by the Netherlands Cancer Registry, location Eindhoven, and alive at questionnaire

invitation, were eligible. 130 patients completed the EORTC QLQ-CIPN20 on average 3.5 years after diagnosis (74% response). Data on chemotherapy regimens, treatment response at time of the survey and dose reductions of chemotherapy were extracted from the medical files. All patients gave written informed consent.

Results: Patients received treatment with one (49%) or more (42%) neurotoxic agents, including thalidomide (50%), bortezomib (48%), lenalidomide (39%) and/or vincristine (7%). Overall, 54% of patients reported at least one neuropathy symptom ('quite a bit' or 'very much' combined). The most reported neuropathy symptoms during the past week were trouble getting an erection (46% of men), tingling toes/feet (30%), numbness in toes/feet (20%), tingling fingers/hands (19%) and trouble opening jars/bottles (19%). There were no differences in neuropathy symptoms between patients who received treatment with only one *vs* a combination of more neurotoxic agents. Patients who received chemotherapy in the past 3 months reported a trend of more experienced neuropathy (64%), compared to patients treated more than 12 months before (50%) (p=0.12). In addition, they reported significantly more severe neuropathy with worse sensory scale scores (mean 21 *vs* 12; p=0.01), respectively. Patients with relapsed or progressive MM (n/N=35/130) received treatment in the past 3 months in 31%, and 51% of patients reported CIPN. Of them 34% reported the highest (4th quartile) and therefore worst sensory subscale scores. A dose modification of chemotherapy was applied in 72% of patients, of whom 50% received a dose reduction because of neurotoxicity. Dose modifications did not seem to adversely affect treatment response rates.

Summary and Conclusions: CIPN is a common dose-limiting side effect reported by more than half of patients with MM. The neuropathy is less severe 12 months after last treatment. However, patients with MM receive neurotoxic treatment throughout a considerable period of life after diagnosis. Regardless of the poor prognosis, 31% of patients with relapsed or progressive MM were still actively treated with chemotherapy, with a high neuropathy symptom burden, which may negatively affect their HRQOL.

E1255

FRONTLINE INDUCTION THERAPY FOR MULTIPLE MYELOMA (MM) IN REAL-WORLD CLINICAL PRACTICE: THIRD INTERIM ANALYSIS OF THE MULTINATIONAL, OBSERVATIONAL EMMOS STUDY (NCT01241396)

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Background: There is a lack of objective data on variation in treatment practices and outcomes for MM between countries.

Aims: The prospective, non-interventional EMMOS study was designed to capture real-world data regarding treatments for MM at different stages of the disease.

Methods: Consenting adult patients (pts) initiating any new line of therapy for MM, irrespective of treatment line at study entry or type of therapy, were eligible for inclusion in the EMMOS registry. A multi-staged site/pt recruitment model was applied to minimize selection bias; enrolment was stratified by country, region, and practice type. At baseline, pts' medical and disease features, treatment history, and remission status were documented. Prospective data regarding treatment, efficacy, and safety (treatment-emergent adverse events [TEAEs]) were electronically captured every 3 mos until 2 yrs after the last pt enrolled. Responses were investigator-assessed; no predefined response criteria were mandated. Here we report results from the third interim analysis of the study, with a focus on frontline therapy in MM pts who had undergone stem cell transplantation (SCT) at the time of data extraction.

Results: Between October 2010 and October 2012, 2353 pts were enrolled in 22 countries in Europe and Africa. Enrolment by practice type was 44% academic, 29% regional, 16% private, and 11% local clinics. The date of data extraction for the present analysis was 29 December 2014, at which time 772/2353 pts had undergone SCT in any treatment line. In the SCT population: pts' median age at MM diagnosis was 57.0 yrs (range, 25-74); 58% of pts were male; 13% had severe renal impairment; and 71% had bone lesions. In 679 pts with available staging data, 69% had ISS stage II/III disease (89% Salmon-Durie stage 2/3). Median duration of follow-up from diagnosis was 40 mos (range, 4-345) and from study entry was 28 mos (range, 0.4-46). The majority of pts (658/772; 85%) underwent only a single SCT procedure during the course of their disease. 534/772 (69%) pts were recorded (retrospectively or prospectively) as having received SCT in frontline. Prospective treatment data were available for 385 SCT pts, of whom 297 (77%) had received SCT upfront and 88 (23%) had received SCT in later lines. As part of their frontline treatment, 293 (76%) pts had received bortezomib-based combinations and 92 (24%) had received non-bortezomib combinations. Response rates to frontline therapy are summarized in the Table 1. Rates of higher-quality responses (very good partial response or better [≥VGPR]) appeared higher with bortezomib-based compared with non-bortezomib-based frontline therapy (65% *vs* 49%). Safety data were analyzed in the 293 pts who had received bortezomib-based combinations in frontline. The most common TEAEs were nausea (19%), diarrhea (18%), and pyrexia (17%). Peripheral sensory neuropathy and neuropathy peripheral were each reported in 28 (10%) pts. 12 (4%) pts experienced ≥1 serious TEAEs that were considered to be at least possibly related to bortezomib.

Table 1. Best response to frontline therapy (response-evaluable population, n=309)*.

	≥VGPR		PR		SD/MR		PD	
	n	%	n	%	n	%	n	%
Btz-based combinations (n=235)	152	65	64	27	14	6	5	2
Non-Btz-based combinations (n=74)	36	49	28	38	10	14	0	0
Total (all frontline combinations; n=309)	188	61	92	30	24	8	5	2

*Response data were missing for 76/385 SCT pts with available prospective data on frontline therapy received; this included 58/293 pts who received bortezomib-based combinations and18/92 pts who received non-bortezomib combinations as frontline therapy.
Abbreviations: Btz, bortezomib; MR, minimal response; PD, progressive disease; PR, partial response; SD, stable disease; VGPR, very good partial response.

Summary and Conclusions: Data from this real-world observational study appear to reflect experience in the prospective clinical trial setting. Bortezomib-based therapy was commonly used as part of frontline treatment in pts undergoing SCT in routine clinical practice, and produced substantial response rates. No new safety signals for bortezomib were observed.

E1256

ANALYSIS OF OVERALL SURVIVAL IN MULTIPLE MYELOMA PATIENTS WITH ³3 LINES OF THERAPY INCLUDING A PI AND AN IMID, OR DOUBLE REFRACTORY TO A PI AND AN IMID USING REAL WORLD DATA

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Background: Novel agents under evaluation for multiple myeloma (MM) treatment show promising initial results. Interpretation of these findings is challeng-

ing due to limited information about the natural history of MM in relapsed patients, particularly in patients treated with recently approved agents. Previously, an International Myeloma Working Group study determined the outcomes of patients refractory to bortezomib and at least 1 IMiD (Kumar S *et al.* Leukemia 2012; 26: 149). Since then pomalidomide (IMiD) and carfilzomib (PI) have been approved for relapsed and refractory MM in the United States (US).
Aims: To use real-world data to define the treatment landscape and natural history of heavily pretreated MM by identifying a cohort of patients with MM double refractory to a PI and IMiD or who had received ≥3 lines of therapy (LOT; including a PI and IMiD).
Methods: The data source was the IMS LifeLink: IMS Oncology Electronic Medical Records (EMR) Database (IMS Health Incorporated, all rights reserved) comprising US patients only. Cohort identification of the initial population included patients from the index period of 2000-2011, excluding those with prior cancer diagnoses. Approximately 88% of patients were diagnosed with MM in 2006 or later. Patients who received ≥3 LOT (including a PI and IMiD) and had shown disease progression within 60 days of completion of the most recent regimen, or had disease refractory to both a PI and IMiD were identified as the target population. MM drugs considered for regimen analysis included PIs (bortezomib, carfilzomib), IMiDs (thalidomide, lenalidomide, pomalidomide), and chemotherapy/steroids (melphalan, cyclophosphamide, dexamethasone). Median overall survival (OS) was assessed based on age; gender, ECOG status at last line of therapy; degree of refractoriness; or prior LOT (≥6 *vs* <6).
Results: Of screened 4,030 patients with multiple myeloma, 101 were excluded for prior cancer diagnoses, 2,918 patients were available for regimen analysis, and 500 met the criteria for the target population. Median ages at diagnosis and eligibility were 66 and 70 years, respectively. At eligibility, 52% were male and 26% had an ECOG score ≥2. Of the 500 patients, 177 received ≥3 prior LOT including a PI and IMiD. The remaining 323 (65%) patients met the criteria of double refractory disease, 22% of whom were triple (n=61) and quadruple (n=9) refractory. Overall, patients received a median of 3 (range, 1-25) prior LOT. All patients received 1 LOT after designation of eligibility, 298 received 2 LOT, and 169 received 3 LOT. Exposure to novel agents was 22% (carfilzomib, 16%; pomalidomide, 6%) at first LOT with >80% as a single agent. Median OS for the full eligible population of 500 patients was 239 days. Median OS was significantly higher in patients aged <65 *versus* ≥65 years (289 *vs* 204 days; P <0.01), but was not significantly different by gender (male, 228 days *vs* female, 260 days). Median OS was 228 days for double refractory patients, 154 days for triple/quadruple refractory, and 350 days for those that received ≥3 prior LOT including a PI and IMiD but were not double refractory. Median OS was 239 days for ECOG score ≤2 *versus* 87 days for ECOG score >2 (P <0.0001). Median OS was 274 days in patients with ≥6 prior LOT compared with 190 days in those with <6 prior LOT (P=0.09).
Summary and Conclusions: These real-world data confirm poor outcomes in patients relapsed or refractory to current MM treatments and highlight the unmet clinical need for novel therapeutic agents for the treatment of heavily treated patients.

E1257

DETECTING EARLY RELAPSE IN MULTIPLE MYELOMA AFTER ASCT: USEFULNESS OF IMMUNEASSAYS

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Background: There are new tools for accurate follow-up and diagnosis assessment in Multiple Myeloma (MM) patients. While the Free Light chain immunoassay (FLC) (Bindingsite, Birmingham, UK) is part of the mandatory response assessment according to IMWG-criteria and recently one of the criteria for diagnosis, the role of the Heavy/Light Chain immunoassay (HLC), is still under investigation with promissory results. Relapses in MM patients are frequent and there is an especial interest in select therapy for consolidation/maintenance after Autologous Stem Cell Transplantation (ASCT) and to detect early relapse to optimize the therapy. We hypothesized that the combination of these techniques could permit to detect early biological (non-symptomatic) relapses (EBR) in these kind of patients.
Aims: To analyze the usefulness of HLC and FLC to detect EBR in MM after ASCT in our hospital.
Methods: A retrospective study was performed including all consecutive patients treated in our center, following these criteria: Diagnosed of secretory MM, upfront-therapy including ASCT between May 2011-August 2014, assessed with our protocol including FLC, HLC, serum and urine electrophoresis (SPE, UPE) with immunofixation (IFX), previous to ASCT, after 12 weeks and every 3 months later with a minimum follow-up of 6 months after ASCT. EBR was defined as an increase of 25% on M-protein (any amount for patients on CR/SR) and/or an increase of >o=20mg/dl for FLC, and/or 25% increase on involved HLC with abnormal ratio. For urine, an increase >500mg/24 hrs of involved free-chain protein.
Results: Fifty-five patients were registered. Median follow-up 21 months. MF ratio: 29/26, mean age 59.5 y (33-71). Immunoglobulin subtype: IgG-Kappa: 41.8% (23), IgG-Lambda: 23.6% (13), IgA-Kappa: 16.4% (9), IgA-Lambda: 7.3% (4), Bence-Jones-Kappa: 3.6% (2), Bence-Jones-Lambda: 7.3% (4). Durie-Salmon Stage: IA: 13.5% (7), II-A: 32.7% (17), III-A: 44.2% (23), III-B: 9.6% (5), missing-data 3 case. All patients received Bortezomib based therapy and MEL200 as conditioning regimen. Status pre-ASCT: minimal response: 12%, Partial Response (PR): 50.0%, very-good-PR (VGPR): 28.0%, complete response (CR): 6% and string response (SR): 4.0%. After ASCT, evaluation reveals that 13.0% achieved SR, 13.0% CR, 30.4% VGPR and 39.1% PR. During follow-up, 27/50 (54.0%) patients who achieved at least PR after ASCT, had a clinical relapse/progress, median PFS 24 months (19.8-28.1). EBR were detected in 19/27 relapsed patients at median time 7 (2-19) months before symptomatic relapse. The EBR were detected by FLCr (31.6%), HLCr (21.0%), FLC+SPE (10.5%), FLC+IFX (5.2%), FLC+HLC+SPE (15.8%), FLC+HLC+SPE+UPE (15.8%).
Summary and Conclusions: Both FLC and HLC are useful tools to detect EBR, in our cohort in more than 50% of patients ahead other techniques. A review of the criteria to re-introduction of therapy and research on this field are warranted.

E1258

MYELOMA MULTIPLE IN PATIENTS AGED ≥80 YEARS, A GROUP IN CONSTANT GROWTH: EXPERIENCE OF A SINGLE CENTER

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Background: In the last decades, the median age at diagnosis of MM cases and the proportion of very old patients (>80 years) have increased. However they are underrepresented in clinical trials.
Aims: The objective of this study is to analyze the clinical features and outcome of very elderly patients with MM and the impact of therapeutic advances in this group of patients.
Methods: We conducted a retrospective analysis of patients with MM diagnosed at HGUV who were 80 years of age or older. The characteristics evaluated included ISS staging, hemoglobin, calcium, renal function, M-protein, cytogenetic study and bone disease. We review treatment, response rate (RR), early mortality and overall survival (OS), and we compare the outcome with those of patients from 65 to 79 years old.
Results: Among 311 patients with MM diagnosed at HGUV from January 1990 to December 2014, 70 (22.5%) were ≥80 years (median age 82.5 years, range 80-93 years) and 166 (53.4%) between 65-79 years, 46% (n=32) males and 54% females (n=38). The proportion of ≥80 years patients increase from 18% in 1990-1999 to 22.4% in 2000-2009 and to 25.6% in 2010-2014. Compared to 65-79 years, octogenarian had more frequently ISS 2-3 (73 *vs* 89.6%, p=0.02) and renal impairment (56.9%vs67.2%;p=0.35), with a similar incidence of severe RI (21.5% and 22.4% respectively). No significant differences were observed in hemoglobin, calcium, and bone disease between both groups. Cytogenetic data were available for 43 patients (63.2%). The incidence for del 17p was 2.4%, 4.9% for t(4;14) and 14.6% for del (13) in ≥80 years group and 4.5%, 9.1% and 6.8% respectively in 65-79 years. There was no significant differences in proportion of smoldering MM between ≥80 years old patients (13.3%) and 65-79 years group (17.7%)(p=0.29). Data from front line therapy were available for 59 patients. Fifteen patients (25.3%) were considered unable to tolerate standard therapy, 4 (6.7%) received steroid alone and 11 (18.6%) only supportive therapy. As first line treatment, 24 patients received melphalan-prednisone (MP) and 13 bortezomib-based regimens (BBR) (with MP:6, Dexamethasone:5 or bendamustine:2). Overall RR (≥partial response) was 44% in ≥80 years. RR were higher in 65-79 years group (60.25%). The median follow up was 27 months in the younger group and 15 months in ≥80 years. Early mortality (≤2 months) was 17.14% in octogenarians and 11.44% in the 65-79 years group. (p=0.236) The median OS was 18 months for ≥80 years and 35 months for 65-79 years group (Log Rank p=0.009). However, there was no significant difference when we excluded patients without active treatment due to comorbid conditions or the smoldering MM. In the subset of 65-79 years, patients treated with VMP had better OS compared with MP (42 *vs* 29 months; p=0,026). However in the ≥80 years group no significant difference in OS between patients treated with MP and BBR was observed.
Summary and Conclusions: Our study evidences that octogenarian MM patients shows shorter OS compared with old patients between 65-79 years. The high rate of ≥80 years patients who are not fit for standard treatment (25%), the high frequency of early mortality (17%) and advanced ISS staging have a negative impact on survival. We have not observed an improvement on OS with BBR in octogenarian patients, however the number of patients was low. Beside the fact that we need more clinical trials that include octogenarian patients, we think that these patients are frail and need an especial approach in terms of treatment and management.

E1259

RESPONSE AND EARLY RELAPSE ASSESSMENT IN MULTIPLE MYELOMA TREATED WITH BORTEZOMIB: ROLE OF HEAVY/LIGHT-CHAIN IMMUNEASSAY

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Background: Multiple Myeloma (MM) is characterized by multiples relapses, for that reason the objective of MM therapy is to achieve the most deeper and sustained response; actual approaches included a consolidation and/or a maintenance period with a close monitorization to assess the relapses and identified the best moment to re-start the therapy. The routinely follow-up for minimal residual disease in MM is based in the M-protein quantification by serum and urine electrophoresis (SPE, UPE) with immunofixation (IFX). The incorporation of Serum Free Light Chain Assay (FLC), and the quantification of paired clonal and non-clonal immunoglobulins (HLC) in serum, offers the possibility to assess the response to therapy more accurately detecting early non-symptomatic (biological) relapses (EBR).
Aims: To analyze the usefulness of HLC and FLC during MM follow-up to detect EBR in patients who received Bortezomib-based first line therapy in MM patients in our center.
Methods: Since January 2008 we have incorporate in the protocol for M-components assessment the quantification of FLC, later the HLC; both at baseline and in follow-up of MM. We have analyzed these parameters in all consecutive patients diagnosed as secretory MM who complete at least 4 cycles of Bortezomib-based upfront therapy between Jan 2008-Jun 2014 in our center.
Results: A total of 135 MM patients started bortezomib-based therapy. Median follow-up 25 months. Females: 40.7%, mean age 69.6 y (32-91). Subtype: IgG-Kappa: 38.2%, IgG-Lambda: 9,8%, IgA-Kappa: 17.9%, IgA-Lambda: 15,4%, IgDL: 1.6%, Bence-Jones-Kappa: 6,5%, Bence-Jones-Lambda: 8,9%, oligosecretory: 1.6%. Durie-Salmon Stage: IA: 9.8%, IB: 1.6%, II-A: 27.6%, IIB: 8.9%, III-A: 21.1%, III-B: 21.1%. ISS: I: 25.2%, II: 32.5%, III: 23.6%. The 21.1% of patients were not included in the analysis of response by uncompleted treatment. Response at end of therapy: minimal response: 5.1%, PR: 44.3%, VGPR: 16.5%, CR: 14.4% SR: 7.2%, Failure: 12.5%. During follow-up 65.7% patients, who achieved at least PR had clinical relapsed/progressed, in 86.9% of them, a previous EBR were detected at a mean of 4.4 months before; methods who detected BER: FLCr (28.8%), HLCr (13.5%), FLC+SPE+IFX (9.6%), FLC+IFX (5.8%), FLC+HLC+SPE (28.8%), FLC+HLC+SPE+UPE (5.7%) Median PFS 20 months (12.8-27.1) Biological PFS: 18 m (12.1-23.8).
Summary and Conclusions: Both FLCr and HLCr are sensitive and précised tools to perform MRD assessment response in MM, in our cohort a 42.3% of EBR were detected ahead of other techniques. More investigations on the role of EBR for therapy initiation are necessary.

E1260

MAJOR CARDIOVASCULAR EVENTS AND COMORBIDITIES IN PATIENTS NEWLY DIAGNOSED WITH MULTIPLE MYELOMA

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Background: Multiple myeloma (MM) is a common hematological cancer in the United States and 62% of patients (pts) diagnosed with MM are aged ≥65. While cardiovascular diseases (CVDs) are prevalent in the older population, the prevalence of CVD and CVD risk factors in pts with newly diagnosed MM (NDMM) have not been well described. In a Swedish study comparing the risk of arterial thrombosis (coronary artery disease and cerebrovascular disease) between 18,627 MM pts diagnosed between 1958 and 2006 relative to 70, 991 controls matched by age, sex, and county of residence, the risk of arterial thrombosis was significantly increased at 1, 5, and 10 years of follow-up (hazard ratio at 1 year=1.9; 95% confidence interval, 1.8-2.1). Mechanisms by which MM pts may be at increased risk of arterial thrombosis are not well understood and thus it is important to understand the prevalence of CVD comorbidities in NDMM pts.
Aims: To describe and characterize the prevalence of prior CVD comorbidities including major arterial events, in NDMM pts and to compare the prevalence of these comorbidities in pts <75 and ≥75 years of age.
Methods: This was a descriptive analysis of NDMM pts aged ≥18 years with Medicare/Medicare Part D coverage in the SEER-Medicare database from July 1, 2007 to December 31, 2009. Pts had continuous enrollment 6 months prior to and 3 months after diagnosis and did not have renal disease requiring dialysis. Comorbidities were identified using International Classification of Diseases, 9th Revision-code-based algorithms.
Results: We identified 3,920 NDMM pts, of whom 1,937 (49.4%) were female, 2,770 (70.7%) were White, and 3,662 (93.4%) were ≥65 years at diagnosis (1,457 (37.2%) were ≥age 75). A Charlson comorbidity score of 0 (lowest risk for 10-year mortality) was present in 2,544 (64.9%) pts. Overall, 212 (5.4%) and 130 (3.3%) NDMM pts had a previous diagnosis of acute coronary syndrome or myocardial infarction (MI) and prior diagnoses of stroke or transient ischemic attack (TIA) were noted in 179 (4.6%) and 123 (3.1%) pts, respectively. Heart failure (HF) was noted in 706 (18.0%) pts, atrial fibrillation in 382 (9.7%) pts, and conduction disorders in 323 (8.2%) pts. Of conditions associated with increased cardiovascular risk, 2,256 (57.6%) had hypertension, 2,109 (53.8%) had hyperlipidemia, and 706 (18.0%) had diabetes.
Most CVD comorbidities were more prevalent among those aged ≥75 (Table 1).

Table 1. Comorbidities Present in Multiple Myeloma Patients at Diagnosis by Age <75 and ≥75 in the SEER-Medicare Database between July 1, 2007 and December 31, 2009 (N=3,920).

	Overall N=3,920	<75 years N=1,851	≥75 years N=2,069
Comorbidity	**N (%)**	**N (%)**	**N (%)**
Acute coronary syndrome/unstable angina	212 (5.4)	87 (4.7)	125 (6.0)
Acute myocardial infarction	130 (3.3)	46 (2.5)	84 (4.1)
Atrial fibrillation	382 (9.7)	104 (5.6)	278 (13.4)
Stroke	179 (4.6)	66 (3.6)	113 (5.5)
Transient ischemic attack	123 (3.1)	46 (2.5)	77 (3.7)
Conduction disorders	323 (8.2)	88 (4.8)	235 (11.4)
Diabetes	706 (18.0)	363 (19.6)	343 (16.6)
Heart failure	706 (18.0)	231 (12.5)	475 (23.0)
Hyperlipidemia	2,109 (53.8)	990 (53.5)	1,119 (54.1)
Hypertension	2,256 (57.6)	994 (53.7)	1,262 (61.0)

Summary and Conclusions: While the background prevalence of major arterial events (acute coronary syndrome, MI, stroke, TIA) was low, there were large numbers of pts with other CVD comorbidities at MM diagnosis. Existing HF at MM diagnosis was noted in 23% of those ≥75 years of age. Recognition of these underlying CVD comorbidities may influence clinical management of NDMM and may assist in understanding the pathogenesis of arterial thromboembolism in these pts.

E1261

ASSESSING TREATMENT RESPONSE IN MULTIPLE MYELOMA: IMPLICATIONS OF USING BONE MARROW PLASMA CELL CONTENT AS A MARKER OF DISEASE BURDEN

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Background: Unlike the majority of malignancies, myeloma tumor burden can be assessed either directly by the tumor cell content per bone marrow examination, or indirectly by the quantity of paraprotein being secreted by the plasma cells. Contemporary response criteria primarily place primacy on the quantitation of serum or urine monoclonal protein, or should these be inadequate, serum free light chain levels. Bone marrow plasma cell content (BMPC) is currently a secondary metric employed when the aforementioned are non-applicable, as in non-secretory myeloma, as well as to characterize deep responses such as complete response.
Aims: To study the correlation between marrow plasmacytosis and monoclonal protein levels, differential treatment effects, and possible implications of any discrepancies.
Methods: We identified 845 patients with newly diagnosed MM treated at Mayo Clinic who demonstrated measurable disease by serum or urine M protein (1 gm/dl and 200 mg/24 h, respectively) or by serum FLC (dFLC >=10 mg/ml); 628 (74%) were evaluable by BMPC criteria. Comparisons were made between (i) treatment responses based on IMWG criteria and BMPC change, (ii) variations of response with respect to specific regimens, and (iii) the effect on survival outcomes.
Results: The median age of the group was 60.5 y; 59% were male, and 58% were alive at the time of analysis. Initial therapy included conventional chemotherapy (13%), dexamethasone (13%), IMiD based (44%), proteasome inhibitor (PI) based (19%), or PI-IMiD combination (11%). When using IMWG criteria, 74% demonstrated partial response, ranging between 58% for conventional therapies to 94% with PI-IMiD combinations. Similarly, when using BMPC criteria for PR per >=50% decrease, 73% of the evaluable patients achieved PR. Overall, 484 (80%) of the 604 patients who were evaluable by both BMPC and M protein demonstrated concordant results by BM and M protein/FLC measurement. Of the remaining 120 patients, 50 demonstrated PR solely by BMPC criteria, while 70 achieved PR by M protein/FLC measures only. When measurable BMPC was defined as >=20%, 679 patients were evaluable by both measures, and the concordance rate remained at 80%. The ratio between serum M protein reduction (%) and the BMPC reduction (%) was compared between conventional and novel therapies. The ratio was significantly lower with conventional therapies (median 0.83) when compared to novel therapies (median 1.02), p <0.01. This suggests

that the BMPC% does not decrement proportionately to the degree of decrease in M protein with novel therapies, which could be consistent with a cytostatic rather than a cytotoxic effect. The median OS for those with a concordant PR by the two measures was superior compared to those who were discordant or not in a PR by either system (p=0.02).
Summary and Conclusions: The current convention of identifying PR by specifying BMPC reduction by >50% with a baseline of >=30% is concordant with M protein criteria for PR in 80% of this cohort. Using a lower threshold of 20% BMPC at baseline had no demonstrable change in PR concordance. Superior median OS was associated with PR concordance between both M protein and BMPC assessments.

E1262

PATIENT EXPERIENCE WITH LIGHT CHAIN AMYLOIDOSIS: A SURVEY FROM THE AMYLOIDOSIS RESEARCH CONSORTIUM

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Background: Light chain (AL) amyloidosis is caused by an accumulation of misfolded proteins, resulting in dysfunction of vital organs (*e.g.*, heart, kidneys, liver, soft tissue). The protean clinical features of AL amyloidosis reflect its systemic nature, and initial symptoms are often nonspecific (*e.g.*, weight loss, fatigue). Consequently, a correct diagnosis is frequently made only after the disease has become advanced. Therapy is directed at the plasma cells that produce the pathogenic proteins. In advanced disease, treatment may be associated with significant adverse effects. 20% to 30% of patients die within 3 to 6 months of initiating therapy. Diagnosing the disease and accessing appropriate therapy pose significant challenges, and there is a paucity of literature depicting the patient experience. This not only impacts patients' quality of life (QoL) but likely also their survival. Such information may identify unmet clinical needs and ways to improve patient care and disease outcomes.
Aims: The primary aims of this study were to identify the challenges in establishing a diagnosis of AL amyloidosis and to gain insight into the AL patient experience.
Methods: Patients, family members, and caregivers were invited to participate in an anonymous online survey through email and social media channels of the Amyloidosis Foundation and an amyloidosis awareness group on Facebook. The 16-question survey was developed by the authors and was available to participants online from January 29 to February 5, 2015. Eight follow-up questions were sent via email to those who responded.
Results: A total of 507 persons completed the survey (patients, 57%; family members, 34%; caregivers, 8%). Most responders were female (63%) and between 50 and 69 years of age (63%). Average age at diagnosis was 57 years. Initial symptoms included fatigue, shortness of breath, weakness, neuropathy, swelling of the legs, and enlarged tongue. The organs most frequently involved included the heart (36%) or kidney (28%); 21% reported multiple organ involvement. Time from initial symptoms to correct diagnosis was more than 1 year in 38% of responders; 31% met with 5 or more physicians before a correct diagnosis was made (Figure 1). Diagnosis occurred at 144 institutions. 62% of responders were evaluated at an amyloidosis center, and 43% received treatment at an amyloidosis center. Almost half (40%) the responders reported receiving no disease-specific information or education. Clinical trial information was provided to only 24%. Almost half (46%) the responders would consider enrolling in a clinical trial if they felt well informed; yet 45% did not know how to enroll. Difficulty tolerating treatment was reported by 54% (93/173) of responders. Following therapy, no improvement in QoL was reported by 30%, with some improvement and definite improvement reported by 40% and 30%, respectively (175 QoL responses). Updated results will be presented.

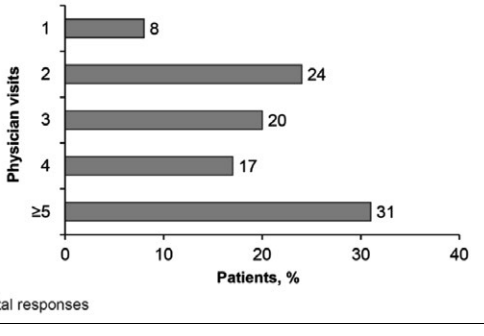

Figure 1. Number of physician visits before establishment of a diagnosis.

Summary and Conclusions: A correct diagnosis of AL amyloidosis often requires numerous physician visits to different medical specialists and often occurs when disease is advanced. The responses obtained in this patient survey highlight the challenges experienced by patients with AL amyloidosis. These data may identify opportunities to educate patients and physicians in order to expedite diagnosis, facilitate appropriate disease management and access to clinical trials, and ultimately improve patient survival.

E1263

POMALIDOMIDE+LOW-DOSE DEXAMETHASONE IN PATIENTS WITH RELAPSED OR REFRACTORY MULTIPLE MYELOMA AND RENAL IMPAIRMENT: PHASE 1 PHARMACOKINETICS AND SAFETY

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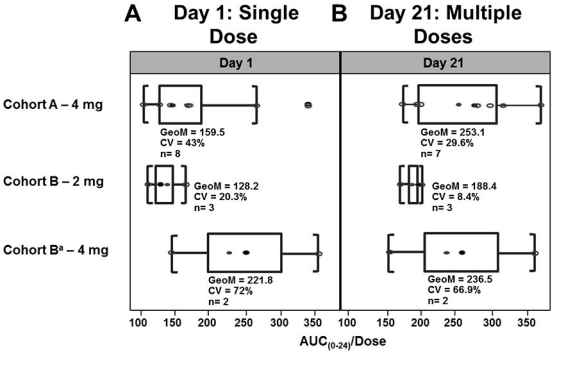
Background: Twenty percent to 40% of patients (pts) with multiple myeloma (MM) exhibit renal impairment (RI), which is associated with poor outcomes (Eleutherakis-Papaikovou *et al.*, *Leuk Lymphom,* 2007; Knudsen *et al.*, *Eur J Haematol,* 2000). Two percent to 13% of these patients require dialysis (Blade *et al.*, *Best Pract Res Clin Haematol*, 2005). More than 95% of pomalidomide (POM) is hepatically metabolized, and <5% is renally excreted as the parent drug; hydrolysis products and phase I metabolites of POM are less potent with respect to antimyeloma and immunomodulatory effects (Hoffmann *et al.*, *Cancer Chemother Pharmacol*, 2013). POM in combination with low-dose dexamethasone (LoDEX) has shown efficacy in pts with refractory or relapsed and refractory MM (RRMM) and moderate RI (creatinine clearance [CrCl] <30-44 mL/min; Siegel *et al.*, *Blood,* 2012; Weisel *et al.*, *J Clin Oncol,* 2013). However, POM+LoDEX has not yet been fully studied in pts with severe RI (CrCl <30 mL/min; serum creatinine ≥3 mg/dL) because these pts were excluded from prior trials.
Aims: MM-008 is a multicentre, open-label phase 1 study designed to assess pharmacokinetics (PK) and safety of POM+LoDEX in RRMM pts and normal or severely impaired renal function with or without dialysis.
Methods: Pts with RRMM (≥1 prior treatment [Tx]) and normal kidney function or mild RI (creatinine clearance [CrCl] ≥60 mL/min; Cohort A-control arm), severe RI (CrCl <30 mL/min) not requiring dialysis (Cohort B), and severe RI requiring dialysis (Cohort C) were eligible. Cohort A received POM 4 mg, and Cohort B received POM 2 or 4 mg on days 1-21 of a 28-day cycle, following a 3+3 dose-escalation design. Cohort B results informed the 4-mg dosage used in Cohort C. All cohorts received DEX 40 mg (20 mg for pts aged >75 yrs) on days 1, 8, 15, and 22. Tx continued until disease progression or unacceptable toxicity. Dose-limiting toxicities (DLTs) were defined as: grade (Gr) 4 neutropenia, febrile neutropenia, Gr 4 thrombocytopenia involving ≥30% decrease in platelet count from baseline and requiring >1 platelet transfusion, Gr 3 thrombocytopenia with significant bleeding (requiring hospitalization and/or platelet transfusion), Gr 4 infection, or ≥Gr 3 other non-hematologic toxicity related to POM. Serial plasma samples were analyzed to generate PK parameters.
Results: Current data for 15 treated pts were available (8 in Cohort A, 3 in Cohort B at 2 mg, and 4 in Cohort B at 4 mg). Median age was 66 yrs (range, 46-76 yrs), 60% (9/15 pts) were male, all had Eastern Cooperative Oncology Group performance status 0 (6/15 pts) or 1 (9/15 pts), and a median time from diagnosis of 3.8 yrs (range, 0.6-12.5 yrs). No DLTs were reported for any cohort in cycle 1. The most common Gr ≥3 adverse events (AEs) were neutropenia (47%), anemia (40%), infection (33%), and fatigue (13%). Median relative dose intensity was consistent across cohorts (0.9-1.1). Mean dose-normalized exposure ($AUC_{0\text{-}24}$) is shown in the Figure 1. One patient in Cohort A discontinued due to dyspnea and 1 discontinued due to pulmonary fibrosis; 1 in Cohort B 4 mg discontinued due to rash. No deaths have occurred during Tx or follow-up visits at 28 days post-Tx discontinuation.



[a] 3 patients in cohort B, 4 mg, were evaluable for pharmacokinetics; only 2 patients were evaluable for pharmacokinetics for each time point.
$AUC_{(0\text{-}24)}$, area under the plasma concentration-time curve from time of dosing (0) to 24 hr; CV, coefficient of variation; GeoM, geometric mean.

Figure 1.

Summary and Conclusions: The ongoing prospective trial MM-008 is examining the PK and safety of POM+LoDEX in pts with RRMM and severe RI. Preliminary PK data demonstrate similar mean dose-normalized exposure at a clinical dose of 4 mg POM in pts with severe RI and no or mild RI. Preliminary safety data and tolerability in this RRMM population are encouraging.

E1264

ALLOGENEIC STEM CELL TRANSPLANTATION FOR MULTIPLE MYELOMA: 15-YEAR EXPERIENCE AT A SINGLE CENTER

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Background: Allogeneic stem cell transplantation (allo-SCT) may cure multiple myeloma (MM) but its role is still under discussion mainly for two reasons: the high morbidity and mortality related to the procedure (TRM) and the impressive results that can be attained with the new drugs.
We present our series of 29 patients with MM and an allo-SCT (2000-2015).
Aims: We present our results with Allo-SCT in a selected population and its posibility to control the disease.
Methods: *Patients:* N=29 (16 female, 13 male). Median age at diagnosis: 45 years (range 21-56). ISS-1: 9 patients (31%), ISS-2: 7 patients (24.1%), ISS-3: 13 patients (44.8%). Eighteen patients had previously received an autologous SCT (62%). The median time from diagnosis to allo- SCT was 494 days (range 122-1,668). State of the disease pre-SCT: complete remission (CR): 7 (24.1%), very good partial remission (VGPR): 10 (34.4%), partial remission (PR): 9 (31%), stable disease (SD): 2 (6.9%), progressive disease (PD) 1 (3.4%). *Transplantation procedure:* The conditioning regimen was myeloablative in 5 patients (17.2%); non-myeloablative in 24 (82.7%). Sixteen patients (55.1%) had received radiotherapy as part of the conditioning regimen. Type of donor: Related Adult Donor (RD): 21 (72.4%), Unrelated Adult Donor (UD): 5 (17.2%), Umbilical Cord Blood: 1 (3.4%), Haploidentical donor: 2 (6.9%).
Results: Response at day 100+: CR: 14 (48.2%), VGPR: 4 (13.8%), PR: 4 (13.8%), SD: 1 (3.4%), PD: 3 (10.4%), Not Evaluable: 3 (10.4%). The cumulative incidence of grade II-IV acute GVHD was 34.4% (10 patients), 7 of which received an allo-SCT from RDs and 3 from UDs. The incidence of moderate-to-severe chronic GVHD was 38% (11 patients). Two patients presented an overlap syndrome. Eleven patients progressed (38%) and were treated with a combination of anti-MM drugs and donor lymphocyte infusion. Ten patients died (34.4%), 6 from transplant-related complications (20.6%) and 4 because of MM progression. Nineteen patients are alive at the time of this report (65.5%), 10 of them in continuous complete remission (34.4%) having not required any further treatment for their disease. Their current ECOG performance status is: 0-1: 12 patients (63.2%), ≥2: 7 patients (36.8%). The median overall survival (OS) from diagnosis is 2,619 days (range 539-6,195). The median overall survival from allo-SCT is 1,951 days (range 25-5,963).
Summary and Conclusions: Allo-SCT when performed in a selected population of MM patients, has a low TRM even when using UD. A significant proportion of patients remain disease-free for a long time. Patients progressing may revert to a CR. The long OS of our series suggests a role of allo-SCT in the control of the disease.

E1265

TUMOR GROWTH IS ASSOCIATED WITH INCREASED MCD VALUES AND TISSUE EXPRESSION OF ENDOGLIN IN MULTIPLE MYELOMA

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Background: In multiple myeloma (MM) malignant plasma cells interact with bone marrow (BM) microenvironment's components and modify it, favoring in multiple manners tha expansion of the clone. The genesis of new blood vessels, in order to provide the suitable conditions for the expanding malignant mass is a dynamic and complex pathological phenomenon with a major role in tumor growth, invasion and disemination.
Aims: The aim of the present study was to evaluate the expression of endoglin in the BM of MM patients. An effort was made to correlate this marker with mast cell dencity (MCD), the angiogenic cytokine angiopoietin-2(angiop-2) and plasma cell nuclear antigen (PCNA).
Methods: BM microvascular dencity (MVD) was estimated by endoglin using immunohistochemical methods in 57 patients with MM up to diagnosis. MCD was evaluated with the same method. Circulating levels of angiop-2 were measured by ELISA in the same group of patients. Plasma cell proliferation index, PCNA was estimated with immunochemical methods. The same parameters were measured in 20 age- and sex-matched healthy controls.
Results: We found that endoglin-MVD along with the MCD and serum levels of angiop-2 and PCNA were increased significantly in patients with MM in comparison to control group ($p<0.001$ in all cases). Angiop-2 serum levels were also higher in advance disease stages($p<0.001$). Similarly CD105 expression, MCD, PCNA values were significantly increased in parallel with the stage of the disease. CD105 expression correlated significantly with MCD($r=0.566$, $P<0.001$), angiop-2($r=0.399$, $p<0.002$) while there was a trend to correlate with PCNA($r=0.0228$, $p>0.08$). All the measured parameters were higher in pre-treatment group in comparison with the post-treatment group($p<0.001$ in all cases).
Summary and Conclusions: Our results showed that angiop-2 and CD105 expression which reflect BM neovascularisation are increased in MM patients and correlate positively with MCD values. This findings indicate that CD105 and MCD could be used as potential tumor markers for disease severity and as possible target for MM treatment.

E1266

LONG-TERM OUTCOMES OF LENALIDOMIDE PLUS LOW-DOSE DEXAMETHASONE (RD) IN CHINESE PATIENTS (PTS) WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA (RRMM): MM-024 EXTENDED ACCESS PROGRAM (EAP)

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Background: In China, the Rd regimen is approved for use in relapsed/refractory multiple myeloma (RRMM) pts who have received ≥ 1 prior treatment (Tx). Approval was based on the efficacy and safety of Rd demonstrated in the MM-021 study, a phase 2 multicenter registration trial in which 199 pts were enrolled (Hou, J Hematol Oncol 2013), and the global MM-009/MM-010 trials (Dimopoulos; Weber, N Engl J Med 2007). MM-024 is an EAP initiated to provide continued Rd Tx for pts enrolled in MM-021, and as a follow-up on the long-term safety of the Rd regimen.
Aims: To present updated safety and efficacy outcomes from the MM-024 EAP.
Methods: MM-024 comprises of Tx and safety follow-up phases. Eligible pts for the Tx phase had participated in the MM-021 study, were still on Tx for ≥1 year (yr) and were progression-free. Pts received the same regimen of Rd as in MM-021 (lenalidomide 25 mg/day on days 1-21, and low-dose dexamethasone 40 mg/day on days 1, 8, 15, and 22 of each 28-day cycle). Starting doses of Rd were the same as the last doses received in MM-021 unless dose adjustments were required prior to rollover, per protocol. Tx was continued until progressive disease (PD) or withdrawal due to toxicities, and pts were followed for a maximum of 5 yrs (including 1 yr spent in MM-021). Pts who discontinued Tx in MM-021, and those who discontinued the MM-024 Tx phase were entered into safety follow-up phase. The primary endpoint was safety; secondary endpoints included progression-free survival (PFS) and overall survival (OS). All efficacy endpoints were measured from the enrollment date of MM-021.
Results: Of the 199 pts in the MM-021 trial, 80 transferred into the MM-024 study (data cutoff November 5, 2014); 41 pts entered the EAP Tx phase, 15 of whom are still receiving Tx. Overall, 26 pts discontinued Tx: 23 due to PD; 2 due to death; and 1 was lost to follow-up. Median follow-up for the treatment cohort was 43.3 months (mos) from initial enrollment in MM-021. In the safety population (n=80), median age was 59 yrs (range 35-76) and 28.8% of pts were aged >65 yrs. Most pts (80.0%) had Durie-Salmon stage III disease at baseline. All pts had received prior Tx for MM, median number was 4. A total of 6.3% of pts had undergone surgery and 3.8% of pts had received radiation Tx; 71.3% of pts had received prior bortezomib, 73.8% had received prior thalidomide, and 51.3% had received both. In the treatment cohort (n=41) median PFS was 36 mos, median OS has not been reached. In the safety population (n=80), 60% of pts had grade (Gr) 3-4 treatment-emergent adverse events (TEAEs); among the most common TEAEs (all grades) were anemia (58.8%), decreased neutrophil count (47.5%), upper respiratory tract infection (33.8%), neutropenia (32.5%), fatigue (23.8%), and thrombocytopenia (18.8%). Gr ≥3 TEAEs included neutropenia (20.0%), decreased neutrophil count (13.8%), anemia (11.3%), pneumonia (8.8%), and decreased white blood cell count (7.5%) (Table 1). No TEAEs led to Tx discontinuation; however, TEAEs led to lenalidomide dose reduction (7.5%), interruption (42.5%), or both (18.8%). There were 2 second primary malignancies reported, one of which occurred in the MM-021 study (solid duodenal tumor and nasopharyngeal carcinoma).
Summary and Conclusions: After 43.3 mos of follow-up in the treatment cohort, median PFS was 36 mos; in the MM-021 final analyses (cutoff date September 26, 2012) median PFS was 8.3 mos. The MM-024 safety profile was comparable to MM-021. This indicates long-term use of Rd is well tolerated and remains an effective Tx in Chinese pts with RRMM who have received multiple prior Tx.

Table 1. Gr 3-4 TEAEs reported in ≥3% of pts.

	MM-024 (N = 80)	MM-021 (N = 199)
Hematologic AEs		
Neutropenia	16 (20.0)	50 (25.1)
Anemia	9 (11.3)	52 (26.1)
Thrombocytopenia	5 (6.3)	29 (14.6)
Leukopenia	5 (6.3)	19 (9.5)
Nonhematologic AEs		
Pneumonia	7 (8.8)	26 (13.1)
Upper respiratory tract infection	5 (6.3)	8 (4.0)
Fatigue	4 (5.0)	8 (4.0)
Hypocalcemia	3 (3.8)	5 (2.5)
Hypokalemia	3 (3.8)	14 (7.0)
Investigations		
Decreased neutrophil count	11 (13.8)	17 (8.5)
Decrease white blood cell count	6 (7.5)	14 (7.0)
Decrease platelet count	4 (5.0)	14 (7.0)
Discontinuation due to AEs	0	18 (9.0)

All values n (%).
TEAE, treatment-emergent adverse event.

E1267

STAT3 EXPRESSION IS ASSOCIATED WITH POOR SURVIVAL IN YOUNG PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA

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Background: The signal transduced and activator of transcription 3 (STAT3) is a key signaling molecule implicated in the regulation of growth and malignant formation, and is considered as prognostic marker in various type of cancer.
Aims: In this study, we investigated the prognostic significance of STAT3 expression in young patients with newly diagnosed multiple myeloma (MM).
Methods: Ninety-four patients under the age of 65 years were enrolled between June 2005 and March 2013. Dual immunochemical staining (IHC) for phosphotyrosine-STAT3 (PY-STAT3) and CD138 on paraffin-embedded bone marrow sections at diagnosis was performed to evaluate tumor cell-specific PY-STAT3 expression. Patients with PY-STAT3 expression were classified into two groups according to diffuseness of IHC staining (a cut off value -30%): weak or strong.
Results: PY-STAT3 was detected in 10 patients (10.6%) at diagnosis and three showed strong-expression. Compared to PY-STAT3 negative patients, PY-STAT3 positive patients show higher C-reactive protein and serum calcium level at diagnosis. Over a median follow up of 38.9 months, PY-STAT3 positivity had predictive value for progression-free survival (PFS) (*P*=0.001) and overall survival (OS) (*P*=0.003). In addition, the median PFS was significantly shorter for strong-positive patients than for other patients. (8.7 months in strong-positive *vs* 11.1 months in weak-positive *vs* 23.0 months in negative patients, respectively, *P* <0.001). In 60 patients who received front-line autologous stem cell transplantation, PY-STAT3 positive patients showed poor PFS compared to PY-STAT3 negative patients (4.2 *vs* 19.2 months, *P*=0.013). In the multivariate analysis, PY-STAT3 expression was an independent prognostic factor for PFS (relative risk (RR) of 2.706, *P*=0.014) and OS (RR of 3.091, *P*=0.044) in young patients with MM (Figure 1).

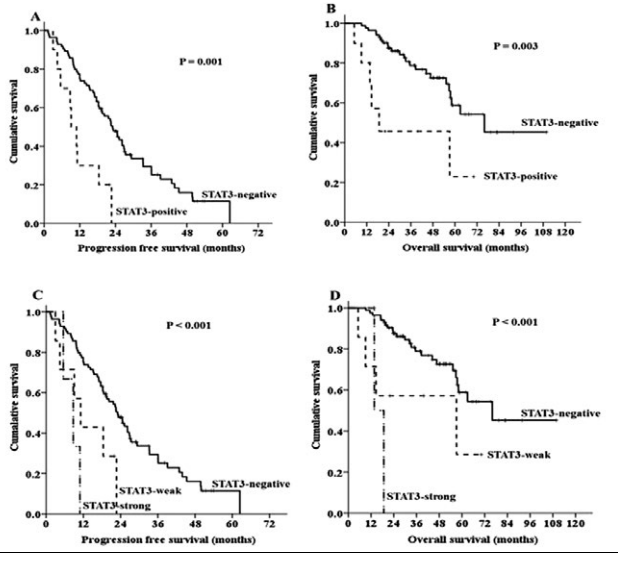

Figure 1. Kaplan-Meier survival curbes for progression-free survival and overall survival according to PY-STAT3-positivity (A, B) and diffuseness of PY-STAT3 expression (C, D).

Summary and Conclusions: These data suggested that PY-STAT3 positivity using dual IHC method was significant prognostic marker in young patients with MM.

E1268

HEPATITIS B REACTIVATION IN MULTIPLE MYELOMA PATIENTS WITH RESOLVED HEPATITIS B UNDERGOING CHEMOTHERAPY

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Background: Despite increasing reports of hepatitis B virus (HBV) reactivation in multiple myeloma (MM), HBV reactivation in patients with resolved hepatitis B [hepatitis B surface antigen (HBsAg)-negative/anti-hepatitis B core antigen antibody (anti-HBc)-positive] is still poorly characterized.
Aims: The aim of this study was to clarify its frequency and risk factors.
Methods: A total of 230 MM patients with resolved hepatitis B were retrospectively reviewed for HBV reactivation and biochemical flare.
Results: During a median 2.4 years of follow-up, HBV reactivation was diagnosed in 12 patients (5.2%). The cumulative rates of HBV reactivation at 2 years and 5 years were 5% and 8%, respectively. A baseline anti-HBs-negative status (*P*=0.033) and high-dose therapy/autologous stem cell transplantation [HDT/ASCT (*P*=0.025)] were significant risk factors that were positively associated with HBV reactivation. In subgroup analysis of patients treated with HDT/ASCT (n=127), a baseline anti-HBs-negative status was the only significant risk factor for HBV reactivation (hazard ratio, 4.64; 95% CI, 1.47-14.7; *P*=0.009).
Summary and Conclusions: These data show that evaluation of anti-HBc is needed for MM patients, and suggest that monitoring of HBV DNA should be considered for patients with resolved hepatitis B undergoing HDT/ASCT, especially those who are anti-HBs-negative.

E1269

POTENTIAL THERAPEUTIC TARGETS IN PLASMA CELL DISORDERS: A FLOW CYTOMETRY STUDY

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Background: The discovery of new targets for tailored therapy is a major improvement in oncology and tools for rapid and reliable detection of those targets are essential. Clinical trials show the benefit of recently developed therapeutic antibodies against antigens on malignant B-cells. Although the occurrence of these antigens on malignant plasma cells is sporadic, targeting those antigens is a therapeutic option in patients with plasma cell disorders.
Aims: The aim of this study was to assess plasma cell disorders cases for biomarkers that have exhibited promise in targeted cancer therapy.
Methods: We retrospectively analyzed patients with amyloid light-chain (AL) amyloidosis (n=30), monoclonal gammopathy of unknown significance (n=9), smoldering myeloma (n=17), newly diagnosed symptomatic multiple myeloma cases (n=25) and multiple myeloma cases in progressive stage of disease (n=22) that were diagnosed in our clinic from Mai to October 2014. An analysis of CD52, CD20, CD30, CD22, CD27, CD38, CD81, CD138 and SLAMF7 expression on bone marrow plasma cells was performed by multiparametric flow cytometry. Furthermore we studied FISH analysis data of all patients for t(11;14) in order to correlate immunophenotyping and genetic parameters.
Results: The expression frequency of CD52, CD30 and CD22 on plasma cells was similar to other studies (12-35%, 0-19% and 0-8%, respectively). Unexpectedly, we observed a high CD20 expression frequency on plasma cell in AL-amyloidosis up to 37% of all examined cases. t(11;14) correlated positively with CD20 expression on plasma cells in AL-amyloidosis (p=0.016). Furthermore, we found that the expression level of SLAMF7 is decreased in advanced plasma cell disorders (p=0.025) and that a diminished expression level of SLAMF7 is associated with low expression of CD27 and CD81 on malignant plasma cells in newly diagnosed multiple myeloma.
Summary and Conclusions: This study provides a contribution to targeted therapy options in plasma cell disorders. Particularly, it puts an emphasis on anti-CD20 antibodies as therapeutic agents in AL-amyloidosis. Regarding the therapeutic options of the SLAMF7 antibody elotuzumab these data advise that target expression diminished during the course of the disease and that analysis of SLAMF7 expression before application of elotuzumab might help to estimate the efficacy of elotuzumab in clinical trials.

E1270

SERUM LEVELS OF FMS-LIKE TYROSINE KINASE LIGAND IN MULTIPLE MYELOMA PATIENTS ARE CORRELATED WITH MARKERS OF TUMOR PROGRSSION

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Background: Fms-like tyrosine kinase 3 (FLT3) ligand (FLT3-L) is a potent hematopoietic cytokine that affects the growth and differentiation of progenitor and stem cells both *in vivo* and *in vitro*. It is probably involved in early B cell development.
Aims: The aim of the present study was to estimate the levels of FLT3L in serum of patients with multiple myeloma(MM) and its relationship with parameters of tumor progression such as Ki-67, the bone marrow infiltration and B-cell activating factor (BAFF)
Methods: We studied 52 newly diagnosed MM patients with mean age 67±8 years, of them 27 had IgG myeloma, 21 IgA and 4 light chain. According to ISS 10 were in stage I, 18 in stage II and 24 in stage III. 18 age and sex matched healthy volunteers were used as control group. All assays were performed at the end of the study, in order to avoid interassay variability. The detection of biological parameters in the serum was performed by a solid-phase sandwich ELISA. Immunohistochemical methods were used in order to determine Ki-67 proliferation index(Ki-67 PI).
Results: Our results indicate that the serum levels of FLT3L and BAFF and the the expression of Ki-67 were significantly higher in patients with MM compared to controls ($p<0.001$ for all cases). Moreover, the serum levels of FLT3L were significantly different among the three stages of disease, with higher values in advancing disease stage ($p<0.001$). Additionally, a positive correlation was found between FLT3L and Ki-67 ($p<0.001$) and BAFF ($p<0.003$), while there was a tendency to correlation between FLT3L and bone marrow infiltration ($p<0.07$)
Summary and Conclusions: Our results showed that FLT3L serum levels were increasd in parallel with ISS stage. The correlation with parameters of disease activity supports the involvement of FLT3L in the mechanisms of promotion of MM growth. Its prognostic impact remains to be proven.

E1271

THE ROLE OF DIAGNOSTIC DELAY IN MULTIPLE MYELOMA: "A DELAY PARADOX"

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Background: The delay in the diagnosis of cancer can influence the outcome. Little is known about the impact of diagnostic delay in multiple myeloma (MM).
Aims: The aim of this study was to prospectively explore the impact of the diagnostic delay in the outcome of newly diagnosed symptomatic MM patients (NDMM). The primary objective was to describe the time in months (m) between the first MM-related symptom and the diagnosis, and between diagnosis and treatment initiation. The secondary objective was to determine whether times to diagnosis and treatment influenced overall survival (OS).
Methods: The dates for the first MM-related symptom, date of diagnosis and date of the induction therapy were recorded for all NDMM in our population-based MM registry, along with variables of prognostic interest. The OS curves were estimated by the Kaplan-Meier method.
Results: 215 consecutive NDMM patients were included in the study, 111 males (51,6%) and 104 females, median age 65 years (12-89). Median OS was 31.3 (21.7-40.9) months (m). There were no significant differences in survival according to sex. The median of diagnostic delay was 4 m (0.2-80). Mean delay for men was 5.5 m and 6.6 m for women (p=ns). Patients with less than 4m of delay had an OS of 19.8 m (11.4-28.2) whereas for those with delay of 4 m or higher, OS was 50.3 m ($p<0.001$). According to sex, OS for patients with <4 *vs* ³4 m was 18.8 *vs* 80.9 m (p=0.001) and 20.4 *vs* 42.7 m (p=0.088), for men and women respectively. Median time from diagnosis to treatment was 13 days. When comparing prognostic factors in both groups, we found that patients with delay of <4 m had ISS1 in 21.3% *vs* 34% (p=0,07), mean serum creatinine 2.3 *vs* 1.6 mg/dL (p=0.03) and mean lactate dehydrogenase (LDH) 338 *vs* 240 U/L (p=0.009). No statistically differences were found for free light chain involved-uninvolved ratio or high risk cytogenetic abnormalities. The percentage of patients with isolated anemia, isolated renal failure or a combination as MM-defining event was higher in the group of <4 m of delay: 64.3%, 57.1% and 59.1% respectively.
Summary and Conclusions: We conclude that the apparent worse prognosis in patients with early diagnosis (less than 4 m) is due to the presence in this group of patients of poorer prognostic factors, in particular a deeper renal impairment, higher levels of LDH and more advanced ISS stage. Early diagnosis in MM seems to be associated to specific MM-defining events and a more aggressive disease.

E1272

SALVAGE AUTOLOGOUS STEM CELL TRANSPLANTATION FOR RELAPSED MULTIPLE MYELOMA

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Background: High-dose chemotherapy followed by autologous stem cell transplantation (ASCT) is a standard frontline therapy for multiple myeloma (MM). Therapeutic options for patients (pts) with relapsed MM after ASCT include novel agents, conventional chemotherapy or salvage ASCT (sASCT), with no standard of care.
Aims: To evaluate the efficacy and safety of novel agents incorporated into sASCT.
Methods: We retrospectively analyzed 66 MM pts who relapsed after up-front single or double ASCT and received sASCT at four italian centres.
Results: Induction therapy in preparation to up-front ASCT (single 67%, double 33%) consisted in conventional chemotherapy (35%) and was thalidomide-based in 58% of pts. Best high-quality response to up-front ASCT included CR (43%) and VGPR (32%). Median age at sASCT was 60 years. Median progression free survival (PFS1) from upfront ASCT to relapse was 44 months. 72.7% of pts received sASCT at first disease progression. Re-induction regimens were bortezomib (bort)-based (84.1%) and IMiDs-based (15.9%). Responses to re-induction therapy included: CR 17.5%, VGPR 30.2%, ≥PR 82.5%. ORR was higher and median time to response was shorter with bort-based in comparison to IMiDs-based regimens, also adjusting for the treatment received as first line (88.2% *vs* 60%, p=0.049; 2.2 *vs* 4.2 months, p=0.01, respectively). 64% of pts already had harvested stem cells for sASCT while 24 pts needed a further peripheral blood stem cells (PBSC) mobilization. 41.7% of them received G-CSF plus plerixafor. Overall, the median number of PBSC harvested was 3.5 (IQR: 2.9-4.9)x 10^6 CD34+/kg. High-dose melphalan was the standard regimen before sASCT and was used at 200 mg/sm in 64.4% of pts. Responses to sASCT included: CR 43.9%, VGPR 33.3%, ≥PR 93.9%. 29% of pts improved from <CR before sASCT to CR after sASCT (p=0.0001). With a median follow up of 2 years after sASCT, 39 pts (59.1%) experienced progression. Median PFS from sASCT (PFS2) was 17.3 months. PFS2 was significantly shorter in pts with PFS1 ≤24 months (9.6 *vs* 18.3 months, p=0.003), in pts who did not receive sASCT at first disease progression (9.7 *vs* 18.3 months, p=0.03), in pts with EMD (9.9 *vs* 18.3 months, p=0.008) and in pts who received reinduction therapy with an IMiDs-based regimen (9.9 *vs* 18 months, p=0.01), also adjusting for treatment received as first line. In multivariate analysis PFS1 ≤24 months (HR 0.21, CI 0.08-0.56) and the presence of EMD (HR 6.6, CI 1.8-24.2) were associated with a shorter PFS2. 23 pts (35%) died after sASCT, mainly due to disease progression (74%). TRM at day+100 was 3%. Median OS from ASCT (OS1) and sASCT (OS2) was 166 and 43 months, respectively. OS2 was significantly shorter in pts with PFS1 ≤24 months (14.2 *vs* 58.3 months, p=0.003), in pts who did not receive sASCT at first disease progression (13.7 *vs* 58.3 months, p=0.008), in pts with EMD (13.9 *vs* 58.3 months, p=0.03) and in pts who failed CR after sASCT (30 months *vs* not reached, p=0.006). In multivariate analysis PFS1 ≤24 months (HR 0.26, CI 0.09-0.80) and CR after sASCT (HR 0.27, CI 0.09-0.80) were associated with a shorter OS2.

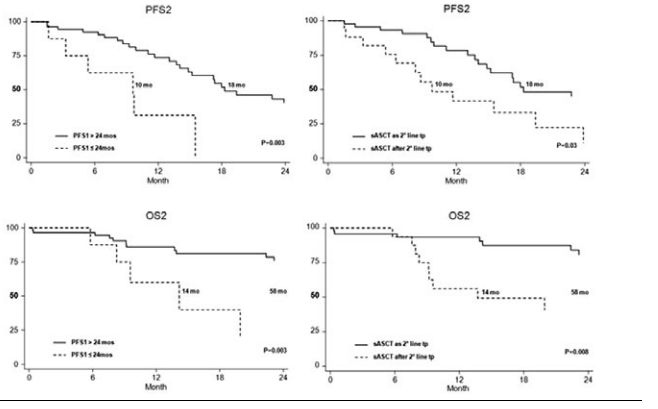

Figure 1.

Summary and Conclusions: Novel agent-based sASCT is a safe and effective procedure for relapsed MM. TRM is similar to that observed in the up-front setting. Patients who are more likely to benefit from sASCT are those in first relapse, with PFS1 >24 months and achieving CR.

E1273

BENCE JONES PROTEINURIA IN SMOLDERING MULTIPLE MYELOMA AS PREDICTOR MARKER OF PROGRESSION TO SYMPTOMATIC MULTIPLE MYELOMA

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Background: Smoldering multiple myeloma (SMM) is a plasma cell disorder with no evidence of myeloma-related symptomatology (hypercalcemia, renal insufficiency, anemia or bone lesions). SMM has a risk of progression to symptomatic multiple myeloma (MM) of approximately 10% per year. However, this risk is not uniform and several prognostic factors for the progression to MM have been identified at diagnosis of SMM. The proportion of bone marrow plasma cells (BMPC), the serum monoclonal protein level and the serum immunoglobulin free light chain ratio (FLC) were identified by the Mayo Clinic Group; the proportion of bone marrow aberrant plasma cells assessed by flow cytometry plus immunoparesis were described by the Spanish Myeloma Group. According to recommendations made by International Myeloma Working Group (IMWG), a 24-h urine protein electrophoresis and immunofixation to identify the presence of Bence Jones (BJ) proteinuria should be performed at diagnosis. BJ proteinuria is a myeloma feature associated with renal function and tumor burden as well. However, there is lack of evidence about the role of BJ proteinuria in SMM as predictor marker of progression to MM.
Aims: The purpose of this study was to investigate the role of the presence of Bence Jones proteinuria at diagnosis in SMM as predictor of progression to MM.
Methods: We reviewed the medical records of all patients diagnosed with SMM in Castilla-León (western region of Spain), between 1983 and 2013, according to the IMWG criteria defined in 2003. The primary endpoint was time to progression to MM.
Results: A total of 152 patients with SMM were included in the analysis. The median age at diagnosis was 70 years (range: 34-90). The serum M-protein at diagnosis ranged from 1 to 2.8 g/dL (median, 2.5). Seventy percent of SMM patients were Ig G subtype. The proportion of bone marrow plasma cells ranged from 1% to 55% (median, 14). Bence Jones proteinuria was detected at diagnosis in 41 patients (27%) and the average amount of BJ proteinuria was 280 mg in 24h urine. We identified 36 high-risk SMM patients(24%) with serum M-protein greater than 3g/dL and more than 10% BMPC. Fifty eight patients (38%) had more than 95% phenotipically abnormal plasma cells by flow cytometry plus immunoparesis. The serum FLC ratio was assessed at diagnosis in 18 patients and it was abnormal (<0.26 or >1.65) in 15 patients (83%). FISH at diagnosis was available in 41 patients. Four of them (10%) had high-risk cytogenetic abnormalities: *t(4;14)* or *del 17p*. The median follow-up for survivors was 65 months. Progression to symptomatic MM occurred in 74 patients (49%). The median time to progression (TTP) to MM in the whole series was almost 6 years. SMM with BJ proteinuria had a significantly shorter median TTP to active disease as compared with patients without BJ proteinuria (21.7 months *vs* 88.3 months ;HR: 2.6, 95% CI: 1.6-4.4; p=0.001). The progression risk at 2 years in the BJ group of SMM was 51%. Multivariate analysis selected BJ proteinuria at diagnosis as an independent variable for progression to symptomatic MM (HR: 2.47, IC 95%: 1.32-4.63; P=0.005). We identified 3 risk categories according to amount of BJ proteinuria: 0 mg per 24h; up to 500 mg/24h; or greater than or equal to 500 mg/24h, with a median TTP of 7, 3 and 1.1 years, respectively (HR: 2.4; 95% IC (1.6-3.64); p <0.001).
Summary and Conclusions: The presence of Bence Jones proteinuria at diagnosis in SMM patients is associated with significantly higher risk of progression to active MM (51% risk of progression at 2 years). Moreover, the presence of 500 mg or more of BJ proteinuria can be considered as a marker for the identification of ultra high risk SMM.

E1274

RE-TREATMENT *VS* CHANGE THERAPY IN FIRST RELAPSE: POST-HOC ANALYSIS OF 476 PATIENTS WITH MULTIPLE MYELOMA (MM) INCLUDED IN TWO PROSPECTIVE TRIALS

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Background: In patients with MM, the choice of salvage therapy at first relapse between re-treatment with the same drug of induction or change class of drug is widely empirical and subjective even if PFS1 duration is usually highly thought of.
Aims: To analyze the outcome in terms of 2ndPFS and PFS2 of first salvage therapies in patients with MM included in 2 prospective trials (VMP *vs* VMPT-VT and MPR *vs* Mel-200) by comparing patients re-treated with the same new-drug used in induction or with other new-drug class.
Methods: We analyzed 476 patients relapsed after first-line therapy that underwent salvage therapies based on Bortezomib (126 pts), Lenalidomide (142 pts), Thalidomide (36 pts), chemotherapy (63 pts), autotransplant (92 pts) and allogeneic transplant (7 pts). Out of these 476 patients, 311 meet the inclusion criteria for this study that was re-treatment or change therapy with Bortezomib-based or Lenalidomide-based therapy without transplantation.
Results: In the elderly group (257 pts), salvage therapy included bortezomib (15%, 2ndPFS=8.8 months), lenalidomide (50%, 2ndPFS=16.6 months), thalidomide (12%, 2ndPFS=8.6 months), chemotherapy (21%, median 2ndPFS=6.6 months). As regard younger patients (219 pts), 44% received bortezomib (median 2ndPFS=7.2 months), 7% received IMiDs (median 2ndPFS=14.3 months), 41.5% bortezomib followed by autotransplant (media 2ndPFS=21.5 months), 3% received allotransplant (median 2ndPFS=35.7 months). Out of 311 patients considered for comparison of re-treatment *vs* change therapy, 52 patients were re-treated (ptsR: 37 Bortezomib-based and 15 Lenalidomide-based) whereas 259 changed therapy (ptsC: 162 Lenalidomide-based, 97 Bortezomib-based). The 2 groups of patients were comparable for the main prognostic factors (age, ISS stage, cytogenetic, renal function, plasmocitoma and maintenance) and for follow-up (median 57.3 *vs* 56.2 months). PtsR received salvage therapy at a median time of 30.4 months (4.2-64.4 months; <12 months=4 pts; 12-24 months=12 pts; >24 months=36 pts) while ptsC after a median time of 25.1 months (1.2-63.9 months; p=0.001). In the whole population, median 2ndPFS and PFS2 was 23.1 and 39.0 months, respectively. Median 2ndPFS of ptsR was 8.8 months compared with 12.7 months of ptsC (p=0.038). Median PFS2 of ptsR was 39.9 months compared with 38.8 months of ptsC (p=0.584). Splitting PFS1 duration in two periods, in patients with a PFS1 duration up to 27 months, change of therapy allowed 2ndPFS and PFS2 significantly longer compared with those of re-treatment (2ndPFS: 21 ptsR=5.3 *vs* 164 ptsC=10.2 months; HR=1.9, 95%CI=1.2-3.1, p=0.006; 3yrsPFS2: ptsR=20% *vs* ptsC=34%, HR=1.5, 95%CI=1.0-2.5, p=0.080) whereas, when PFS1 was longer than 27 months, 2ndPFS and PFS2 became similar between the two therapeutic options (2ndPFS: 31 ptsR=16.3 *vs* 95 ptsC=16.6 months, HR=1.2, 95%CI=0.6-2.1, p=0.614; 3yrsPFS2: ptsR=90% *vs* ptsC=92%, HR=1.1, 95%CI=0.6-1.9, p=0.779).
Summary and Conclusions: Our data suggest that changing therapy seems to be preferable at least until PFS1 is longer than that expected by a given regimen while re-treatment may be consider behind this time to save drugs for subsequent relapses. However, these results should be confirmed by a randomized trial comparing these two strategies, at least for relapses occurring between 2 or 3 year after diagnosis.

E1275

AL AMYLOIDOSIS AND QUALITY OF LIFE OUTCOMES

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Background: Patients with light chain amyloidosis (AL) often have delayed diagnosis and present with significant symptomatology; this in turn results in decreased quality of life (QOL). With improving treatment options providing longer survival, it is becoming increasingly important to assess treatment toxicity and QOL. However there is a paucity of data in the literature of QOL in AL patients. At Mayo Clinic, we employ a "Hematology Patient Reported Symptom Screen" (HPRSS) at each visit, which is a three question assessment of fatigue, pain and overall QOL.
Aims: Our objective was to determine if health related quality of life metrics are prognostic in AL.
Methods: We performed a retrospective chart review of newly diagnosed patients with AL seen at our institution between 2009 and 2014 to collect clinical data and HPRSS scores. HPRSS is scored on a scale of 0-10; 10 being the worst for fatigue or pain, and 10 being the best overall QOL. The scores are collected at each visit, and analyzed at diagnosis, 3 months, 6 months and 1 year. There were 322 patients identified with evaluable HPRSS scores. Only those patients who died within the first year or had at least 12 months of follow-up were included (n=305).
Results: The median scores (interquartile range) for fatigue, pain, and QOL at diagnosis were 6 (3,7), 2 (0,5), 5 (3,8), respectively. The median overall survival (OS) for the entire cohort was 39.1 months, and 145 patients died in the first year. There was a significant difference in baseline HPRSS between the 1-year or better survivors and the early death patients in the domains of *fatigue* (5 [IQR 3, 7] *versus* 7 [IQR 5, 8], p<0.0001) and *QOL* (6 [IQR 4, 8] *versus* 5 [IQR 3, 7], p=0.006), but not in the pain domain (2 [IQR 0, 5] *versus* 2 [IQR 0.5]). There were significant baseline differences in the early death group for alkaline phosphatase, bilirubin, creatinine, and Mayo stage. On univariate analysis both *fatigue* and *QOL* were prognostic for OS. On multivariate which included Mayo staging and autologous stem cell transplant, only baseline *fatigue* remained an independently prognostic value. In order to compare change in HPRSS scores over time, we limited the subsequent analyses to those 126 patients who had both baseline and 12 month measurements. QOL scores improved significantly 6 [IQR 3.5, 8] à 7 [IQR 5, 8] between the two measurements, two sided Wilcoxon signed rank p=0.01, but there were no significant changes in fatigue scores (5 [IQR 2,

5]à 4.5 [IQR 3, 6], p=NS) or pain scores (2 [IQR 0,4]à 1.5 [IQR 0, 4], p=NS) over time. Approaching the data slightly differently, one could score whether patients HPRSS scores *worsened* by 1 point, remained *stable*, or *improved* by 1 point over time. The respective proportions were: for fatigue, 37%, 20%, and 43%; for QOL, 31%, 17%, and 52%; and for pain, 36%, 25%, and 39%. There was no difference in OS based on change or lack of change of QOL or fatigue, but patients whose pain worsened had a significant better OS. (Figure 1)This was predominantly driven by those 67 patients who did not undergo ASCT and presumably received bortezomib based therapy.

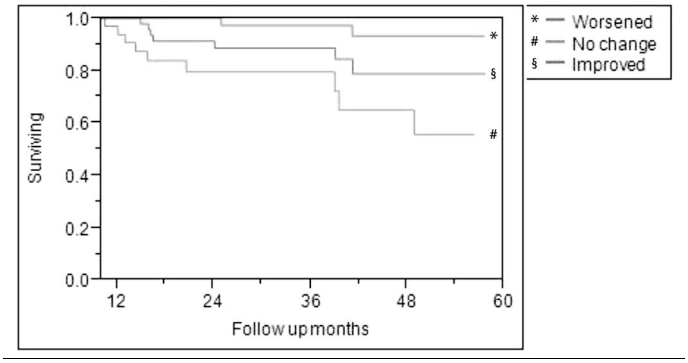

Figure 1. Change in HPRSS scores from diagnosis to 12 month of pain.

Summary and Conclusions: Patient reported fatigue and QOL are important predictors of outcomes in newly diagnosed AL patients. These simple measures can be incorporated in to routine clinical practice and should be considered an important end point for future clinical trials.

E1276

THE ROLE OF INITIAL 18F-FDG PET/CT IN PATIENTS WITH POEMS SYNDROME

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Background: In POEMS syndrome, the identification and biopsy of an osteosclerotic lesion or a lymph node typical of Casleman's disease is essential to establish the diagnosis and plan appropriate treatment.
Aims: The aim of this study is to evaluate the usefulness to detect and select lesions for the diagnostic biopsy in patients suspected POEMS syndrome
Methods: We reviewed 21 patients with POEMS syndrome retrospectively. The 16 patients of them received an (18)F-FDG PET/CT scan using a Discovery DSTe PET/CT scanner (GE medical Systems, Milwaukee, WI). All patients also had undergone bone Ga-scintigraphy.
Results: We reviewed 21 patients with POEMS syndrome retrospectively. The 16 patients of them received an (18)F-FDG PET/CT scan using a Discovery DSTe PET/CT scanner (GE medical Systems, Milwaukee, WI). All patients also had undergone bone Ga-scintigraphy.
Results: The median age was 47.5 year and the ratio of male to female was 1.9. The typical 5 features of peripheral neuropathy (100%), organomegaly (74%), endocrinopathy (74%), M protein (100%), and skin change (84%) were observed. Sclerotic bone lesions (81%) and extravascular volume overload (84%) were also commonly observed. The high serum VEGF level at diagnosis was detected in all tested patients (n=17). Although most cases of sclerotic bone were detected by plainx-ray or Ga-scintigraphy, 5 of 17 cases were detected by only (18)F-FDG PET/CT scan. Plasmacytoma was detected in 7 patients (33%). Except one case, plasmacytoma were revealed by (18)F-FDG PET/CT scan. Multiple lesions of plasmacytoma were detected in two patients. One patient who did not take (18)F-FDG PET/CT scan showed bone pathology on Ga-scintigraphy and discovered plasmacytoma by excisional biopsy. Castleman's disease was suspected on the (18)F-FDG PET/CT scan in two patients (10%) and one case was pathologically confirmed by biopsy. Of 16 patients who had undergone (18)F-FDG PET/CT scan, 12 patients showed abnormal bone lesions or hypermetabolic lesions. Sclerotic bone was seen in 11 patients, abnormal hypermetabolic focus suspected plasmacytoma was seen in 6 patients, and multiple lymphadenopathy with FDG uptake was seen in 2 patients. Plasmacytoma was confirmed by biopsy in five patients and Castleman's disease in 1 patient. In two cases of plasmacytoma, (18)F-FDG PET/CT scan revealed hypermetabolic abnormalities, which were not detected by Ga-scintigraphy or plainx-ray. As the first line treatment, 12 patients received autologous stem cell transplantation (ASCT), 5 patients received chemotherapy with or without radiation and four patients received high-dose steroid, radiation or immune modulators only. Two of five patients with chemotherapy underwent ASCT as second line treatment. The median follow-up duration was 43.4month (11.2-99.8). Four-year overall survival rate was 74.5%. Six of seven patients with plasmacytoma received local radiotherapy with systemic therapy (Table 1).

Table 1. Characteristics of 16 patients who had undergone (18) F-FDG PET/CT scan.

Patient No.	Age	Sex	The Finding by PET-CT	Biopsy proof	Site of biopsy	Results
1	63	M	Abnormal intense FDG uptake with osteoblastic activity in L5, left S1 and right iliac crest	Yes	lumbar spine	Plasmacytoma
2	33	F	No definite abnormal focus			
3	35	F	Bone destruction in the right ileum shows intense FDG uptake	No	Rt.ilium	
4	41	M	No definite abnormal focus			
5	43	F	Sclerotic lesion in Rt.ischium and sacral bone			
6	46	M	Osteosclerotic lesion involving iliac bone with intense FDG uptake, suggestive of malignancy	Yes	iliac bone	Plasmacytoma
7	49	M	Extensive pathologic lymphadenopathy and Multiple osteoblastic or sclerotic lesions in axial skeleton			
8	41	M	Multiple well-enhancing (r/o multicentric Castleman's disease) and multiple small osteosclerotic lesions in the axial skeleton	No		
9	44	M	Multiple osteosclerotic lesions in the axial skeleton and left femur			
10	57	F	No definite abnormal focus			
11	52	M	Multicentric Castleman's disease	Yes	Retropancreatic lymph node	Castleman's disease
12	28	M	Multiple sclerotic bony lesions			
13	46	M	No definite abnormal focus			
14	68	M	Osteolytic lesion in the Lt ischium with intense FDG uptake, suggestive of malignancy	Yes	Lt. iliac bone	Plasmacytoma
15	26	M	Osteolytic lesion in the right acetabulum shows intense FDG uptake, suggestive of malignancy	Yes	acetabulum	Plasmacytoma
16	59	F	Osteolytic and sclerotic lesion involving sacrum, suggestive of malignancy	Yes	sacrum	Plasmacytoma

Summary and Conclusions: (18)F-FDG PET/CT scan is useful in detecting and selecting bone lesions and accompanying plasmacytoma or Castleman's disease for the reliable diagnosis of POEMS syndrome. Furthermore, this finding has potential clinical application in therapy evaluation and follow-up as well as diagnosis.

E1277

TRIAL EFFICACY *VS* REAL WORLD EFFECTIVENESS IN FIRST LINE TREATMENT OF MULTIPLE MYELOMA

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Background: Large randomized clinical trials (RCT) are the foundation of the registration of newly developed drugs. A potential problem with RCTs is that the inclusion/exclusion criteria will make the population different from the actual population treated in real life. Hence, it is important to understand how the results from the RCT can be generalized to a general population.
Aims: The primary aim of the present study was to assess the generalizability of the large 1st line RCTs in Multiple Myeloma (MM) to the Nordic setting and to understand potential difference and magnitude in outcomes between RCTs and patients treated in standard care in the Nordics.
Methods: A retrospective analysis was performed on an incident cohort of 2960 MM-patients from 24 hospitals in Denmark, Finland, Norway and Sweden. The database contained information on patient baseline characteristics, treatments and outcomes. Data from relevant 1st line MM RCTs was selected from the treatment MP (Waage, A., *et al.*, Blood. 2010], MPT (Waage, A., *et al.*, Blood. 2010) and VMP (San Miguel, J.F., *et al.*, N Engl J Med, 2008) and baseline characteristics were compared to newly diagnosed Nordic MM treated patients. Potential difference in response and overall survival (OS) was estimated by adjusting the RWE population to the RCT population using matching adjusted indirect comparisons. Patients were matched on age (median approximated to mean), gender, calcium, beta2-microglobulin and ISS score 3. These variables were selected because they were reported in all trials and have previously been identified as having prognostic value.
Results: Patients in the Nordic database treated with MP (n=880) had a response rate of (PD, NR, PR, VGPR, ≥nCR) of (13%, 39%, 38%, 6%, 4%). After matching (n=347), the response rate was slightly worse (12%, 43%, 36%, 6%, 3%). This can be compared to the response rate from the RCT of (7%, 53%, 33%, 3%, 4%). OS for Nordic MP treated patients was 2.67 years (2.25-3.17). After matching the OS was 3.37 years (2.86-3.96) and this can be compared to the trial with OS 2.40 years (2.23-2.66). Patients treated with MPT (n=283) in the Nordic countries had a response rate of (5%, 14%, 52%, 20%, 9%). After matching (n=179) the response rate was slightly changed to (6%, 20%, 50%, 13% 11%). The corresponding RCT response results were 14%, 29%, 34%, 10%, and 13% respectively. OS for Nordic MPT treated patients was 4.15 years (3.73- 4.74). After matching the OS was 4.28 years (3.98-NA) years and compared to 2.42 years

(2.08-3.17) OS observed in the corresponding trial. Patients treated with VMP (n=59) in the Nordic countries had a response rate of (4%, 5%, 40%, 18%, 33%). After matching (n=31) the response rate was improved to (8%, 11%, 28%, 8%, 45%). This corresponding response rates shown in the trial are 1%, 23%, 33%, 8%, and 33% respectively. OS for Nordic MP treated patients was 4.86 years (3.79-NA). After matching the OS was 4.86 years (4.86-NA) and this can be compared to the trial with OS 4.70 years.

Summary and Conclusions: Surprisingly Nordic treated MM patients do very well compared to, and even better than, patients treated in RCTs. Since the OS for all tested treatments improves after matching to the RCT baseline characteristics, patients recruited to the RCTs seems to be a bit better than ordinary Nordic patents. The database used in the present study, and the used method, can be valuable for generalizing the results to the Nordic setting and estimating potential difference for future RCTs and Nordic MM treated patients. Future research should include different data cuts to see whether the analyses are biased by differences subsequent treatments applied in RCTs and clinical practice.

E1278

HEAVY/LIGHT CHAIN IMMUNOPARESIS AS A NOVEL MARKER OF POOR OUTCOMES IN SYSTEMIC AL AMYLOIDOSIS

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Background: Monoclonal free light chain (FLC) levels together with cardiac biomarkers represent the basis of current risk-stratification models for AL amyloidosis, with high-risk patients demonstrating poor survival. However there exists a need to identify new prognostic biomarkers that help assess outcomes, particularly in patients surviving initial therapy. Suppression of heavy/light chain (HLC) immunoglobulins has been shown to add prognostic value in plasma cell dyscrasias, but similar studies in AL amyloidosis are lacking.

Aims: To assess the frequency of HLC immunoparesis in AL patients and contrast the results in relation to outcome.

Methods: The study included 170 patients with systemic AL amyloidosis seen at the National Amyloidosis Centre (London, UK). Serum samples were tested nephelometrically for FLC and heavy/light chain (HLC, *i.e.* IgGκ, IgGλ, IgAκ, IgAλ, IgMκ and IgMλ) concentrations using Freelite® and Hevylite® assays, respectively (The Binding Site Group Ltd, UK); and total immunoglobulins (IgG, IgA and IgM). Immunoparesis was defined either by total immunoglobulin (Ig) measurements as the concentration of any Ig class below the lower limit of normal (*i.e.* in g/L: IgG<6, IgA<0.8, IgM<0.5; total Ig suppression), or by HLC immunoassays as levels of any HLC isotype below the lower limit of their respective reference range (*i.e.* in g/L: IgGκ<4.03; IgGλ<1.97; IgAκ<0.48; IgAλ<0.36; IgMκ<0.29; IgMλ<0.17). Cardiac involvement was defined by the presence of amyloid deposits in tissue biopsies and/or N-terminal pro-brain natriuretic peptide (NTproBNP) levels >332 ng/L. Survival studies were carried out using Kaplan-Meier curves with the log rank test used to indicate significance. Median survival for all patients was 26.2 months, and for patients with cardiac involvement 14.8 months.

Results: HLC measurements identified immunoparesis of at least one HLC isotype in 145 (85%) patients; and severe immunoparesis, defined as two or more HLC isotypes suppressed by at least 50% below normal levels, in 29 (17%) patients. High monoclonal FLC levels (>180 mg/L, n=82(61%)) associated with shorter survival in all patients (median OS 14.8 *vs* 43.1 months; p=0.05), but not in a landmark analysis of 127 patients alive at 6 months (p=0.33). By contrast severe HLC immunoparesis associated with shorter survival in the latter group of patients alive at 6 months (20.2 *vs* 42.8 months; p=0.09); with results approaching significance. In a subset analysis of 89 patients with cardiac involvement and alive at 6 months, severe HLC immunoparesis strongly impacted outcome (median survival 8.8 *vs* 29.9 months, p=0.007) (Figure 1) whereas monoclonal FLC levels reported no significant association (p=0.23). Systemic immunoparesis as determined by total Ig measurements offered no prognostic information in any patient-group.

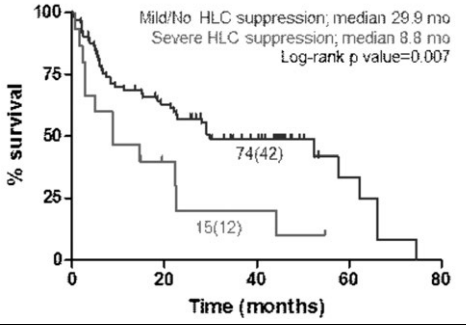

Figure 1.

Summary and Conclusions: HLC suppression is a common occurrence in AL amyloidosis and may add to current models for the prognostication of patients.

E1279

ELEVATED FACTOR VIII LEVELS CARRY A POOR OVERALL SURVIVAL IN NEWLY DIAGNOSED SYSTEMIC LIGHT CHAIN (AL) AMYLOIDOSIS PATIENTS

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Background: Immunoglobulin light chains form amyloid fibrils and are deposited in different tissues in systemic light chain (AL) amyloidosis. Von Willebrand Factor (vWF) is a multimeric adhesive glycoprotein which promotes platelet adhesion to the subendothelium at sites of vascular injury and platelet-platelet interactions under high shear-rate conditions. VWF acts as a carrier for FVIII, acting to protect FVIII from rapid proteolysis.

Aims: To examine the coagulation factors, specifically FVIII, VWF antigen and anti-thrombin in newly diagnosed systemic AL patients, and to assess the prognostic utilities of these investigations.

Methods: One hundred patients with suspected AL amyloidosis referred to the National Amyloidosis centre, London were prospectively examined between May and December 2013. All patients underwent a detailed assessment for organ involvement as per standard protocol. In addition, patients had blood samples taken for baseline activated partial thromboplastin time (APTT), prothrombin time (PT), thrombin time (TT), clauss fibrinogen, factor assays, protein C, protein S and anti-thrombin levels, in conjunction with the Royal Free Hospital based bleeding questionnaire. All assays were carried out using an ACL TOP™ coagulometer [Instrumentation Laboratory (IL), Bedford, USA], using each patient's frozen platelet poor plasma aliquots.

Results: The analysis included 70 newly diagnosed AL patients of the 100 suspected systemic AL referrals. Examining this group, 41% male and the median age 66.1 years (range 56.4-75), cardiac (70% with Mayo stage 3 in 40%), renal (79%), liver (39%), PNS (13%) and ANS involvement (13%). Single organ involvement was present in 27%, with greater than 2 organ involvement in 39%. The bleeding questionnaire reported a bleeding score of 1 in 18.6% and greater than 3 in 7%, with a variable duration of symptoms ranging from 1-60 months. The sites of bleeding included cutaneous (n=17), oral (n=7), epistaxis (n=5), haemarthrosis (n=1), muscle haematoma (n=1) and following surgery (n=2). Coagulation screen abnormalities were present in 28.6% (excluding patients on warfarin), with prolongation of the thrombin time in 48.6%. Interestingly, of 14 patients with an albumin less than 25mg/L, 57% had a low anti-thrombin level, and by univariate analysis this factor was associated with survival (HR: 0.96, 95% CI 0.94-0.98, p<0.005). One striking finding was 94% and 91% had elevated factor VIII and von Willebrand factor antigen, with median levels of 260 IU/dL (range 120-630) and 252 IU/dL (range 101-745) respectively. The plasma metalloproteinase (ADAMTS 13) was normal in all patients. Examining the prognostic significance of both VWF antigen and FVIII; a FVIII >280 IU/dL was associated with a poor overall survival outcome, median survival 11.8 *versus* 17.1 months (p=0.013), illustrated in Figure 1.

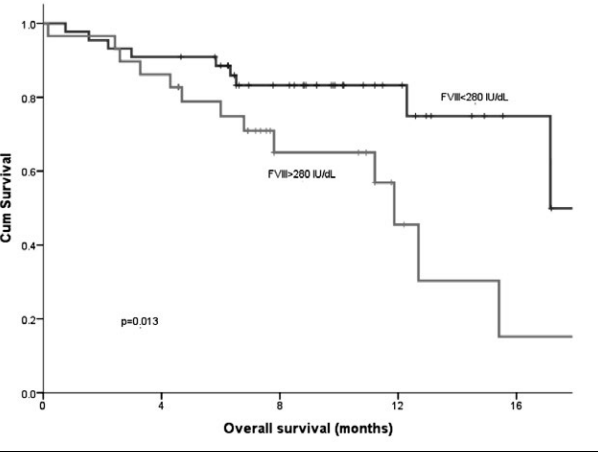

Figure 1.

Summary and Conclusions: In newly diagnosed patients with systemic AL amyloidosis, greater than 90% of patients had elevated VWF antigen and FVIII levels, which may suggest increased platelet-platelet interactions within the endothelium or potential sites of vascular injury that occur with immunoglobulin

amyloid fibrils interacting at these sites. Examining these coagulation factors independently, a FVIII>280IU/dL was associated with a significantly poor outcome. Low anti-thrombin levels also statistically negatively impacted survival.

E1280

FIRST REPORT OF A PROSPECTIVE STUDY ON WHOLE-BODY DYNAMIC CONTRAST ENHANCED MRI IN NEWLY DIAGNOSED MYELOMA PATIENTS TREATED WITH UP-FRONT AUTOLOGOUS TRANSPLANTATION: DESCRIPTIVE STUDY AT BASELINE

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Background: Bone disease is the hallmark of multiple myeloma (MM). MRI is the most sensitive non-invasive imaging technique for detection of bone marrow involvement. It provides relevant information on the pattern of marrow infiltration (focal and/or diffuse). Recent studies showed an independent prognostic value of baseline whole-body MRI. Whole-Body Dynamic Contrast Enhanced MRI (WB-DCE-MRI) provides functional parameters reflecting MM angiogenesis, useful for patient staging and response assessment. Like PET/CT, WB-DCE-MRI could be useful to evaluate MRD and to predict long-term outcomes.
Aims: To report the results of Whole Body Dynamic Contrast Enhanced MRI (WB-DCE-MRI) in patients with newly diagnosed MM with CRAB criteria at baseline. First results of the multi-centric cohort EVALICEMM (NCT01171430).
Methods: Patients were prospectively enrolled in this multi-centric IRB approved study to investigate the role of WB-DCE-MRI to identify skeletal and extra-skeletal lesions at baseline, and to assess with a second and third WB-DCE-MRI, interim response (before high-dose therapy) and response at the end of treatment (one month after autologous stem cell transplant). T1 and T2 signal intensity of focal lesions and bone marrow were analyzed together with the DCE-MRI quantitative enhancement values of the bone marrow (BMEmax) and of focal lesions (FLEmax). Extra-skeletal lesions and existence of epidural lesions were also noted. We here report on the main findings at baseline.
Results: The main characteristics of the 79 patients enrolled between July 2010 and January 2014 who underwent WB-DCE-MRI at diagnosis are as follows: median age of 59 y (range: 54-62), isotype IgG in 61%, IgA in 19% and light-chain in 20% of cases, ISS stage I, II and III respectively in 45, 37 and 18% of patients, median value of β2M was 3.5 mg/l (range 2.6-4.9) and adverse cytogenetic features (deletion 17p or t(4;14)) were observed in 13 of the 64 documented cases. At baseline WB-DCE-MRI, 52 patients (66%) presented with diffuse bone marrow involvement together with focal lesions, 12 with diffuse bone marrow involvement (15%) and 15 with focal lesions alone (19%). There was a significant correlation between T1/T2 signal intensity abnormalities and the dynamic enhancement parameters, both regarding focal lesions and bone marrow, with BMEmax significantly higher in patients with diffuse involvement (186% *vs* 91%, p<0.001). Mean FLEmax in active lesions was 285%. Six patients (8%) presented with extra-skeletal abnormalities (spleen n=3, muscle n=2, lung n=1) and thirteen patients (17%) had epidural lesions. A correlation was observed between BMEmax and International Staging System (p=0.003) and between BMEmax and β2M level (p=0.0017). No significant correlation was observed between BMEmax and adverse cytogenetic features.
Summary and Conclusions: At baseline, functional parameters derived from WB-DCE-MRI are well correlated to T1/T2 bone marrow signal abnormalities. BMEmax appears to be correlated to ISS stage and to β2M level. Extra-skeletal findings are present in 8% of patients which should advocate whole body imaging.

E1281

PROGNOSTIC IMPACT OF SERUM FREE LIGHT CHAIN (SFLC) EVALUATION IN PATIENTS WITH NEWLY DIAGNOSED SYMPTOMATIC MULTIPLE MYELOMA (MM) RECEIVING BORTEZOMIB (BTZ) -BASED REGIMENS

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Background: sFLC assay is an important tool for the diagnosis and monitoring of MM, however data on prognostic significance in symptomatic MM treated with novel agents are still limited.
Aims: We performed a retrospective analysis aimed at investigating the role of sFLC evaluation over time as predictor of outcomes in newly diagnosed symptomatic MM receiving btz.
Methods: Patients treated with a first line btz-based regimen and who had sFLC assay serially evaluated were selected. sFLC assay (Freelite; The Binding Site, Birmingham, UK) was performed by BN II nephelometer (Date Behring, Deerfield, IL, USA).
Results: 150 patients were available for analysis, 90 (60%) received btz incorporated into a program of auto transplant and 60 (40%) combined with conventional chemotherapy. One-hundred and nineteen patients (79%) had a Ig secreting MM, 28 (19%) a light chain only MM and 3 (2%) a non-secretory disease. At baseline, 128 patients (85%) showed an abnormal sFLC ratio (sFLCR). After treatment, 71 patients (47%) achieved a complete response (CR) and 113 (75%) showed a normalization of sFLCR: of these 69 (61%) were in CR whereas 44 (39%) had a monoclonal-protein (M protein) still detectable by electrophoresis or immunofixation (p<0.001). With a median follow-up of 31 months (mo), 59 patients (39%) progressed and 27 (18%) died. The median time to progression (TTP), progression free survival (PFS) and overall survival (OS) were 44, 39 and 75 mo, respectively. At baseline, an involved/uninvolved sFLCR ≥100 (categorized as high) resulted as the most powerful value for discriminating outcomes of survival and was observed in 43% of patients. High and low sFLCR groups were equally distributed between transplant and non-transplant regimens (p=0.109). High baseline sFLCR correlated with Bence Jones isotype (p<0.001), ISS stage III (p<0.001), higher bone marrow infiltration (p=0.003), higher creatinine (p=0.018) and beta-2-microgloblin level (p<0.001), lower haemoglobin concentration (p=0.003), and presence of del(13q) (p=0.001); whereas no correlation was found with t(4;14) or del(17p). High sFLCR, compared to sFLCR <100, was associated with a lower probability to normalize sFLCR after treatment (67% *vs* 81%, p=0.046), and shorter TTP (35 *vs* 50 mo, p=0.003) and PFS (32 *vs* 50 mo, p=0.008), whereas the OS was similar in the two groups (81% *vs* 76% at 3 years, p=0.36). Conversely, survival outcomes were significantly superior in patients who achieved a normalization of sFLCR in comparison with those who failed this objective with median TTP of 49 *vs* 18 mo, (p<0.0001), median PFS of 49 *vs* 17 mo (p<0.0001) and median OS of 75 *vs* 43 mo (p<0.0001), respectively. Moreover, achievement of sFLCR normalization maintained its prognostic value for TTP (p=0.003) and PFS (p=0.003), along with achievement of CR (p=0.032 and p=0.018, respectively) and ISS I/II (p=0.001 and p<0.001, respectively), by Cox regression analysis. Finally, an involved/uninvolved sFLCR ≥100 was observed in 38% of patients at relapse and was associated with earlier start of salvage therapy, compared with sFLCR <100 (89% *vs* 64% at 3 mo, p=0.0426).
Summary and Conclusions: High sFLCR at baseline was associated with shorter TTP and PFS, and at relapse with a short-lasting treatment-free phase. Conversely, normalization of sFLCR after treatment was a robust prognostic indicator of longer disease control, irrespective of achievement of conventional CR. Evaluation of sFLC, in addition to M protein, allows to better define the tumour burden throughout the course of the disease.

E1282

EVALUATION OF THE CHROMOSOMAL ABERRATION PATTERN IN IMMUNOGLOBULIN LIGHT CHAIN AMYLOIDOSIS

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Background: Chromosomal aberrations of plasma cells are well recognized as important pathogenetic and prognostic factors in multiple myeloma, whereas it has remained unclear in immunoglobulin light chain (AL) amyloidosis in particularly Asian patients.
Aims: The purpose of this study is to identify prognostic cytogenetic risk factors by interphase FISH in AL amyloidosis cohort patients in Korea.
Methods: A total of 184 patients with systemic AL amyloidosis were retrospectively analyzed from April 1995 to August 2014 in Korea.
Results: Cytogenetic testing by interphase fluorescence *in situ* hybridization (FISH) had been performed in 80 (44%) patients with AL amyloidosis, and 29 (36%) patients had abnormal FISH as follows; most common abnormality is gain of 1q21 (18%) followed by t(11;14), deletion 13, t(4;14), deletion 17, t(14;20) and t(14;16). Patients with abnormal FISH results had more bone marrow plasma cells (median 16.5 *vs* 30%, p=0.011). Median overall survival (OS) was significantly longer in patients without gain of 1q21 (39.2 months) as compared to in patients with gain of 1q21 (12.2 months, p=0.043). There was no statistical significantly difference, but patients with t(11;14) or deletion 13 had a shorter median OS (18.9 *versus* 36.3 months, p=0.367 or 15.2 *vs* 34.7 months, p=0.517).
Summary and Conclusions: In conclusion, our results suggest that detection of gain of 1q21 is an adverse prognostic factor in patients with AL amyloidosis. And further prospective and large studies of FISH test are warranted.

E1283

IMPACT OF NF-KB EXPRESSION IN THE PROGNOSIS OF MULTIPLE MYELOMA PATIENTS TREATED WITH BORTEZOMIB IN FRONTLINE REGIMENS

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Background: Nuclear factor κappa B (NF-kB) is a heterodimeric transcription factor that promotes transcription of several anti-apoptotic growth factors, proteins, and cytokines, after its nuclear translocation. NF-kB is normally present in the cytoplasm in association with its inhibitor, IkB. Bortezomib inhibits degradation of Iκ-B and therefore blocks NF-kB activity. As NF-κB is highly expressed in myeloma cells, its inhibition by bortezomib promotes their apoptosis. Bortezomib is frequently included in the frontline regimens to treat multiple myeloma (MM) patients. However, the prognostic impact of NF-kB expression levels in plasma cells from newly diagnosed MM patients, treated with bortezomib, is largely unknown.
Aims: To analyze the expression levels of NF-kB in CD138+/CD19- and CD138+/CD19+ plasma cells from MM patients, at diagnosis, and to determine its impact in response and in overall survival of patients treated with bortezomib as frontline regimen.
Methods: We evaluated 24 newly diagnosed MM patients, between April 2010 and April 2013, treated with bortezomib (+ dexamethasone,±cyclophosphamide) as first-line regimen. NF-kB expression levels were analyzed with monoclonal antibodies by flow cytometry in CD138+/CD19+ and CD138+/CD19- plasma cells from bone marrow samples collected at diagnosis. NF-kB expression levels are mentioned in mean intensity of fluorescence (MIF). Response evaluation was determined according to International Myeloma Working Group response criteria. For statistical analysis, *software* IBM SPSS *Statistics* v22 was used.
Results: Twenty-four patients (42% male) were studied, with a median age of 61 (41-75) years; 21/24 (88%) patients presented response to bortezomib. We analyzed NF-kB expression levels in CD138+/CD19+ and CD138+/CD19- plasma cells in patients with and without response to bortezomib (1 CR, 4 VGPR, 16 PR). According to our results, patients with response to bortezomib presented higher NF-kB expression levels in CD138+/CD19- plasma cells (26,2±1,7 MIF *vs* 19,7±1,8 MIF; p=0,032). In CD138+/CD19+ plasma cells, we didn't find differences in NF-kB expression levels (38±2,3 MIF e 33,6±5,6 MIF, respectively). Based on these results, we searched for a prognostic impact of NF-kB expression levels in MM patients with response to bortezomib. Among these patients, median NF-kB expression levels in CD138+/CD19- and CD138+/CD19+ plasma cells were 26,2±1,7 MIF and 38±2,3 MIF, respectively (p=0,0001). We also found that NF-kB expression levels higher than or equal to 25 MIF in CD138+/CD19- plasma cells are associated to a longer survival: median survival not reached compared with 26,1 months (95% CI; 22,4-29,8); p=0,022, for NF-kB expression levels lower than 25.
Summary and Conclusions: Plasma cells from MM patients with different phenotypes present distinct NF-kB expression levels. Higher NF-kB expression levels in CD138+/CD19- plasma cells are associated with increased response rates to bortezomib and with a benefit in overall survival. These results suggest that NF-kB expression levels in CD138+/CD19- plasma cells from MM patients might be considered a potential biomarker for response to bortezomib and prognosis.
This work was supported by CIMAGO (project nº 23/09).

E1284

CIRCULATING BCMA BINDING TO ITS LIGAND BAFF PREVENTS NORMAL ANTIBODY PRODUCTION IN MULTIPLE MYELOMA PATIENTS

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Background: A hallmark of multiple myeloma (MM) is the low levels of uninvolved immunoglobulin (Ig) levels. B-cell maturation antigen (BCMA) is a receptor expressed in mature non-malignant and malignant B lymphocytes, including plasma cells. We previously demonstrated that BCMA is present in the serum of MM patients and that its levels predict overall survival (Sanchez *et al.* Br J Haematol 2012).
Aims: We hypothesized that circulating BCMA binds to its ligands, preventing normal plasma cell development and antibody production in MM patients.
Methods: BCMA-Fc and control Ig were obtained (R&D Systems). Human BCMA and mouse BAFF, and mouse plasma IgA, IgA and IgM levels were measured with ELISA (R&D Systems & Bethyl Laboratories). rhBCMA-mBAFF complexes were determined using an ELISA. Plates were pre-coated with a monoclonal mouse anti-BAFF capture Ab. Plasma samples were incubated and an anti-human-BCMA detection Ab was added. Human serum IgA and IgG levels were determined in MM patients using nephelometry (Immage 800, Beckman Coulter). Hevylite® Assays (Binding Site) were used to quantify the levels of heavy-light chain isoform pairs.
Results: To determine the effects of human BCMA on plasma Ig levels in immune competent mice, rhBCMA-Fc or control Ig-Fc (100 mg) was injected into C57 Bl or Balb/c mice. rhBCMA-Fc resulted in significant decreases in IgA, IgG and IgM levels. Decreases in IgA levels were observed when compared to baseline levels on days 4 and 6 (P=0.0031 and P=0.0064, respectively), and the controls (P=0.0087 and P=0.0221). Mouse IgG levels also showed a reduction compared to baseline (P=0.0023), the Ig-Fc (P=0.0014) and control (P=0.0129) groups. IgM levels showed similar decreases when compared to the untreated (P=0.0001) and Ig-Fc (P=0.0088) groups. We determined if rhBCMA-mBAFF complexes formed *in vivo*. Complexes were detected by ELISA at high levels in plasma from mice dosed with BCMA-Fc, whereas none were found in samples in control Ig-Fc or untreated mice. Next, we determined the relationship of serum BCMA levels to uninvolved Ig levels in MM pts. For pts with IgA (n=134) or IgG (n=313) MM, higher BCMA levels (≥100 ng/ml) correlated with below normal levels of uninvolved IgG in IgA MM and uninvolved IgA in IgG MM, whereas lower BCMA levels (<100 ng/ml) correlated with normal uninvolved levels (P <0.0001). Using the Hevylite Assay, similar results were observed BCMA levels compared to uninvolved IgG isoforms in both pts with involved IgG lambda (n=62, P=0.0006) and IgG kappa (n=117, P <0.0001) MM.
Summary and Conclusions: We demonstrate the formation of circulating BCMA-BAFF complexes in MM, and administration of recombinant BCMA to normal mice results in marked reductions in their antibody levels. We also show that BCMA levels inversely correlate with uninvolved Ig levels in MM pts. Thus, the lack of normal antibody production in MM pts results in part from circulating BCMA binding its ligands, preventing production of normal antibody-producing cells.

E1285

BORTEZOMIB CONSOLIDATION FOLLOWING ASCT FOR MULTIPLE MYELOMA (MM) IMPROVES RESPONSE DEPTH, WITH ACCEPTABLE TOXICITY AND PRESERVED QUALITY OF LIFE

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Background: Consolidation following autologous stem cell transplantation (ASCT) may improve depth of response and prolong time to next treatment, but there is no established standard of care.
Aims: To explore the effect on response depth, progression free survival (PFS) and quality of life (QOL) in patients on a single arm phase 2 post ASCT bortezomib consolidation trial (BCT)
Methods: Bortezomib-naïve patients with at least stable disease were enrolled 3 to 4 months following ASCT, and received Bortezomib at 1.3mg/m^2 (17 started with IV, the rest with SC) on days 1, 8, 15, 22 of a 4-week cycle, maximum 8 cycles. Disease response (IMWG) was assessed at 3, 6 and 12 months post ASCT. Minimal residual disease (MRD) by multiparametric flow cytometry was assessed at the same time points from patient 15 onwards. Toxicity and QOL (EORTC-QLQ-C30) were assessed at each cycle and end of treatment. PFS and OS were measured from date of registration and estimated using the Kaplan-Meier method.
Results: 40 patients were recruited December 2009-March 2014, 22 (55%) were male. Median age was 61 years (range 43-69), isotypes were: 22 (59%) IgG, 9 (24%) IgA, 1 (3%) IgD, 5 (14%) LC only, 1 NS and 2 unknown. Induction regimens pre-ASCT were thalidomide (33 patients, 83%), idarubicin and dexamethasone (5 patients, 13%), and unknown in 2 (5%). All patients received melphalan 200mg/m^2 conditioning for ASCT. At trial entry, median time from diagnosis and from ASCT were 11.0 months (8.6-18.1), and 3.4 months (2.7-4.3) respectively. Of the 40 patients enrolled, 1 withdrew before treatment, 1 progressed in cycle 1, and 2 stopped treatment in/after cycle 1 (treatment related adverse events); 36 patients (90%) completed more than 1 cycle of treatment. A further 10 (25%) patients stopped treatment early, 5 due to toxicity (4 neuro-toxicity and 1 elevated alkaline phosphatase), 4 due to patient/physician choice, and 1 for disease progression. 21 (53%) patients had a dose delay, 15 (38**%**) patients had a dose modification, and 28 (70%) at least one dose omitted. A median of 8 cycles of trial treatment were received. After a median follow up of 30.0 months, 25 patients (63%) are alive without progression, 13 (33%) are alive having progressed, and 2 (5%) have died of MM. BCT improved response depth in assessable patients who completed more than 1 cycle of treatment (N=34): 4 (12%) ≥CR, 19 (56%) VGPR, 10 (29%) PR and 1 (3%) SD pre-treatment, increasing to 7 (21%) ≥CR, 20 (59%) VGPR, 4 (12%) PR, 1 (3%) PD and 2 (6%) N/A at 12 months post ASCT; 11 patients (32%) improved response. Median PFS is 35.5 months (95% CI, 27.5-43.5), with a 2 year PFS of 77.7% (95% CI, 62.8-92.6). 23 patients had MRD assessed at 3 or 6 months post ASCT, of whom 18 had follow up samples at 12 months (end of consolidation). Of these, 9 were MRD+ at the earlier time point, and 4 of these converted to MRD- at 12 months. All MRD- patients remained MRD- at 12 months. Eleven (28%) patients experienced a total of 15 grade 3 adverse events; 6 (neuropathy), 4 (infection), 1 (fatigue), 1 (elevated alkaline phosphatase), 1 (hearing impairment), 2 (haematological). In addition 1 patient had a grade 4 infection (cycle 1, treatment discontinued) and 1 grade 4 back pain. EORTC-QLQ-C30

scores for global health status, and physical, emotional and social functioning did not change significantly throughout treatment.
Summary and Conclusions: Consolidation therapy using weekly Bortezomib post ASCT is well tolerated and associated with a deepening of response, including MRD- rate. This post-ASCT strategy deserves further study.

E1286

SUPERIOR EFFICACY OF VTD OVER VCD BEFORE AND AFTER AUTOLOGOUS STEM-CELL TRANSPLANTATION IN NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS

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Background: A triplet bortezomib-based induction regimen and autologous stem-cell transplantation (ASCT) are the current standard of care for young, newly diagnosed, multiple myeloma (MM) patients (pts). However, due to the lack of randomized trials comparing different regimens incorporating bortezomib, the treatment choice is ultimately based upon physician's preference or single center's policy. Bortezomib-thalidomide-dexamethasone (VTD) has been approved by EMA in this setting. Bortezomib-cyclophosphamide-dexamethasone (VCD) is an alternative to VTD, although efficacy results have not been backed by phase III studies.
Aims: To compare the efficacy of VTD *vs* VCD before and after ASCT.
Methods: 107 pts who were randomized to the VTD arm of the GIMEMA-MMY-3006 study were compared with an equal number of Italian pair mates who were treated with VCD and ASCT as part of the EMN02 trial. Baseline case matching criteria were age (±2 years), ISS stage (1 *vs* 2 *vs* 3), presence of t(4;14) and/or del(17p) positivity. Induction treatment consisted of three 21-day cycles of either VTD (bortezomib 1.3 mg/m^2 on d 1, 4, 8, and 11; thalidomide 100-200 mg daily; dexamethasone 40 mg on d 1, 2, 4, 5, 8, 9, 11, and 12) or VCD (bortezomib and dexamethasone as in VTD; cyclophosphamide 500 mg/m^2 on d 1 and 8). Melphalan dose prior to ASCT was 200 mg/m^2 (Mel-200). Response was evaluated according to IMWG criteria.
Results: In comparison with VCD, VTD yielded a higher rate of ≥VGPR both before (38% *vs* 60%, p=0.001) and after (63% *vs* 78%, p=0.014) ASCT, including a significantly higher probability of CR (5% *vs* 19%, p=0.001, and 14% *vs* 39%, p<0.001, respectively). Pts with t(4;14) and/or del(17p) had a significantly higher probability to achieve ≥VGPR after VTD induction therapy as compared to those receiving VCD (86% *vs* 43%, p=0.004). In pts with advanced ISS stage (2-3), the superior efficacy of VTD over VCD was retained both before ASCT (CR: 25% *vs* 5%, p=0.002; ≥VGPR: 67% *vs* 38%, p=0.001) and after ASCT (CR: 43% *vs* 11%, p<0.001; ≥VGPR: 85% *vs* 64%, p=0.007). Any grade 3-4 hematological toxicity was higher with VCD *vs* VTD induction therapy (12% *vs* 5%). Grade ≥2 peripheral neuropathy (PN) (NCI CTCAE, version 3.0) was slightly, but not significantly, higher for pts on VTD as compared to those on VCD (12% *vs* 9%, p=0.19). However, when the threshold of grade ≥3 PN was considered, the difference between the two groups significantly favoured pts treated with VCD (2% *vs* 7%, p=0.004), although it is worth noting that only a single pt on VCD and 2 pts on VTD discontinued treatment due to PN. No significant difference in the median dose of CD34+ cells/kg infused to support Mel-200 was observed between pts reveiving VTD or VCD (4x10^6 *vs* 4.5x10^6/kg).
Summary and Conclusions: The triplet VTD induction therapy was associated with significantly higher pre-ASCT CR and ≥VGPR rates compared to VCD, confirming that VTD is one of the most active bortezomib-based induction regimens in preparation for subsequent ASCT. The superior efficacy of VTD over VCD was confirmed also after ASCT and was retained across subgroups of pts with ISS stage 2-3 and a high-risk cytogenetic profile [t(4;14) and/or del(17p)]. Data from the current analysis need to be confirmed by randomized studies designed to prospectively compare VTD *vs* VCD induction therapy before ASCT.

E1287

IMPACT OF SERUM ALPHA 1-ACID GLYCOPROTEIN (AAG), A POTENTIAL PATIENT SELECTION MARKER FOR FILANESIB, ON SURVIVAL AND RESPONSE TO CONVENTIONAL THERAPIES IN PATIENTS WITH RELAPSED MULTIPLE MYELOMA (RMM)

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Background: Serum AAG (orosomucoid) is an acute-phase reactant protein that has been shown to correlate with disease stage as well as overall survival (OS) in patients with multiple myeloma (MM). More recently, it has been proposed as a response marker for patient outcome to filanesib (ARRY-520), a novel kinesin spindle protein inhibitor undergoing evaluation for the treatment of MM. To date, RMM patients with serum AAG concentrations >110 mg/dL have not demonstrated objective responses to single-agent filanesib, while the response rate for patients below this cut point is approximately 23%. It is hypothesized that this lack of response may be caused by AAG binding to filanesib, reducing free concentrations of serum filanesib, while having no appreciable effect on other MM drugs.
Aims: The current study evaluated the prognostic value of AAG for OS, and its ability to predict response in RMM patients treated with standard MM regimens.
Methods: AAG levels in plasma samples from patients with RMM who had received at least one prior therapy (median 4 prior lines; range, 1 to 14) were measured in a central laboratory (ICON Laboratory Services) using a validated assay (Randox Laboratories). Patients were followed for objective response to their next line of therapy and for overall survival. The relationship between AAG concentrations and treatment outcomes were assessed with univariate and multivariate analyses.
Results: AAG levels were measured in plasma from 247 patients (60% male, median age 62 yrs) with RMM who had subsequent follow-up for OS. The median time of plasma collection was 30 months after diagnosis of MM (range, 0.5 to 280 months). At the time of analysis, 60% of patients had died and the median OS from date of plasma collection was 27.2 mos. The median plasma AAG level was 81.1 mg/dL (range 25-266); 20% had high AAG (>110 mg/dL, the cutoff used for filanesib study). AAG status correlated with the International Staging System (ISS) prognostic index and other prognostic factors for MM (*e.g.*, LDH, β2M, number of prior regimens). In a univariate analysis, AAG status was prognostic for OS: median OS was 16 mos for those with high AAG compared with 30 mos for those with low AAG (hazard ratio=1.5; p=0.04). However, in a multivariate analysis that included other prognostic factors, the effect of AAG on OS was not significant (HR=1.3, p=0.2). Importantly, there was no correlation between patient AAG concentrations and objective response (≥PR) to standard therapies initiated within 60 days following AAG measurement that included stem cell transplant, treatment with proteasome inhibitors and/or immunomodulatory agents (overall response rate=59% *vs* 55%, respectively, for patients with low or high AAG, p=0.80).
Summary and Conclusions: In this retrospective study in patients with RMM, plasma AAG concentration was not an independent prognostic factor for OS or predictive of response to conventional MM therapies. These results suggest that the value of AAG as a predictor for response may be unique to treatment with filanesib amongst current myeloma therapies. This may be due to direct binding of AAG to filanesib. The predictive and prognostic nature of AAG as a patient exclusion biomarker for filanesib treatment is being evaluated in an ongoing randomized study (NCT01989325).

E1288

BORTEZOMIB FOR THE TREATMENT OF MULTIPLE MYELOMA: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Background: Multiple myeloma is a plasma cell malignancy accounting for approximately 1% of cancers diagnosed and 12% of haematological malignancies. The first-in-class proteasome inhibitor, bortezomib, is commonly used in all myeloma disease settings. We conducted a systematic review and meta-analysis to evaluate the accumulated clinical evidence of bortezomib treatment of myeloma.
Aims: To assess the clinical benefits and side effects of bortezomib compared to other therapies, doses, administration methods and treatment schedules. Primary objectives were to assess effects of bortezomib on overall survival (OS) and progression-free survival (PFS). Secondary objectives included response rates (RR), adverse events (AE), treatment-related death (TRD) and health-related quality of life (HRQoL).
Methods: We searched MEDLINE, the Cochrane Central Register of Controlled Trials and EMBASE (till end of Nov 2014), conference proceedings and clinical trial registries for randomised controlled trials (RCTs). Two review authors independently extracted outcomes data and assessed risk of bias. Hazard ratios (HR) and their confidence intervals (CI) were extracted for OS and PFS and Odds Ratios (OR) for RRs, AEs and TRD. Trial authors were contacted if summary statistics were missing. Log-rank statistics were estimated if not available.
Results: Sixteen relevant RCTs involving 5630 patients were identified and

12 trials included in the meta-analysis. Four trials of different doses, administration and treatment schedules were reviewed qualitatively. Overall risk of bias was considered to be generally low. We estimated OS benefit from 4118 patients (Peto OR=0.77 (95% CI: 0.69 to 0.86, P <0.00001) and PFS benefit from 4344 patients (Peto OR=0.67 (95% CI: 0.61 to 0.72, P <0.00001) in favour of bortezomib. Meta analysis of complete and overall RR also produced statistically significant results in favour of bortezomib. Subgroup analyses by disease setting revealed improvements in all outcomes. For therapy setting, a statistically significant benefit was observed in all outcomes except OS following consolidation therapy. Bortezomib treatment led to statistically significant increased risks of thrombocytopenia, neutropenia, gastro-intestinal toxicities, peripheral neuropathy, infection and fatigue. A greater risk of cardiac disorders was observed in one comparison group (bortezomib *vs* no bortezomib with different background therapy or *vs* other agents), however, no evidence of increased risk of TRD. Only 4 trials analysed HRQoL and could not be meta-analysed.
Summary and Conclusions: This systematic review and meta-analysis demonstrates myeloma patients receiving bortezomib benefit in terms of OS, PFS and RR. Bortezomib should be considered a standard therapy for myeloma. However, we are unable to draw conclusions regarding optimal combination therapy containing bortezomib. Further evaluation of the newer proteasome inhibitors and more studies of HRQoL are also needed.
This abstract is based on a protocol of Cochrane Review published 11/11/2013, Issue 11. Upon completion and approval, the final version is expected to be published in the Cochrane Database of Systematic Reviews (www.thecochranelibrary.com).

E1289

CAN OVERALL SURVIVAL BE IMPROVED IN ELDERLY MULTIPLE MYELOMA PATIENTS?

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Background: The ongoing introduction of novel therapies for MM provides clinicians and patients various treatment options in numerous lines of treatment. Understanding the current treatment practices and the outcomes in different age groups is of vital importance to understand whether these new treatment should be used in patients from all age groups and where options for treatment optimization exist.
Aims: (i) To describe treatment patterns and corresponding outcomes in terms of response, time to next therapy (TTNT) and overall survival (OS) of Nordic Multiple Myeloma (MM) patients with a focus on age. (ii) To investigate whether age affected the patients' probability of being treated with a conventional (*e.g.* melphelan prednisone) or a novel treatment (bortezomib, thalidomide or lenalidomide) and whether this treatment choice affected OS in the elderly.
Methods: A retrospective analysis was performed on incident 2960 MM-patients from 24 hospitals in Denmark, Finland, Norway and Sweden. The database contained information on patient baseline characteristics such as age, gender, international staging system (ISS) stage, albumin, creatine, MM type and response, TTNT and OS over eight lines of treatment. The patients were stratified over three age groups (<65, 65<75 and ≥75) for which baseline characteristics, treatments, response, TTNT and OS were reported. To validly compare the effectiveness (OS) of novel *vs* conventional treatments in first line, propensity score matching was used and also multivariate cox-regressions.
Results: Patients were on average 67 years old, 48% were male and 28%, 41% and 31% in ISS stages I, II and III, respectively. The chance of being treated with a conventional therapy increases with age in all lines of therapy. In first line, 13% in younger patients <65 years and 72% of elderly patients ≥75 years were treated with a conventional. Patients seemed to respond better on novel therapies. In first line, patients aged ≥75 years treated with a conventional treatment, novel treatment and stem cell transplantation had a 9%, 28% and 69% probability on a ≥very good partial response respectively. Patients' ≥75 with no response or progressive disease on first line had a median OS of 1.54 years whereas patients in the same age group with (near) complete response had a median OS of 3.27 years. For patients aged ≥75 years, a matched treatment comparison using propensity scoring showed significantly longer OS for novel therapies 3.61 (2.66-6.44) years *versus* conventional therapies 2.14 (1.78-2.57) years. Multivariate cox-regression models gave a similar result with an estimated hazard ratio of 0.45 (0.24-0.98).
Summary and Conclusions: This paper shows that in this incident cohort elderly patients are significantly more likely to be treated with a conventional MM therapy than younger patients, while elderly patient do significantly benefit from novel therapies in first line compared to conventional therapies in terms of longer OS. Hence, there is a potential to improve therapy for and further increase the OS among elderly MM patients with novel treatment options.

E1290

PROGNOSTIC IMPACT OF CIRCULATING PLASMA CELLS ON SURVIVAL OF PATIENTS WITH MULTIPLE MYELOMA

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Background: The prognosis of patients with plasma cell leukemia (PCL) is extremely poor. In multiple myeloma (MM) not fullfiling criteria of PCL, the finding of plasma cells in peripheral blood is a marker for highly proliferative disease. The current definition of PCL requires the presence of an absolute peripheral blood plasma cell count >2x 10^9/L and >20% PC in differential white cell count. Recently, it has been suggested a lower cut-off of ≥5% or ≥0.5x 10^9/L of nucleated peripheral blood cells to redefine this entity.
Aims: To investigate whether the presence and number of circulating plasma cells by morphology can be used as a predictor marker of survival in patients with MM not fulfilling PCL criteria, trying to find the threshold to a new definition of PCL.
Methods: Three hundred and seventy patients (male/female 203/167; median age 69 years, range 28-90) diagnosed with symptomatic MM or primary PCL following the International Myeloma Working Group criteria between January 2008 and December 2012. For inclusion, peripheral blood smears at diagnosis had to be available for review. This study was conducted in four University Hospitals from Catalonia. Clinical data including age, gender, Durie-Salmon and ISS stage, initial treatment and follow up were collected from medical records. Patients were treated according to local protocols. Wright-Giemsa stained peripheral blood smears were systematically reviewed. A minimum of 100 nucleated cells per smear were counted. Patients were classified in three groups: *MM-low* group defined as <5% or <0.5x10^9/L circulating plasma cells, *MM-high* group defined as 5% to 20% and/or 0.5x10^9/L to 2x10^9/L circulating plasma cells, and PCL defined as >20% and/or >2x10^9/L circulating plasma cells.
Results: Of the 370 patients studied, 357 (96.5%), 9 (2.4%) and 4 (1.1%) were included in the *MM-low*, *MM-high* and PCL groups, respectively. Patients in the *MM-high* group had lower platelet counts (median 91x10^9/L *vs* 214x10^9/L, p <.0001) and higher bone marrow plasma cells at diagnosis (median 50% *vs* 39%, p=0.031) than the *MM-low* group. Median survival of patients in the *MM-low*, *MM-high* and PCL groups were 52 (95% CI 42.4-61.5), 7 (95% CI 4.0-9.9) and 12 (95% CI 3.1-20.8) months, respectively (Figure 1). *MM-high* group was associated with shorter survival (Hazard ratio 4.34, 95% CI 2.16-8.74) independently of age, Durie-Salmon stage and International Staging System. Within the *MM-low* group, no differences were found between patients with 0% circulating PC (N: 292, median survival 52 months, 95% CI 40.6-63.3) and 1% to 4% circulating PC (N: 65, median survival 53 months, 95% CI 31.7-74.2).

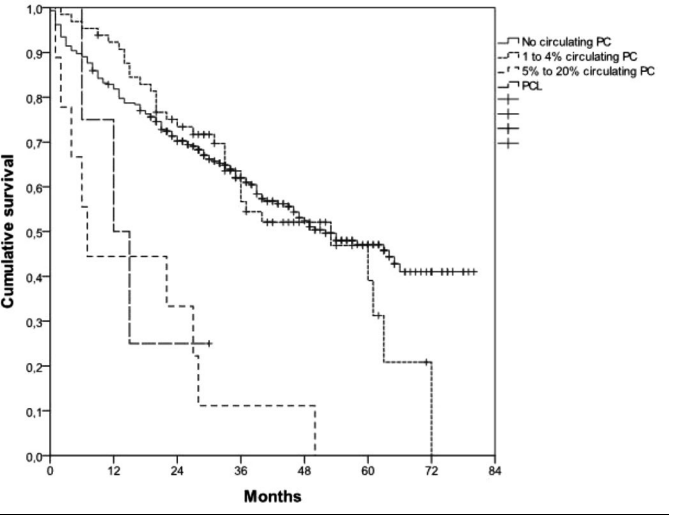

Figure 1.

Summary and Conclusions: The presence of either >5% or >0.5x10^9/L circulating plasma cells in patients with MM has similar adverse prognostic impact that PCL as currently defined. Despite the limited number of cases, our findings support the perception that the definition of PCL should be revisited. The proposal for this new threshold for PCL definition deserves confirmation in other independent study.

E1291

A POPULATION BASED STUDY OF NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS IN LATVIA (2007-2009 YEARS)

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Background: Multiple myeloma (MM) is the second most common hematologic malignancy found in adult population. Specific end-organ damage caused by the disease, such as renal disease, anemia and bone lesions are associated with worse prognosis of MM patients.
Aims: To identify primary diagnosed MM patients from National Center of Hematology with primary diagnosed MM between 2007 and 2009 and to analyze 5-year survival rate.
Methods: This retrospective 3-year study included 162 patients from National Center of Hematology with primary diagnosed MM between 2007 and 2009. Statistical analyses were performed using Graph Pad Prism 5, SPSS statistical software (version 20). Survival curves were analyzed accordingly Kaplan-Meier method and statistically compared using the log-rank test. 5-year survival rate were analyzed for all group and for some subgroups. The survival factors were analyzed without taking in to account the therapy received. During the time period (2007- 2009), the novel agents in Latvia were not State paid and Velcade was available only as a third treatment line. Autologus stem cell transplantation was applied for just 12 patients.
Results: We have analyzed dates of 162 patients (n=66 (41%) males, n=96 (59%) females), with an age range from 48 to 85, mean age (Mean±SD) was 67.49±9.02 years. 47.5% of the patients were older than 70 years. The most frequently reported symptoms were bone pain (51%) and fatigue (25%). MM in 7% was diagnosed during routine blood tests. Most of patients (56%) were in stage III (*Salmon-Durie* staging system), but 25% were in II stage and 19% in I stage. M-protein in serum mean concentrations was 32.42±23.42 g/l (range 0-104), but in patients with III stage it was-48.83±25.36 g/l. Bone lesions had 56% (n=105) patients. >3 lytic bone lesions with vertebral fractures were detected in n=68 (42%) cases. Renal disease was proved in n=77 (48%) cases (glomerular filtration rate (GFR) <60 ml/min/1.73m^2). 12 patients with MM needed hemodialysis. 3 patients were with extramedullary involvement. Five-year survival rate in analyzed group was 25.9%.The median survival period for all patients was 30.3 month (95% CI 24.8-35.9 month). Statistically better survival was in patients under 70 years of age (p <0.001). The 5-year survival rate was 41.2% of patient <70 years, but in patients >70 years it was 9.1%. The most important survival factor was stage of disease (*Salmon-Durie*). Better survival was proved with early stage of MM. We didn't find statistically significant survival difference between 2nd and 3rd stage (p=0.274). Patients with 1st stage of disease 5 year survival rate was 51.6%, but 2nd-22% and 3rd-18.9%. The second most important survival factor was renal disease with statistically significant worse survival (p<0,001). The 5-year survival rate in patient group with renal disease was 32.9%, but without it-18.2%. We found statistically significant survival difference between groups according to hemoglobin level. Patients with Hb <85 g/L had statistically significant worse survival, than with Hb level 85 to 100g/L (p=0.016) and with Hb >100g/L (p<0.001). 5-year survival rate in patients with Hb <85 g/L was 12.2%, but with Hb 85 to 100 g/L-22.2% and with Hb >100 g/L-34.5%.
Summary and Conclusions: 1) 5-year survival rate for patients with primary diagnosed MM from 2007 till 2009 in Latvia was 25.9%. 2) Important survival factors for patients with MM were found to be age more than 70 years, advanced MM stage (*Salmon-Durie*), renal disease and anemia. 3) MM in Latvia was diagnosed at late stage (56% patients were at 3rd stage) with bone lesions in 65% of cases and renal failure (48%).

E1292

OUTCOME OF NEWLY DIAGNOSED SYMPTOMATIC MULTIPLE MYELOMA IN VERY ELDERLY PATIENTS (AGED 80 YEARS OR MORE)

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Background: Multiple Myeloma (MM) is a plasma cell neoplasm typical of the elderly, with a median age at diagnosis of 65 years. The increase in median age in western countries has led to an increase in the incidence of this disease; in addition the introduction of novel agents, such as the immuno-modulatory drugs thalidomide and lenalidomide, and the proteasome inhibitor bortezomib, has considerably changed the therapeutic scenario both for young and elderly patients with MM.
Aims: The aim of our study is a retrospective analysis of the outcome of very elderly (80 years or more) patients with newly diagnosed symtomatic MM to determine the characteristics of this subset of patients.
Methods: We collected data from 47 very elderly (80 years or more) patients (M/F: 23/24) diagnosed and treated from January 2008 to December 2014.
Results: Median follow-up was 31 months (range 6-98) after diagnosis. Median age at diagnosis was 83 years (range 80-89) and PS was <2 in 37 cases (79%). One or two concomitant diseases requiring specific treatments were present in 29 patients (62%), and 3 or more concomitant diseases were present in 8 patients (17%). MM was IgG lambda in 9 patients, IgG k in 26 patients, IgA k in 5 cases, IgA lambda in 4 cases, and micromolecular in 3 cases. According to the ISS, 17 patients were classified as III stage, 10 as II stage and 20 patients as I stage. Bone lytic lesions were present at diagnosis in 34 patients (72%), representing the most CRAB feature. Anemia (median value: 8.4 g/dL) was present in 32 patients (68%); hypercalcemia was present in 4 patients (8%) and renal failure was present in 2 patients (4%). First line therapy was bortezomib (once-weekly administration)/dexamethasone in 10 patients (21%), melphalan/prednisone +/- thalidomide in 9 patients (19%) and bortezomib/melphalan/prednisone in 28 patients (60%). According to IMWG response criteria, 19 patients (40%) achieved CR, 12 (26%) achieved PR, 5 (11%) achieved VGPR, and 8 (17%) achieved stable disease; 3 patients (6%) experienced progressive disease. Hematologic toxicity was infrequent but usually weak/moderate (grades 1 & 2 on the WHO scale) and 24 patients received erythropoiesis-stimulating agents. Extrahematologic toxicity was observed in 17 patients (36%), and neuropathy was the most common adverse event for treatment. 25 patients (53%) had at least one disease progression since diagnosis and were therefore switched to second-line therapy. The median time to first disease progression was 21 months (range 6-58) since start of first-line therapy. Second line therapy was bortezomib (once-weekly administration)/dexamethasone in 5 patients, and lenalidomide/dexamethasone in 20 patients. 5 patients received bendamustine/dexamethasone as third line of treatment in disease progression. 31 patients (66%) are still alive for a median overall survival of 22 months (range 7-92). 14 patients died due to disease progression and 2 died due to causes not related to MM.
Summary and Conclusions: A study in a larger series of patients is warranted but our experience showed that no upper age limit should be applied for the administration of new drugs with MM; these treatments could be offered to very elderly patients, including those with severe concomitant diseases. A study in a larger series of patients is warranted but our experience showed that no upperage limit should be applied for the administration of new drugs with MM; these treatments could be offeredto very elderly patients, including those with severe concomitant diseases.

E1293

INTENTION TO TREAT *VS* AGE OF DIAGNOSIS. ANALYSIS OF BENEFITIAL EFFECT OF NEW AGENTS IN THE TREATMENT OF NEWLY DIAGNOSED MULTIPLE MYELOMA (NDMM)

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Background: Beneficial effect on survival after the introduction of high-dose chemotherapy supported by hematopoietic progenitors and new therapeutic agents (thalidomide, bortezomib and lenalidomide) in the treatment of NDMM patients is recognized by the entire scientific community.
Aims: Evaluate the early introduction the new anti-myeloma agents is short-term survival in NDMM patients.
Methods: Characteristics of patients: n: 322. Median age: 74 years (38-100). Gender: male/female: 192/130. Age at diagnosis (ADx-years): A1 (<65): 82, A2 (66-75): 108; A3 (>75): 132. Exposure to new agents according to ADx in 1st line and before 6 months: A1: 34 and 10 (54%), A2: 37 and 24 (52%) and A3: 22 and 10 (24%) (p.01).
Results: The short-term survival (SV6 & SV12) is lower in patients Exposed *versus* No-Exposed to new agents. SV6 & SV12 in Patients Exposed to new agents is similar among the three age groups. This lack of difference is kept until 24 months. Only statistically significant differences are observed in the analysis at 3 years (SV36m of 81 *vs* 65/58%, p.01; no differences between the 2 groups over 65 years). Landmark: to avoid bias initial selection (characteristics of patients, early mortality, palliative treatments in the "old-era"...) we have performed an analysis excluding patients who had died before 6 months of diagnosis. 72 patients (62 in group NO-Exposed and 10 in the Exposed) were excluded. The SV12, SV24 and SV36 (%) according to age groups (<65, 66-75,>75) were 97, 94, 93 (ns); 91, 82 and 79 (ns) and 84, 72 and 65 (p.02 between groups <and>65). The overall survival was 77 *vs* 56 *vs* 49 months (p.018). In the subanalysis by age and exposure differences in SV short, medium and long term are still maintained, with significant differences.
Summary and Conclusions: Early introduction of new therapeutic agents in NDMM patients influence short-term survival independent of the age.
This therapeutic approach achieves an effect not previously described: the absence of differences in survival by age groups until 36 months from diagnosis. Better post-induction (intensification, consolidation, maintenance) treatment in the group of non-candidate patients could improve these results. Recent studies show the beneficial effect of "continuous" treatment in this group of patients (GEM2005, MM-015, FIRST) (Figure 1).

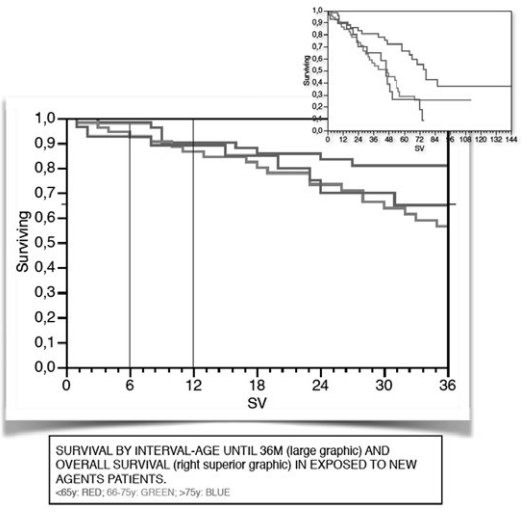

Figure 1.

E1294

SUBCUTANEOUS *VERSUS* INTRAVENOUS BORTEZOMIB: A REAL-LIFE STUDY ON MULTIPLE MYELOMA PATIENTS

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Background: Intravenous Bortezomib (i.v. Bor) has emerged as a standard of care for the treatment of patients (pts) with multiple myeloma (MM). Similar efficacy and better safety profile have been showed with subcutaneous administration of Bor (s.c. Bor).
Aims: To compare efficacy and tolerability of standard marketing patented combination regimens (1° line therapy with VTD/VMP, treatment at relapse with VD) incorporating s.c. *vs* i.v. Bor outside clinical trials.
Methods: After local Ethic Committee approval, we reviewed data of 276 consecutive MM pts treated at our Hematologic division between July 2005 to December 2014. We collected data regarding up-front (VTD/VMP regimen), as well as treatment at relapse (VD regimen) with s.c./i.v. Bor. Standard criteria were applied to evaluate response rate and toxicity.
Results: A) 1° line-VMP: 43 pts were analyzed, 15 with s.c. *vs* 28 with i.v. Bor. Pts received similar number (N°) of cycle (5,5 (range 1-9) *vs* 5 (range 1-9), p=0.605). There were no difference in terms of ORR (³PR, 73% *vs* 63%, p=0.709) as well as of CR+nCR rate (27% *vs* 29%, p=0.53). Grade 3-4 Bor-related toxicity rate were comparable: 11% *vs* 12% respectively for s.c. *vs* i.v. administration (p>0,9). None of the pts in both group developed grade 3-4 Bor-related peripheral neuropathy (PN). B) 1° line VTD (as induction before transplant): 96 pts were analyzed, 15 with s.c. 81 *vs* with i.v. Bor. Pts received similar N° of cycle (4 (range 1-5) *vs* 4 (range 1-6), p=0.9). There were no difference in terms of ORR (³PR, 87% *vs* 84%, p>0.9) as well as of CR+nCR rate (7% *vs* 16%, p=0.22). Grade 3-4 toxicity rates were significantly lower with s.c. Bor (none *vs* 34% respectively for s.c. *vs* i.v. administration (p=0,045)) with none of pts treated with s.c. Bor developing grade 3-4 PN *vs* 7.7% in pts treated with i.v. Bor. C) VD at relapse: 201 pts were analyzed, 28 with s.c. *vs* 173 with i.v. Bor. Pts received similar N° of cycle (4 (range 1-8) *vs* 4 (range 1-9), p=0.27). Pts receiving s.c. Bor had higher ORR (³PR, 69% *vs* 48%, p=0.05), in particular we found a significantly higher rate of CR+nCR (19% *vs* 6%, p=0.02). Grade 3-4 toxicity rates were significantly lower with s.c. Bor (14 *vs* 43% respectively for s.c. *vs* i.v. administration (p=0,045)) with none of pts treated with s.c. Bor developing grade 3-4 PN *vs* 12.8% in pts treated with i.v. Bor.
Summary and Conclusions: This real life data confirms the efficacy and the good toxicity profile of the subcutaneous administration of Bor in all settings of patients.

E1295

THE AMPLIFICATION OF 1Q21 IS AN ADVERSE PROGNOSTIC FACTOR IN CHINESE PATIENTS WITH MULTIPLE MYELOMA

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Background: The prognostic heterogeneity of multiple myeloma (MM) is largely due to different genetic abnormalities. Cytogenetic analysis has revealed that most of MM harbor chromosome aberrations. Amplification of 1q21 is one of the most common chromosomal aberrations.
Aims: In the present study, we examined the amplification of 1q21 in Chinese patients with newly diagnosed multiple myeloma and analyzed the relationship between this genetic event and clinical features of patients.
Methods: Interphase fluorescence *in situ* hybridization (I-FISH) was applied to detect the 1q21 amplification in 86 Chinese patients with newly diagnosed MM.
Results: 1q21 amplification (≥3 red signals) was found in totally 40 of 86 (46.5%) cases, among which 29 with 3copies of 1q21 and 11 with at least 4 copies of 1q21. There was no significant difference in sex, age, International Stage (ISS), Durie-Salmon stage, immunoglobulin subtype, C-reactive protein, erythrocyte sedimentation rate, β2-microglobulin, hemoglobin, platelet number and albumin concentrations among those patients without1q21 amplification, with 3copies of 1q21 and with at least 4copies of 1q21 (p>0.05). Further analysis revealed a significant difference of overall survival (OS) (p=0.047) and progression-free survival (PFS) (p=0.001) among the three arms. Bortezomib could not significantly improve the OS (p=0.626) or PFS (p=0.514) for patients with at least 4 copies of 1q21.
Summary and Conclusions: These findings suggest that 1q21 amplification especially more than 4 copies represents a factor for poor prognosis in MM patients receiving Bortezomib-based regimens.

E1296

USE OF EARLY CHANGES IN GENE EXPRESSION OF PLASMA CELLS MAY PREDICT RESPONSE TO BORTEZOMIB IN PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA

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Background: Maximizing the response rate of first-line therapy (≥VGPR) in multiple myeloma is prognostically important since it has been associated with improved progression-free and overall survival. Molecular changes in plasma cells after treatment with bortezomib caused by the *in vivo* interaction between drug and tumor may be used as a predictor of response to therapy. Studies of gene expression have revealed that certain genes associated with the proteasome (*PSMD4*, *BIRC5*, *KIAA1754*) may represent clinically useful prognostic factors.
Aims: We aimed to examine the use of early changes in the expression of these genes as predictors of the quality of response to first-line bortezomib-based therapy in newly-diagnosed multiple myeloma.
Methods: We studied prospectively changes in the expression of three genes (*PSMD4*, *BIRC5*, *KIAA1754*) induced by bortezomib and their possible correlation with response rates, in 25 patients (16 men, 9 women) with newly-diagnosed multiple myeloma between March 2009 and March 2010. Monitoring continued until January 2014. 19 patients received a combination of bortezomib with dexamethasone (VD) and 6 received a combination of bortezomib, melphalan, and prednisolone (MPV). Bortezomib was administered intravenously with the classic biweekly regimen. Bone marrow aspiration and plasma cell collection was performed at diagnosis (Day 0) and on the seventh day (Day +7) of treatment (*i.e.*, before the third dose of bortezomib). For the purposes of comparison, we analyzed 5 patients who had thalidomide-based treatment (3 patients had thalidomide-dexamethasone, and 2 patients had thalidomide, melphalan, and prednisolone). Selective isolation of CD138+ plasma cells was performed with use of magnetic beads, confirmed with flow cytometry, and followed by the isolation of RNA. We studied gene expression by means of RT-PCR. The observed changes in gene expression were recorded as upregulation (*Up*), downregulation (*Down*), or no change (*No change*). We used changes in the expression of actin gene in order to normalize the variation in the expression of the 3 genes in study. All patients gave written informed consent for the study.
Results: VGPR/CR reached 8 of 30 patients (26.6%), PR 15 of 30 patients (50%), and SD/PD was seen in 7 of 30 patients (23.4%). We found that the changes in the expression of the RNA of the *BIRC5* gene (P=0.001) and *KIAA1754* gene (P<0.001) were associated significantly with the quality of response to bortezomib-based treatment, whereas the changes in the expression of the *PSMD4* gene were not associated with the quality of response to bortezomib (P=0.132). In particular, an upregulation in the expression of *KIAA1754* was associated with the achievement of VGPR/CR, whereas a downregulation in the expression of *BIRC5* was associated with an inferior response. All patients who achieved VGPR/CR had the following *BIRC5*/*KIAA1754* combinations: *Down*/*Up* or *No change*/*Up.* Patients in whom SD/PD was seen had the following *BIRC5*/*KIAA1754* combinations: *Up*/*Down*, *Up*/*No change,* and *No change*/*Down*. Patients who reached a PR usually had *BIRC5*/*KIAA1754* combinations: *Up*/*Up*, *Down*/*Down*, or *No change*/*No change*. We also found that the expression of *PSMD4*, *BIRC5*, and *KIAA1754* was not affected by thalidomide-based treatment.
Summary and Conclusions: We found that the early changes (Day +7) in the expression of *BIRC5* and *KIAA1752* genes were significantly associated with the prediction of response to bortezomib-based treatment, but were not altered

after treatment with thalidomide. These results are specific to the probability of response to first-line bortezomib and, therefore, could be used as part of a risk stratification system for early modification of first-line treatment in patients with newly-diagnosed multiple myeloma. These data may have relevance for the emerging concept of precision medicine.

E1297

ANTI-GRP78 MONOCLONAL ANTIBODY PAT-SM6 IN REFRACTORY AND EXTRAMEDULLARY MULTIPLE MYELOMA: PRECLINICAL AND CLINICAL EVIDENCE FOR A COMBINATORIAL STRATEGY WITH NOVEL AGENTS

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Background: Despite enormous progress in multiple myeloma (MM) therapy the development of effective therapeutic strategies for relapsed and drug refractory patients continues to represent an unmet medical need. We have developed an IgM monoclonal antibody (PAT-SM6) which interferes with Glucose Regulated Protein 78 (GRP78), a heat shock protein frequently reported to be involved in the mediation of drug resistance. In an initial phase I trial with heavily pretreated MM patients, PAT-SM6 treatment showed a disease stabilization rate of 33% when used as single agent. Objective responses according to the IMWG criteria were not seen.
Aims: In this study we further evaluated PAT-SM6 in refractory MM disease.
Methods: We used an *in vitro* drug combination model to investigate the effect of PAT-SM6 in combination with other MM drugs. Immunohistochemical staining with PAT-SM6 and control antibodies on bone marrow specimens and extramedullary lesions was performed. PAT-SM6 was also tested in combination with Lenalidomide (Len) and Bortezomib (BTZ) on the basis of a compassionate use program in one patient with refractory extramedullary MM after informed consent.
Results: Immunohistochemical staining with PAT-SM6 showed stable GRP78 antigen expression throughout various stages of MM including cases with extramedullary disease. Resistance to immunomodulatory drugs (Len) or proteasome inhibitors (BTZ) did not affect GRP78 expression. Previous data showed that treatment with single agent PAT-SM6 reduced tumor load and M protein levels in a 5T33 murine model of aggressive MM in a dose dependent manner. PAT-SM6 combined with MM drugs such as Len, BTZ, Carfilzomib (CFZ) and Dexamethasone (Dexa) showed additive and synergistic effects in some (but not all) tested MM cell lines when analyzed by the Chou Talalay method. Interestingly, synergy was also observed in BTZ, Len and/or Dexa resistant cell lines. A patient with plasmoblastic Len and BTZ resistant MM experienced partial remission of intra- and extramedullary plasma cell tumors after treatment with PAT-SM6 in combination with Len and BTZ. Remission was not durable but repeated biopsies at progression showed preserved target expression at the extramedullary sites giving hope that a prolonged or intensified antibody treatment can sustain remission in future patients.
Summary and Conclusions: The Anti-GRP78 antibody PAT-SM6 showed promising activity *in vitro* and in a late stage MM patient with aggressive tumor biology and drug resistance. Further studies focusing on refractory patients using antibody combinations with novel agents are warranted to elucidate the future role of PAT-SM6 in the treatment of MM.

E1298

ACCURACY OF THE SERUM TOTAL LIGHT CHAIN RATIO AS A PREDICTOR OF ABNORMAL SERUM FREE LIGHT CHAIN RATIOS

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Background: The identification and quantification of serum free light chains (sFLC) with the determination of the kappa/lambda sFLC ratio has been shown to be superior to serum total light chains (sTLC) and to the analysis of the urinary excretion of light chains, in the diagnosis, management and follow-up of plasma-cell dyscrasias. However, the price of the sFLC test can be much higher than that of sTLC (reaching a five-fold difference, in our country). This difference in cost means that the test is still frequently unavailable in lower-income countries, and in smaller Centers in middle-income countries.
Aims: We aim to determine the extent to which the determination of the sTLC and of their ratio can be a surrogate marker for sFLC evaluation, as determined by its sensitivity, sensibility and positive and negative predictive value (PPV and NPV).
Methods: We analysed all samples processed in our Lab between the introduction of the test in 01-09-2005 and 28-02-2015, selecting only those cases with a simultaneous evaluation, in the same blood sample, of total kappa LC, total lambda LC, free kappa LC and free lambda LC; any samples not fulfilling this criterion were excluded. The reference interval used for the sFLC ratio was 0.26-1.65 (inclusive), and 1.35-2.65 (inclusive) for the sTLC ratio. Borderline deviations from the reference interval were defined as a decrease or increase of 10% over the lower and upper limits of the reference interval, respectively.
Results: We obtained 960 samples fulfilling the criteria defined above. The sTLC ratios were abnormal in 76.7% of samples with an abnormal sFLC ratio, but in only 31.3% of samples with a normal sFLC ratio ($p<0.001$). This reflects an inter-rater agreement of 71.5% (Cohen's kappa=0.42, $p<0.0001$). The sensitivity of the sTLC ratio for the prediction of an altered sFLC ratio was, therefore, 76.6% (95% CI: 71.7-81.1%), with a specificity of 68.7% (95% CI: 64.9-72.3%), a PPV of 56.2% (95% CI: 51.5-60.9%) and a NPV of 84.9% (95% CI: 81.5-87.9%). The area under the receiver operating characteristic (ROC) curve (AUC) was 0.73, which represents a diagnostic test with a moderate accuracy. Considering only samples with borderline deviations of the sFLC ratio, as defined above, the percentage of abnormal sTLC ratios decreased to 66.7% (remaining 31.2% in patients with normal sFLC ratios, $p=0.002$). For these borderline cases, concordance decreased to 68.7% (kappa=0.06, $p=0.0008$). Specificity was unaffected, at 68.7%; although the NPV increased to 98.6% (95% CI: 97.0-99.5%), the PPV was only 5.7% (95% CI: 3.0-9.8%). Accuracy was poor, with an AUC of 0.68. When the sFLC ratio was more than twice the upper limit or less than half the lower limit of normal, the sensitivity of the sTLC ratio increased to 83.3% (95% CI: 77.8-87.9%), while the NPV remained acceptable at 91.9% (95% CI: 89.1-94.2%).
Summary and Conclusions: We found that the sTLC ratio is a test with a moderate accuracy at identifying altered sFLC ratios, which are the current gold-standard. Overall, sensitivity, specificity and PPV were fair; the NPV was approximately 85%, which meant that only 15% of patients with a normal sTLC ratio would in fact have an abnormal sFLC ratio. Although the accuracy of the test was poor for borderline samples, its NPV was excellent at approximately 99%. These data suggest that, within the context of very limited monetary resources, the sTLC ratio could optimize the allocation of resources, by helping to exclude those patients who are unlikely to have an altered sFLC ratio, and prioritizing the use of sFLC tests in those patients more likely to have abnormal results.

E1299

CLINICAL FEATURES, PROGNOSIS AND TREATMENT OUTCOME OF THE OF THE EXTRAOSSEUS PLASMA CELL NEOPLASIA: PLASMACYTOMA, MULTIPLE MYELOMA AND PRIMARY PLASMA CELL LEUKEMIA

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Background: Extraosseous localizations of solitary plasmacytoma (ESP), or symptomatic multiple myeloma (EMM) including primary plasma cell leukemia (PCL), are rare presentations of plasma cell neoplasia (PCN).
Aims: The aim of this study was to analyse clinical characteristics, prognosis, and treatment outcome in patients with extraosseous ESP, EMM and primary PCL.
Methods: The study included analysis of the 560 newly diagnosed patients with PCN for the past fourteen years, of which 42 patients (25 males/17 females, median age 57.5 years, range 19-74yrs) had extraosseous PCN. ESP was diagnosed in 24 patients (57.2%), EMM in 12 (28.6%) and PCL in 6 (14.3%). Regarding the type of M protein, the distribution was as following: IgG (50%), light chains (29.4%), IgA (11.8%), IgD (5.9%) and IgM (2.9%). Using the immunofixation electrophoresis (IEF) and serum free-lite test (FLT), low paraprotein concentrations were detected in 27/34 patients (79.4%). Non-secretory disease was confirmed with negative IEF and normal FLT ratio in 8/24 patients (33.3%). The most frequent localizations of the extraosseus PCN were: Oral cavity or pharynx (25%); Paravertebral tumours (16.7%); Muscle infiltration of the thoracal/abdominal wall (13.9%); Salivary glands (8.3%); Lymph nodes (8.3%); and maxillary sinus (8.3%). Less frequent localizations were stomach, colon, and testis found in 1/42pts (2.4%). Majority of the patients with EMM were diagnosed with clinical stage III (CS, Durie&Salmon, 76.2%). Regarding International Scoring System (ISS), 66.7% patients with EMM had ISS1-2, and 33.3% had ISS3. Renal impairment existed in 4 patients (9.5%). Applied treatment was as follows: Radiotherapy (RT, 19% pts); chemotherapy±RT (HT±RT, 54.8%pts); and HT+autologous stem cell transplantation (HT+ASCT, 26.2%pts).
Results: Initial overall treatment response (CR/VGPR/PR) was achieved in 95.2% of analysed patients. In the group of patients with ESP, the 5-year survival was achieved in 90%pts, without reached median overall survival (OS). Among 24 patients with ESP, 41.7% relapsed/progressed within first 4 years of follow-up (range 1-14yrs). PD to symptomatic myeloma occurred in: 4/10pts (40%) treated with RT; and in 6/14pts (42.9%) treated with RT±HT. Median OS of the patients with ESP who progressed to MM was 113 months (95% CI, 70.216-157.469), with 5-year survival achieved in 58% pts, and 50 months of median EFS. Patients with EMM had median OS 84 months (95% CI, 45.194-122.806), and 5-year survival of 62% pts. Median EFS for this group was 48 months (95% CI, 43.467-52.533). The patients with PCL had the worst outcome

with median overall survival (OS) of 36 months (95% CI, 4.564-67.436) (Log Rank 3.864, p<0.05), 5-year survival of 36% pts, and median EFS of 25 months (95% CI, 5.539-44.461, Log Rank 7.923; p<0,05). There was no significant difference in the OS of the patients with EMM/PCL regardless the type of M protein (Log Rank 1.127, p>0,05) or its concentrations (Log Rank 1.708, p>0.05), CS (Log Rank 1.841, p>0,05) or ISS score (Log Rank 3.852, p>0.05), and extraosseal localization (Log Rank 7.210; p>0,05).
Summary and Conclusions: In analyzed group, immature origin of the extraosseus PCN is expressed through low-secretory features of predominantly rare types of M protein. There is no difference in the course of ESP disease regardless the treatment approach while primary PCL, as the principal representative of extramedullary disease retains the most aggressive features despite the introduction of novel therapies.

LB2086

EFFICACY AND SAFETY OF LENALIDOMIDE AND DEXAMETHASONE COMBINATION IN PATIENTS WITH POEMS SYNDROME PRE-TREATED OR INELIGIBLE FOR HIGH-DOSE THERAPY: RESULTS OF A PROSPECTIVE TRIAL

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Introduction: POEMS syndrome is a rare multisystemic disease. Several treatments have been proposed for POEMS even if there is so far no controlled study. Lenalidomide has anti-angiogenic activity through inhibition of VEGF and TNF alpha.
Aims: This study plans to evaluate efficacy and toxicity of Lenalidomide in POEMS. Lenalidomide 25 mg/day was given for 21 days in association with weekly Dexamethasone 40 mg until progression or toxicity. Response to treatment was assessed by a specifically prepared clinical scale which evaluates ten hematological and clinical parameters(clinical response evaluation scale ", CRES, range 0-20), as welll as by the Overall Neuropathy Limitations Scale (ONLS), an expanded MRC sum score, the INCAT sensory sum score and EMG, to evaluate neurological response. The primary end-point was the response to therapy after six monthly cycles of RD. Pts were considered responders if they showed an improvement of at least 1 point in the ONLS scale and at least 3 in the CRES scale.
Methods: From 10/09 to 10/14, we performed a pilot study with Lenalidomide plus dexamethasone (RD) in 20 patients with POEMS - All Pts had a median ONLS score of 4.5 (range 1-8), and monoclonal component (lambda restricted in 88%) - Serum VEGF levels were >1000 pg/ml in all but two pts (88.9%) with a median level of 4043 pg/ml (range 687-13856). Osteoscleoriotic bone lesions were found byx-ray in 12 pts (67%), while Castelman Disease was histologicallly confirmed in 2 pts. Skin changes were observed in 16 pts (89%), hepatosplenomegaly in 14 (78%) pleural effusion, ascites or peripheral edema in 6 pts (33%) and endocrine alterations in 16 (89%).
Results: Response evaluation after 6 months of RD was done in 16 out of 18 pts: 2 pts had dropped out during the first cycle (1 lost to follow-up, 1 for withdrawal of informed consent): 15/18 of included pts (83.3%) completed the 6 cycles of therapy and were improved at six month in the clinical or neurological scale or both, while one patient not improved and suspended the treatment after 4 cycles. 13 pts (72.2%) had improved by at least 3 points in CRES, ranging from 3 to 7 points (mean score at entry 10.36; mean score at six month 7.18; p=0.0006). 10 pts (55.6%) improved by at least one point in the ONLS with an improvement ranging from 1 to 3 points (mean score at entry 4.3; mean score at six month 3.6; p=0.0176), five remained stable and one patient had deteriorated by two points. Six patients are still on treatment, after a median follow-up of 33 months (range 10-52 months)- 5 Pts discontinued treatment for complete response-stable disease after a median of 30 months (range 25-32 months) and only one relapsed 20 months later. In 4 pts the disease progressed after 10, 21, 29 and 35 months. No patient discontinued treatment due to toxicity. Lenalidomide was reduced in 43% of patients mainly for haematological toxicity (grade II-III), and dexamentasone was reduced in all patients, and stopped in 30%. Has not been reported any thrombotic events or secondary neoplasia in patients enrolled.
Summary and Conclusions: Conclusions: This prospective trail confirms that lenalidomide is very effective and well tolerated in the majority of patients, regardless of any previous treatments and it has a prolonged efficacy in POEMS Syndrome.
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Myeloproliferative neoplasms - Biology

E1300

HIGH PREVALENCE OF RAS PATHWAY MUTATIONS IN CMML PATIENTS WITH HIGH COLONY GROWTH

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Background: Some time ago we reported that extensive *in vitro* formation of colony-forming unit-granulocyte-macrophage (CFU-GM) without exogenous growth factors is found in a subset of patients with chronic myelomonocytic leukemia (CMML) *(Geissler K et al. Leuk Res 1988)*. Subsequently we found that this spontaneous myeloid colony formation depends on low endogenously produced granulocyte/macrophage colony-stimulating factor (GM-CSF) concentrations in the culture system *(Geissler K et al. J Exp Med 1996)*. Moreover we have reported that CMML patients with high spontaneous CFU-GM growth (>100/10^5PBMNC) have a worse prognosis compared to patients with low CFU-GM growth *(Sagaster V et al. Ann Hematol 2004)*. The "RASopathies" are a group of genetic syndromes caused by germline mutations in genes that encode components of the RAS signaling pathway including NRAS, KRAS, NF-1, CBL, and PTPN11 *(Niemeyer CM. Haematologica 2014)*. Besides their developmental defects they share a predisposition to JMML. Molecular alterations of these components in murine hematopoietic cells can lead to a CMML like disease *in vivo* and to spontaneous myeloid colony formation *in vitro* due to hypersensitivity of granulomonocytic precursors against GM-CSF *(Wang J et al. Blood 2010)*. The significance of spontaneous CFU-GM growth in man regarding molecular aberration profiles in CMML remains unknown.
Aims: Based on current knowledge we speculated that mutations in the RAS signaling pathway may play a significant role in autonomous CFU-GM formation in CMML.
Methods: In this study we reanalyzed CMML cells from our patients with respect to the presence of molecular aberrations of RAS pathway components and correlated the results with *in vitro* CFU-GM growth. For this purpose we used deep DNA sequencing of 27 CMML associated genes.
Results: Our results show a high prevalence of RAS pathway mutations in CMML patients with high spontaneous CFU-GM formation. Mutations in the components of the RAS pathway were found in 12/15 (80%, NRAS 7, KRAS 1, NF1 2, CBL 2) CMML patients with high colony growth and in 2/9 (22%, NRAS 1, CBL 1) patients with low spontaneous colony formation (p=0.008; Fisher exact test). Mutations of RAS pathway components were mutually exclusive. In all patients with RAS pathway mutations additional mutations were observed in other genes, particularly in components of DNA methylation and/or the spliceosome. Finally, as expected, we found a significant influence of RAS pathway mutations and of high CFU-GM formation, respectively, on overall survival in patients with CMML.
Summary and Conclusions: Our findings suggest that CMML with high spontaneous colony growth is a RAS pathway-dependent malignancy which may have clinical implications concerning prognostication and therapeutic strategies aimed at targeting the hyperactive RAS signaling pathway in these patients.

E1301

ACQUIRED UNIPARENTAL DISOMY OF CHROMOSOME 14 IN MYELOID MALIGNANCIES TARGETS THE IMPRINTED MEG3-DLK1 LOCUS

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Background: Whole or partial chromosomal acquired uniparental disomy (aUPD) is a common finding in myeloid malignancies and typically acts to convert a somatically-acquired heterozygous mutation to homozygosity with loss of the normal allele. Examples are aUPD of chromosomes 4q, 7q and 9p associated with mutations of *TET2*, *EZH2* and *JAK2* respectively. Several other regions of recurrent aUPD have been identified; *e.g.* chromosome 14q aUPD (aUPD14) is seen in about 1% of myeloid malignancies and also in population cohorts of elderly individuals at a frequency of about 0.04%. Indeed, chromosome 14q is the most commonly affected region in such cohorts.

Aims: To identify the molecular target of aUPD14.
Methods: Acquired UPD14 cases identified by SNP array analysis were analysed by whole exome sequencing, *MEG3* methylation by quantitative methylation-specific PCR of bisulphited DNA and *MEG3* and *DLK1* expression by RT-qPCR.
Results: Whole exome sequencing of six aUPD14 cases, of which 3 had been diagnosed with a myeloid neoplasm and 3 had been picked up in a population-based screen of elderly individuals, found no recurrent variants and therefore we considered alternative possibilities. Inherited, constitutional UPD14 is associated with Temple syndrome (maternal UPD) or Wang-Kagami syndrome (paternal UPD), developmental disorders which result from inappropriate expression of genes within the imprinted *MEG3-DLK1* locus at 14q32. The non-methylated maternal chromosome expresses the non-coding *asRTL1, MEG3, MEG8* genes as well as multiple miRNAs and snoRNAs, whilst the methylated paternally inherited chromosome expresses the protein-coding genes *DLK1, RTL1* and *DIO3*. Since *MEG3-DLK1* is deregulated in diverse cancers (including overexpression of *DLK1* in myelofibrosis) and this locus falls within the minimal region of aUPD14, we investigated the possibility that it might be the target of aUPD14. *MEG3* methylation analysis was performed on 22 cases with aUPD14 (21 with myeloid neoplasia, one healthy older individual) and six cases with a myeloid neoplasia and trisomy 14. Of 8 cases with aUPD14 estimated to affect >50% of cells *i.e.* a B-allele frequency >0.75, all 8 showed a methylation value three standard deviations above that of controls indicating loss of the maternal chromosome and gain of the paternal chromosome ($p<0.01$). Loss of maternal chromosome 14 was also apparent in cases with lower levels of aUPD14 and overall the degree of methylation imbalance strongly correlated with the level of aUPD ($r=0.76$; $p=0.0001$). By contrast, no consistent parental bias was seen in cases with trisomy 14. To determine if *MEG3* methylation is more widespread in myeloid malignancies, we examined a further 48 CMML cases with unknown aUPD status and 48 MDS/MPN cases known not to have aUPD14 by SNP array analysis. We identified three additional cases with increased *MEG3* methylation of which two had no evidence of aUPD by microsatellite analysis, thus suggesting alternative mechanisms of *MEG3-DLK1* deregulation. Using RT-qPCR we found increased *DLK1* expression but no consistent change in *MEG3* expression in two cases with aUPD14 compared to controls. Interestingly we also saw altered expression of *DLK1* and *MEG3* expression in myeloid neoplasias without aUPD14, further suggesting that deregulation of the *MEG3-DLK1* locus is not restricted to cases with aUPD14.
Summary and Conclusions: We conclude that aUPD14 is a recurrent abnormality targeting the imprinted *MEG3-DLK1* locus, and that deregulation of this locus may be more widely associated with myeloid malignancy, as well as clonal hemopoiesis in apparently healthy older individuals.

E1302

CIRCULATING ENDOTHELIAL CELLS (CEC) AS A SURROGATE MARKER FOR EVOLUTION IN PH-NEGATIVE MYELOPROLIFERATIVE NEOPLASMS

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Background: The normal circulating endothelial cell (CEC) reference range established in healthy donors is 1-20 CECs/mL blood. Changes in endothelial cells play an important role in a variety of disorders including the classical Ph-negative chronic myeloproliferative neoplasms (MPNs) Polycythaemia vera (PV) and Myelofibrosis (MF). An elevated number of CECs has been described in these diseases but also has lead to a wide variation in the reported range of CECs.
Aims: The aims of the present study were: (1) To compare the quantification of CECs in Ph-negative MPNs using two different platforms, Cell-Search system-Veridex® and Polychromatic Flow Cytometry; (2) to establish variations of CEC numbers in different risk-adapted MPNs according to time from diagnosis (for PV patients) and to DIPPS (for MF patients).
Methods: The study included 49 consecutive patients that were divided into 4 groups: PV up to 10 years from diagnosis (n=10), PV with less than 10 years from diagnosis (n=10), low DIPSS MF (n=9) and high DIPSS MF (n=10). As a control group, 10 healthy blood donors (HC) were also studied. Assessment of the circulating CECs by the Cell-Search system was performed using a semiautomated fluorescence microscope. CD146+ cells were immunomagnetically isolated and labeled with anti-CD105, CD45 and nuclear stain DAPI. A camera is used to scan stained cells and evaluates potential CEC candidates by image analysis software. Assessment of the circulating CECs by flow cytometry was performed. using KDR, CD34, CD45 and CD133 monoclonal antibodies. Each analysis included 1,000,000 events.
Results: The statistical comparison of the CEC numbers showed significant differences between PV patients and MF patients (Mann Whitney U test, $p<0.001$) but also between Low and High DIPPS MF ($p=0.022$). Regarding the flow cytometry population, statistical analyses found significance for the same comparisons: $p<0.001$ between PV patients and MF patients and $p=0.009$ between Low and High DIPPS MF. No statistically significant differences were found between patients with PV for either method. Both PV and MF groups had a significant higher number of CEC compared to healthy donors ($p<0.001$) (Table 1).

Table 1.

GROUP	CellSearch system CD146+CD105+CD45- (CEC/4ml)	Flow Cytometry CD34+KDR+133+ (%)
Control group	10.50 (3-26)	0.0006975 (0.000144-0.002331)
P. Vera up to 10 years	58.50 (17-130)	0.0001345 (0-0.233905)
P.Vera >10 years from diagnosis	64.00 (9-507)	0.0049305 (0-0.05922)
Low DIPPS MF	84.00 (31-288)	0.0277855 (0-0.71932)
High DIPPS MF	268.00 (69-7497)	0.153546 (0.00504-0.595544)

Summary and Conclusions: Our results suggest a role for CEC in the pathogenesis of PV and MF and indicate that: (1) the CEC value could useful for the evaluation of disease progression in PV and MF patients; (2) The standardized method of CEC quantification (Veridex®) correlates with flow cytometry determination of this population.
Acknowledgements: This work was supported by a grant from Novartis.

E1303

Abstract withdrawn

E1304

SUPERVISED MULTI-CLASSIFIER SEPARATION OF THE PH-NEGATIVE MYELOPROLIFERATIVE NEOPLASMS

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Background: Since the discovery of a point mutation in the Janus kinase 2 (JAK2V617F) gene in 2005, the concept of essential thrombocythemia (ET), polycythemia vera (PV) and primary myelofibrosis (PMF) as separate disease entities has been questioned and replaced by the hypothesis of a biological continuum from ET over PV to PMF in JAK2V617F positive patients. Transformations from ET to PV and of both entities to myelofibrosis are frequent and the similarities between JAK2V617F positive ET and PV suggest that overlap between them are more common than previously recognized. Recently, by performing unsupervised Principal Component Analysis (PCA) on gene expression data from these three disease entities, we yielded further support to a biological continuum from ET over PV to PMF with overlap between ET and PV. However, no study has applied supervised classification to explore the molecular classification of ET, PV and PMF which may add information to the clinical diagnosis.
Aims: By using supervised classification methods, the aim of the study was to investigate if ET, PV, and PMF can be separated into distinct groups and to examine if an overlap between these three entities exists.
Methods: Gene expression microarray studies have been performed on whole blood from patients with ET (n=19; 9/10 patients JAK2V617F-/+), PV (n=41; 1/40 patient JAK2V617F-/+), and PMF (n=9; 7/2 JAK2V617F-/+) using Affymetrix HG-U133 2.0 Plus microarrays. A voting procedure involving eleven different support vector machine learning algorithms SVM and KSVM for pattern recognition were chosen for multi-class classification. The top 5-100 genes were used to train the models with SVM/KSVM and leave one out cross validation (LOOCV). A balanced accuracy and a significance level of the classification methods were determined. The gene sets used in the classification procedure were subjected to functional analysis.
Results: In total, the 11 different SVM and KSVM classification algorithms correctly predicted 15 of 19 (79%) patients with ET, 28 of 41 (68%) patients with PV, and 8 of 9 (89%) patients with PMF. The classification models showed a balanced accuracy of 79%. The performance of the classification methods was highly significant ($p=1x10^{-6}$). The results revealed that 4 of 4 misclassified patients with ET were classified as PV of whom 3 were JAK2 positive indicative of a possible transition towards PV. 10 of 13 misclassified patients with PV were classified as ET and the remaining 3 as PMF. One of 9 patients with PMF was misclassified as ET. These results may add more precise molecular phenotype information to the diagnosis. The resulting gene sets used in the classification model included 117, 91, and 92 genes in ET *vs* PV/PMF, PV *vs* ET/PMF, and PMF *vs* ET/PV, respectively. To explore the

biology of the three gene sets using functional analysis, an association with nucleotide binding, inflammation, metabolic process and secretory system was shown in ET *vs* PV/PMF, plasma membrane, metabolic process, signal transduction, and inflammation in PV *vs* ET/PMF, and immune system, signal transduction, secretory system, and transcriptional regulation in PMF *vs* ET/PV.
Summary and Conclusions: We have performed supervised classification of gene expression data from patients with ET, PV, and PMF. Our data show that ET, PV and PMF may be separated into distinct disease entities with overlap between ET and PV providing further support to a biological continuum from ET over PV to PMF. Moreover, our study may add important information to the clinical diagnosis of ET, PV, and PMF.

E1305

SERUM LEVELS OF MATRIX METALLOPROTEINASES AND THEIR TISSUE INHIBITORS ARE ELEVATED IN JAK2V617F MUTATED COMPARED TO CALR MUTATED MYELOFIBROSIS AND TRIPLE NEGATIVE ESSENTIAL THROMBOCYTEMIA PATIENTS

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Background: Myelofibrosis (MF) and essential thrombocythemia (ET) are myeloproliferative neoplasms (MPN) resulting from acquired hematopoietic stem cell mutations. MF and ET patients may harbor $JAK2^{V617F}$, MPLW515K/L or calreticulin mutations ($CALR^{MUT}$). The mechanism by which mutant CALR alters cellular function to result in myeloid proliferation remains unclear. Matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) are involved in tumor neoangiogenesis which plays important role in hematological neoplasias progression and prognosis.
Aims: To investigate expression of RNAm and serum levels of MMPs, TIMPs, and plasma markers of angiogenesis (VEGFA and FGF2) according to $JAK2^{V617F}$ and CALR mutational status in MPN patients.
Methods: Sixty-five MPN patients were studied: 21 primary MF (PMF), 23 ET, and 21 myelofibrosis post-ET (MPET). MPN diagnosis was defined according to the 2008 WHO criteria. $JAK2^{V617F}$, MPLW515K/L and MMP2, MMP9, TIMP1 and TIMP2 gene expression were evaluated in peripheral leukocytes by real time PCR, using TaqMan MGB probes. CALR mutations were analyzed in $JAK2^{V617F}$ and MPL W515K/L negative samples by Sanger sequencing method. Protein levels of MMPs, TIMPs, VEGFA and FGF2 were measured Luminex technology.
Results: The frequencies of $JAK2^{V617F}$ mutated patients were: 52% (N=11) in PMF, 52% (N=12) in ET and 57% (N=12) in MFPET. CALR mutations were detected in 38% (N=8) of PMF, 13% (N=3) of ET and 33% (N=7) of MPET patients. One ET patient presented MPLW515L mutation. We found higher protein levels of MMP9 in PMF $JAK2^{V617F}$ mutated patients (N=11) compared with $CALR^{MUT}$ ones (N=8) (158.3 *vs* 55.8 ng/mL; P=0.023). MFPET $JAK2^{V617F}$ patients (N=12) presented higher levels of MMP9 (131.0 *vs* 84.5 ng/mL; P=0.049), TIMP2 (119.8 *vs* 103.3 ng/mL; P=0.049), VEGFA (139.0 *vs* 11.16 pg/mL; P=0.008) and FGF2 (21.5 *vs* 6.6 pg/mL; P=0.027) compared with $CALR^{MUT}$ (N=8). For ET, we found that triple negatives (N=6) showed lower MMP2 (122.4 *vs* 156.6 ng/mL; P=0.048) and TIMP2 (97.5 *vs* 106.4 ng/mL; P=0.020) levels in comparison with JAK2 mutated (N=12).
Summary and Conclusions: Our data proposes that $JAK2^{V617F}$ mutated patients exhibit higher levels of proteins involved in angiogenesis compared with CALR mutated and triple negative counterparts. These findings suggest that CALR mutated and triple negative patients may present a less aggressive course of the disease than $JAK2^{V617F}$ mutated ones. *FAPESP 2012/12957-5.*

E1306

THE MUTATION OF THE SPLICING GENE SRSF2 PERFORMED BY HRM SCREENING IN THE DIAGNOSIS OF CMML

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Background: Recent works associate the mutation of the splicing gene SRSF2 (PRO95) with chronic myelomonocytic leukemia (CMML), a clonal complex neoplasm that belongs to the group of myelodysplastic syndromes/myeloproliferative neoplasms (MDS /MPN) of the 2008 WHO classification, reaching a prevalence of 40- 50%. This gene is located on chromosome 17q25.1, is involved in the regulation of the stability of DNA and its mutation coexists with others in a high prevalence.
Aims: The aim of this retrospective study was to analyze SRSF2 mutations using high resolution melting (HRM) as a screening method, in a series of patients diagnosed with CMML and its relationship with different variables which will be discussed below.
Methods: We included 96 patients diagnosed and followed up between 2010-2014: CMML (n=36, CMML-1, 78% (n=28), CMML-2, 22% (n=8). FAB: Myelodysplastic 42% (n=15), myeloproliferative 58% (n=21)), MDS and AML (n=50), other (n=10) (3 CLL, 2 thrombocytosis, 1 ITP, 1 HL, 3 MGUS). We used DNA from total bone marrow nucleated cells. The samples were studied by real-time PCR and HRM as a screening method. Primers were described by Terra L.Lasho *et al.* (Blood 2012 120: 4168-4171) with a PCR product of 327 bp. By Melting analysis we obtained 2 possible results, mutated *vs* non mutated. The analysis was done in duplicate, including C +, C- and wild type reference. The positive cases were sequenced by Sanger method. We did not observe false positives or negatives when we sequenced the results obtained by HRM. Statistical analysis was performed by Clopper-Pearson method for the analysis of the prevalence, Log Rank (Mantel-Cox), Breslow (Generalized Wilcoxon), Tarone-Ware method for OS analysis, Mann-Whitney test to compare the medians, student t-test was applied to compare means.
Results: 79.3% (23/29) of patients with CMML and 20.7% (6/29) of the patients with MDS and AML presented SRSF2mut. None of the 10 patients with other diagnosis showed mutations. We observed a significant association (P<0.001) between SRSF2mut and CMML, in our series of patients. A bivariate analysis was performed in the group of 36 patients with CMML, in relation to the presence or absence of SRSF2mut (details on Table 1). SRSF2mut prevalence in this subgroup was 63.89%. The mutational profile observed (n=21) was p.Pro95His (n=10) p.Pro95Leu (n=4) p.Pro95Arg (n=3) 24bp del (n=3). Karyotype at diagnosis was normal in 15 patients. The mean age at diagnosis was 71 years, with no age difference observed in relation to SRSF2mut (SRSF2mut=71.87 years *vs* SRSF2wt=69.54 years). A higher prevalence of SRSF2mut was observed in myeloproliferative FAB variants (71.4% (n=15) (P=0.310)) and type 2 OMS (75.0% (n=6) (P=0.682)). Hemoglobin levels were similar in both groups (10.57 g/dl for SRSF2mut and 11.06 for SRSF2wt). The mean platelet count was lower for patients with SRSF2mut, 125000/ul *vs* 205000/ul for SRSF2wt, (p=0.058). The median survival time was 4 years (Me=5.1 years). The treatment and follow-up were not homogeneous. There was no difference in survival between the two groups (P=0.396). Of interest, 17 patients with CMML-1 WHO progressed to type 2. All of them were SRSF2mut. Three patients progressed to AML, all were SRSF2mut. One patient with CMML-1 (WHO) presented 2 spliceosome mutations (SRSF2mut and SF3B1mut.).

Table 1.

Variable	Results	IC and p-value
SRSF2mut prevalence in LMMC	**63,89% (n=23) SRSF2mut** 36,11% (n=13) SRSF2wt	*IC (95%) = (46,22 – 79,18)* *IC (95%) = (20,82 – 53,78)*
Age at the diagnosis	**Age = 71,03**. σ= 9,14. M_e = 73 (Q1: 67,25 y Q3: 77).	*IC(95%): (67,94 – 74,12)*
SRSF2mut/SRSF2wt and age at the diagnosis (years)	Age - SRSF2mut = 71,87 σ=9,57. M_e= 74 (Q1: 67 y Q3: 79). Age - SRSF2wt = 69,54 σ = 8,47. M_e =72 (Q1: 64,5 y Q3: 75).	*p-value= 0.344*
SRSF2mut and FAB	"D" variant= 53,3% (n=8) **"P" variant= 71,4% (n=15)**	*p-value =0,310*
SRSF2mut and WHO	Type "1"= 60,7% (n=17) **Type "2"= 75,0% (n=6)**	*p-value= 0.682*
OS (days)	**Global= 1471,74** M_e= 1868	
OS (days) and SRSF2mut/SRSF2wt.	SRSF2mut= 1648,58 M_e= 2521 SRSF2wt= 1334,09 M_e= 1868	*p-value=0.396*
SRSF2 and Hemoglobine (g/dl) at the diagnosis.	**SRSF2mut = 10,57.** σ =1,86. M_e= 11 (Q1: 9,5 y Q3: 11,70). **SRSF2wt = 11,06.** σ = 2,00. M_e=11,5 (Q1: 9,75 y Q3: 12.75).	*p-value= 0.466*
SRSF2 and platelets count (ul) at the diagnosis.	**SRSF2mut (n=23)= 125000**. σ =73275. M_e=116000 (Q1: 58000 y Q3: 178000). **SRSF2wt (n=12)= 205000**. σ=166503,47.M_e=191000 (Q1: 65200 y Q3: 275000).	***p-value = 0,058***
SRSF2 in CMML, MDS and AML.	**SRSF2mut:** - **CMML(n=23)= 79,3%** - **MDS and AML (n=6)= 20.7%** **SRSF2wt:** - **CMML (n=13)= 22.8%** - **MDS and AML (n=44)= 77.2%**	***p-value< 0.001***

Summary and Conclusions: We found a higher association between SRSF2mut and CMML in our series of patients than others reported before, maybe it depends on the use of HRM as a screening method. We found also a tendency in the association of myeloproliferative FAB subtypes and type 2 of WHO with SRSF2mut, as well as with a lower platelet count. Whether this mutation is or not associated with increased aggressiveness of the disease should be proved. Based on our results, detection of SRSF2mut by HRM screening could be a useful diagnostic tool in CMML since is a feasible technique that could be easily performed in hematology diagnostic laboratories.

E1307

FINDING MOLECULAR/EPIGENETIC SIGNATURES IN PHILADELPHIA NEGATIVE MPNS: A NOVEL TOOL FOR STRENGTHENING DISEASE STATUS CLASSIFICATION AND TAILORING PERSONALIZED DRUG STRATEGIES

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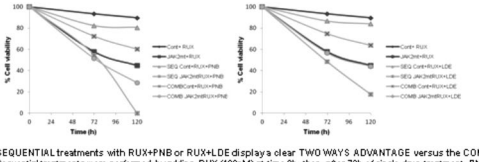
Background: Nowadays, there is a lack of data addressing possible epigenetic alterations and their impact on disease status/evolution in Philadelphia-negative myeloproliferative neoplasms (MPNs). Even so, a significant number of clinical trials are currently using inhibitors of histone deacetylases, but little is precisely known about the impact of histone modifications and chromatin remodeling in MPNs (1). In line, only in a recent study has been shown a correlation between phosphorylated STAT5 and acetylated histone H3 pharmacodynamic readouts, in a preclinical study using JAK2 (V617F)-driven disease models (2). In JAK2 mutant cells, it has been already proposed a prominent role of Akt/mTOR pathways besides Jak/Stat itself (3). Nonetheless, it is plausible to conceive that the pathogenic mechanism operating in these cells might ensue from a complex network of adaptational changes, affecting sequentially other signalling pathways such as Sonic Hedgehog.
Aims: Hence, we propose that in MPNs, it would be crucial to find out combinatorial profiles of molecular/epigenetic targets. Thus, the so-called "Molecular/Epigenetic Signatures", would have the potential to address unresolved questions in the prognosis and therapeutic of MPNs: i) establishing an improved starting diagnostic profile at the molecular/epigenetic level; ii) potential capacity for better disease prognosis; iii) implementation of tailored drug strategies according to patient profiles; iv) novel tool for following up the therapeutic efficacy, providing a guide to address "drug decision making" (including second line strategies).
Methods: Accordingly, we studied molecular/epigenetic signatures in a JAK2V617F CD34+ cell model, based on combinatorial profiles composed by: Akt/mTOR activity; histone deacetylases (HDACs) activity; acetylated-lysine 4 of histone 3 (H_3K_4Ac); and Gli1 expression level, as a master transcription factor in Sonic Hedgehog pathway. In addition, we aimed to test the effect of single and double drug treatments on the proposed combination of markers using Ruxolitinib, a JAK2 inhibitor; Panobinostat, pan-HDACs inhibitor; LDE225, antagonist of the Sonic Hedgehog signalling downstream target Smoothened; BMK120, a class I PI3K inhibitor; and Everolimus, an mTOR inhibitor (all the drugs kindly provided by Novartis Pharma). Moreover, we aimed to address whether sequential drug additions in double-drug treatment strategies would provide significant advantages *versus* classical combinatorial (simultaneous) additions.
Results: The molecular/epigenetic profiles investigated here shed novel signatures with potential to establish a more detailed disease status diagnosis (see supporting Figure for details). Moreover, the molecular/epigenetic signatures correlated well with the impact on cell viability/proliferation of the different drug treatments assayed in our cell model, thus turning out as a potent profiling tool to test the efficacy of different therapeutic strategies. In addition, the sequential drug strategies for Ruxolitinib+Panobinostat, and Ruxolitinib+ LED225, displayed clear advantage *versus* combined (simultaneous) additions, based on a two ways effect: by improving its efficacy reducing cell viability/proliferations capacity, and reducing drug toxicity side effects in control cells by decreasing drug concentrations. Our data could open the pave to solve the aforementioned unresolved questions in the prognosis and therapeutic intervention of MPNs.



SEQUENTIAL treatments with RUX+PNB or RUX+LDE display a clear TWO WAYS ADVANTAGE versus the COMBINED treatment strategy. Sequential treatments were performed by adding RUX (100nM) at time 0h, then, after 72h of single drug treatment, PNB (5nM) or LDE (5nM) were added to cell cultures. Cell samples were subsequently analyzed at 72h and 120h from the starting time. Combined treatments consisted in a direct addition at time 0h of RUX (100nM) plus PNB (20nM) or LDE(10nM) for the total length of 120h. Data displayed supported what was so-called as "two ways advantage" for RUX+PNB and RUX+LED sequential treatments versus the classical combined strategies: i) side toxicity in control CD34+ cells was reduced more than a 25%; ii) increased efficacy of sequential treatments, which requires four times lower concentration of PNB than in the combined approach

Figure 1.

Summary and Conclusions: Our data could open the pave to solve the aforementioned unresolved questions in the prognosis and therapeutic intervention of MPNs.

E1308

TNIP1 AS A NOVEL REARRANGEMENT PARTNER FOR 5Q32 PDGFRB IN EOSINOPHILIA-ASSOCIATED MYELOID NEOPLASM

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Background: A 5q32 *PDGFRB* rearrangement is a diagnostic criteria for a rare distinctive myeloproliferative neoplasm (MPN), often presenting with prominent eosinophilia. Most common is a t(5;12)(q32;p13) resulting in an *ETV6-PDGFRB* fusion mRNA. Variant translocations with a 5q32 *PDGFRB* gene rearrangement present with other fusion genes. In all cases the result is an aberrant, constitutive activation of the tyrosine kinase receptor. To date more than 19 *PDGFRB* rearrangement partners have been reported. The identification of *PDGFRB*-rearranged MPNs is of utmost diagnostic and therapeutic importance, as in the pre-imatinib era acute transformation could occur within a relatively short period of time. *PDGFRB* rearranged MPNs have been shown to be sensitive to tyrosine kinase inhibitors such as imatinib.
Aims: Here we reported on a 76-years-old woman, recently presenting with a MPN with leukocytosis, thrombocytosis, eosinophilia and mastocytosis.
Results: GTG banding of cultured bone marrow resulted in a normal female karyotype. Eosinophilia prompted us to investigate the status of *PDGFRA*, *PDGFRB* and *FGFR1* by FISH analyses. Loss of a distal *PDGFRB* signal on one chromosome 5 was demonstrated using 5q32 *PDGFRB* rearrangement probes, suggesting either an interstitial deletion or an unbalanced translocation involving 5q32 *PDGFRB*. Subsequent FISH analysis using a 5q subtelomere probe was indicative for a deletion. SNP-array analysis (CytoSNP-850k;Illumina) demonstrated an ~897 kb deletion likely with breakpoints within the 5q32 *PDGFRB* and 5q33.1 *TNIP1* genes.
Summary and Conclusions: *TNIP1* encodes TNFAIP3-interacting protein 1, a negative co-regulator of the NF-kB pathway. Loss-of-function mutations in *TNIP1* have been reported in diffuse large B-cell lymphoma (DLBDL), and *TNIP1* splice variants have been implicated in lymphoma and acute myeloid leukemia (AML). The transcriptional orientation of *TNIP1* and *PDGFRB* would allow expression of a 5' *TNIP1-PDGFRB* 3' fusion mRNA, in line with other *PDGFRB* gene fusions. The amino-terminal TNFAIP3-interacting protein 1 moiety that would be present in a TNFAIP3-interacting protein 1-PDGFRB chimeric protein contains several coiled-coil domains, which is canonical for dimerization and constitutive activation of the tyrosine kinase domain of PDGFRB. The patient has started treatment using 400 mg of imatinib daily. We will present on ongoing molecular characterization and treatment-response of the MPN with this novel, cytogenetically cryptic *TNIP1-PDGFRB* gene rearrangement. Our data underscore testing for cryptic rearrangements in MPN with eosinophilia.

E1309

ACTIVATED STAT5 AS NOVEL STEM TARGET IN JAK2 V617F POSITIVE MYELOPROLIFERATIVE NEOPLASMS (MPN)

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Background: Myeloproliferative neoplasms (MPN) represent the most common myeloid neoplasms and have an increasing incidence in the Western World. Three major categories of JAK2 V617F+ MPN have been defined by the WHO: essential thrombocythemia (ET), polycythemia vera (PV), and primary myelofibrosis (PMF). Currently, drug therapies used in JAK2 V617F+ MPN are only able to suppress abnormal cell production for a certain time period but are not able to eradicate the disease, which may be due to resistance of neoplastic stem cells (NSC). However, so far little is known about target expression profiles in MPN NSC. The JAK2-STAT5 pathway has recently been implicated as a potential target-pathway in CML NSC. This pathway also mediates proliferation and survival in MPN cells. It has also been described that mutated JAK2 leads to STAT5 overexpression and STAT5 activation in MPN. However, little is known about expression and function of STAT5 in MPN NSC.
Aims: The aims of our project were to examine the expression of activated STAT5 (pSTAT5) in putative CD34+/CD38- NSC in JAK2 V617F-transformed MPN and to ask whether pSTAT5 serves as a therapeutic NSC-target.
Methods: We analyzed 32 bone marrow (BM) samples obtained from patients with JAK2 V617F+ MPN, 17 BM samples obtained from patients with JAK2 V617F- MPN, and 7 non-reactive BM samples. Expression of pSTAT5 in NSC was determined by flow cytometry. Furthermore, we employed two JAK2 V617F+ cell lines, HEL and SET-2. The effects of JAK2- and STAT5-targeting drugs on proliferation were examined by measuring ^{3}H-thymidine uptake, and drug effects on apoptosis were examined by assessing the levels of activated caspase-3 by flow cytometry. We used two STAT5 inhibitors, pimozide and piceatannol, and four JAK2 inhibitors: AZD1480, TG101348, R763 and ruxolitinib. In select experiments, the effects of STAT5- and/or JAK2 inhibitors on primary MPN cells were examined.
Results: We have detected a significantly higher expression of pSTAT5 in the putative CD34+/CD38- NSC fractions in our JAK2 V617F+ MPN patients when compared to normal CD34+/CD38- BM stem cells. JAK2 V617F-

patients showed no statistically significant difference in pSTAT5 expression in the stem cell compartment compared to normal stem cells. As assessed by flow cytometry, immunocytochemistry and Western blotting, HEL cells and SET-2 cells were found to constitutively express pSTAT5, and that incubation with STAT5- or JAK2 blockers leads to a decrease in pSTAT5 expression. In addition, we found that all JAK2 inhibitors and all STAT5 inhibitors counteract proliferation in HEL cells and SET2 cells, and in most instances these drugs also induced apoptosis in these cell lines (IC_{50} and ED_{50} values are shown in the Table 1). Similar effects were found when the targeting drugs were applied on BM cells isolated from MPN patients. Again, all drugs tested were found to suppress proliferation of MPN cells in a dose-dependent manner. In one experiment, we examined the effects of the JAK2 blockers on CD34+/CD38- cells in a BM sample isolated from a patient with ET. Here, only TG101348 (5 μM, 48 hours) was able to decrease the numbers of NSC significantly, whereas the other JAK2 inhibitors showed only slight effects on CD34+/CD38- NSC.

Table 1.

	piceatannol	pimozide	AZD1480	TG101348	R763	ruxolitinib	
HEL	10 -17.5	5 - 7.5	0.5 - 1	1- 2	1 -2	1 - 2	IC_{50}
SET-2	10 -17.5	5 - 7.5	0.05 - 0.1	0.1 - 0.25	0.01 - 0.05	0.025 - 0.05	(μM)
HEL	>50	10 - 25	>50	2.5 - 5	>50	>50	ED_{50}
SET-2	>50	<10	1 - 2.5	1 - 2.5	2.5 - 5	>5	(μM)

Summary and Conclusions: In summary, our data show that NSC in JAK2 V617F+ MPN express pSTAT5, and that targeting of STAT5 may be a novel interesting approach to control NSC expansion in these malignancies. Whether drug-induced suppression of pSTAT5 in NSC is effective clinically remains to be determined in clinical trials.

E1310

PROGRESSION TO MYELOPROLIFERATIVE NEOPLASMS WAS ASSOCIATED WITH MUTATIONS OF THE JAK2 AND CALR GENES IN PATIENTS WITH IDIOPATHIC LEUKOCYTOSIS AND THROMBOCYTOSIS

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Background: Idiopathic leukocytosis and erythrocytosis are hematological disorders without specific causes. Frequent V617F mutations on the *JAK2* gene have been reported in patients with polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF). We also found *JAK2* V617F mutations in one of 11 patients with idiopathic erythrocytosis. Recently mutations of the *CSF3R, SETBP1,* and *ETNK1* genes have been found in chronic neutrophilic leukemia and atypical chronic myeloid leukemia. Moreover an autosomal mutation was found in the *CSF3R* gene in a family with chronic neutrophilia. However, mutations associated with idiopathic leukocytosis and unexplained thrombocytosis have largely been unknown.
Aims: To elucidate the relevance of gene mutations, we analyzed mutations of the *CSF3R, JAK2, CALR, SETBP1,* and *ETNK1* genes in idiopathic leukocytosis and thrombocytosis.
Methods: Leukocytosis is defined as a total white blood cell (WBC) count more than two standard deviations above the mean, or a value greater than 11,000/μL. Those patients who satisfied the following criteria were included in the study: leukocytosis (predominantly neutrophils); the absence of apparent causes of leukocytosis; and documentation of the leukocytosis over a prolonged period of time. The period of observation was 1 year or longer in most patients. Those patients with unexplained persistent thrombocytosis (greater than 350,000/μL) who did not satisfy the criteria of ET (World Health Organization classification) were included in the study. Neutrophils or mononuclear cells were collected obtaining written informed consent from 16 patients with idiopathic leukocytosis (n=10), thrombocytosis (n=3), PV (n=1), and ET (n=2). Five of the patients were males and 11 were females. Neutrophils from peripheral blood were purified by dextran sedimentation followed by hypotonic lysis and centrifugation with Ficoll-Conray. Mononuclear cells were isolated from bone marrow by Ficoll-Conray gradient centrifugation. Genomic DNA was extracted using the QIAamp DNA blood mini kit (Qiagen, Valencia, CA, USA). Mutations within hot spots of the *CSF3R, JAK2, CALR, SETBP1,* and *ETNK1* genes were analyzed by direct sequencing in both directions using a 3730xL DNA Analyzer (Life technologies, Carlsbad, CA, USA) and/or allele specific polymerase chain reaction analysis. The current study was conducted within the guidelines and with the approval of ethical committee.
Results: *JAK2* V617F mutations were found in one of the 10 patients with idiopathic leukocytosis, one PV patient, and one of the two ET patients. No mutations of the *CSF3R, SETBP1,* and *ETNK1* genes were found in idiopathic leukocytosis, thrombocytosis, PV or ET patients. Type 1 mutations of the *CALR* gene were detected in two of the three unexplained thrombocytosis, and one of the two ET patients. All of the three patients with the type 1 mutations of the *CALR* gene were females. One idiopathic leukocytosis patient with *JAK2* V617F mutation has developed PV, whereas one idiopathic leukocytosis patient without the mutations has developed leukemia. The other 8 patients with idiopathic leukocytosis have a stable disease. One patient with unexplained thrombocytosis developed MF.
Summary and Conclusions: Our study suggests that idiopathic leukocytosis and unexplained thrombocytosis are heterogeneous conditions regarding mutations of the *JAK2* and *CALR* genes and clinical courses.

E1311

TO TAKE HOME: HOW MUCH VALUE IS LOW BURDEN OF JAK2 V617F ALLELE MUTATION?

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Background: The discovery of the JAK2 V617F mutation and its association with distinct haematological and clinical characteristics has prompted studies to assess the predictive value of JAK2 V617F and the allele burden for the most common complications of Mieloproliferative neoplasm (MPN, like Policitemic Vera -PV-, Essential Thrombocitosis -ET-, Myelofibrosis -MF-), and its relationship with prognosis and survival. The studies support the contention that quantification of JAK2 V617F allele burden might convey relevant clinical information in well-defined categories of MPN cases. They also show that a high JAK2V617F allele burden is associated with more severe disease with lower hemoglobine, more prevalent massive splenomegaly, increased rate of venous thrombosis (VT) and higher prevalence of bleeding events.
Aims: In this study we compare clinical and hematologic characteristics of patients with low allele burden with the rest.
Methods: In this retrospective study, we selected peripheral blood samples of 89 patients with Ph-negative MPNs. 45 were men and 44 women, with ages between 27 and 86 years old (mediane 53 years). We analyzed JAK2V617F status, the allele burden and its relationship with hematologic and clinical parameters like bleeding, deep vein thrombosis among others. The cutoff value for the allele burden was set to ≥10% for high and intermediate levels and <10% for low levels. JAK2 V617F mutation analysis was performed through High Resolution Melting and Sanger sequencing. For the statistical analysis, patient's characteristics were compared by Fisher's exact test for categorical variables, Mann-Whitney U test for continuous variables and Shapiro. Wilk to check the normality of the data of quantitative variables. All analyses were two sided, and significance was set at a p- value of 0.05. SPSS statistical package (v. 15.0) was us.
Results: In our series 20.2% of the cases presented low allele burden (PV 1/46, ET 14/34, MF 3/9). The presence of low allele burden was not associated with hematologic parametres compared to high and intermediate burden. In PV, arterial thrombosis was detected in 0% of low buden cases compared to high burden cases with 24% incidencie (p>0,05). On the same way, there was VT in 20% of high burden cases *versus* 0% of low burden cases. On the other hand, arterial thrombosis cases in patients with ET happened in 3% with low budden and 6% with high and intermediate burden; in VT cases, there were 14.3% episodes in low burden and 20% cases with high and intermediate burden. The allele burden was not associated with the necessity of transfusions, phlebotomy or bleeding. In ET, patients with low allele burden presented lower use of hydrea (p=0,035). We found that 16.7% of deaths presented low burden in contrast to 83% with high and intermediate burden.
Summary and Conclusions:-According to our results, PV and TE patients with low JAK2 V617F allele burden presented lower vein and arterial thrombotic risk. - Patients with ET with low allele burden would require with lower frequency hydrea treatment and it is probably relationated to myeloproliferative activity. - We need to increase our series in order to confirm our results, nevertheless, we believe that to get a better insight about the stratification of patients according to mutant allele burden may be important to achieve a personalized and more specific/effective treatment.

E1312

CHRONIC INFLAMMATION BIOMARKERS IN MYELOPROLIFERATIVE NEOPLASMS

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Background: Chronic inflammation contributes to the initiation and pathophysiology of cancers, including myeloproliferative neoplasms (MPNs). Unfortunately the new JAK1/2 inhibitors act only on symptoms and currently there is no cure for MPNs. The recent JAK1/2 inhibitor trials in MPNs unexpectedly showed that reducing inflammation can be more beneficial to patients than targeting gene mutants. Among chronic inflammation biomarkers, TGF-β and IL-6 have a central role, possessing both immunomodulatory and fibrogenic activities. TGF-β upregulates IL-6 and the levels of these cytokines are increased in MPNs.
Aims: We aim to evaluate the pro-inflammatory TGF-β, IL-6, NF-kB and anti-inflammatory IL-10 signaling pathways in MPNs.
Methods: We analyzed 63 patients with essential thrombocythemia (ET), 92 with polycythemia vera (PV), and 50 with primary fibrosis (PMF), and per 10

patients with secondary erythrocytosis and healthy controls. To evaluate the proposed signaling pathways in CD34+ hematopoietic progenitors, of 20 MPN patients, microarray analysis was used.
Results: Statistically significant leukocytosis was observed in PV *vs* secondary erythrocytosis and in JAK2V617F mutation positive *vs* negative PMF patients. Moreover, thrombocytosis was generally present in MPNs, while JAK2V617F allele burden significantly decreased the level of thrombocytosis in ET and PMF. Regarding TGF-β signaling related genes of hematopoietic progenitors: transcription factor Dp-1 (TFDP1) was significantly decreased in PMF, while cyclin-dependent kinase inhibitor 2B (CDKN2B) was increased in PV *vs* controls. Also, bone morphogenetic protein receptor, type II (BMPRII) was enhanced in ET and PV, whereas TGF-β receptor 1 in all MPNs. Regarding IL-6 signaling pathway related genes: interleukin 6 signal transducer (IL6ST) and mitogen-activated protein kinase kinase 1 (MAP2K1) were increased in ET and PV *vs* healthy controls. Considering NF-kB signaling related genes: tumor necrosis factor receptor superfamily (TNFRSF1A) and TNFRSF1A-associated via death domain (TRADD) were increased in PMF and PV, respectively. In addition, TNFRSF1A had more prominent overexpression in JAK2V617F mutation positive patients than in MPN patients with no JAK2V617F mutation, reaching statistical significance in ET. Analysis of IL-10 signaling pathway related genes revealed significant increase of biliverdin reductase A (BLVRA) and heme oxygenase 1 (HMOX1) in PV and ET, respectively.
Summary and Conclusions: The presented inflammation biomarkers will provide a better understanding of molecular mechanisms implicated in the development and progression of MPNs.

E1313

THE PRESENCE OF THE JAK2V617F POINT MUTATION AFFECTS OSTEOCLAST FUNCTION IN PATIENTS WITH CHRONIC MYELOPROLIFERATIVE NEOPLASMS

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Background: Myeloproliferative neoplasms (MPN) patients are at increased risk for femoral fracture and the development of osteoporosis, compared to age-matched population groups (1). The JAK2V617F is a gain of function point mutation that occurs frequently in MPN patients and deranges many cellular properties of their hemopoietic cells.
Aims: Therefore we speculate that genetically modified JAK2 can slightly modify osteoclast homeostasis, a gain of function that can be implicated in increased bone resort ion and deregulation of the hematopoietic stem cell niche in MPN patients.
Methods: Peripheral blood was drawn from 18 newly diagnosed MPN patients (14 essential thrombocythemia, 2 primary myelofibrosis, 2 polycythemia vera diagnosed according to 2008 WHO criteria) and from 4 age-matched normal donors (ND). Osteoclast (OCL) forming assays were started from $2x10^5$ positively selected monocytes. OCL were formed also under the presence of the pan-JAK Inhibitor AG-490. Genomic DNA was extracted from the formed osteoclasts and the JAK2V617F/JAK2WT genomic DNA ratio was calculated by using the Taqman based JAK2MutaQuant Kit (IPSOGEN). Comparisons between groups were performed by using the Mann-Whitney for unpaired and the Wilconxon Rank test for paired samples.
Results: OCLs formed from monocytes derived from heterozygous for the JAK2V617F mutation (+/-) MPN patients, were significantly more compared to those from the JAK2 wild type(WT) MPN patients and to ND as well [OCL per 10.000 monocytes, Median Value of osteoclasts (MV) (min-max) was for JAK2V617F (+/-) MPN patients: 960 (159-3674), for JAK2 WT MPN patients: 204 (81-1286), p=0.03, and for ND was: 322 (215-522) and p=0.05 when comparing JAK2V617F(+/-) to ND, Mann-Whitney test]. The ratio of JAK2V617F/JAK2WT genomic DNA was increased in OCLs compared to the input monocyte cells. The median enrichment of OCLs for the JAK2V617F mutated clone was 12% (3.3%>26% and p<0.01, Wilconxon Rank test). Compared to ND OCLs from patients with JAK2V617F (+/-) are more susceptible to JAK2 inhibition (Mann-Whitney test, p=0.03) but this was not statistically significant for JAK2 WT MPN patients (Mann-Whitney test, p=0.1).
Summary and Conclusions: The acquisition of the JAK2V617F point mutation in hematopoietic cells capable for osteoclastogenetic differentiation provides them with increased OCL forming ability but these OCLs are concurrently becoming more vulnerable to JAK2 kinase inhibition. This deregulation in osteoclast homeostasis can contribute to osteoporosis development in MPN patients and probably in the mobilisation of hemopoietic progenitor cells towards the periphery.

E1314

INTERLABORATORY EVALUATION OF TARGETED NEXT GENERATION SEQUENCING FOR MYELOPROLIFERATIVE NEOPLASMS ASSOCIATED MUTATIONS

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Background: Classic myeloproliferative neoplasms (MPN) include polycythemia vera, essential thrombocythemia and primary myelofibrosis and share an association with molecular abnormalities such as JAK2V617F or CALR mutations. Numerous somatic mutations have been identified in MPN patients including mutations in signaling pathways, in epigenetic or splicing regulators, and in oncogenes. Although the relationship between mutations and the pathophysiology of MPN is not clear, some mutations are important for diagnostic classification whereas others have prognostic relevance, making more pregnant the need for high throughput sequencing approaches. Next generation sequencing (NGS) is capable of quickly allowing the assembly of a single multi-gene panel relevant to MPN, but as NGS becomes a clinical tool, a full understanding of the variables affecting sequencing analysis output is required.
Aims: In this multicentric study, we evaluated different NGS platforms 1) for their robustness in the identification of mutations relevant to MPN diagnosis, and 2) by comparing quantitative data for these variants.
Methods: Four laboratories participated in this study. All of them analysed 14 DNA samples from MPN patients. Two NGS platforms (MiSeq-Illumina (n=3), PGM-Ion Torrent (n=1)) with different exon sequencing approaches (amplicon sequencing (TSCA-Illumina (n=2), AmpliSeq-Lifetech (n=1) or Haloplex-Agilent (n=1)) were evaluated. The gene panels slightly differed between groups, but a common set of 10 genes was present in each center: *JAK2, MPL, ASXL1, TET2, EZH2, IDH1, IDH2, DNMT3A, LNK, SRSF2*. Sequencing data were analysed on each platform using local pipelines (VariantStudio-Illumina (n=2), SureCall-Agilent (n=1) and IonReporter-life tech (n=1)) and compared between laboratories with variant allele frequencies (VAF)>2% and depth>50X filters.
Results: Comparative analysis of all labs found 32 mutations, all validated by Sanger sequencing or allele-specific PCR, on 8 common genes. Twenty four (75%) of these mutations on 6 genes were detected by all platforms with VAF between 12% and 85%. The discrepancies between labs focused on 2 genes: *ASXL1* and *SRSF2*. PGM platform missed all deletions or duplications in *ASXL1* gene (4/4). Low coverage was observed for SRSF2 hotspot point mutations in 2 platforms (AmpliSeq-PGM and TSCA-MiSeq). Three labs have integrated *CALR* gene on their panel design, only the lab using SureCall analysis identified large deletions in this gene. We observed a tight correlation of JAK2V617F VAF (23% to 85%, n=9) among the different platforms (standard deviation average (SDA): 2.4, range: 0.37-3.94). In addition the different NGS technologies were well correlated with qPCR results (r^2=0.86). Excepted for 2 frameshift variants in *DNMT3A* and *ASXL1*, an excellent reproducibility was observed in quantitative values of VAF (n=32 mutations) among participants (SDA: 4.35, range: 0.37-19.5).
Summary and Conclusions: This inter-laboratory comparison demonstrates that NGS is a reliable technology for mutations screening in MPN and would permit a rapid molecular characterization. Moreover, we observed an excellent accuracy of quantitative evaluation of VAF in particular with comparable results between NGS and qPCR for JAK2V617F, and a good reproducibility regardless platform characteristics and chemistry approaches. In summary, although customized optimization to enhance variants detection remains necessary (low depth, bioinformatics tools for indel detection), this comparative assessment of NGS pipelines gives important insights into sequencing and analysis strategies for routine hospital transfer.

E1315

A 7- GENE SIGNATURE DEPICTS THE BIOCHEMICAL PROFILE OF EARLY PREFIBROTIC MYELOFIBROSIS

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Background: The Philadelphia-negative myeloproliferative neoplasms include essential thrombocythemia (ET), polycythemia vera (PV) and primary myelofibrosis (PMF). Recent studies have shown that a large proportion of patients classified as ET actually have prefibrotic myelofibrosis (pre-MF). Because prognosis and likely in the future also treatment of both entities are different, it is important to distinguish between them. According to the WHO classification, bone marrow histology is a major component of the distinction between ET

and pre-MF. However, the differential diagnosis between these two entities can be challenging.
Aims: We aimed at identifying a simple gene signature-composed of a few genes-which were able to distinguish true ET from pre-MF.
Methods: Gene expression profiles have been generated on whole blood from 17 patients with ET using Affymetrix HG-U133 2.0 Plus microarrays. Seven carefully chosen genes were used to develop a supervised classification model to predict the signature of ET patients. The biochemical variables lactate dehydrogenase (LDH) and leukocyte count were used to divide patients into subgroups, taking into account that the leukocyte count may be elevated in ET and pre-MF due to other factors than the myeloproliferation per se (*e.g.* inflammation and other cancer), whereas the LDH is elevated in myeloproliferative cancer only as a result of clonal myeloproliferation provided that other reasons are excluded (*e.g.* liver disease and hemolysis). In addition, we a priori assumed the leukocyte count to be normal in "genuine" ET (no increase in granulopoiesis), whereas pre-MF is often associated with an elevated leukocyte count and thrombocythemia. Bone marrow biopsies from 14 of the 17 patients were evaluated by 4 independent hematopathologists.
Results: Based on the LDH and leukocyte values, 9 patients were assigned to the ET group and 8 patients were assigned to the pre-MF group; however, the composition of patients in the LDH and leukocyte groups differed. Using LDH as the clinical variable, the resulting 7-gene signature including MPO, CEACAM8, CRISP3, MS4A3, CEACAM6, HEMGN, and MMP8 correctly predicted 8 of 8 patients in the pre-MF group (sensitivity 100%) and correctly classified 8 of 9 patients in the ET group (specificity 89%) and showed a balanced accuracy of 94%. Using the leukocyte value as the clinical variable, the 7-gene signature resulted in 62% sensitivity, 78% specificity, and a balanced accuracy of 70%. The performance of the 7-gene signature using LDH as the clinical variable was tested against the bone marrow evaluation from the hematopathologists showing a concordance of 71%, 79%, 62%, and 38%. Gene ontology analysis and literature research show that the 7 genes are involved in key processes associated with the pathophysiology of MPNs. CRISP3, MMP8 and MPO are neutrophile granula proteins that play important roles in immune and inflammatory responses. CEACAM6 and CEACAM8 are involved in cell adhesion, cellular invasiveness, angiogenesis, and inflammation. MS4A3 is a hematopoietic cell cycle regulator, and HEMGN plays an important role in differentiation, proliferation and hematopoietic development.
Summary and Conclusions: Supervised classification was able to distinguish between ET and pre-MF with a balanced accuracy of 94%. Our findings suggest that classification using LDH as the clinical variable may add support to the clinical diagnosis of these two entities. Prospective studies of a larger cohort of patients are being planned to substantiate LDH as a simple parameter in the distinction between ET and pre-MF.

E1316

PLATELET ANALYSIS INCREASES THE DETECTION OF MUTATIONS IN TRIPLE-NEGATIVE ESSENTIAL THROMBOCYTHEMIA PATIENTS

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Background: Mutations in *JAK2* (50-60%), *CALR* (15-30%) and *MPL* (1-5%) have been identified in patients with essential thrombocythemia (ET). However, there is still a percentage of ET patients (10-30%) who are wild-type for mutations in these genes when isolated granulocytes or peripheral blood are analyzed. This subgroup of patients called "triple-negative" has not been extensively studied with regard to the presence of *JAK2, CALR* and *MPL* mutations in platelets.
Aims: To analyze *JAK2*V617F, *CALR* and *MPL* mutational status in platelets from a cohort of triple negative ET patients.
Methods: From a whole cohort of 236 ET patients consecutively diagnosed in a single institution, we included 33 ET patients (14%) lacking *JAK2*V617F (determined by quantitative allele-specific PCR), *MPL* exon 10 mutations (W515, S505; analyzed by Sanger sequencing) and *CALR* exon 9 mutations (analyzed by PCR followed by fragment analysis) in granulocytes.
Mutational analysis. Platelet RNA was extracted with Trizol and 1ug total RNA was reverse transcribed. The mutational analysis of *JAK2*V617F was performed by quantitative allele-specific real time PCR. Analysis of exon 10 of the *MPL* gene (S505, W515) was performed by next generation sequencing (NGS) (454 GS Junior, Roche), with a median coverage of 1335x (range 497-4863). Mutations were confirmed by CAST-PCR. The mutational analysis of exon 9 of the *CALR* gene was performed by PCR, using a 6-carboxyfluorescein labeled reverse primer, followed by fragment analysis in a Genetic Analyzer 3500DX (Applied Biosystems) or by NGS deep sequencing (454 GS Junior) with a median coverage of 1326.5x (range 607-1686).
Results: *JAK2*V617F was detected in 3 out of 33 (9.1%) patients analyzed, with allele burdens of 16%, 20% and 33%. *MPL* mutations (W515L) were detected in 3 out of 33 patients by NGS. Two cases with *MPL* W515L allele burden of 12% and 26% in platelets were confirmed by CAST-PCR. The third case with only a 3% of mutant allele burden was not confirmed. Interestingly, the coexistence of *JAK2*V617F and *MPL*W515L was observed in one case with a mutant allele burden of 16% and 12% respectively. Regarding *CALR,* two cases showed deletions in exon 9 by NGS with allele burdens of 1% and 2%. These mutations were not confirmed by fragment analysis.
Summary and Conclusions: In summary, we have confirmed the presence of *JAK2*V617F and/or *MPL* mutations in 4/33 (12%) ET patients previously considered as triple-negative. Platelet analysis increases the sensitivity of driver mutation detection in this subset of ET patients when compared to conventional analysis on whole blood or isolated granulocytes.
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E1317

BONE MARROW STROMA MEDIATED PROTECTION OF MPN CELLS FROM RUXOLITINIB- AND VORINOSTAT-INDUCED APOPTOSIS REQUIRES MAPK-JNK AND PI3K-AKT/PKB SIGNALING PATHWAY ACTIVATION

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Background: The classical BCR-ABL-negative myeloproliferative neoplasms (MPN) are characterized by increased proliferation of hematopoietic precursors in the bone marrow resulting in elevated numbers of terminally differentiated cells. Despite the recent advances in the understanding of the biology of MPN, there is still no curative treatment for MPN except for bone marrow transplantation. The discovery of JAK-STAT constitutive activation in the majority of MPN patients led to the development of clinical studies targeting MPN with Ruxolitinib, a JAK1/2-specific inhibitor. However, despite achieving significant reductions in splenomegaly and symptomatic improvement in MPN patients, JAK inhibition failed to induce complete remissions and eradicate the malignant clone. In alternative, histone deacetylase inhibitors have shown success in the treatment of several hematological malignancies but their efficacy in MPN is limited.
Aims: Given that both Ruxolitinib and the HDAC inhibitor Vorinostat fail to eradicate the neoplastic clone in MPN patients, we investigated the hypothesis that the bone marrow stroma may confers resistance to these drugs by preventing their cytotoxic effects on neoplastic cells.
Methods: We made use of a co-culture system in which MPN cell lines (SET-2, HEL and UKE-1) are placed in direct contact with the HS-5 bone marrow stromal cell line or its conditioned media. Upon exposure of MPN cells to the bone marrow microenvironment and following treatment with different drugs, the cells are harvested to analyze cellular viability, gene expression by quantitative-PCR and signaling pathway activation by Western-blot.
Results: The treatment of the SET-2 cell line with both Vorinostat and Ruxolitinib promoted apoptosis and decreased proliferation. However, apoptosis was significantly abrogated when SET-2 cells were cultured in the presence of a stromal layer of HS-5 cells or HS-5 conditioned medium. The stroma protective effect was maintained for up to 6 six days and was also dependent on drug concentration. The bone marrow stroma protective effect correlated with altered expression in MPN cells of genes associated with inflammatory processes, apoptosis and proliferation (*CDKN1A*, *IER3*, *BIRC3*, *TNFRSF8*, *TNFRSF9*, *COX2*, *IL1B*), and with the activation of signaling pathways important for cellular homeostasis, such as PI3K-PKB/Akt, MAPK-JNK, JAK-STAT and NF-κB as shown by increased phosphorylation of PKB/Akt (Ser473); GSK3α/β (Ser9/Ser21); S6 (Ser235/236); STAT3/5 (Tyr705/Tyr694) and p65/RELA (Ser536). Importantly, the pharmacological inhibition of PI3K-PKB/Akt and MAPK-JNK signaling pathways completely abrogates the protective effect bone marrow stroma on SET-2 cells.
Summary and Conclusions: Overall, we show that bone marrow stroma protects MPN cells from the cytotoxic effects of two pharmacological agents of different classes: Vorinostat and Ruxolitinib. This protective effect is likely achieved, through the up-regulation of genes associated with apoptosis (*IER3, BIRC3*, *TNFRSF9*) and relies on the activation of pro-survival signaling pathways such as PI3K-PKB/Akt and MAPK-JNK. We did not observe any effects of the bone marrow stroma on proliferation or differentiation, suggesting that the main effect of bone marrow stromal cells is to prevent apoptosis of the neoplastic cells. Our results identify a possible cell non-autonomous mechanism by which Ruxolitinib fails to eradicate the neoplastic clone in MPN patients and may indicate novel therapeutic targets for MPN.

E1318

FREQUENCY AND ALLELE BURDEN OF CALR MUTATIONS IN CHINESE WITH ESSENTIAL THROMBOCYTHEMIA AND PRIMARY MYELOFIBROSIS WITHOUT JAK2 V617F OR MPL MUTATIONS

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Background: CALR mutations are detected in about 50% of persons of pre-

dominately European descent with essential thrombocythemia (ET) or primary myelofibrosis (PMF) with wild-type alleles of JAK2 and MPL. The role of CALR mutations in the aetiology of ET and PMF is unknown and correlates between CALR mutation, mutant allele burden and clinical features are being studied.
Aims: To study the frequency of CALR mutations and clinical correlates in Chinese populations.
Methods: We studied 1088 Chinese with diverse myeloproliferative neoplasms including ET (N=234) and PMF (N=50) without JAK2^{V617F} or MPL exon 10 mutations. Detection of CALR exon 9 mutations was done by PCR amplification followed by fragment length analysis and direct sequencing.
Results: CALR mutation was detected in 53% (95%CI, 46-60%) of subjects with ET and 56% (95% CI, 41-70%) of subjects with PMF. 152 CALR mutations were identified clustering into 15 types including deletions (N=8), insertions (N=3) and complex indels (N=4). We also identified 9 new mutation (Figure1). Mean (±SD) mutant allele burden was 31±12% (range, 0.5-69%). Persons with PMF had higher CALR mutant allele burdens than those with ET (38±8% *vs* 29±12%; *P*<0.001). Amongst persons with CALR mutations, those with PMF had different clinical features from those with ET.
Summary and Conclusions: These data may be useful for diagnosing ET and PMF in Chinese who are about 40% of all persons with ET and PMF and for monitoring therapy-response. They also highlight similarities and differences in CALR mutations between Chinese and persons of predominately European descent with these diseases.

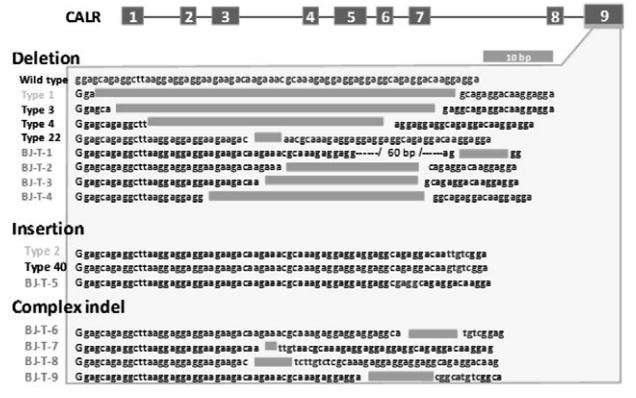

Figure 1. Genomic positions of the 15 mutation types.

E1319

GENE AND PROTEIN EXPRESSION ANALYSES OF THE MTOR SIGNALING PATHWAY IN MYELOPROLIFERATIVE NEOPLASMS

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Background: The main target of activated phosphoinositide-3-kinase (PI3K)/AKT is the serine/threonine kinase mammalian target of rapamycin (mTOR). JAK inhibition, used in therapy of myeloproliferative neoplasms (MPNs), abolished the activation of JAK/STAT and PI3K/mTOR signaling pathways.
Aims: We intend to reveal gene and protein markers of MPN pathogenesis, with an accent to PI3K/AKT and mTOR pathway members and related genes.
Methods: Using cDNA microarray analysis of 25.100 unique genes, we studied the gene expression profile of hematopoietic CD34$^+$ progenitor cells and granulocytes obtained from peripheral blood of subjects with essential thrombocythemia (ET), polycythemia vera (PV) and primary myelofibrosis (PMF). The microarray analyses of the hematopoietic progenitor cells and granulocytes were performed from 20 MPN subjects: JAK2 positive ET, PV, PMF subjects, and JAK2 negative ET, PMF subjects. The granulocytes for proteomic studies were pooled in 4 groups: PV with JAK2 mutant allele burden above 80%, ET with JAK2 mutation, PMF with JAK2 mutation and ET/PMF with no JAK2 mutation.
Results: The number of differentially regulated genes was about two fold larger in hematopoietic CD34$^+$ progenitors compared to granulocytes. Thirty-six genes (including *RUNX1, TNFRSF19*) were persistently highly expressed, while 42 genes (including *FOXD4, PDE4A*) were underexpressed both in hematopoietic CD34$^+$ progenitors and granulocytes. Using proteomic studies, significant upregulation was observed for MAPK and PI3K/Akt signaling regulators that control myeloid cell apoptosis, differentiation and proliferation (GADD45B, CRIP1, SIPA1). When the status of mTOR signaling pathway related genes was analyzed, PI3K/Akt regulators were preferentially upregulated in hematopoietic progenitors of MPNs, with downregulated major components of the protein complex EIF4F.
Summary and Conclusions: Molecular profiling of hematopoietic progenitors and granulocytes of MPN determined gene expression patterns beyond their recognized function in disease pathogenesis that included dominant upregulation of PI3K/AKT signaling.

E1320

THE ANTIPROLIFERATIVE EFFECT OF SIMVASTATIN IS ASSOCIATED WITH EPIGENETIC MECHANISMS IN NEOPLASTIC MAST CELLS

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Background: There is growing evidence that statins like simvastatin show antileukemic activities (Bockorny and Dasanu, 2015). A multitude of experimental studies have established their pro-apoptotic, anti-proliferative, anti-invasive, and radiosensitizing effects. Although several cellular pathways activated by statins have been identified, the precise mechanisms by which statins inhibit neoplastic cell growth remain elusive and likely vary with the type of malignancy. Statins are 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors that act on the mevalonate pathway and inhibit synthesis of cholesterol, geranylgeranyl pyrophosphate (GGPP) and farnesyl pyrophosphate (FPP). Consequently, they prevent posttranslational isoprenylation of small GTPases, and inhibit their activity. In neoplastic cells, activated RAS-GTPase, the founding member of the gene family, down-regulates the expression of the pro-apoptotic gene FAS via epigenetic DNA-methylation by DNMT1 (Thaler *et al.*, 2013).
Aims: The aim of this study was to investigate possible epigenetic mechanisms, which are responsible for the antiproliferative activity of statins on neoplastic mast cells.
Methods: Here we performed *in vitro* analyses from human mast cell leukemia lines (HMC 1.1. and HMC 1.2.) following 3 days of simvastatin-treatment. For comparative reasons a bisphosphonate-based inhibitor of the mevalonate pathway (ibandronate) and a demethylating agent (decitabine) were used in pharmacological concentrations. Modulation of gene expression from selected genes, were analysed from gene chip data (Affymetrix) with respective bioinformatics tools (Pathvisio and GSEA) and confirmed and via real time PCR and immune blotting.
Results: Dose response curves showed a significantly lower sensitivity of HMC 1.2 (EC50: 16.8 µM) as compared to HMC 1.1 (EC50: 3.5 µM). It remains to be established, if this is associated with the Kit D816V mutation, which is only present in HMC 1.2. Treatment with simvastatin upregulated the microRNA612 and down-regulated the epigenetic key enzymes *DNMT1* and *HDAC2.* This was associated with down-regulation of the repair mediator *TYMS* (thymidilate synthase) and the associated metabolic pathway (one carbon/folate metabolism, see Figure 1) as well as the *BRCA* genes, the latter being just observed with simvastatin but not with ibandronate or decitabine, thus indicating a specific effect of statin on DNA-repair.

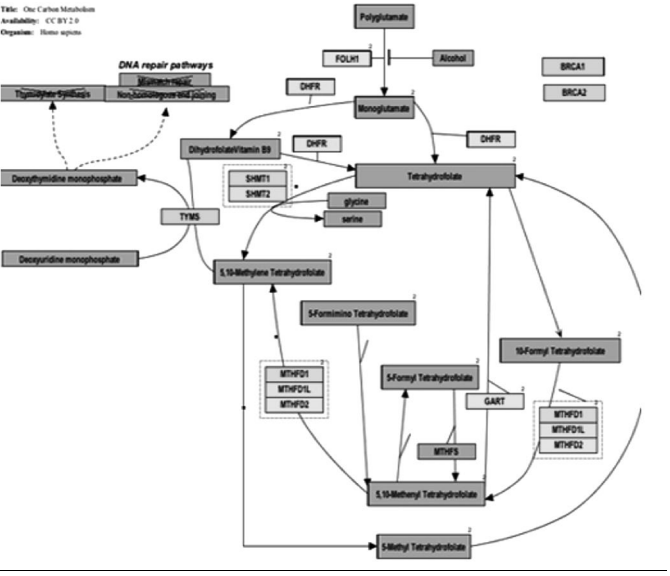

Figure 1. One carbon metabolism pathway (green: down-regulation, red: up-regulation).

Summary and Conclusions: In conclusion this study shows that the antiproliferative effect of statins is associated with principal epigenetic mechanisms including DNA-demethylation, histone modification as well as expression of microRNA in mast cell leukemia lines, thus confirming similar data from solid cancer cell lines (Karlic *et al.*, 2015). It remains to be elucidated, whether statin-mediated reduction of mediators for DNA repair is a possible cause or a consequence of the observed epigenetic and antiproliferative mechanisms.

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E1321

CD30 EXPRESSION ON NEOPLASTIC MAST CELLS IN SYSTEMIC MASTOCYTOSIS-CORRELATION OF ASSESSMENT BY IMMUNOHISTOCHEMISTRY AND MULTIPARAMETER FLOW CYTOMETRY WITH RESPECT TO CLINICAL PARAMETERS

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Background: Systemic mastocytosis (SM) is a rare disease and clinical presentation is highly variable from taking rather indolent to very aggressive courses, seldom even with the presentation of mast cell leukemia. CD30 (Ki-1 antigen) expression on neoplastic mast cells (MC) evaluated by immunohistochemical staining (IH) on bone marrow (BM) biopsies has been reported to be present in patients with more aggressive variants of MC. When assessed by multiparameter flow cytometry (MFC) it might also contribute to improved diagnostic accuracy. Only scarce data exists on correlation of CD30 expression detected by MFC with clinical SM parameters.
Aims: To compare CD30 expression on MC of patients with SM detected by either MFC or IH and to correlate results with patient characteristics and disease characteristics as well as with cytogenetics (CG) determined by chromosome banding analysis and with molecular genetics (MG).
Methods: We studied BM of 60 patients with SM by MFC. CD30 expression was quantified by a five color staining assay with monoclonal antibodies against CD30, CD45, CD117, CD2 and CD25. Aberrant MC were defined as CD117/CD45 positive cells with coexpression of CD2 and/or CD25. Mean fluorescence intensities (MFI) of CD30 were determined in MC and correlated to CD30 MFI in lymphocytes to derive CD30 index. For 22 patients data on MC infiltration and CD30 expression by IH was assessed while CG and MG were done in 29 and 50 patients, respectively. *KIT*D816V mutation was analyzed using melting curve-based RNA mutation analysis applying PNA clamping according to Sotlar *et al.* [Am J Pathol. 162: 737-746, 2003]. Expression of CD30 by MFC was correlated to results of CD30 expression by IH and results of CG and MG.
Results: 26 patients were male and 34 female. Median age was 61 years (20-81 years). Aberrant karyotypes were found in 3 patients while 26 had a normal karyotype. *KIT*D816V mutation was found in 46/50 patients. In 6/60 patients concurrent hematological non-mast cell disease (AHNMD) was diagnosed. Mean (±SD) MC infiltration was 20%±26% (range, 1.5%>85%) by IH and 0.5%±2.3% (range, 0.01%>17%) by MFC. Mean (±SD) CD30 index was 20.5±24.3 (range, 3-154), mean (±SD) CD30 expression by IH was 9%±17% (range, 0%>70%). Percentages of MC infiltration detected by IH and MFC correlated significantly (p=0.002, r=0.819). No correlation of MFC CD30 index with age, sex, concomitant AHNMD, grade of MC infiltration or percentage of CD30 positive MC by IH was found. Interestingly, we detected a trend to higher CD30 index in patients with *KIT*D816V mutation (21.0±26.4 *vs* 10.9±9.4, n.s.) and a trend to lower CD30 index in patients with abnormal karyotype (11.6±10.3 *vs* 13.8±8.5, n.s.).
Summary and Conclusions: CD30 expression on neoplastic MC in patients with SM is a dynamic parameter and can be assessed by MFC. CD30 expression may be stronger in patients harbouring *KIT*D816V mutations compared to those who do not. Patients with abnormal cytogenetics show a trend to lower CD30 expression on MC. The expression of CD30 on neoplastic MC in patients with SM should be further analyzed by MFC and IH in combination to substantiate the present findings.

E1322

INCIDENCE OF CALR MUTATIONS IN PATIENTS WITH CEREBRAL VENOUS THROMBOSIS WITHOUT OVERT CHRONIC MYELOPROLIFERATIVE NEOPLASM

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Background: In Myeloproliferative neoplasms (MPN) including Essential Thrombocythemia (ET), Polycythemia Vera (PV) and Myelofibrosis thrombosis is the main cause of morbidity and mortality. Venous thromboses are observed in about 2 to 15 % of patients according to the MPN subtype. Venous thromboses at unusual sites are not exceptional and even typical of MPN as reported affecting the splanchnic territory in 5-10 % of the patients with PV or ET and the cerebral circulation reaching up to 1 % in ET. The JAK2V617F mutation is present in 95% of cases with PV and 60% of cases with ET or Myelofibrosis. With the discovery of this mutation, the diagnosis of MPN was greatly facilitated and it was shown that a MPN at an early stage or even at a latent stage may be responsible for the splanchnic thrombosis in a significant proportion of patients. By contrast, the JAK2V617F mutation was very rare in patients with cerebral venous thrombosis (CVT) and no patent signs of MPN, as we previously reported (Bellucci *et al.*, 2008) in a series of 87 patients. Recently, mutations in the *CALR* gene were reported in the majority of patients having MPN with non-mutated JAK2.
Aims: We looked for the presence of JAK2V617F and *CALR* mutations in patients with CVT and no hematological signs of patent MPN in order to see whether CVT may reveal MPN at a latent stage in a particular subgroup of patients.
Methods: From September 2007 to April 2014, 167 patients with CVT were included in our study: a patent MPN was carefully ruled out. Hematocrit was checked to be repeatedly below 52 % in men and 48 % in women and the platelet count to be repeatedly below 400G /L in order to rule out PV and ET respectively, according to the WHO classification. Diagnosis of CVT was based on magnetic resonance imaging (MRI) combined with MR venography and/or helical cerebral CT venography. We looked for the JAK2V617F mutation using an allele specific real-time PCR assay (Mutaquant®, Qiagen) and for the *CALR* mutations using PCR followed by fragment analysis, which also allowed to calculate the mutant allele burden.
Results: The JAK2V617F mutation was detected in 2 patients. In patient 1 (male, 56 years old) the allele burden was low at 0.5 %: an antiphospholipid syndrome was evidenced requiring antivitamin K therapy. After a follow-up of 32 months no MPN was detected. In patient 2 (female, 21 years old) the JAK2V617F mutation was also low at 3.2 %. CVT occurred concomitantly to oestroprogestative intake. Blood counts were still normal 10 months after the diagnosis of CVT. In 2 patients a type 1 *CALR* mutation (p.L367fs*46) was detected. Patient 3 (male, 47 years old) had a venous recurrent thromboembolic disease with a first deep venous thrombosis 2 years before the CVT and a recurrence 22 months later. Patient 4 (female, 19 years old) presented with CVT after oestroprogestative intake. The mutant *CALR* allele burden was low, at 10 and 12% in patient 3 and 4 respectively. No MPN and no thrombophilic abnormality were observed after a follow-up of 22 and 11 months in patient 3 and 4 respectively.
Summary and Conclusions: The incidence of the JAK2V617F and *CALR* mutations in patients with CVT without overt MPN was very low in this study. In this large cohort of 167 patients JAK2V617F mutation occurred in 2 patients (1.2%) and *CALR* mutations in 2 other patients. Interestingly all allele burdens were low suggesting that these patients may be at an early stage of the disease which may justify a prolonged follow-up. We do not recommend a systematic research of these mutations in such patients but it may be helpful in some particular cases with CVT without any etiology.

E1323

COMPARISON OF THREE DIAGNOSTIC METHODS TO DETECT CALRETICULIN MUTATIONS IN MYELOPROLIFERATIVE NEOPLASMS

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Background: Calreticulin (*CALR*) mutations have recently been reported in 70-84% *JAK2V617F*-negative myeloproliferative neoplasms (MPN), and this detection has become necessary to improve the diagnosis of MPN.
Aims: The aim of our study was to compare in parallel on a large cohort of ET patients three diagnostic methods [High Resolution Melting (HRM) analysis, product sizing analysis and Sanger sequencing] in the detection of *CALR* mutations.
Methods: Based on the 2008 WHO diagnostic criteria, a total of 298 ET patients from the hematology laboratory, University hospital in Dijon, France, were retrospectively studied.
Results: Altogether, a *JAK2V617F* or a *MPL* exon 10 mutation was observed in 179 (60%) and 13 (4.5%) patients, respectively. Finally 83 ET patients (33 males, 50 females) with a mean age of 63 y at the time of diagnosis (ranging from 20 to 95 years) diagnosed from 2005 to 2013 did not harbor any mutation and were considered as "double-negative" and thus tested using three molecular methods for a *CALR* mutation. Using the HRM method, a positive curve was noted in 54 patients, whereas no *CALR* mutation was observed in 28. Of note, in one patient there was no DNA amplification. Using the product sizing analysis, a *CALR* mutation was noted in 55 ET patients, and no mutation was

seen in 28. Finally, using Sanger sequencing, 50 ET patients harbored a *CALR* mutation, whereas no DNA amplification was noted in 4 samples. Taking altogether, a *CALR* mutation was noted in 56 patients and was of type 1 (leading to a deletion of 52 bp) or type 2 (leading to an insertion of 5 bp) in 28 (49%) and 20 (35%) patients, respectively. The 8 (16%) remaining mutations were 2 deletions of 46bp, 1 deletion of 1bp, 1 deletion of 2bp, 1 deletion of 25bp, 1 deletion of 33bp, 2 indel (1 insertion of 4bp and deletion of 2 bp and 1 insertion of 5bp and deletion of 1 bp). A false negative test was observed in 1, 2 and 2 in product sizing analysis, HRM method and Sanger sequencing, respectively. The 2 false negatives cases in HRM had either negative curves (patient #1) or curves with low amplitude (patient #2) contrary to the product sizing analysis that showed the presence of one (patient #1) or several mutant peaks (patient #2). Of note, in patient #2 the sequencing analysis showed a 25bp deletion associated with an intron mutation. The 2 false negative cases in sequencing analysis had both positive curves in HRM and mutant peaks in product sizing analysis however with low allelic burden (13% and 8% respectively). The false negative case in product sizing analysis had a deletion of 1bp that was not well separated from the wild type peak in product sizing analysis despite the HRM curves were positive and the sequencing analysis showed a deletion of 1 bp. The sensitivity for the HRM, the product sizing analysis and Sanger sequencing was 96.4%, 98.2% and 89.3% respectively, whereas the specificity was 96.3%, 100% and 100%. In our cohort, the product sizing analysis was the most sensitive method with an easy interpretation, while the HRM was sometimes difficult to interpret. On the other hand, when large series of samples were tested, HRM provided results in a short delay in comparison to the other methods that required more time. Finally the sequencing method, which is the reference method, had the lowest sensitivity but allowed to describe precisely the type of the mutations.

Summary and Conclusions: In routine laboratory activity, product sizing analysis and HRM are globally similar to detect the *CALR* mutations and may be used as first-line screening tests, followed by Sanger sequencing if positive to confirm and to determine the type of the mutations.

E1324

ALTERNATIVE SPLICING VARIANT COULD BE RESPONSIBLE OF MORGANA UNDER-EXPRESSION IN ACML

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Background: Morgana/chp-1 is a chaperone protein regulating ROCK activity and it is involved in centrosome duplication and tumorigenesis. Morgana +/- mice develop with age a fatal transplantable myeloproliferative disease similar to aCML, presenting centrosome amplification as well as cytogenetic abnormalities in bone marrow (BM). Notably, low morgana expression levels were found in the BM of aCML-affected patients and in a portion of CML Philadelphia positive patients. To assess whether morgana gene (CHORDC1) mutations can be responsible for low morgana expression levels in aCML patients we screened exome sequencing data obtained; no mutation were detectable suggesting that more complex regulatory mechanisms are involved in Morgana underexpression.
Aims: The aim of this study was to understand the mechanism responsible of Morgana reducted expression in aCML in order to identify a new pathway druggable to restore Morgana levels.
Methods: RNAs extracted from 9 primary aCML bone marrow samples collected at diagnosis after informed consent was reverse transcripted in c-DNA using random examers. The amplification products derived from Morgana gene were purified using Gel Extraction System and sequenced with ABI Prism 3100 Capillary Genetic Analyzer (Applied Biosystems). To evaluate the amount of the wild type and alternative transcript, we used two specific TAQMAN probes, one able to recognise only the wild type form and one other able to recognise both the transcripts. Protein analysis were performed by immunohistochemistry (IHC) experiments on formalin-fixed, paraffin-embedded bone marrows. Exome sequencing was used to extend to a larger portion of aCML patients and to analyze another pathology (AML) in order to verify the specificity of this inactivation.
Results: In addition to the main Morgana mRNA we found the co-existence of another transcript; direct sequencing of amplification products confirmed the presence of an alternative transcript (AS) which lacks the entire exon 3 of Morgana; this phenomenon produces a change on the correct reading frame and as consequence after 9 nucleotide the generation an earlier STOP codon. The product of this new transcript isn't a functional protein. Next we decide to quantify this alternative transcript by using one probe able to recognize both the transcripts and one probe spanning the exons 4 and 5 of Morgana and so able to recognize only the wild type form. We observed that unlike the group of healthy donors all the aCML where Morgana wild type is low have an high presence of alternative transcript. Surprisingly in the little percentage of aCML with normal Morgana level the alternative splicing is not significantly expressed. The generation of this AS colud be the mechanism responsible of Morgana down-expression in aCML; in fact all the patients with high alternative trascript shown very low level in Morgana protein, analysed by IHC. All these data where confirmed by exome sequencing, where the alternative transcript is observed on a large portion of aCML while in 10 AML tested it is not detectable, suggesting the specificity of this type of regulation in this myeloproliferative neoplasm
Summary and Conclusions: In this preliminary work, we identify one Morgana AS responsible of aCML reduced Morgana expression. This could be a consequence of mutations of those genes that are involved in the regulation of the spliceosome even in the absence of spliceosome gene mutations, alternative splicing of specific genes has also been reported with important biological consequences and potential therapeutic opportunity.

E1325

MUTATION ANALYSIS OF JAK2, MPL, CALR, ASXL1, TET2, IDH1, IDH2, DNMT3A, AND SF3B1 IN MYELOPROLIFERATIVE NEOPLASM

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Background: Myeloproliferative neoplasms (MPNs) are blood diseases characterized by clonal hematopoiesis, chronic excessive production of differentiated blood cells and increased risk for thrombosis and secondary leukemic transformation, which included polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF). JAK2 or MPL mutations are found in 50-70% of patients with PMF or ET, whereas calreticulin (CALR) mutations account for the majority of the remaining cases. However, MPN patients often showed different clinical phenotype despite having the same mutation, and other somatic mutations have been identified in some patients with MPN.
Aims: We evaluated incidence and clinical implication of several frequent mutations in Korean MPN patients, including *JAK2 V617F*, *JAK2* exon 12, *MPL, CALR*, *ASXL1*, *TET2*, *IDH1*, *IDH2*, *DNMT3A*, and *SF3B1*.
Methods: We enrolled 87 Philadelphia negative MPN patients whose archived peripheral blood or bone marrow sample collected at the time of diagnosis or first referral were available. Mutations were analyzed by direct sequencing and clinical data was collected retrospectively.
Results: We recruited 21 (25.8%) PV, 54 (59.6%) ET, and 12 (13.5%) PMF patients. *JAK2 V617F* was the most frequently detected mutation accounting for 69.0% (n=60) of MPN; 81.0% (n=17) of PV, 66.7% (n=36) of ET, and 58.6% (n=7) of PMF patients (Figure 1). *JAK2* exon12 mutation was detected in 1 PV patient. *CALR* mutations were found in 30.8% (n=8) of JAK2 mutation negative MPN. It was not observed in PV, but one ET patient had *CALR* and *JAK2 V617F* mutation concurrently. Mutations in *ASXL1, TET2*, *DNMT3A*, and *SF3B1* were also found in 9 (10.3%), 8 (9.2%), 4 (4.6%), and 1 (1.1%) MPN patients, respectively, and these mutations were found exclusively with *CALR* mutation. No *MPL, IDH1* nor *IDH2* mutation was detected in this cohort. *JAK2 V617F* mutation was associated with the absence of symptom, high leukocyte count, and low erythropoietin level with statistical significance. Patients with *CALR* mutation had lower leukocyte count and hemoglobin level, and higher platelet count than patients who had *JAK2 V617F* mutation only, or had neither *CALR* nor *JAK2 V617F* mutation. *JAK2 V617F* and *CALR* mutation did not affect overall survival significantly. But patients with *JAK2 V617F* mutation and without *CALR* mutation seemed to have worse survival than patients without *JAK2 V617F* mutation or with *CALR* mutation.

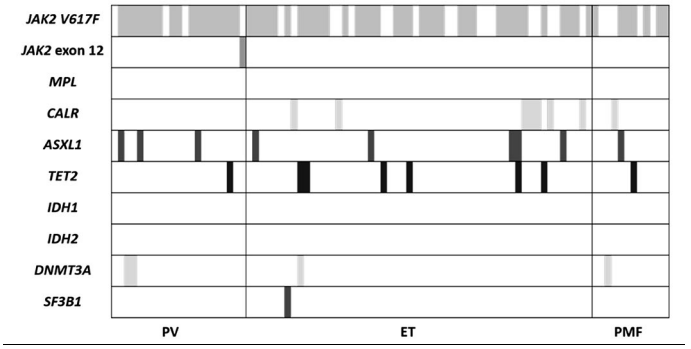

Figure 1. Mutational profile of 87 myeloproliferative disease.

Summary and Conclusions: It is first data of *JAK2 V617F*, *JAK2* exon 12, *MPL, CALR*, *ASXL1*, *TET2*, *IDH1*, *IDH2*, *DNMT3A*, and *SF3B1* mutation in Korean population. To validate of these clinical implication and prognostic value, extension of patient number and follow up period would be warranted.

E1326

MARKERS OF CLONAL HEMATOPOIESIS IN PH-NEGATIVE MYELOPROLIFERATIVE NEOPLASMS

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Background: In 2013 several researchers published data describing recurrent mutations-insertions and deletions-in 9th exon of *CALR* gene among patients (pts) with Philadelphia -negative myeloproliferative neoplasms (Ph(-)MPN). Mutations were not found in pts with polycythemia vera (PV). Frequency of *CALR* mutations in pts with essential thrombocythemia (ET) and primary myelofibrosis (PMF) is from 20 to 50%, respectively. Studies in mouse models showed JAK/STAT pathway activation in cells expressing mutated *CALR*. It has been observed that mutations in genes *CALR, JAK2, MPL* are mutually exclusive. Hence, mutations in 9th exon of *CALR* gene can be regarded as a new marker of clonal hematopoiesis like *JAK2* and *MPL* mutations. Recent retrospective studies have shown that *CALR*-ins/del burdening associated with milder disease and increased overall survival (OS) compared with cases *JAK2*(+) or *MPL*(+) Ph(-)MPN.
Aims: The goal of our study was to determine frequency of *JAK2, MPL, CALR* mutations in Ph(-)MPN pts and to analyze OS in groups with different clonal markers and triple-negative.
Methods: We examined 182 pts with Ph(-)MPN: 76 pts with PV, 63 pts with ET and 43 pts with PMF. The detection of V617F mutation of *JAK2* for each patient was done. *JAK2V617F*-negative pts with PV underwent the analysis of mutations in 12th exon of *JAK2* with direct sequencing. For *JAK2*V617F-negative pts with ET and PMF investigation of 9th exon of *CALR* was carried out through high resolution melting (HRM) followed by direct sequencing of mutated samples. *JAK2*(-) and *CALR*(-) samples were tested for *MPL 515* codon mutations with PCR-RFLP method. Karyotype research was done for patients with available bone marrow samples.
Results: *JAK2V617F* mutation was determined in 74/76 (97,3%) PV pts, in 26/63 (41,27%) ET pts and in 21/43 (48,83%) PMF pts. 2/76 (2,7%) pts with PV had mutations in 12th exon of *JAK2*. 515 codon mutations of *MPL* observed in 3/43 (6,97%) cases of PMF and in 3/63 (4,76%) cases of ET. Frequency of *CALR* mutations among pts with PMF and ET was 20,63% (13/63) and 13,95% (6/43), respectively. There was no reliable differences in OS in groups of pts with *JAK2*(+), *MPL*(+), *CALR*(+) and without mutations (p=0.127) (fig.1), but it should be mentioned that 4 of 6 pts, who died pts had unfavorable karyotype (+7, +8, del(5q), complex). It was not taken into account when determining OS. Interestingly, 3-year OS in *CALR*-mutated group was 100% while OS in *MPL*(+) and triple-negative group was 67% and 80%, respectively.

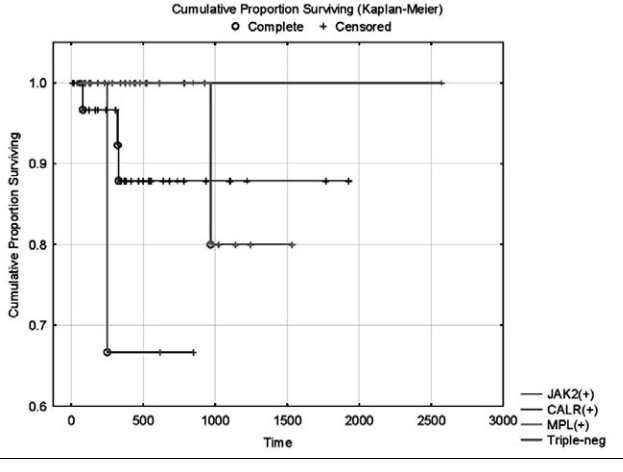

Figure 1.

Summary and Conclusions: *CALR* mutations are highly specific marker of clonal hematopoiesis along with *JAK2* and *MPL* mutations. However, genetic testing of clonal markers is insufficient. Only complex molecular genetic and cytogenetic analysis of tumor cells has crucial importance for diagnosis and prognosis of Ph(-)MPN.

E1327

JAK2V617F MUTATION IN COMBINATION WITH ASXL1, DNMT3A, TET2, U2AF1 AND RUNX1 VARIANTS IS ASSOCIATED WITH SEVERE CLINICAL PHENOTYPES IN PRIMARY MYELOFIBROSIS

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Background: The past decade has witnessed a significant progress in the understanding of the molecular pathogenesis of myeloproliferative neoplasms (MPN). A large number of genes have now been implicated but their relative importance, interactions and implications for prognosis remain unknown.
Aims: The aim of this study was to use a panel of mutations shown to be associated with MPN to determine their relative importance and to identify driver mutations and novel variants to elucidate the pathogenesis and predict survival in patients with MPN in a multiracial country.
Methods: We performed targeted sequencing on normal controls and patients with MPN from 3 different races (Malay, Chinese and Indian) in Malaysia who were diagnosed with polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF) according to the 2008 WHO diagnostic criteria for MPNs. Patients from 3 tertiary-level hospitals were recruited prospectively over 3 years and informed consents were obtained. DNA was extracted from peripheral blood myeloid cells at diagnosis and high-throughput sequenced using an Ion Torrent PGM and AmpliSeq targeting platform, with a panel of 26 MPN-related genes.
Results: 22 patients (9 PV, 8 ET, 5 PMF) and 3 normal controls were recruited into the study. 9 of 22 (40.9%) patients were Malay men and the median age of the study population was 58y (range 26-75y). 19 of 22 (86.4%) patients harbored the $JAK2^{V617F}$ mutation which was detected in 9 (100%) PV, 5 (62.5%) ET and 5 (100%) PMF patients; 7 of the 19 $JAK2^{V617F}$ positive patients (2 PV, 2 ET and 3 PMF) also harbored potentially pathogenic variants involving the *ASXL1, DNMT3A*, *TET2*, *U2AF1*, and *RUNX1* genes. 2 of the 3 $JAK2^{V617F}$ negative ET patients had *MPL* exon 10 and *TET2* mutations. Several novel variants involving the *DNMT3A, TET2, RUNX1, SH2B3* and *CHEK2* genes were identified in the study. The 3 PMF patients with $JAK2^{V617F}$ mutation in combination with *ASXL1, DNMT3A*, *TET2, U2AF1* and *RUNX1* variants presented with severe clinical phenotypes, with marked anaemia, elevated leucocyte counts, hyperferritinaemia, massive splenomegaly of >15cm, hepatomegaly and debilitating constitutional symptoms. DIPSS-plus score was intermediate-2 for 1 Chinese patient and high for 2 Malay patients who both died within 2 years from diagnosis. None of these 3 patients developed thrombosis. However, 5 patients (3 PV and 2 ET) in the study who developed thrombosis all had the $JAK2^{V617F}$ mutation only. 3 ET patients developed bleeding. 2 of these patients had $JAK2^{V617F}$ mutation alone and 1 patient had a combination of *MPL exon 10* and a *TET2* variant.
Summary and Conclusions: MPN in Malaysia is found predominantly in older Malay men with a higher incidence of $JAK2^{V617F}$ positivity in the different subtypes when compared to other studies. PMF patients who harbor the $JAK2^{V617F}$ in combination with *ASXL1, DNMT3A, TET2, U2AF1* and *RUNX1* putatively pathogenic variants are found to have more severe clinical phenotypes with very poor prognosis. Further studies are required to explore this finding and the novel variants found in this study further.

E1328

GATA-1, BUT NOT FOG-1, FLI-1 OR CALR, IS UP-REGULATED IN ESSENTIAL THROMBOCYTHEMIA INDEPENDENTLY FROM JAK2 AND CALR MUTATIONS

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Background: GATA-1 is the founding member of the GATA transcription factor family and it is essential for cell maturation and differentiation within the erythroid and megakaryocytic lineages. We and others have demonstrated that elevated GATA-1 expression is found in the bone marrow of Essential Thrombocythaemia (ET) patients, independent of JAK2V617F and CALR mutations. GATA-1 is able to coordinate lineage specification through its ability to bind both DNA and protein partners that include; Friend of GATA (FOG-1) and the Friend leukaemia integration 1 (FLI-1) transcription factors. FOG□1 is vital for megakaryocyte and erythroid□lineage commitment and its expression largely overlaps spatiotemporally with that of GATA-1. FLI-1 is an ETS family member that is expressed at high levels in megakaryocytic progenitors. In conjunction with GATA-1, FLI-1 targets those genes responsible for megakaryopoiesis. Calreticulin (CALR) is a calcium-binding protein involved in signaling and protein expression that is believed to be responsible for clearing misfolded proteins and involved in expression regulation. CALR mutations reported in myeloproliferative neoplasms create translation frameshifts in exon 9 which truncate the C-terminal calcium binding domain and create a novel C-terminal peptide. Initial

reports support CALR mutations as early and disease-initiating mutations that favor expansion of the megakaryocytic lineage. CALR mutations are mutually exclusive with JAK2 or MPL mutations.
Aims: We wanted to study the expression of GATA-1 in peripheral blood (PB) of patients with ET, together with FLI-1, FOG-1 and CALR, trying to identify if there is a common altered pathway and/or any correlation with JAK2 and CALR mutational status.
Methods: PB specimens were collected from 36 patients diagnosed with ET, 17 JAK2 mutated (47%), 4 CALR (11%) mutated, 1 MPL mutated (3%) and 14 with no molecular abnormalities, and compared with a cohort of healthy volunteers. Samples were enriched for the mononuclear fraction by Ficoll separation. Total RNA was extracted and analysed by Real Time PCR for GATA-1, FOG-1, FLI-1 and CALR expression relative to the housekeeping gene GAPDH using the $2^{-\Delta\Delta CT}$ method.
Results: We confirmed the data obtained in bone marrow demonstrating that GATA-1 is significantly up-regulated in ET patients also in PB and that GATA-1 overexpression is independent from JAK2V617F and CALR mutations. However, the transcription factors FOG-1 and FLI-1 do not appear to be subject to the same regulatory control in ET as that of GATA-1, remaining at the same level of expression as the controls. Interestingly we also found a significant downregulation in CALR mRNA comparing with controls and this is indipendent from CALR mutation as well.
Summary and Conclusions: These results suggest that GATA-1 is specifically deregulated in ET. GATA-1 overexpression is isolated and indipendent from its co-factor FOG-1 or FLI-1. Very interestingly CALR is downregulated in ET samples indipendently from CALR mutation or JAK2 matation, however more data are required to better understand the mechanisms of this deregulation.

Myeloproliferative neoplasms - Clinical

E1329

PEGYLATED INTERFERON TREATMENT IMPROVES SURVIVAL IN POLYCYTHEMIA VERA PATIENTS: A SINGLE CENTER EXPERIENCE

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Background: Polycythemia vera (PV) is a myeloproliferative disease with a reported median survival of 14-15 years (Tefferi *et al.*, 2014). The outcome is mainly affected by thrombo-embolic events and evolution to myelofibrosis (MF) and acute myeloid leukemia (AML).
Aims: The primary aim of the study was to evaluate the incidence of thrombosis, MF and AML according to prognostic factors and therapeutic strategies in a large series of PV patients. The secondary aim was to evaluate the outcome and the variation in JAK2 allele burden in the patients treated with pegylated interferon-alpha (peg-IFN-α).
Methods: Since 1995, 227 patients were consecutively diagnosed with PV at the "A.O.U. Città della Salute e della Scienza" hospital of Turin. Hydroxyurea (HU) was the first line treatment for the patients who required cytoreductive therapy. Since 2006, we treated with peg-IFN-α the younger patients (age<65 years) at high thrombotic risk or intolerant/resistant to phlebotomy. The use of alchilant agents was limited to the oldest patients who were refractory/resistant to HU.
Results: Median age at diagnosis was 66 years old and median follow-up was 8 years (13 to 244 months). Eleven% of patients presented with a thrombotic event at diagnosis and no correlation with the blood count was found. Sixty-nine% of patients were at high thrombotic risk for age or history of thrombosis, however only the 13.3% of the whole cohort had a major thrombotic event during follow-up. Major and minor thrombosis were statistically associated with age over 60 and thrombotic history (p=0.034) but not with other cardiovascular (CV) risk factors (hypertension, diabetes, smoking, hyperlipidemia) and the treatment. Overall survival (OS) was longer than what reported in other studies, with 90% and 58% of patients still alive at the median follow-up of 8 years and at the maximum follow-up of 17 years, respectively. Regarding treatment, 97% of patients received anti-platelet (89%) or anticoagulant agents (8%) and 86% of patients received cytoreductive therapy, 88% with HU. Twenty-four patients were treated with peg-IFN-α for at least 3 months (range 3-110) and their survival was longer than patients treated with HU (p=0.034) or with alkylating agents (p=0.0023) (100% alive at the last follow-up). Even if peg-IFN-α was available only from 2006, patients treated with peg-IFN-α had a median follow-up from diagnosis similar to patients treated with HU (86 *vs* 97 months). Of note, median age at diagnosis in the peg-IFN-α group was 53 and only 11% of the patients had previous thrombosis. However, at multivariate analysis the treatment with peg-IFN-alfa was confirmed to positively impact on survival, regardless of the age and the thrombotic history. Moreover, a reduction of JAK2 quantitative burden was observed in 58% of the patients treated with peg-IFN-α, an encouraging data considering that the median treatment duration was still relatively short (median 26 months). Overall peg-IFN-alfa was fairly tolerated, with a discontinuation rate of 25%, as described in other studies (Quintas-Cardama *et al.*, 2006). Fifteen% of patients developed MF, a slightly higher percentage than what reported by Tefferi *et al.* in 2014, but in line with data previously published (Cervantes *et al.*, 2008). The median MF-free survival was 17,8 years. AML evolution occurred in 5.7% of patients only.
Summary and Conclusions: Our study confirmed some known prognostic factors for thrombotic occurrence (age and thrombotic history) but no other CV risk factors, probably due to the positive role of the anti-platelet therapy.
Peg-IFN-α is a promising therapy in PV, not only to control symptoms, but also in a quantitative reduction of JAK2 mutation and OS improvement.

E1330

TERT RS2736100_C POLYMORPHISM AS PREDISPOSITION FACTOR FOR MYELOPROLIFERATIVE NEOPLASMS

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Background: The TERT rs2736100 single nucleotide polymorphism was found to be a susceptibility factor for a variety of cancers (lung, glioma, bladder), and recently for sporadic and familial myeloproliferative neoplasms.
Aims: The aim of our study was to investigate the role of TERT rs2736100 and JAK2 rs12343867 polymorphisms in a large cohort of Hungarian MPN

patients regarding their frequency and their effect on clinical characteristics.
Methods: LightCycler melting analysis was applied to identify the risk allele of TERT rs2736100_C and JAK2 rs12343867_C tagging 46/1 haplotype, in 209 polycythemia vera (JAK2 V617F positive PV), 281 essential thrombocytosis (ET), 94 primary myelofibrosis (PMF) patients and 400 healthy individuals.
Results: In the ET cohort 148 (53%) JAK2 V617F, 95 (34%) CALR and 9 (3%) MPL gene mutation positive and 29 (10%) triple negative cases; while in the PMF cohort 51 (54%) JAK2 V617F, 25 (27%) CALR and 7 (7%) MPL gene mutation positive and 11 (12%) triple negative cases were identified. Both genetic variants showed increased allele frequencies in MPN patients compared to controls (TERT rs2736100: 62.7±2.8% *vs* 48.8±3.5%, p<0.0001; OR [±95%CI]: 1.7 [1.5-2.1]; JAK2 rs1234386: 45.7±2.9% *vs* 29.8±3.2%, p<0.0001; OR [±95%CI]: 2.0 [1.6-2.4]. Beside the allelic model, the difference remained significant in all tested models (dominant, recessive, genotypic). Carriership of the TERT rs2736100_C allele (dominant model) increased MPN susceptibility equally in JAK2 V617F or CALR positive MPN patients (OR=2.8 [1.9-4.1] in JAK2 V617F positive, OR=2.3 [1.3-4.1] in CALR positive groups, p=0.5 for JAK2 V617F *vs* CALR positive groups comparison) as well as in PV, ET or PMF subgroups. In contrast, the effect of the JAK2 rs12343867_C allele was more pronounced in the JAK2 V617F positive group (OR=3.5 [2.6-4.8] in JAK2 positive, OR=1.6 [1.0-2.4] in the CALR positive groups, p=0<0.001 for JAK2 V617F *vs* CALR positive groups comparison). Combined hetero- or homozigosity conferred even higher risk for MPN (combined heterozigosity: OR: 6.0 [3.4-10.4], combined homozigosity: OR: 9.6 [4.4-21.1]). In the whole MPN cohort, TERT rs2736100_C carriers displayed higher white blood cell count (9 *vs* 11 G/L, p=0.019) compared to homozygous AA patients. The frequency of different complications (splenomegaly, venous and arterial thrombosis, myelofibrotic or leukemic transformation) were not associated with the investigated TERT polymorphism either alone or in combination with the JAK2 haplotype.
Summary and Conclusions: In the present study, in an independent large cohort, we confirmed that, TERT rs2736100_C polymorphism predisposes to the development of MPN regardless of the molecular background or disease type.

E1331

PATIENT SELF-CARE INTERVENTIONS: DO THEY IMPACT MPN-RELATED FATIGUE?

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Background: The Philadelphia chromosome negative myeloproliferative neoplasms include essential thrombocythemia (ET), polycythemia vera (PV), and myelofibrosis (MF). Fatigue is a prominent feature of these disease subtypes which leads to reduced quality of life and impaired daily functioning. We have previously evaluated the results of our initial phase I project evaluating fatigue among MPN patients (Emanuel et. al. Blood 2013;a1595). Among the 879 online respondents, patients most often employed exercise, diet, social interaction, nutrition, rest, and relaxation techniques to reduce their fatigue. Despite our previous efforts, little is known to date about the degree of relief obtained from these specific fatigue-alleviating strategies.
Aims: The aim of this study is to evaluate fatigue-alleviating strategies in MRF.
Methods: A 70-item internet-based survey was developed and hosted by the Mayo Clinic Survey Research Center. The survey was promoted online via multiple MPN-related webpages including the *MPN Forum*, *MPN Net*, *MPN Research Foundation*, and *MPN Voice* during late February to March of 2014. The MPN-SAF including the 10-item Brief Fatigue Inventory (BFI) was used to assess disease burden (Blood. 2011 Jul 14;118(2):401-8). Fatigue was characterized using a set of questions to evaluate timing, frequency, duration, triggers, and impact on daily activities. Patients were queried as to whether they had utilized individual interventions to alleviate fatigue and were asked to rate the success of each intervention on a 1 (not at all successful) to 5 (very successful) scale.
Results: Fatigue was prevalent and severe among survey respondents (BFI 24-hour worst fatigue item: score ≥1 95.3%, mean score 6.2 (SD=2.7)). Average BFI score was 4.4 (SD=2.4). In selecting one or more times of day when fatigue is most noticed, patients most often noticed their fatigue in the evening (44.0%) or afternoon (42.4%), compared to the morning (17.7%) or continuously throughout the day (23.4%). Fatigue triggers included physical work (58.8%), stress (48.5%), exercise (41.6%), medications (25.2%), intellectual work (24.3%), eating (16.9%) and sexual activity (6.6%). Approximately half of patients felt that their fatigue was "about the same" over the last 6 months (48.7%). However, large portion of patients felt that their fatigue had worsened (40.7%), with only a minority of patients feeling that their fatigue had improved (10.7%). Fatigue affected aspirations (64.5%), travel (61.0%), long term planning (58.3%), and vacation planning (57.2%). Most patients felt that fatigue limited their normal daily activities (69.1%). Numerous self-care strategies were implemented in order to reduce fatigue, including setting priorities, postponing essential activities, exercise, and naps. Of these interventions, patients most often felt that scheduling activities during peak energy times, pacing activities, labor saving devices, and setting priorities were most efficacious in reducing their fatigue (greater than 70% of patient attempting these strategies felt them to be "very" or "somewhat" successful). Moderate/severe fatigue (categorized as BFI score ≥4) was present more frequently in those who did not exercise as compared to those who reported exercising at least once per week [188/248 (75.8%) *vs* 671/1181 (56.8%); p<0.001]. BFI score as compared to exercise frequency per week is displayed in Figure 1. Exercise appears to have a dose impact, as each level of physical activity is associated with lower fatigue. All interventions except exercise and volunteer activities were attempted by a significantly higher proportion of patients with moderate/severe fatigue than those with none/mild fatigue level.

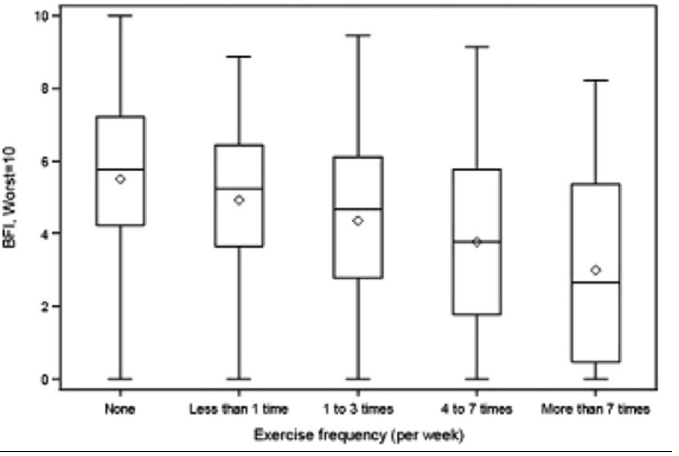

Figure 1. Box plot for BFI score by exercise frequency.

Summary and Conclusions: Overall patients with MPNs experience disabling fatigue that greatly lowers their quality of life. This is the first published summary evaluating the effectiveness of non-pharmacologic interventions utilized to alleviate fatigue among MPN patients. The unique role of exercise in alleviating MPN related fatigue needs further prospective investigation. We plan to use the results of this survey to inform an at-home interventional trial to investigate the impact of a variety of fatigue reduction techniques.

E1332

Abstract withdrawn

E1333

ULTRA-DEEP SEQUENCING (UDS) ALLOWS MORE SENSITIVE DETECTION OF THE D816V AND OTHER KIT GENE MUTATIONS IN SYSTEMIC MASTOCYTOSIS

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Background: According to the World Health Organization (WHO) classification, the diagnosis of Systemic Mastocytosis (SM) relies on bone marrow (BM) examination and is based on a major and four minor criteria. The somatic 'autoactivating' point mutation D816V in the KIT receptor gene is one of the minor criteria, founded in the great majority of patients (90%) and it plays a central role

in the pathogenesis of the disease. Indolent Systemic Mastocytosis (ISM) is the most common variant of SM, characterized by a very low MC burden and associated with very different clinical pictures. A highly sensitive diagnostic methods for D816V detection are required to assure an appropriate diagnosis and to reduce false-negative results. The recent development of "ultra-deep amplicon sequencing" (UDS) technologies has opened the way to a more accurate characterization of molecular aberrations with higher sensitivity of screening for known and unknown mutations.

Aims: Our aims were: i) to set-up and optimize a UDS-based mutation screening strategy of the KIT gene on the Roche GS Junior Instrument; ii) to test the sensitivity of our UDS assay to detect the D816V mutation; iii) to investigate the presence of additional KIT mutations in SM.

Methods: We decided to take advantage of a next generation sequencing approach to perform an UDS KIT gene mutation analysis on 24 bone marrow (BM) samples from patients whit ISM that were negative for the D816V mutation by Sanger Sequencing which has a sensitivity of 20%. Fusion primers were designed to generate ten partially overlapping amplicon covering the whole KIT transcript (exons 1-21) by RT-PCR. To determine the lower detection limit of our UDS-assay, serial dilutions of the HMC-1 cell line (harboring the D816V mutation) into an unmutated K562 cell line in ratios such as to simulate the following mutation loads were sequenced: 50%, 37.5%, 25%, 12.5%, 5%; 2.5%, 1.25%, 0.5%, 0.25%.

Results: UDS of cell line dilutions showed a high accuracy of D816V mutation detection and linearity of mutation calling over the entire range down to 0.25%. The UDS technology allowed to detected the D816V mutation, below the lower detection limit of Sanger Sequencing, with an abundance from 0.5% to 11%, in 15/24 ISM patients. Two additional sequence variations were detected in a large proportion of patients. These two variations included a 3bp in-frame deletion in exon 15 (GenBankx06182.1: c.2164_2166delAGC; p.S715del) found in 12/24 patients and a 12bp in frame-deletion in exon 9 in all patients, whit an abundance ranging from 83% to 97% (GenBankx06182.1: c.1550_1561delGTAACAACAAAG; p.G510_K513del). Previously published studies indicate that the KIT Gly-Asn-Asn-Lys$^{510\text{-}513+/-}$ alternatively spliced located immediately downstream to the extracellular KIT domain and KIT Ser$^{715+/-}$, an interkinase KIT domain, are expressed in normal human hematopoietic cell, leukemic cell lines, acute myeloid leukemia blast and GISTs and represent rather a splice variant of KIT transcript. Interestingly our results showed the presence of the transmembrane domain M541L (GenBankx06182.1: c.1642A>C; p.Met541Leu) KIT-activating mutation in exon 10, with an abundance of 50%, in addition to D816V, in 3/24 ISM. This mutation is known to retain sensitivity to imatinib mesylate (Figure 1).

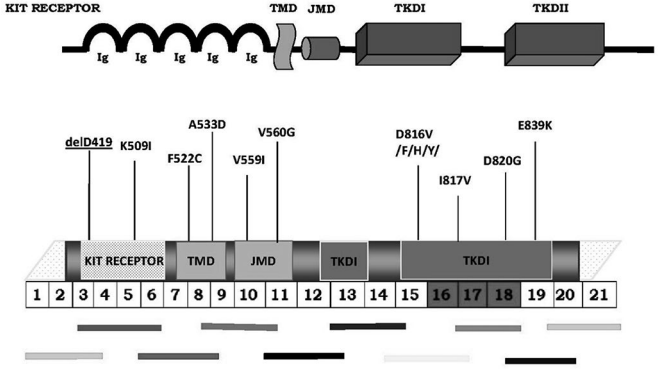

Figure 1.

Summary and Conclusions: Our preliminary results suggest that our-UDS based KIT gene mutation screening assay might be a reliable and sensitive alternative to conventional sequencing methods for the detection of the D816V. We are now planning to investigate whether the greater sensitivity of UDS allows to detect the D816V mutation in peripheral blood mononuclear cells from patients with a suspected clonal mast cell disorder. These results could represent a starting point to plan other extensive studies to better understand the exact role of KIT receptor alterations in SM. Supported by ELN, AIL, AIRC, PRIN, progetto Regione-Università 2010-12 (L. Bolondi), FP7 NGS-PTL project.

E1334

RUXOLITINIB *VS* BEST AVAILABLE THERAPY IN PATIENTS WITH POLYCYTHEMIA VERA TREATED IN THE RESPONSE STUDY: A SUBGROUP ANALYSIS OF HYDROXYUREA- AND NON-HYDROXYUREA-TREATED PATIENTS

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Background: Ruxolitinib (RUX) is a JAK1/JAK2 inhibitor that proved superior to best available therapy (BAT) in controlling hematocrit (HCT) and improving splenomegaly and symptoms in patients (pts) with polycythemia vera (PV) treated in the RESPONSE study. Because RESPONSE enrolled pts who had an inadequate response to or unacceptable side effects with hydroxyurea (HU), we performed a subgroup analysis to explore the efficacy of non-HU treatment options in the BAT arm.

Aims: An exploratory analysis to compare key efficacy parameters of RUX with HU and non-HU BAT in pts with PV who were resistant to or intolerant of HU by modified ELN criteria.

Methods: RESPONSE is an open-label phase 3 study; pts with splenomegaly (≥450 cm^3 by MRI) who had HU-resistant disease or HU intolerance by modified ELN criteria were randomized 1:1 to RUX 10 mg bid or BAT. BAT included single-agent HU, interferon, anagrelide, immunomodulators, pipobroman, or no medication. HU was chosen based on the treating investigators' judgment that these patients could derive some benefit in the absence of more suitable alternative treatments. All pts received low-dose daily aspirin. The primary composite endpoint was the proportion of pts who achieved both HCT control without phlebotomy (PBT) from wk 8 to 32 (with ≤1 PBT postrandomization and prior to wk 8) and a ≥35% reduction in spleen volume from baseline at wk 32 by MRI. The proportion of pts who achieved a complete hematologic response (CHR) at wk 32 was a key secondary endpoint. Symptoms were assessed using the Myeloproliferative Neoplasm Symptom Assessment Form (MPN-SAF). Pts in the BAT arm could cross over to RUX starting at wk 32 if they had disease progression or did not achieve the primary endpoint. The primary analysis occurred when all pts reached wk 48 or discontinued.

Results: Overall, 110 pts were randomized to RUX and 112 to BAT. In the BAT arm, 66 pts received HU as their initial treatment, and 45 pts received non-HU treatment as their initial BAT (interferon, n=13; anagrelide, n=8; immunomodulators, n=5; pipobroman, n=2; no medication, n=17); 1 BAT pt did not receive study drug and was excluded from this analysis. Six pts changed BAT. Non-HU treatment pts were pooled for analysis because few pts received any individual treatment option as their initial BAT. Demographic characteristics were generally well balanced across groups. The primary composite endpoint was achieved in 1.5% of HU and 21% of RUX pts; no non-HU BAT pts achieved a primary response. Larger proportions of pts receiving RUX achieved spleen volume reductions, HCT control, CHR, and symptom responses than pts receiving HU- or non-HU BAT (Table 1). The proportion of pts who had PBT from wk 8 to 32 was substantially lower in the RUX arm than among pts receiving HU or non-HU treatments in the BAT arm.

Table 1. Efficacy in patients who received RUX, BAT, HU BAT, and non-UH BAT.

n (%)	RUX (n=110)	BAT[a] (n=112)	HU BAT (n=66)	Non-HU BAT (n=45)
Primary response at week 32	23 (21)	1 (1)	1 (2)	0
Spleen volume reduction ≥35%	42 (38)	1 (1)	1 (2)	0
HCT control	66 (60)	22 (20)	15 (23)	7 (16)
CHR at week 32	26 (24)	10 (9)	7 (11)	3 (7)
Phlebotomies from week 8 to 32	(n=106)	(n=109)	(n=65)	(n=44)
≥1	21 (20)	68 (62)	41 (63)	27 (61)
≥3	3 (3)	22 (20)	10 (15)	12 (27)
Symptom response[b]	(n=74)	(n=81)	(n=49)	(n=32)
≥50% reduction in MPN-SAF total symptom score at week 32	36 (49)	4 (5)	2 (4)	2 (6)

[a] 1 patient was randomized but did not receive study drug.
[b] In patients with scores at both baseline and week 32.

Summary and Conclusions: In the phase 3 RESPONSE study in pts with PV who had an inadequate response to or unacceptable side effects from HU, RUX treatment is effective in controlling HCT without PBT, decreasing spleen volume, and improving PV-related symptoms when compared with HU and non-HU standard treatment options.

E1335

RISK OF PREGNANCY COMPLICATIONS AND EFFECT OF DIFFERENT TREATMENTS IN WOMEN WITH ESSENTIAL THROMBOCYTHEMIA: A RETROSPECTIVE MONOCENTER ANALYSIS OF 62 PREGNANCIES.

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Background: A high rate of obstetric complications occurs in women with

essential thrombocythemia (ET), with a consistent high risk of early fetal loss and a probability of live births around 60%. A variety of therapeutic strategies has been proposed, with a risk-driven intensity of treatment. The role of the JAK2 V617 mutation as predictor of pregnancy complications and possible therapeutic driver is controversial.

Aims: To assess in a retrospective cohort of ET patients the JAK2 V617F-related risk of pregnancy complications and the efficacy of different treatments during antepartum and postpartum periods.

Methods: We analysed 62 pregnancies occurred in 38 women with ET (median 2 pregnancies per woman, range 1-3) during the years 2001-2014. The median age of the patients at diagnosis was 29 years (range 18-41); the age at conception was >35 years in 33 pregnancies (53%). Nineteen women (50%) carried the JAK2V617F mutation. Two of them had hepatic vein thrombosis and TIA before first conception, respectively. One terminated pregnancy, one blighted ovum and one miscarriage due to Turner syndrome were excluded from further analysis. Antepartum treatment consisted of low molecular weight (LMWH)+aspirin (ASA) in 32 pregnancies, ASA in 14, LMWH in 6; seven pregnancies were left untreated. Interferon was administered during 9 pregnancies. The impact of different antepartum therapeutic strategies was estimated by a multivariate proportional hazards regression model over the weeks of gestation. Puerperium periods were defined as 6 weeks after delivery at ≥20 week of gestation and were treated with LMWH in 45 of 51 cases.

Results: Among the evaluable pregnancies the rate of live births was 83% (49/59); miscarriage occurred in 8, stillbirth in 2, abruptio placentae with neonatal death in 1, intrauterine fetal growth retardation in 6. Overall, 17 obstetric complications (OC) were considered ET-related (17/59, 29%) and occurred in 7 cases during LMWH+ASA (22%), in 2 during ASA (14%), 3 during LMWH (50%), and 5 in the untreated pregnancies (71%). No thrombosis complicated antepartum-periods. Antithrombotic treatment reduced by 88% the risk of OC in comparison *versus* untreated pregnancies (odds ratio, OR 0.12, 95%CI 0.02-0.69). The rate of OC was 43% in pregnancies of JAK2 V617F-positive women (13/30) and 14% in the pregnancies of the JAK2 V617F-negative women (4/29) (OR 4.77, 95%CI 1.33-17.18); however after exclusion of the untreated pregnancies the risk associated with the JAK2 V617F mutation was no more significant (OR 3.33, 95%CI 0.85-13.00). A multivariate proportional hazards regression model including age >35 yrs, JAK2 V617F mutation, and antepartum ASA, LMWH, and interferon, retained only ASA as a variable associated with the outcome (OR for complications 0.28, 95%CI 0.10-0.80, p=0.01). Among the 6 untreated puerperium periods, one was complicated by cerebral vein thrombosis (17%), whereas no thrombosis occurred during the remaining puerperium periods treated with LMWH.

Summary and Conclusions: In ET patients JAK2 V617F is associated with an increased risk of OC, which was prevented by treatment; namely, in this cohort antepartum ASA was highly effective in preventing ET-related OC; therefore, LMWH should be reserved only to women with additional risk factors for venous thromboembolism independent of ET. The rate of puerperium-related venous thrombosis is high and prompts LMWH prophylaxis.

E1336

ANALYSIS OF PHENOTYPE AND OUTCOME IN ESSENTIAL THROMBOCYTHEMIA WITH CALR AND JAK2 MUTATIONS

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Background: The *JAK2* V617F mutation, the thrombopoietin receptor mutation *MPL* W515K/L and the Calreticulin (*CALR*) mutations are mutually exclusive in essential thrombocythemia (ET) and support a novel molecular categorization of ET. The most recently discovered *CALR* mutations account for up to 30% of ET cases.

Aims: In a retrospective study, we have examined the clinical phenotype and outcome in a Belgian cohort of 165 ET patients in relation to their mutational status.

Methods: A cohort of 107 *JAK2* V617F negative ET cases diagnosed at several Belgian hospitals was collected and analyzed for *CALR* and *MPL* mutations. We also collected a control cohort of 58 *JAK2* V617F positive ET diagnosed in the University Hospitals Leuven and sequenced for *CALR* mutations. The median follow-up of the whole cohort of 165 patients was 8 years with a range of 1-34 years.

Results: 62 (65.3%) *JAK2* V617F/*MPL* W515K/L negative patients were carrying an indel in *CALR*, including 37 cases (59.6%) of Type 1 (c.1092_1143del) and 19 cases (30.6%) of Type 2 (c.1154_1155insTTGTC) indels. We have also identified one ET patient positive for both *CALR* and *JAK2* V617F mutations. We compared the hematological and clinical features between *CALR* +ve patients and *JAK2* V617F +ve patients. This revealed that *CALR* mutations are associated with younger age, male gender, higher platelet counts, lower leukocyte counts, lower erythrocyte counts, hemoglobin and hematocrit ratio. Dividing the *CALR* +ve group into Type 1 and Type 2 showed that *CALR* Type 1 was associated with male gender and higher platelets compared to *JAK2* V617F +ve patients. *CALR* mutant patients had a better overall survival than the *JAK2* V617F positive patients. However, in the group of patients ≤60 years old *CALR* +ve group had a better overall survival over *JAK2* V617F +ve group. Nonetheless, the group of patients older than 60 years showed no difference in the overall survival between the *CALR* +ve group and the *JAK2* V617F +ve group. In our cohort, *CALR* +ve patients present a higher risk of progressing to MF, but no difference in myelofibrosis-free survival or leukemia-free survival was found (Figure 1).

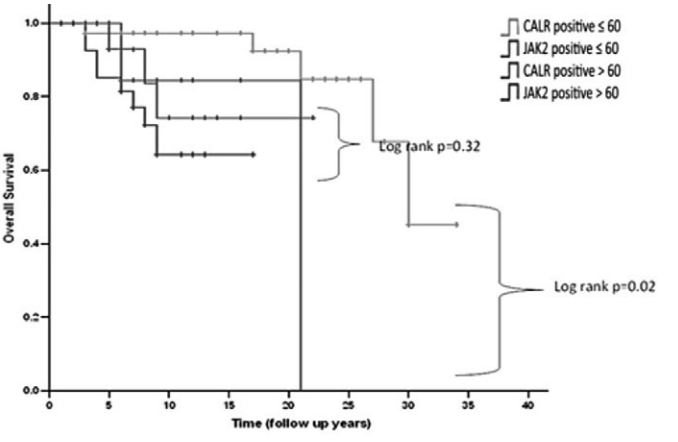

Figure 1.

Summary and Conclusions: Our study confirms that *CALR* +ve ET is phenotypically distinct from *JAK2* V617F +ve ET, with differences in the clinical presentation and the disease course. *CALR* was also associated with better overall survival, restricted to ET patients less than 60 years old. Our study extends the growing body of evidence that *CALR* mutated ET has a distinct phenotypic and hematological profile compared to the *JAK2* V617F +ve group and corroborates the observations from other similar studies.

E1337

MUTATIONAL STATUS AND CARDIOVASCULAR COMPLICATIONS IN PATIENTS WITH STRICTLY WHO 2008-DIAGNOSED ESSENTIAL THROMBOCYTHEMIA: A MONOCENTRIC STUDY

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Background: Patients with essential thrombocythemia (ET) may carry JAK2, MPL, or a calreticulin gene (CALR) mutation. The somatic mutations in CALR are mutually exclusive with JAK2 and MPL mutations and is the second most mutated gene in ET. Mutant CALR is a result of frameshift mutations, caused by exon 9 deletions (type-1) or insertions (type 2). About 10% of ET patients are still now triple negative (3NEG) for these mutations.

Aims: In the current study, we looked for phenotypic or prognostic differences between ET patients stratified on the basis of their mutational status, the IPSET-thrombosis (IPSET-T) score.

Methods: The study involved 183 patients (median follow-up 10.3 years [range 0.2-31]) with a diagnosis of ET strictly fulfilling the 2008 WHO criteria. All these patients are regularly followed twice a year at our Department. The study was approved by the institutional Ethics Committee of Padua Hospital. In agreement with the IPSET-T score, we assessed our patients' thrombotic risk based on: patient's age at diagnosis; cardiovascular (CV) risk factors; prothrombotic conditions and mutational status. Real-time quantitative PCR for JAK2 and direct sequencing for MPL and CALR mutations were used. Cox proportional hazard regression model was used for multivariable analysis. MPL-mutated patients were not considered in the statistical analysis.

Results: Patients stratified by molecular status, IPSET-T score and cardiovascular events during follow-up are summarized in Table 1. The rate of thrombosis during follow-up was 0.5% pats/year for low-risk patients, 2.3% for intermediate-risk cases, and 5.1% for the high-risk group. After stratifying patients by mutational status, the thrombosis rate was 4.2%, 1.6% and 0.7% patients/year, respectively, for JAK2V67F, CALR (1.6% pats/y for CALR-1 and 1.5% pats/y for CALR-2) and 3NEG patients. In multivariable analysis adjusted for sex and age at diagnosis the presence of JAK2V617F mutation maintains a negative prognostic impact in predicting thrombosis both when compared with CALR mutations (HR 2.6; 95% CI 1.2-5.5; p=0.013) and when compared with triple negative (HR 5.3; 95% CI 1.3-22.5; p=0.022); no differences were demonstrated comparing CALR and 3NEG patients. The patients distribution in agreement with IPSET-t score was similar in CALR type 1, type

2 and 3NEG patients. Hemorrhages occurrence was similar in all mutational groups.

	JAK2	*MPL*	*CALR type 1*	*CALR type 2*	3NEG
N (%)	114 (62.3%)	3 (1.6%)	25 (13.6%)	19 (10.4%)	22 (12%)
Mean age at diagnosis (y)	53.9 ± 16.1	62.6 ± 6.3	50.8 ± 16.2	51.4±14.9	41.2 ± 16.2
Mean WBC at diagnosis (x10^9/L)	8.7 ± 2.4	7.3 ± 0.1	7.7 ± 2.1	7.5 ± 4.1	7.9 ± 2.9
Mean hemoglobin at diagnosis (g/L)	140 ± 15	135 ± 11	130 ± 13	133 ± 13	133 ± 14
Mean platelets at diagnosis (x10^9/L)	706 ± 226	726 ± 234	830 ± 205	963 ± 474	751 ± 236
IPSET-T score					
low-risk	0 (0%)	1 (33.3%)	18 (72%)	17 (89%)	18 (81%)
intermediate risk	26 (22.8%)	1 (33.3%)	3 (12%)	2 (22%)	3 (13.6%)
high risk	88 (77.2%)	1 (33.3%)	4 (16%)	0 (0%)	1 (4.5%)
Patients with at least a thrombotic event (%)	47 (41.2%)	1 (33.3%)	5 (20%)	3 (15.8%)	2 (0.9%)
Patients with at least a bleeding event (%)	21 (18.4%)	0 (0%)	6 (24%)	4 (21.1%)	3 (13.6%)

Table 1.

Summary and Conclusions: It is known that ET patients carrying JAK2 mutation have a higher thrombotic risk compared to other ET having higher hemoglobin and WBC counts. CALR mutated patients, in contrast, are associated with higher platelet and lower hemoglobin and WBC counts. In our cohort, *CALR* mutations as well as the absence of any known mutation (3NEG) segregate with factors associated with a lower thrombotic risk. Interestingly, all CALR type 2 and 84% of CALR type 1 have a low-intermediate IPSET-T score and have a congruously lower rate of thrombosis during follow-up. Therefore, *CALR* mutation does not have a negative impact on thrombotic risk. We did not obtain a significant difference in hemorrhagic events occurrence. Our data provide evidence that evaluation of JAK2, MPL, and CALR mutation status is not only important for diagnosis but also for the evaluation of thrombotic risk.

E1338

CLINICAL SIGNIFICANCE OF CIRCULATING MICROPARTICLES IN PH-MYELOPROLIFERATIVE NEOPLASMS (MPN)

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Background: Microparticles (MPs) are small membrane vesicles that are classified a as follow: platelet-derived MPs (PMPs), endothelial MPs (EMPs), red blood cell MPs (RMPs) and tissue factor MPs (TF^+MPs) based on their origins. Philadelphia chromosome-negative myeloproliferative neoplasms (Ph^-MPN) are disorders characterized by abnormal hematopoiesis, thrombosis and JAK2V617F mutation. Although MPs are considered as biomarkers reflecting procoagulant state in cancer patients, whether they exist in the patients with Ph^-MPN remains unclear.
Aims: Our objective in this study was to measure the variation of the four types of MPs in the patients with MPN and to Figure out their correlations with JAK2V617F mutation and some clinical complications, especially thrombosis and splenomegaly.
Methods: Ninty-two patients with MPN were enrolled in this study, including 60 essential thrombocythemia (ET), 20 polycythaemia vera (PV), and 12 primary myelofibrosis (PMF). 30 healthy volunteers were selected as normal controls. Venous blood wasanticoagulated with sodium citrate (1:9). Plasma samples were measured by flow cytometry for RMPs, PMPs, TF+MPs and EMPs with phycoerythrin (PE)-conjugated monoclonal antibodies CD235a, CD61, CD142, and CD62E, respectively. Forward scatter was set in scale using fluorescent microspheres of 0.8μm and standard fluorescent microbeads (0~0.8μm) in diameter were used to set the microparticle gate. Data were presented as mean and standard deviation. Meanwhile, genomic DNA was extracted from mononuclear cells and amplified by allele specific polymerase chain reaction (PCR).
Results: (1) Levels of RMPs, PMPs, EMPs and TF^+MPs in patients with Ph^-MPN was(135.2±291.60)/μl, (960.7±1539.1)/μl, (808.8±1244.5)/μl and (103.2±303.6)/μlrespectively; they were all significantly higher than control group (P<0.05). Moreover, levels of all four types of MPs in PMF group demonstrate significantly higher than PV group (P<0.05), and RMPs in PMF group was significantly higher than ET group (P<0.05).(2) Ph^-MPN patients with thrombosis complication showed higher levels of all four types of MPs than those without thrombosis complication(P<0.01). (3) Ph^-MPN patients with splenomegaly showed higher levels of all four types of MPs than those without splenomegaly (P<0.01). (4) All four types of MPs in the JAK2V617F mutation group were higher than the group in which those patients without mutation (P<0.05).
Summary and Conclusions: Ph^-MPN patients reveals higher levels of all four types of MPs than normal controls, especially in patients complicated with thrombosis or splenomegaly. MPs in PMF patients possess more obvious increase than PV and ET groups. Patients with JAK2V617F mutation showed higher levels of the four types of MPs than that without JAK2V617F mutation. Consequently, MPs play an crucial role in the pathogenesis of Ph^-MPN, and the MPs released may promote the formation of thrombosis.

E1339

MATRIX METALLOPROTEINASE 2,3,9,10 AND 13 GENE MUTATIONS IN MYELOPROLIFERATIVE DISEASES

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Background: Clonal myeloid disorders develop as a result of an acquired mutation in the multipotent stem cells or early precursor cells. Matrix metalloproteinases (MMP) are proteolytic enzymes that play role in arrangement of tissues, morphogenesis, wound healing and maturation. Additionally, they are consigned in pathological pathway of tumor invasion, angiogenesis and metastasis. The genetic polymorphisms may influence the functions of these enzymes.
Aims: In this study the association of myeloproliferative diseases (MPD) with polymorphisms in MMP2, MMP3, MMP9, MMP10 and MMP13genes were investigated.We aimed to show is there any relationship with MMPs and disease prognosis and fibrosis of bone marrow.
Methods: Fiftyseven patients and 22 control cases were included in the study. The study group was composed of 28 patients with polycythemia vera (PV), 12 patients with essential thrombocytosis (ET) and 17 patients with secondary polycythemia (SP) patients. The ET and PV patients were diagnosed according to 2008 WHO diagnostic criteria and they were either receiving medical therapy and/or phlebotomy. DNA isolation was done from peripheral blood samples by PureLink TM Genomic DNA Kit. MMP2 gene-735 C>T (rs2285053), MMP3 gene -1612 5A → 6A (rs35068180), MMP9 gene Gln279Arg (rs17576 G>A), MMP10 gene +180G>A and MMP13 gene -77A>G polymorphisms were investigated. Polymerase chain reaction products of the investigated gene region were observed in%3 agarose gel. Genotype was determined by DNA sequencing after each polymorphism was recognized.
Results: The ratios of heterozygote patients for MMP2 were 39.3%in PV, 47.1% in SP, 41.7% in ET and 27.3% in control groups. Similarly, the ratios of heterozygote patients for MMP3 were 38.3% in PV, 47.1% in SP, 33.3% in ET and 68.2% in control groups, respectively. There was not any significant difference between groups regarding MMP2 and MMP3 mutations. The MMP9 polymorphism was most commonly reported in ET group (50%) while there was not any MMP9 polymorphism reported in the control group (p=0.001). There was not any statistically significant difference between groups regarding the MMP10 polymorphisms. Heterozygote patients for MMP13 were determined as 71.4% in PV, 70.6% in SP, 75.0% in ET and 50.0% in control groups. Heterozygote MMP13 patients were statistically significantly more common in PV and ET groups than the control group (p=0.001).
Summary and Conclusions: We have determined that MMP9 gene Gln279Arg and MMP13 gene -77A>G polymorphysms were associated with ET while MMP13 -77A>G polymorphysms were associated with PV. The MMP gene polymorphysims were not associated with SP development. Increased number of megakaryocytes and increased thrombocyte outflow in ET patients may be associated with increased MMP9 polymorphisms. On the other hand, MMP13 polymorphisms may be associated with the acceleration of bone marrow fibrosis. Further studies are warranted to determine the association of MMP9 and MMP13 polymorphisms in patients with MPD.

E1340

KARYOTYPE OF CIRCULATING PROGENITOR CELLS OF MYELOID LINEAGE COULD BE RELATED TO CLINICAL COURSE OF MYELOFIBROSIS

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Background: Granulocyte colony stimulating factor (G-CSF) is known to stimulate mitosis of myeloid progenitors as well as non-committed stem cells that could harbour hidden mutations and give rise to new pathological clones in myelofibrosis (MF), especially leukemic. The level of circulating stem cells is elevated during the disease progression. However, the total level of these cells is low in peripheral blood (PB), and most of them don't proliferate without specific stimulation. Unfavorable karyotype of bone marrow (BM) is negative prognostic feature of the pathological process in MF. Karyotype peculiarities are revealed only in dividing cells using conventional cytogenetic methods, but not in stem cells, and the information obtained during the investigation is limited to BM, whereas the pathological cells are also presented in blood, spleen and other organs. A lot of driver point mutations with impact on total survival of the patients with MF are revealed. For instance, mutation as *CALR* gene is related to favorable prognosis, and mutation of *ASXL1* gene is related

to poor outcome of the disease. However, there are no data confirming correlation of unfavorable karyotype with mutation of *ASXL1* or other genes in MF.
Aims: The aim of the study was to examine cytogenetic properties of myeloid progenitor cells, circulating in the peripheral blood of patients with myelofibrosis.
Methods: The study group consisted of 34 patients with confirmed diagnosis of both idiopathic, post-polycythemia vera, and post-essential thrombocythemia MF. The PB was cultivated for 24 h with G-CSF in order to obtain mitoses of circulating stem cells and to stimulate divisions of blast cells and promyelocytes. Control analysis were performed in non-stimulated BM using standard approaches. G-method of differential staining was used for cytogenetic studies of both PB and BM.
Results: Cytogenetic abnormalities were revealed in 47% of patients. The spectrum of the most common anomalies obtained in this study included deletions and translocations of chromosome 1, deletions of 5q and 20q, trisomies of chromosomes 3, 8, 9, 12, monosomies of chromosomes 3, 5, 7, 9, 11, 13, 15, 17, and polyploidy. Karyotype of G-CSF-stimulated PB matched the BM karyotype in most patients. Although there were some differences in proportions between abnormal clones in the cases of mosaic karyotypes. Polyploidy was more characteristic for the BM and was observed in 30% of cases that included single polyploid metaphases, whereas incidence of polyploidy in the PB was 6%. Most of the polyploidy metaphases were presented as tetraploid ones, however, near-tetraploid, near-triploid and octaploid were also revealed in some cases. The study of G-CSF-stimulated blood was successful for the majority of patients in whom cytogenetic studies of BM failed. High mitotic index according to cytogenetic analysis of PB with concomitant chromosome abnormalities were accompanied by IPSS score >=2 and DIPSS score >=3 for other clinical indicators. If BM aspiration for cytogenetic analysis was unsuccessful, but cytogenetic abnormalities were found in the study of PB, the disease course was unfavorable, because of appearance of transfusion dependency, significantly increased leukocytosis with increasing changes in leukogram, or even transformation to acute leukemia and death. The follow-up within 1 year showed that the cytogenetic changes in PB in some cases were preserved or further evolved with the appearance of additional chromosomal abnormalities and worsening of the patient's condition.
Summary and Conclusions: Cytogenetic studies of PB in MF using *in vitro* stimulation with G-CSF could complement cytogenetic analysis of BM and determination of the mutation status. The spectrum of anomalies obtained during cytogenetic analysis of both G-CSF-stimulated PB and BM aspirate are very similar. Therefore, the cytogenetic analysis of an *in vitro* G-CSF-stimulated PB may be an acceptable alternative to repeated BM punctures in cases of significant fibrosis that makes BM difficult or impossible to aspirate. Cytogenetic abnormalities in the blood of patients in our group were associated with unfavorable course of MF. Collection of PB for cytogenetic analysis in dynamics may become potential additional criterion for monitoring the effectiveness of treatment and the disease progression. The absence of need to repeat BM puncture is an additional factor that could contribute to greater adherence of the patients to diagnostic and treatment procedures. Regular monitoring of cytogenetic changes could help searching for chromosomal loci with candidate genes involved in the disease progression.

E1341

IPSET-THROMBOSIS BETTER IDENTIFIES THROMBOSIS FREE SURVIVAL

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Background: Essential thrombocythemia (ET) is the most common among the myeloproliferative neoplasms (MPNs). For better predicting the occurrence of thrombotic events, an International Prognostic Score of Thrombosis for ET (IPSET-Thrombosis) was recently developed.
Aims: We aimed to investigate the validity of IPSET-Thrombosis in a Turkish patient cohort, and to compare the efficacy of IPSET-Thrombosis and conventional risk scoring systems in predicting thrombosis free survival.
Methods: We retrospectively evaluated the clinical characteristics and risk factors for thrombosis in 112 Turkish patients. Median thrombosis free survival and Harrell C-Concordance indexes were calculated for both conventional and IPSET-Thrombosis.
Results: Median age of 112 patients included in the study was 61 (27-90) years at the time of diagnosis. When patients were stratified according to the conventional risk stratification system, 43.8 % of patients were in low risk and 56.2% were in high risk group. 22.4% of low risk and 42.9% of high risk patients had an at least one occasion of thromboembolic event. When patients were stratified according to the IPSET-Thrombosis, 33% were in low risk, 26.8% were in intermediate risk and 40.2% were in high risk group. Considering IPSET-Thrombosis risk groups 5.4% of low risk, 26.7% of intermediate risk and 66.2% of high risk patients had an at least one occasion of thromboembolic event. Regarding IPSET-Thrombosis risk groups, 10 year thrombosis free survival was 86.8% for low risk, 39.4% for intermediate risk and 32.9% for high risk groups (p<0.001). Harrell C-concordance indexes of conventional and IPSET-Thrombosis were 0.60 and 0.77 respectively (Figure 1).

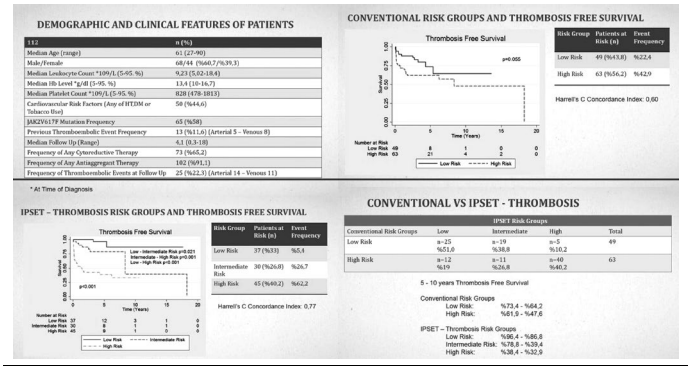

Figure 1.

Summary and Conclusions: By validating the reproducibility of IPSET-Thrombosis in Turkish ET patients, we conclude that IPSET-Thrombosis identifies thrombosis free survival better than the conventional risk stratification system.

E1342

EFFICACY OF RUXOLITINIB IN MYELOID NEOPLASMS WITH PCM1-JAK2 FUSION GENE

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Background: In September 2014 Schwaab *et al.* published two cases of myeloid neoplasms associated with *PCM1-JAK2* and *BCR-JAK2* fusion genes treated with ruxolitinib. While they reported a very good initial response for both cases, relapse occurred after 18 and 24 months respectively. The authors concluded that the response of myeloid disorders with *JAK2* fusion genes to ruxolitinib is short-lived, but that ruxolitinib may be an important bridging therapy prior to ASCT.
Aims: To report the follow-up of our two cases of myeloid neoplasms/Chronic eosinophilic leukemia (CEL) with a *PCM1-JAK2* fusion gene treated with Ruxolitinib.
Methods: We monitored at 3, 6, 12, 24 and 36 months the number of metaphases with t(8;9) through cytogenetics, the proportion of rearranged nuclei through FISH, and the amount of PCM1-JAK2 fusion transcript through quantitative real time PCR analysis.

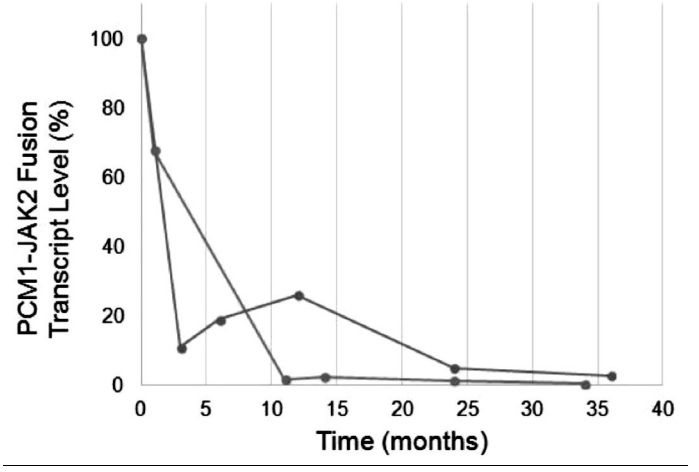

Figure 1. The two lines represent the fold difference in transcript levels normalized to the baseline sample considered as 100%.

Results: The first case was a 72-year-old male with a PCM1-JAK2 positive CEL who gradually obtained a complete cytogenetic remission over a period of 15 months of therapy with ruxolitinib at a dose of 10- 20 mg bid. The hematological course of this patients beyond 15 months, under continued treatment with ruxolitinib (10 mg, bid) was uneventful, with moderate anemia ((Hb>120 g/L) not requiring blood transfusions, with normal leukocytes and platelet counts and normal eosinophil counts. Consecutive cytogenetic and FISH studies on bone marrow showed complete cytogenetic remission. In addition, the meas-

urement of disease burden by real-time quantitative RT-PCR showed a 2log decrease at 34 months as compared with the disease burden at the start of ruxolitinib. The last reevaluation was done three months before his death to unrelated cardiac problems (septic endocarditis) 36 months after the start of ruxolitinib, without- evidence of relapse. The second case was a 31-year-old female affected with CEL. She started Ruxolitinib in 2011 at 15 mg BID and obtained a complete clinical remission. She obtained a reduction of aberrant metaphases and aberrant nuclei with t(8;9) and a marked reduction of the PCM1-JAK2 fusion transcript. She is still alive in complete hematological remission. Both patients therefore achieved durable complete hematologic remissions and cytogenetic response lasting for 3 year in each case respectively. Figure 1 shows the marked reduction of the PCM1-JAK2 fusion transcript.
Summary and Conclusions: These cases demonstrate that the response of myeloproliferative neoplasms with PCM1-JAK2 fusion genes can be long-lived, without use of ASCT.

E1343

CLINICAL FEATURES OF JAPANESE POLYCYTHEMIA VERA AND ESSENTIAL THROMBOCYTHEMIA PATIENTS HARBORING CALR, JAK2V617F, JAK2EX12DEL, AND MPLW515L/K MUTATIONS

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Background: Myeloproliferative neoplasms (MPNs) are characterized by the monoclonal proliferation of one or more lines of myeloid cells due to hematopoietic stem cell abnormalities. MPNs involve hyperplasia of differentiated blood cells and an increased risk of thrombosis, and can progress to leukemia. The risk of complication of polycythemia vera (PV) and essential thrombocythemia (ET) by thrombosis in Japanese patients is clearly lower than in western populations, suggesting that genetic background such as race may influence the clinical features.
Aims: This study aimed to clarify the relationship between genetic mutations and haplotypes and clinical features in Japanese patients with PV and ET.
Methods: This study prospectively analyzed 74 PV and 303 ET patients who were diagnosed at facilities which agreed to participate in this study. Comparisons were made with 232 normal controls. Mutation biased polymerase chain reaction and direct sequencing were used to assess *JAK2V617F*, *JAK2Ex12del, MPLW515L/K* mutations, and the single nucleotide polymorphisms, which are located near the *JAK2V617F* mutation, to determine *JAK2* 46/1 haplotype. *CALR* mutations were analyzed by direct sequencing of exon 9. Statistical analyses were performed with SPSS software (version 12.1.4). Linkage analysis was performed with haploveiw ver.4.2.
Results: Genetic mutations in PV patients included *JAK2V617F* (93.2%; 69/74), *JAK2Ex12del* (5.4%; 4/74), and *MPL* mutations (1.4%; 1/74). Genetic mutations in ET patients included *JAK2V617F* (60.9%; 180/294), *CALR* (16.0%; 47/294), and *MPL* (3.7%; 11/294) mutations. There were 57 ET patients (19.4%) who did not have any of these mutations. There were no clinical differences, including *JAK2V617F* allele burden, between PV patients harboring the various genetic mutations. However, *CALR* mutation-positive ET patients had a significantly lower WBC count (p=0.003), Hb value (p<0.001), Ht value (p<0.001), and NAP score (p=0.015), and significantly more platelets (p=0.003), relative to *JAK2V617F*-positive ET patients and ET patients with no mutations. Compared to Type 1 *CALR* mutation (p.L367fs*46 deletion)-positive ET patients, those with Type 2 mutations (p.K385fs*47 insertion) had a significantly higher platelet count (p=0.048). The single nucleotide polymorphisms rs12340895, rs10974944, rs12343867, and rs1159782, which are located near JAK2V617F, exhibited linkage disequilibrium between PV patients, ET patients, and normal controls. Compared to normal controls, the frequency of the *JAK2* 46/1 haplotype was significantly higher among patients with *JAK2V617F* (p<0.001), *JAK2Ex12del* (p<0.001), or *MPL* mutations (p<0.001), whereas no significant difference was found among *CALR* mutation-positive patients. The cumulative incidence of thrombosis from diagnosis of PV and ET was 9.6% and 9.0%, respectively, after 10 years. The cumulative incidence of thrombosis showed an increasing trend among PV patients aged ≥60 years (p=0.098), but no significant difference was found in any of the other parameters (history of thrombosis, WBC count, NAP score, 46/1 haplotype, various genetic mutations. In ET patients, the cumulative incidence of thrombosis was significantly higher among those with a history of thrombosis (with history: 18.0% in 10 years *vs* no history: 7.0% in 10 years; p=0.043). *JAK2V617F*-positive patients had a higher cumulative incidence of thrombosis (12.3%) relative to those with *CALR* mutations (6.25%), the *MPLW515L/K* mutation (0%), or no mutation (0%), although the differences were not significant.
Summary and Conclusions: The incidence of developing thrombosis after an ET or PV diagnosis was about 9% each, which are lower than those observed in western countries. We did not find a significant correlation between *CALR* mutations and the 46/1 haplotype. *JAK2V617F*-positive ET patients and *CALR* mutation-positive patients may have different mechanisms of occurrence and clinical features of ET, suggesting the potential need for therapy stratification in the future.

E1344

BONE MINERAL DENSITY IN PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA AND POLYCYTHEMIA VERA

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Background: Several systemic inflammatory diseases are associated with osteoporosis and increased risk of fractures which at least in part may be caused by low bone mineral density (BMD). Chronic inflammation is today a model for cancer development and may also play an important role in the pathology of chronic myeloproliferative neoplasms (CMPN). Patients with essential thrombocythemia (ET) and polycythemia vera (PV) have an increased risk of fractures in comparison with a matched background population as shown in population based studies.
Aims: The aim of this study was to assess BMD in CMPN patients in a clinical cross sectional study.
Methods: Patients with ET or PV according to WHO 2010-criteria *International Classification of Diseases,* 10th revision were recruited from the Department of Haematology, Odense University Hospital, Denmark. Dual energyx-ray absorption (DXA) was used to measure BMD at the hip and the spine L1-L4. Patients were compared with individuals from a cohort of healthy, Danish, Caucasian (n=499) recruited from the general population of the municipality in Odense. Each CMPN patient was individually matched with a subject from this cohort on age, sex, weight and height.
Results: 46 patients and 46 controls were included, 55% were females and 45 were men. Mean age was 56 years. The results of BMD measurements were presented in the Figure 1 demonstrating that BMD measurement in both hip and spine were comparable with the reference group.

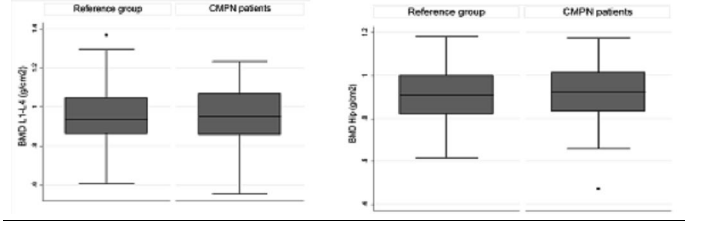

Figure 1.

Summary and Conclusions: This study assessed BMD in CMPN patients and compared results with healthy individuals. Results of the BMD measurements in both hip and spine were comparable with the reference group. This study does not explain the increased risk of fractures found in CMPN patients. BMD alone may not be accurate in predicting fractures in CMPN, and our results calls out for further studies with advanced imaging modalities or assessment of biochemical bone turn over markers.

E1345

PREVALENCE OF EOSINOPHILIAS (EOS), IDIOPATIC HYPEREOSINOPHILIA AND HYPEREOSINOPHILIC SYNDROME (HES) IN A LARGE (ONE MILLION) POPULATION IN NORTH ITALY (THE ROMAGNA GREATER AREA)

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Background: The Eosinophilias (Eos) encompass a broad range of nonhema-

tologic (secondary or reactive) and hematologic (primary, clonal) disorders. Hypereosinophilia has been defined as a peripheral blood eosinophils (Eo) count greater than 1,500/mm^3 The HES is characterized by marked unexplained blood and tissue Eos and defined (1975) by Chusid's criteria: (1) blood Eo ≥1500/mm3 for longer than 6 months, (2) lack of evidence for parasitic, allergic, or other known causes of Eos, and (3) presumptive signs or symptoms of organ involvement. Disease prognosis relies on identifying the origin of Eos. The 2008 WHO establishes a semimolecular classification of eosinophilic disorders (ED) based on the presence of recurrent molecular alterations (PDGFRA, PDGFRB, or FGFR1), or of other clonal markers, and where HES is a diagnosis of exclusion. The incidence and prevalence of ED and HES is not well characterized, and also the frequency of PDGFRA rearrangement is really unknown, with a high median rate of 23% from 8 clinical studies, that probably depends from the selection bias of patients.

Aims: The aim of this study was to investigate the prevalence of ED in the Greater Romagna Area (AVR), an homogeneous large geographic area served by the Italian National Health System (INHS).

Methods: The Hub Laboratory (HL) of AVR serves more than one million (1.124.866 in 2012) inhabitants living in an area of about 5000 square km in North of Italy, that provides Laboratory Medicine service for all general practitioners and for all the hospitals of the INHS. It includes hematology laboratory, and genetic sections. The results of all the tests carried out by the HL since 2009, including the differential cell counts (DIFF), are stored in the LIS database. We downloaded from the LIS the DIFF results obtained in 2012 and selected the cases with ≥1.5x10^9/L Eo among the 574.380 unique individuals with at least one DIFF. In order to verify if the first Chusid criteria for HES was satisfied we searched for other DIFF requests in the semesters before and after the selected cases and for Eo ≥1.5x10^9/L in these DIFFs. For this total cohort of possible HES patients, we performed further data extraction from LIS with the intent to exclude the more frequent causes of secondary Eos (*e.g.* total IgE, fecal parasites, CRP, vitamin B12, bone marrow smears and cytogenetic). Moreover we matched the clinical records (InfoClin, LOG80, ONAMB) of the uncertain cases for final diagnosis.

Results: Of 574.380 unique individuals with one DIFF in 2012, 452 satisfied the first Chusid's criteria, and on these cohort we will performed our investigations. Here we report the preliminary results on 44 patients (10% of the total cohort). We excluded 27 patients with secondary Eos (61,3%), and 15 asymptomatic patients no further investigated despite a persistent mild Eos (<2.5x 10^9/L), and finally we identified 2 patients with sign and symptoms of HES, with a prevalence in the AVR of 0.034%. Of note, the 2 patients were male, and died in 3 and 15 months without evaluation of the presence of molecular rearrangements.

Summary and Conclusions: The low prevalence of HES (<0.05%) is confirmed by these preliminary data. With the completion of this analysis we will achieve a better characterization of ED and, since we are investigating half of the resident population of AVR, also the real prevalence of these rare diseases will be known, including PDGFRA positive clonal disease, primary HES, but probably with the exclusion of the ED with specific tissue Eos without peripheral Eos.

E1346

A SCORING SYSTEM BASED ON LEUKOCYTE ALKALINE PHOSPHATASE ACTIVITY AND PERIPHERAL GRANULOCYTE PRECURSOR PERCENTAGE PREDICTS JAK2 V617F MUTATION IN PATIENTS WITH PRIMARY MYELOFIBROSIS

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Background: Patients with primary myelofibrosis (PMF) may carry mutations of *JAK2*, *MPL* or *CALR* (calreticulin) gene. The genetic subtypes of PMF are different as regards clinical course, disease progression and overall survival. The mutation in the *JAK2* gene consisting of a valine-to-phenylalanine change at position 617 (*JAK2* V617F) was reported in half and more of the patients. This mutation increases JAK2 kinase activity and is significantly associated with higher blood cell count, granulocyte activation and thrombosis risk.

Aims: Since increased leukocyte alkaline phosphatase (LAP) expression is considered a granulocyte activation marker and variable LAP levels are found in PMF, the aims of our study were to evaluate LAP activity in PFM patients searching for possible correlations among LAP score, *JAK2* V617F mutation status, and clinical-pathological features; moreover, in patients treated with a JAK1/2 inhibitor, to determine possible correlation between LAP levels and degree of response to therapy.

Methods: We evaluated LAP activity by cytochemistry in peripheral blood smears from 266 PMF patients at diagnosis, whereas *JAK2* V617F mutation status was assessed by polymerase chain reaction (PCR). On the basis of a ROC curve analysis we defined a simple scoring system to predict *JAK2* V617F mutation with high sensitivity and specificity. Then, we prospectively tested this score system in a new cohort of 105 PFM patients.

Results: *JAK2* V617F mutation was identified in 162 patients (61%). In these patients LAP scores were significantly higher than in nonmutated cases (P<0.0001), without difference in relation to the allele burden. Score values above the normal range were observed only in patients carrying the mutation. No correlation was found between LAP score and white blood cell or platelet count, hemoglobin concentration, splenomegaly, IPSS risk group, while there was a significant inverse correlation between LAP score and peripheral granulocyte precursor percentage. A ROC curve analysis allowed us to identify a LAP score of 100 (AUC=0.81, 95% CI 0.76-0.86) and a peripheral granulocyte precursor percentage of 10 (AUC=0.80, 95% CI 0.64-0.79) as optimal cut-off to discriminate mutated patients with good sensitivity and specificity (range 84-98%). On the basis of these variables, we awarded 0 points (LAP score <100, or peripheral granulocyte precursors ≥10%), 1 point (peripheral granulocyte precursors <10%) or 2 points (LAP score ≥100), and developed a simple scoring system to predict *JAK2* V617F mutation. All cases with a score of 3 carried the mutation, while 94% of patients with a score of 0 showed a wild-type gene. Then, we prospectively tested this score system in a new cohort of 105 PFM patients obtaining superimposable results. Nineteen PMF patients, 16 carrying the *JAK2* V617F mutation and 3 nonmutated, were treated with the JAK1/2 inhibitor Ruxolitinib. Eleven patients had a ≥50% reduction in spleen volume, 2 cases achieved a modest decrease in spleen size, whereas in 6 cases no spleen response was observed. In all cases, LAP score significantly decreased during treatment (P=0.02); however, no correlation was found between LAP levels or peripheral granulocyte precursors percentages and response to therapy.

Summary and Conclusions: We confirmed the association between LAP levels and *JAK2* V617F mutation; moreover, we suggest a very simple, low expensive and reproducible method based on old techniques to predict this gene mutation.

E1347

RUXOLITINIB IN CHRONIC MYELOMONOCYTIC LEUKEMIA AND SYMPTOMATIC SPLENOMEGALY

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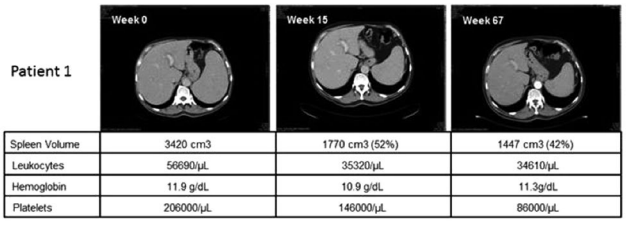
Background: In patients with myelofibrosis the JAK1/2 inhibitor ruxolitinib has been shown to reduce splenomegaly and constitutional symptoms. Efficacy was also seen in patients without the JAK2 V617F mutation suggesting that some drug effects may be due to a general attenuation of cytokine signaling (*Ostojic A et al. Drugs Today 2011*). In patients with chronic myelomonocytic leukemia (CMML) symptomatic splenomegaly and constitutional symptoms may be also a clinical problem and there is no generally accepted standard treatment for the myeloproliferative form of CMML. *In vitro* findings by us (*Geissler K et al. J Exp Med 1996*) and others (*Padron E et al. Blood 2013*) suggest that divergent molecular aberrations in CMML seem to converge within the GM-CSF signaling pathway. Since JAK2 is a sentinel kinase in this pathway (*Hansen G et al. Cell 2008*) ruxolitinib may be an attractive drug in CMML.

Aims: Clinical observations from case reports may be helpful to support treatment concepts.

Methods: We report our findings in 2 CMML patients with symptomatic splenomegaly and constitutional symptoms receiving ruxolitinib off label.



Computed tomographies and spleen volumes at different time points are shown. Spleen volumes were calculated using the formula S Vol = 30 + 0·58 (width x length x thickness) (*Prassopoulos P et al. Eur Radiol 1997*). Numbers in brackets give the percentages of spleen size as compared to the spleen volumes prior to ruxolitinib treatment (week 0). * Patient 2 had gastrointestinal bleeding at that time.

Figure 1. Effect of ruxolitinib on spleen volumes in two patients with chronic myelomonocytic leukemia.

Results: Patient 1 is a 75-year-old man with CMML-1 since 10/2011, JAK2 V617F mutation, progressive splenomegaly and constitutional symptoms refractory to hydroxyurea. After informed consent ruxolitinib (2x20mg/d) was started in 5/2013. Within 1 week constitutional symptoms subsided. CT confirmed a reduction in spleen volume of 48% at week 15 (Figure 1). Initiation of

ruxolitinib was associated with a clear reduction of white blood cell (WBC) counts without clinically relevant changes in platelet counts and hemoglobin values. The patients response regarding splenomegaly and constitutional symptoms is continuing without a clear decline in JAK2 V617F allele burden (94.6% at week 0, 89.7% at week 67). Patient 2 is a 72-year-old man with CMML-1 and mutations in NRAS, and SRSF2, respectively. Because of rapidly increasing WBC counts (from 25990/mm^3 to 62920/mm^3 within 6 weeks), symptomatic splenomegaly and constitutional symptoms the patient received ruxolitinib (2x20mg/d) in 8/2012 after providing consent. Constitutional symptoms disappeared within 1 week. A reduction of spleen volume of 38% at week 15 and of 48% at week 21 of ruxolitinib therapy was observed (Figure 1). Initiation of ruxolitinib was followed by a stabilization of WBC counts with no major hematological toxicity. Spontaneous relative to stimulated *in vitro* CFU-GM formation, which has been shown by us to be GM-CSF-dependent (*Geissler et al. J Exp Med 1996*), decreased from 101% before ruxolitinib to 36% at week 5. After 8 months the patient lost his response to ruxolitinib and was treated with azacitidine. Having achieved a second transient response he died 22 months after diagnosis due to progressive disease.

Summary and Conclusions: We show here for the first time spleen response and disappearance of constitutional symptoms by ruxolitinib in 2 patients with CMML. The response in patient 1 which was seen without reduction in the JAK2 allele burden as well as culture data obtained during ruxolitinib therapy in patient 2 are in line with the assumption that inhibition of GM-CSF-dependent signaling may be at least in part responsible for these effects. Ruxolitinib may be an interesting molecule to be systematically studied in patients with CMML.

E1348

CLINICAL DIFFERENCES IN ESSENTIAL THROMBOCYTHEMIA PATIENTS WITH AND WITHOUT THROMBOTIC COMPLICATIONS

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Background: Thrombosis is the main factor causing disablement in essential thrombocythemia (ET) patients. Currently, the connection between increased blood platelets and leukocytes values, as well as *JAK2V617F* mutation presence and the risk of thrombotic events in ET is well-known.

Aims: The aim of this study is to analyze clinical characteristics in ET patients with and without thrombotic complications.

Methods: The subject for this study were 218 patients who had been diagnosed at the Russian Research Institute of Hematology and Transfusiology outpatient department over a period of 10 years (2003-2013). The ET patients were divided into 2 groups: those with thrombosis (Thr+) (n=67) and those without (Thr-) (n=151). During the study, the following parameters were observed: age, sex ratio, blood test data, and the frequency of *JAK2V617F* mutation. The Mann-Whitney U test for quantitative variables and the Chi-square test with Yate's contingency correction were used for statistical analysis. Statistical significance was set at the p<0.05 level.

Results: In group Thr+ the following thromboses were observed: arterial 38 (56.7%), venous 21 (31.4%), and both 8 (11.9%). The majority of ET patients with thrombosis had had thrombotic events before ET was established and 7 of them developed it during the follow-up (median follow-up=24 months). Other variables were: group Thr+ median age 58.1 and group Thr- 59.6 years; sex ratio in group Thr+ 44 (65.7%) female and 23 (34.3%) male, in group Thr- 117 (77.5%) female and 34 (22.5%) male (p=0.09). No statistical differences were found in blood count analysis values at the point of ET detection. Mean values (standard deviations): in group Thr+ hemoglobin-14.1 (20) g/dL, WBC-10.10 (3.75)x10^9/L, platelets-932 (407)x10^9/L and group Thr- hemoglobin-13.9 (16) g/dL, WBC-9.86 (3.56)x10^9/L, platelets-914 (358)x10^9/L. *JAK2V617F* mutation was detected in 84% of the ET patients in group Thr+ and in 48% of those in group Thr- (p=0.004). The risk of thrombosis according to the IPSET-thrombosis scale in group Thr+ was as follows: low-7.6%, intermediate-20.9% high-71.5% and 42.3%, 37.8%, 19.9% in group Thr- (p<0.0001).

Summary and Conclusions: ET patients show difference in thrombotic events development. Risk factors for thrombosis include: age>60 years, cardiac risk factors, thrombotic complications in patient case history, and *JAK2V617F* mutation. *JAK2V617F* mutation and IPSET-thrombosis scores assist in individualizing therapeutic strategy. This helps provide dynamic thrombosis prevention, extend the patient's life duration, and improve their quality of life.

E1349

LONG-TERM RESULTS OF PREDNISONE TREATMENT FOR THE ANEMIA OF MYELOFIBROSIS

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Background: Corticosteroids are generally considered to have modest activity in the treatment of the anemia of myelofibrosis (MF), but the available data are limited.

Aims: To retrospectively evaluate the efficacy and long-term outcome of single-agent prednisone, usually given after failure of other therapies, in MF patients with severe anemia. In addition, to analyze the pre-treatment clinical variables associated with the response to such therapy.

Methods: Among 511 patients diagnosed with primary MF (PMF: 345) or MF evolving from a previous essential thrombocythemia (ET) or polycythemia vera (PV) (post-ET/PV MF: 166) from 1978 to 2014, thirty cases (PMF: 27, post-ET MF: 3) were eligible for the study. Initial prednisone dose was 0.5-1 mg/kg daily, with progressive tapering to the minimum effective dose in responders. Responses were assessed according to the revised International Working Group for Myelofibrosis Treatment and Research (IWG-MRT) consensus criteria (Tefferi *et al.,* Blood 2013).

Results: Median follow-up was 17.4 months (interquartile range [IQR]: 5.5-29.1). Twelve patients (40%) achieved anemia response according to the IWG-MRT criteria, after a median time of 1.1 months (IQR: 0.9-1.6) on treatment. The median daily dose to attain the response was 45 mg (IQR: 22.5-60), and the median daily maintenance dose in responders was 10 mg (IQR: 6-15). Median response duration was 12.3 months (IQR: 9.3-29.7). At univariate analysis, constitutional symptoms and more than 2% circulating blasts at treatment start had borderline statistical significance for a lower probability of response. A platelet increase >50x 10^9/L was observed in 3 out of 11 patients (27%) with baseline counts <100x 10^9/L. Median survival from prednisone start was significantly longer in anemia responders (5.0 years, 95% CI: 3.5-6.5, *versus* 1.5 years, 95% CI: 0.2-2.8; P=0.002).

Summary and Conclusions: Prednisone has clear therapeutic activity in the anemia of MF and should be considered in patients failing conventional anemia-treating agents.

E1350

CLINICAL CHARACTERISTICS OF CALR MUTATIONS IN JAPANESE PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA

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Background: Recurrent *CALR* mutations were recently identified in myeloproliferative neoplasms patients without mutated *JAK2* or *MPL*.

Aims: We analyzed these three mutations in Japanese patients with essential thrombocythemia (ET) in our single institute and elucidated clinical characteristics of patients with *CALR* mutation.

Methods: Genomic DNA was isolated from granulocytes in peripheral blood of 66 patients with ET. Mutational analyses of *JAK2*V617F, *JAK2* exon 12, *CALR* exon 9, and *MPL* exon 10 were performed with quenching probe method or Sanger sequencing.

Results: Our ET cohort (male/female, 35/31; age, 16 y.o.-85 y.o.; median age, 63 y.o.) consisted of 30 (45.5%) patients with *CALR* exon 9 frame shift mutation, 24 patients (36.4%) with *JAK2*V617F, 2 patients (3.0%) with *MPL*W515, and 10 patients (15.2%) without these three mutations (triple negative). From genomic analysis of *CALR* mutations, all of them were heterozygous and the details of *CALR* mutation type were as follows: type 1, 52-bp deletion (n=15, male/female, 9/6); type 2, 5-bp TTGTC insertion (n=11, male/female, 9/2); type 4, 34-bp deletion (n=2, male/female, 1/1); novel frame shift deletions (c.1100_1152del53insG and c.1097_1130del34) (n=2, male/female, 1/1). One of novel mutations is a single amino acid variant of type 1 mutation and the other one is 2 amino acid variant of type 4 mutation. Next, we compared the clinical parameters between *CALR*-mutated ET and *JAK2*-mutated ET. *CALR* mutations were detected in the majority of male patients and relatively younger female ET patients, while frequency of *JAK2*V617F mutation was higher in elder female patients. Platelet counts of *CALR*-mutated patients tended to be higher than those of *JAK2*-mutated patients (1013x 10^9 /L *vs* 778x 10^9 /L, p=0.05). A progressive increase in the number of platelets (>300x 10^9 /year) was observed in 7 out of 30 *CALR*-mutated ET, but in only one out of 24 *JAK2*-mutated ET. However, thrombotic events were not observed in 30 *CALR*-mutated patients, while they did occur in 6 out of 24 *JAK2*-mutated patients and 3 out of 10 triple negative patients. Significant difference was not observed between type 1 and type 2 *CALR* mutations, regarding clinical characteristics because patient numbers were small. Disease progression was observed in three out of eleven ET cases

with *CALR* type 2 mutation about twenty years after diagnosis of ET: two patients developed severe secondary myelofibrosis (MF), and one patient developed acute myeloid leukemia (FAB M7). All of them were male with type 2 *CALR* mutation. Both of two secondary MF patients had cytogenetic abnormalities including 1q21 and 6p23. One of them is still alive after allogeneic bone marrow transplantation, but the other patient died of hemorrhagic cerebral infarction due to tumor embolism. A patient with secondary leukemia contained complicated karyotype including 1q32 abnormality and died of chemorefractory leukemia (Table 1).

Table 1. ET with transformation to severe MF or acute leukemia.

Age (y.o.)	Sex	ET onset (y.o.)	Transformation	Mutation type	Karyotype
39	M	20	Secondary MF	*CALR* type2	der(6)t(1;6)(q21;p23)
61	M	38	Secondary MF	*CALR* type2	dup(1)(q21q32), add(6)(p23)
62	M	40	Acute myeloid leukemia (M7)	*CALR* type2	44, XY, add(1)(q32), der(5)t(5;14)(q13;q11.2), der(8)t(8;8)(p12;q11.2), add(10)(p11.2), -14, -17, -18, -22, +2mar /42, idem, -Y, -4, -7, +8, add(9)(q34)

Summary and Conclusions: Platelet counts were higher and tended to increase at a higher rate in patients with *CALR*-mutated patients than in those with *JAK2*-mutated patients. However, incidence of thrombotic events was significant lower in *CALR*-mutated patients. Disease progression was observed in three male patients with type 2 *CALR* mutation and cytogenetic abnormalities including 1q. It is reported that PMF patients with type 2 *CALR* mutation exhibit comparably worse survival than those with type 1 *CALR* mutation. Although patient numbers are limited, combination of *CALR* type 2 mutation and specific cytogenetic abnormalities might be related to disease progression.

E1351

SIMULATION IN CONTINUING EDUCATION: IMPROVING HEMATOLOGIST EVIDENCE-BASED DECISIONS FOR MYELOPROLIFERATIVE NEOPLASM MANAGEMENT

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Background: Due to the rarity of the disorders, many hematologists, especially those in the community setting, lack experience in diagnosis, treatment and monitoring of myeloproliferative neoplasms (MPN). Prompt, accurate diagnosis and appropriate initiation of treatment are important because polycythemia vera (PV) and myelofibrosis (MF) are associated with shortened life expectancy, serious complications, and impaired quality of life.
Aims: Underlying clinical practice gaps and educational needs were identified, and a study was conducted to determine whether online, simulation-based educational interventions could improve competence and performance of hematologists in managing patients with MPN.
Methods: A cohort of US-practicing hematologists who participated in simulation-based educational interventions was evaluated. The interventions consisted of 4 cases presented in a platform that allowed learners to choose from numerous lab tests and assessment scales as well as thousands of diagnoses, treatments, and monitoring approaches. Participant clinical decisions were analyzed using artificial intelligence technology, and instantaneous or delayed clinical guidance was provided employing current evidence-based and expert faculty responses. To assess the impact of simulation-based education on clinical decisions, each participant's decisions were collected after clinical guidance and compared with that same individual's baseline data. A 2-tailed paired T-test was used to determine statistical significance.
Results: The assessment sample consisted of 284 hematologists who made at least 1 clinical decision within the simulation and proceeded to the final, debrief section. As a result of clinical guidance provided through simulation, significant improvements were observed in several areas of management of patients with MPN, specifically: In PV: -11% improvement in the diagnosis of PV (62% post intervention *vs* 56% baseline, *P*<.013); -109% improvement in the selection of appropriate initiation of therapy (JAK2 inhibitor/interferon alpha) for a patient with PV refractory to hydroxyurea (48% post intervention *vs* 23% baseline, *P*<.001); -79% improvement in MPN symptom monitoring for a patient with PV (50% post intervention *vs* 29% baseline, *P*<.001); In MF: -29% improvement in the diagnosis of primary MF (63% post intervention *vs* 49% baseline, *P*<.002); -124% improvement in the selection of appropriate initiation of therapy (JAK2 inhibitor) for a patient with primary MF (60% post intervention *vs* 27% baseline, *P*<.001); -75% improvement in MPN symptom monitoring for a patient with MF being managed with a JAK2 inhibitor (40% post intervention *vs* 23% baseline, *P*<.001).
Summary and Conclusions: This study demonstrated the success of simulation-based educational interventions on improving the evidence-based practice patterns of hematologists in the management of patients with MPN. Despite the marked improvement in competence and performance, hematologist education needs specific to accurate MPN diagnosis, treatment selection, and symptom monitoring remain. The education gaps uncovered during this intervention and the evolving treatment landscape outside of the United States lay a foundation for future global education initiatives to bridge education gaps in MPN.

E1352

CLINICAL CHARACTERISTICS OF POLYCYTHEMIA VERA PATIENTS, RECEIVING DIFFERENT TYPES OF THERAPY

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Background: Achievements of fundamental sciences in study the pathogenesis of Polycythemia Vera (PV) made possible to create drugs with target mechanism of action. It is important to determine indications for target drugs with consideration their high cost and possible side effects.
Aims: Aim of study was to investigate clinical features of PV, receiving different types of therapy.
Methods: 252 patients (pts) (147 female, 107 male), median age 59 years (range 20-86), median of observation 6.1 year. Pts were divided into 4 groups: phlebotomy and erythrocytapheresis (Phleb/Aph), therapy with hydroxyurea (HU), with interferon-alpha (IFN), combined therapy with hydroxyurea and interferon-alpha (HU+IFN). The endpoints were: clinical and laboratory features, treatment outcomes, thrombosis incidence and overall survival (OS). Responses to therapy were evaluated according to ELN criteria. Statistical analysis was conducted with Kruskal-Wallis ANOVA and Chi-square tests. Survival analysis was performed by Kaplan-Meier method with log-rank test.
Results: The Phleb/Aph group consisted of 36 pts with median age 57 years. Baseline complete blood count (CBC): HB 18.6 g/dl (13.1-23.8), RBC $6.78x10^{12}$/l (5.36-9.30), HCT 57.6 (46.9-75.0), WBC $8.8x10^{9}$/l (4.3-16.4), PLT $337x10^{9}$/l (143-796). Splenomegaly was observed in 17%. Phleb/Aph procedures performed with rate from 0.10 to 9.7 (mean 5.1) procedures per year. Complete response (CR) was achieved in 5.5%, partial response (PR) in 67%, no response (NR) was seen in 27.5% of patients. The outcome in myelofibrosis (MF) was observed in 2.8%. Thrombotic episodes occurred in 19%. The HU group included 173 pts (median age 61 years). CBC data: HB 18.7 g/dl (13.94-25.6), RBC $7.22x10^{12}$/l (5.22-10.29), HCT 59.3 (47.1-82.0), WBC $12.5x10^{9}$/l (3.6-64.8), PLT $535x10^{9}$/l (136-1642). Splenomegaly was observed in 35%. Mean daily dose of HU was 0.7 (0.3-1.5) g. 7.5% achieved in CR, 76.5% had PR and NR in 16%. The MF transformation was observed in 3.5%. Thrombotic incidence was in 6.6%. The IFN group consisted of 11 pts with median 53 years. CBC: HB 17.6 g/dl (13.5-21.5), RBC $6.66x10^{12}$/l (5.90-7.48), HCT 54.2 (43.0-64.2), WBC $12.3x10^{9}$/l (3.6-23.7), PLT $562x10^{9}$/l (312-1087). Splenomegaly was detected in 64%. Mean weekly dose of IFN was 8.27 (4-12) million IU. CR was achieved in 36%, PR in 36%, NR was seen in 28%. The outcome in MF was observed in 28%. Thrombotic complications did not occurred. The HU+IFN group consisted of 32 pts with median age 51 years. CBC: HB 19.0g/dl (13.2-22.3), RBC $7.45x10^{12}$/l (5.17-9.99), HCT 60.4 (48.8-72.8), WBC $11.0x10^{9}$/l (5.1-21.0), PLT $549x10^{9}$/l (171-1189). Splenomegaly was observed in 53%. Mean daily dose of HU was 0.8 (0.5-1.5) g, mean weekly dose of IFN was 8.27 (6-30) million IU. CR wasn't achieved in this group, PR had 75% and NR in 25%. The outcome in MF was seen in 6%. Arterial thrombotic episodes occurred in 6%. Statistically significant differences between groups were obtained: age of patients (p=0.006), RBC(p<0.01), HCT (p<0.01), WBC (p<0.01), PLT (p<0.01), splenomegaly (p=0.04), response to therapy (p<0.01). Groups didn't differ in the frequency of postPV-MF (p=0.23), the overall frequency of thrombosis (p=0.19), arterial (p=0.56) and venous (p=0.34) thrombosis, and OS (p=0.29).
Summary and Conclusions: PV is one of the most frequent myeloproliferative neoplasms with various clinical symptoms. The choice of therapy depends on the patient's age and clinical symptoms. The most of PV patients don't have a clinically significant response to therapy or achieve only a partial response. This PV population might have perspective of improved treatment results and OS with administration of targeted drugs in future.

E1353

COMPLETE HEMATOLOGIC CONTROL WITH RUXOLITINIB IN PATIENTS WITH POLYCYTHEMIA VERA (PV) RESISTANT TO OR INTOLERANT OF HYDROXYUREA

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Background: Ruxolitinib (RUX) is a JAK1/JAK2 inhibitor that has shown superiority over standard therapy for the treatment of PV for patients (pts) with an inadequate response to or unacceptable side effects from hydroxyurea (HU). RUX was well tolerated and effective in controlling hematocrit (HCT), decreasing splenomegaly, and improving symptoms. PV is a trilineage disease, and in addition to elevated HCT, pts often have elevated white blood cell (WBC) and platelet (PLT) levels.
Aims: To explore the efficacy of RUX *vs* best available therapy (BAT) in controlling HCT, WBC, and PLT levels in RESPONSE.
Methods: Pts with PV who were HU-resistant/intolerant by modified ELN criteria and required phlebotomy (PBT) for HCT control were randomized 1:1 to RUX 10 mg bid or BAT. The primary composite endpoint was the proportion of pts who achieved HCT control without PBT from wk 8-32 (with ≤1 PBT wk 0-8) and a ≥35% reduction in spleen volume from baseline (BL) at wk 32. Pts on BAT could cross over to RUX after wk 32. In this preplanned analysis of a wk 80 data cutoff, hematologic parameters were assessed by BL level (HCT, >45% and ≤45%; WBC and PLT, by BL quartile [Q1-Q4]).
Results: Pt characteristics (RUX, n=110; BAT, n=112) were well balanced between groups. Median HCT was 43.3% *vs* 44.0% (RUX *vs* BAT); despite having HCT control with PBT within 2 wk of randomization, many pts had HCT >45% at BL (RUX, 25% [n=28]; BAT, 22% [n=25]); PLTs (median, 397 *vs* 444×10^9/L) ranged from: RUX, 100-1852×10^9/L (Q1, 250; Q3, 650); BAT, 78-1604×10^9/L (Q1, 261; Q3, 654); and WBCs (median, 16.5 *vs* 16.3×10^9/L) from: RUX, 3.0-67.0×10^9/L (Q1, 11.1; Q3, 22.5); BAT, 2.9-82.8×10^9/L (Q1, 10.4; Q3, 23.1). At data cutoff, 82.7% of RUX pts remained on treatment and no pts remained on BAT (median exposure, 111 and 34 wk). As previously reported, significantly more RUX pts achieved the primary composite endpoint (21% *vs* 1%; *P*<.0001) and complete hematologic response (CHR) at wk 32 compared with BAT (24% *vs* 9% *P*=.003; Vannucchi *NEJM* 2015). These improvements were durable in RUX pts, with probabilities of 0.96 and 0.69 of maintaining a primary response and CHR at wk 80, respectively. In comparison to BAT pts, more RUX pts achieved WBC ≤10×10^9/L (RUX: BL, 21%; wk 32, 41%; BAT: BL, 21%; wk 32, 31%) and PLT ≤400×10^9/L (RUX: BL, 51%; wk 32, 61%; BAT: BL, 46%; wk 32, 41%). For pts with uncontrolled HCT (>45%) at BL despite PBT (Figure 1A), mean HCT in the RUX arm declined to <45% while mean HCT for BAT-treated pts remained mostly >45% and above RUX-treated pts. For pts whose HCT was <45% at BL, mean values in the RUX arm were also consistently lower than in the BAT arm. Pts with near normal WBC or PLT counts at BL (Q1, Q2) had levels that remained at or near BL values over the course of treatment. For pts with higher WBC or PLT counts at BL (Q3, Q4), mean values improved with RUX and were lower than with BAT. Pts with the highest WBC counts at BL (Q4) had the largest reductions (Figure 1B), with mean values in the RUX arm approximately 15×10^9/L or lower from wk 8 onward and those in the BAT arm >25×10^9/L.

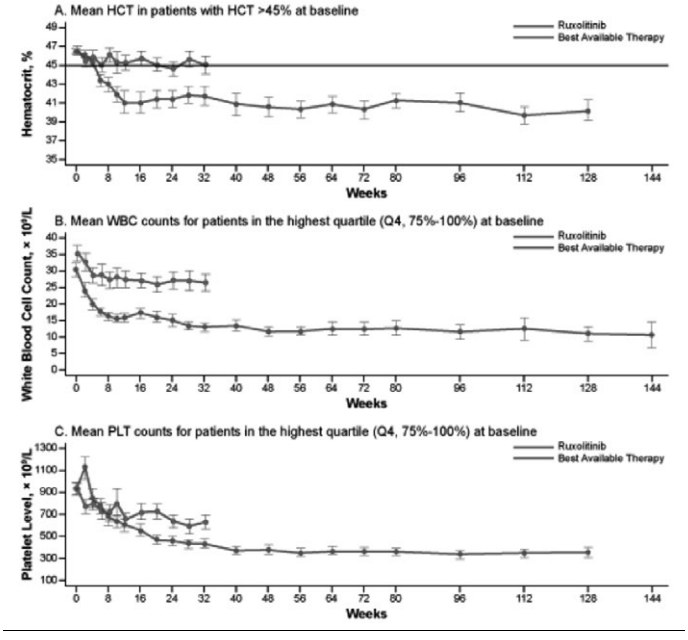

Figure 1. Mean (+/- standard erros) HCT, WBC, and PLT levels by baseline categories and time.

Similar results were seen in pts with the highest PLT counts at BL (Figure 1C), with mean values in the RUX arm reaching <500×10^9/L from wk 20 onward while those in the BAT arm remained elevated.
Summary and Conclusions: In RESPONSE, long-term RUX treatment led to control of HCT, WBC, and PLT levels, with the largest reductions for pts with the most elevated values at BL. RUX provided durable and comprehensive hematologic control in pts with PV who had an inadequate response to or unacceptable side effects from HU.

LB2091

PHASE 1/2, DOSE-ESCALATION STUDY OF ORAL NS-018 IN PATIENTS WITH PRIMARY MYELOFIBROSIS (PMF), POST-POLYCYTHEMIA VERA MF (POST-PV MF), OR POST-ESSENTIAL THROMBOCYTHEMIA MF (POST-ET MF)

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Background: NS-018 is an oral, selective, small molecule inhibitor of Janus kinase 2 (JAK2)
Aims: The purpose of this study is to determine the safety and tolerability of orally administered NS-018 in patients with PMF, post-PV MF, or post-ET MF
Methods: This multicenter, Phase 1/2, 3+3 dose-escalation study of NS-018 enrolled patients with IPSS intermediate-1, intermediate-2, or high risk PMF, post-PV MF, or post-ET MF. Patients were ≥18 years, ECOG PS ≤2 and required therapy. NS-018 was dosed orally QD or BID in 28-day cycles. Responses were assessed according to IWG consensus criteria, changes in spleen size by manual palpation and QOL with the Myelofibrosis Symptom Assessment Form (MF-SAF). The Phase 1 portion of the study is completeand is presented here.
Results: Patients: 48 patients were enrolled across 10 cohorts. Patient characteristics: 37 PMF, 5 post-PV MF, 6 post-ET MF; median age (range) 69.5yrs (38-83); M/F:29/19; 35 JAK2V617F+; and 22 previously treated with a different JAK2 inhibitor. Dosing and tolerability: Dose levels included 75, 125, 200, 300 and 400 mg QD and 100, 200, 250, 300 and 400 mg BID. Systemic exposures based on Cmax were approximately dose proportional across the tested range from 75 to 400 mg. At 400 mg QD/BID, NS-018 was not well-tolerable-based on the proportion of drug-related neurologic AEs. Dizziness (Grade2 and 3), peripheral neuropathy (Grade1), headaches(Grade1), disturbance in attention (Grade1), vertigo (Grade1), dysesthesia (Grade1), and nervous system disorder (Grade1) were observed at 400 mg BID. Dizziness (Grade1 and 3), paresthesia (Grade1), disturbance in attention (Grade1), and aphasia (Grade1) wereobserved at 400 mg QD. At NS-018 doses <400 mg, 300 mg QD was better tolerated than 250 mg or 300 mg BID, with a lower rate of neurologic AEs and discontinuations due to AEs. For the 300 mg QDcohort (n=15), the most frequent drug-related AEs across all grades were decreased platelet count (≥Grade 3, 20%); Grade 3 related AEs were platelet count decreased (13%) and dizziness (7%); and Grade 4 related AEs were platelet count decreased (7%). Overall, 31 patients (65%) discontinued treatment due to AEs (n=9), progressive disease (n=11), MD/patient decision (n=9), or no clinical benefit after 6 cycles (n=2). The AEs leading to discontinuation included thrombocytopenia (Grade 4), transient ischemic attack (Grade 2), muscle contraction involuntary (Grade 2), dizziness (Grade 1), insomnia (Grade 1),neutropenia (Grade 3), electrocardiogram QT prolonged (Grade 3), hemolytic anemia (Grade 3), dizziness (Grade 2), dysphagia (Grade 2), nervous system disorder (Grade 1), and hemolysis (Grade 3). Responses: Of 36 evaluable patients with baseline splenomegaly ≥5 cm and treatment for ≥1 cycle, 20 (56%) patients showed ≥50% reduction in spleen size (confirmed for ≥8 weeks in 16 patients). In addition, 12 patients showed splenic clinical improvement (CI) for ≥8 weeks, 4 patients hemoglobin CI, and 1 patient platelet CI. Median (range) time to splenic response and duration of response: 1 cycle (1-12) and 6 cycles (1-25). Splenic reductions ≥50% are shown in the Table 1 for the specified patient groups. Changes in MF-SAF and PK and PD results will be presented.

Table 1.

	Splenic reduction (in evaluable patients)
All doses	20/36 (56%)
300mg QD	5/9 (56%)
300mg BID	3/6 (50%)
Prior different JAK inhibitor treated	9/19 (47%)

Summary and Conclusions: The RP2D dose was 300 mg QD. This dose provided a durable dosing schedule,an acceptable safety profile,was associated with splenic size reduction and clinical improvement. Phase 2 is ongoing and includes patients previously treated with a different JAK2 inhibitor.

Non-Hodgkin & Hodgkin lymphoma - Biology

E1354

XRCC1 399GG GENOTYPE PREDICTS LONG LASTING CR AND SIGNIFICANTLY LONGER OVERALL SURVIVAL IN RESISTANT LYMPHOMA TREATED WITH BENDA-BEAM AND ASCT

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Background: We demonstrated (Visani *et al.*, Blood 2011, Blood 2014) the efficacy of a new conditioning regimen with bendamustine, etoposide, cytarabine, and melphalan (Benda-BEAM) prior to autologous stem cell transplant (ASCT) in heavily pretreated lymphoma patients (72% CR at 41 months after ASCT; 3 years PFS 75%). Biological markers predicting response to Benda-BEAM would significantly impact the clinical decision making. Cytotoxic activity of Bendamustine is enhanced by inhibition of the base excision repair (BER) DNA-damage response pathway, suggesting that the BER enzymes play a key role in the repair of DNA damage caused by Bendamustine. Accordingly, we evaluated the impact of the BER family genetic variants, in lymphoma patients receiving the Benda-BEAM conditioning regimen pre-ASCT.
Aims: To evaluate the prognostic significance ofxRCC1 G399A,xRCC3 C241T and ADPRT T2444C non-synonymous genetic variants on clinical outcome of Hodgkin (HD) and non-Hodgkin (NHL) lymphoma patients treated with BeEAM-ASCT.
Methods: All 43 patients with resistant/relapsed NHL (n=28) or HD (n=15), enrolled in the clinical trial (EUDRACTnumber2008-002736-15), were analyzed. Peripheral blood samples were available for somatic DNA extraction and genotyping analysis. Three Single Nucleotide Polymorphysms (SNPs) of BER genes [XRCC1 399 (rs25487 G/A, Arg/Gln),xRCC3 241 (rs861539 C/T, Thr/Met), ADPRT T2444C (rs1136410 T/C, Val/Ala)] were analyzed by PCR-HRM (High Resolution Melting) assay and restriction digests of PCR products. X^2 test and Fisher's exact test were used to analyze the association between the pathological features and SNPs. All the candidate genotypes were evaluated to identify a potential correlation with overall survival (OS) using long-rank test and Cox regression model. For all the target genes, the distribution of genotypes was checked for Hardy-Weinberg equilibrium.
Results: In univariate analysis, thexRCC1 399 GG andxRCC3 241 TT genotype were associated with significantly longer overall survival (OS), if compared with the other genetic variants (p=0.035, p=.0.025). Multivariate analysis confirmed the prognostic role ofxRCC1 GG on OS (p=0.005). Interestingly, all patients carryingxRCC1 399 GG genotype reached CR, independently from the disease type (HD and NHL), thus suggesting a possible positive predictive role for thexRCC1 399 GG genotype in influencing both CR and OS of heavily pretreated lymphoma patients. On the other hand, CR rate for patients bearing thexRCC1 399 AA or GA genotype were 75% and 84%, respectively. Analyzing pre-treatment features and genetic variants, we observed that patients carrying the ADPRT 2444 CC or CT genotype had a chemoresistant disease, whereas with TT genotype had a chemosensitive disease. Interestingly, the disease status at transplant was a strong predictor of both PFS and OS in the clinical trial. On the other hand, no association was found between the disease type or others clinical features and SNPs in BER genes.
Summary and Conclusions: xRCC1 399GG genotype is predictive for longer duration of overall survival in lymphoma patients treated with Benda-BEAM conditioning pre-ASCT. Moreover, the presence ofxRCC1 399 GG genotypes was associated with the achievement of CR in 100% of patients carrying this genotype.xRCC1 399 genotype could, thus, represent a biomarker in patients with lymphoma, treated with Bendamustine BEAM regimen and ASCT.
Acknowledgements: supported in part by AIL Pesaro Onlus.

E1355

FUNCTIONAL DEFECTS OF T CELLS OF LYMPHOMA PATIENTS AFTER DIFFERENT CHEMOTHERAPY REGIMENS ACTIVATED BY THE BISPECIFIC ANTIBODY AFM11

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Background: Patients who suffer from hematological malignancies like B cell lymphoma have to undergo chemotherapy. There is a high risk for a relapse or

a refractory disease for older patients and patients suffering from MCL. Unfortunately, there are only poor options for younger patients after autologous Stx transplantation and for older patients who are not eligible for transplantation. In this situation new immunotherapy and antibodies gain in importance. One of them is the bispecific T cell engager CD19-CD3 antibody AFM11. The T cells system of the patients seems to play a critical role in response or non-response to T cell engaging antibodies.
Aims: We try to evaluate the T cell system of the patients after chemotherapy (R-Benda, R-CHOP, HD-BEAM). As a preclinical study we explore the potential of the TandAb antibody AFM11 in heatlhy donors and lymphoma patients to describe the function and possible defects of patients T cells after chemotherapy.
Methods: We examine the blood of 32 patients with different lymphoma and chemotherapies 6 weeks after their last chemotherapy. 17 healthy donors donated blood for the control group. The lymphocytes are analyzed by multicolor FACS staining. Absolut cell counts are calculated by the percentages in the lymph gate in relation to the lymphocytes absolute number in the central lab. For quantification of cytokines of the AFM11 engaged T cells CBA technology was used. Proliferation assays were performed with CFSE dilution technique. For lytic activity a FACS based lysing assay was performed with Annexin and PI readout. The effector to target ratio was decreased for higher sensitivity of this assay to a ratio of 1:25 (E:T).
Results: A full immune recovery of the immune system of lymphoma patients six weeks after chemotherapy is not verifiable. We find less CD3 cells (median 200 cells/µl -chemo group *vs* 1200cells/µl-healthy), NK cells (median 48-chemo group *vs* 222 cells/µl-helathy) and no B cells in comparison to healthy donors. Regulatory T cells-known as suppressor cells- are much higher in the R-Bendamustin and HD-Beam group. These findings can be relevant for the treatment of patients with a T cell engaged antibodies like bispecific antibodies with one CD3 component. To clarify the function of the T cells we performed standardized T cell assays with AFM11. There is no difference between healthy donors and patients regarding the proliferation of T cells after AFM11 activation. T cells of patients after R-CHOP and HD BEAM therapy produce high amounts of IL10 (average: 30pg/ml) in comparison to the R-Benda group (average: 10pg/ml). The IL2 production of patients T cells treated with R-Bendamustin and HD -BEAM was reduced from 700pg/ml (healthy donors and R-CHOP) to 300pg/ml. The specific lyse is 25% reduced in patients after chemotherapy without any significance difference in the subgroups of the different chemotherapy protocols. To investigate differences between the subgroups and for a better sensitivity of the lysing assay we decreased the effector to target ratio from 1:2 to 1:25. With this step we see reduced lysing capacity of patients T cells after R-Bendamustin and HD BEAM treatment.
Summary and Conclusions: T cells of lymphoma patients after different chemotherapy regiments are reduced and have functional defects. This may affect the *in vivo* response to bispecific antibodies like AFM11. If this findings correlate with the *in vivo* response will be evaluated in an ongoing phase I trial with AFM11 in lymphomas.

E1356

DESIGN AND DEVELOPMENT OF A NOVEL, HIGHLY POTENT AND SELECTIVE PI3K-DELTA INHIBITOR, CPL-302-215, AS POTENTIAL TREATMENT OF HEMATOLOGIC MALIGNANCIES

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Background: The phosphatidylinositol-3-kinase δ (PI3Kδ) has emerged as a promising target for the treatment of many hematological malignancies, such as chronic lymphocytic leukemia (CLL) and non-Hodgkin lymphomas (NHL). Recent clinical trials have demonstrated that Idelalisib, a specific PI3Kδ inhibitor, achieved significant antitumor activity in CLL and NHL. However, Idelalisib can have serious side effects, *i.e.* hepatotoxicity, severe diarrhea or colitis, pneumonitis, and intestinal perforation. Therefore, there is an urgent need to develop PI3Kδ inhibitors which entail diminished adverse events.
Aims: The aim of the study was to investigate the therapeutic potential of a novel PI3Kδ inhibitor CPL-302-215 in hematologic malignancies.
Methods: Using knowledge-based drug design approach we have designed a novel small molecule PI3Kδ inhibitor, CPL-302-215, structurally different from other known δ-selective inhibitors. To establish the activity and the selectivity of the compound among class I of PI3K lipid kinases and selected protein kinases, we have used kinase activity assay, ADPGlo™. Additionally, the biological potency and selectivity of a developed inhibitor was evaluated in a cell viability assays and Western blot analysis in a number of hematological cell lines. Finally, using an *in vivo* mouse model a bioavailability, as well as microsomal stability was assessed.
Results: Our results indicate that CPL-302-215 inhibits PI3Kδ kinase with low nanomolar concentrations (IC_{50}=5,3nM) comparable to Idelalisib (IC_{50}=4,1nM). Additionally, CPL-302-215 exhibits exceptional selectivity over γ, β and α isoforms of PI3K (2080, 2000, and 63-fold, respectively) in comparison to Idelalisib (27, 100 and 117-fold, respectively). Moreover, CPL-302-215 does not inhibit protein kinases from the Celon Pharma's selectivity panel at the concentration of 1µM. In cell-based viability assays CPL-302-215 inhibits some NHL cell lines. Western blot analysis has shown that CPL-302-215 significantly inhibits phosphorylation of Akt in Raji cell line after anti-IgM stimulation (IC_{50}<50nM). Additionally, in a PI3Kγ-specific cell line RAW 264.7, compound selectivity has been confirmed by showing no effect on Akt phosphorylation after C5a stimulation up to the concentration of 5µM in opposite to Idelalisib which reduced phosphorylation of Akt in 5µM. Analysis on microsomes (mouse, rat, dog and human), showed that CPL-302-215 is showing virtually no CYP-mediated metabolism. Therefore, a pharmacokinetics study was conducted to characterize CPL-302-215 disposition in mice plasma. Orally (30mg/kg) and intravenously (10mg/kg) administered CPL-302-215 displayed attractive bioavailability and reached appreciably high concentrations in plasma (p.o. AUC=5,157µg/ml*h^{-1}, Tmax=0,5h, Cmax=3,038µg/ml; i.v. AUC=2,492µg/ml*h^{-1}, Cmax=1,053µg/ml; F=82,82%). Currently, the therapeutic potential of CPL-302-215 on primary cells from CLL patients is being evaluated.
Summary and Conclusions: We have designed CPL-302-215-a very potent and selective PI3Kδ inhibitor, both in the kinase- and cell-based assays. Preclinical data from NHL cell lines indicate the therapeutic potential of CPL-302-215 in hematologic malignancies. Moreover, our compound is very stable on various species and has attractive bioavailability in mice. The activity and unique selectivity of CPL-302-215 qualifies it for the consideration as a drug with wide therapeutic window and limited side effects in clinical use.

E1357

NR4A1 AND NR4A3 POSSESS FUNCTIONAL REDUNDANCY BY REGULATING PRO-APOPTOTIC GENES IN AGGRESSIVE LYMPHOMAS.

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Background: Recently, we described a significant down-regulation of NR4A1 (Nur77) and NR4A3 (Nor-1) -two members of the orphan nuclear receptors acting together as critical tumor suppressor genes in acute myeloid leukemia- in aggressive lymphoma. NR4A1 over-expression proved its pro-apoptotic function in aggressive lymphoma cells and its lymphoma suppressive properties *in vivo* was demonstrated in axenograft mouse model.
Aims: Since the role of down-regulated NR4A3 in aggressive lymphomas is unknown, we aimed to investigate the function of NR4A3 in lymphoid malignancies.
Methods: For functional characterization NR4A3 was over-expressed in a SuDHL4 lymphoma cell line by using an inducible lentiviral construct followed by various apoptotic assays and by axenograft mouse experiment. To further compare the transcriptional activity as nuclear receptor of NR4A3 to NR4A1, both receptors were separately over-expressed in aggressive lymphoma cell lines (Karpas-422 and SuDHL4 as GCB- cell line, RI-1 and U2932 as ABC-cell lines) followed by mRNA expression analysis of intrinsic (BIK, BID, BMF, Noxa, BAK, Bax, Puma, Bim, Bcl-2, Bcl-X and Mcl-1) and extrinsic (FasL, Fas, Trail, DR4 and DR5) apoptotic genes.
Results: Induction of NR4A3 expression led to a significantly higher proportion of induced SuDHL4 cells undergoing apoptosis as demonstrated by DNA cleavage, Annexin V staining and increased caspase 3-7 activity suggesting functional redundancy to NR4A1 in aggressive lymphomas. To test the tumor suppressor function of NR4A3 *in vivo*, the stably transduced SuDHL4 lymphoma cell line was further investigated in the NOD scid gamma (NSG) mouse model. Induction of NR4A3 in SuDHL4 abrogated tumor growth in the NSG mice, in contrast to vector control- and uninduced SuDHL4 cells, which formed massive lymphoid tumors. Interestingly, mRNA expression analysis of apoptotic genes in aggressive lymphoma cells demonstrated that NR4A1 and NR4A3 over-expression induced Trail, Bim, Puma, BIK, BID and BAK in a similar pattern.
Summary and Conclusions: Our data demonstrate that NR4A3 has pro-apoptotic properties which are functionally redundant to NR4A1 and define NR4A3 as novel tumor suppressor in aggressive lymphomas.

E1358

CD3+/CD8+/CD16+/CD56-/CD57+ T-LGLL REPRESENTS A DIFFERENT SUBSET WITH DISTINCT CLINICAL AND BIOLOGICAL FEATURES

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Background: T-Large Granular Lymphocyte Leukemia (T-LGLL) is a chronic and heterogeneous lymphoproliferative disorder characterized by clonal expansion of CD3+ Large Granular Lymphocyte (LGL) with effector memory phenotype (CD3+/CD8+/CD57+/CD45RA+/CD62L-), although rare forms of CD4+ LGLL are described and characterized by CD4+/$CD8^{-/dim}$ expression. Most patients present an indolent course but can become symptomatic due to the

development of cytopenia, in particular neutropenia. At last, somatic activating STAT3 mutations were discovered in approximately 30-40% of T-LGLL patients.
Aims: Aim of this study is the characterization of different clinical and biological features of T-LGLL patients through evaluation of clinical, namely the presence of neutropenia, and biological features including phenotypic analysis, the presence of STAT3 mutation, activation and expression of its target Mcl-1.
Methods: In 87 patients with diagnosis of T-LGLL, LGLs were analysed for the presence of neutropenia and by flow cytometry using antibody for CD3, CD4, CD8, CD16, CD56, CD57; DNA samples from these 87 patients were available for STAT3 mutation analysis through Sanger sequencing and PCR ARMS for Y640F and D661Y mutations. Western Blotting analysis for pSTAT3 Tyr 705, STAT3 and Mcl-1 level was performed.
Results: A cohort of 87 patients affected by T-LGLL was studied by FACS analysis; 56/87 (64%) patients were CD3+/CD4-/CD8+ T-LGLL (CD8+ T-LGLL) while 31/87 (36%) patients were CD3+/CD4+/CD8$^{-/dim}$ T-LGLL (CD4+ T-LGLL). STAT3 exon 21 somatic mutation analysis with Sanger sequencing and PCR ARMS analysis was then performed and 30 out of 87 (34%) patients resulted mutated (21 with Y640F, 7 with D661Y, 1 with D661V and 1 with N647I). All mutated patients were in CD3+/CD4-/CD8+ group while all CD3+/CD4+/CD8$^{-/dim}$ patients resulted wild type. Additional FACS analysis with CD16, CD56 and CD57 antibodies was performed in CD3+/CD4-/CD8+ cohort identifying four distinct subgroups: CD16+/CD56-/CD57±subgroup (33/56, 59%), CD16-/CD56-/CD57+ subgroup (5/56, 9%), CD16-/CD56+/CD57+ subgroup (15/56, 27%) and CD16+/CD56+/CD57+ (3/56, 5%). Interestingly, 27/28 mutated patients were in the CD16+/CD56-/CD57±subgroup while only one mutated patient fall in the CD16-/CD56-/CD57+ subgroup. Patients with CD3+/CD8+/CD16+/CD56-/CD57±phenotype presented higher level of pSTAT3 Tyr705 than other CD8+ T-LGLL and CD4+ T-LGLL patients, but not significantly differences in STAT3 and Mcl-1 level were found. Finally, mean ANC (absolute neutrophil count) in CD3+/CD8+/CD16+/CD56-/CD57±was significantly lower than the remaining CD8+ and CD4+ patients (800±547 *vs* 2744±1046, p<0,01).
Summary and Conclusions: In conclusion, a subgroup of patient characterized by high frequency neutropenia, STAT3 exon 21 mutation and STAT3 activation has been identified among the whole population of CD3+/CD8+/CD16+/CD56-/CD57±T-LGLL. Interestingly, no significant differences in STAT3 level and his downstream target Mcl-1 were found among different cell subsets. Taken together, these data may suggest a causal relationship between STAT3 mutation and activation and the development of neutropenia. Further studies are in progress to clarify the pathways involved in this setting.

E1359

STAT5B MUTATION ANALYSIS IN A COHORT OF T-LGL LEUKEMIA PATIENTS

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Background: T-cell large granular lymphocyte (T-LGL) leukemia is a heterogeneous disorder characterized by chronic expansion of cytotoxic T lymphocytes. The World Health Organization (WHO) recognizes within its classification only the indolent T-LGL leukemia (T-LGLL). However, several cases of aggressive T-LGLL have been reported, presenting symptoms of acute illness, hepatosplenomegaly, lymphadenopathy, lymphocytosis, anemia or thrombocytopenia. In these cases leukemic LGLs typically displayed a CD3+/CD8+/CD56+ immunophenotype. Recently, activating STAT mutations have been discovered in T-LGLL patients, STAT3 mutations in 30-40% of T-LGLL indolent patients, while STAT5b mutations were found only in very few cases, but these latters being more frequently detectable in patients with severe clinical course.
Aims: We analyzed STAT5b mutation frequency in a series of 52 patients affected by T-LGLL, with the aim to characterize the associated clinical and biological features.
Methods: Immunophenotypic characterization was obtained by flow cytometer analysis. STAT3 and STAT5b mutations were analyzed by Sanger sequencing. FACSAria cell sorter was used to purify T-LGLs depending on their cell surface markers. T-cell Receptor rearrangement was analyzed on DNA by PCR. Activation of STAT3 and STAT5b was evaluated by Western Blot analysis.
Results: Fifty-one patients showed the classical indolent T-LGLL, with neutropenia, as the relevant clinical feature in 15 patients. One case showed an aggressive clinical course presenting high leucocytosis with a doubling time of 4 weeks, fatigue and moderate hepatosplenomegaly; no neutropenia were present at the diagnosis. Mutation analysis did not reveal any STAT5b mutations in all the indolent T-LGLL. On the other hand, two STAT5b mutations (N642H and Y665F) were demonstrated in the aggressive case of T-LGLL. STAT5b mutations resulted mutually exclusive with STAT3 mutations, reported in 17 cases. By Flow analysis, all the patients with indolent T-LGLL have a LGL clone expressing CD57 antigen. In STAT5b mutated patient proliferating cells were CD3+/CD8+/CD56+/CD57-ve. By selecting for CD16 and CD158b antigens expression in LGLs of this patient, three LGL subsets were recognized, namely a) CD158b+/CD16-, b) CD16+/CD158b- and c) CD16-/CD158b- subsets. Performing mutation analysis on sorted cells, CD158+/CD16- cell subset resulted STAT5b wild type, CD16-/CD158b- subset showed both N642H and Y665F mutations and the small CD16+/CD158- subpopulation carried Y665F mutation. By evaluation of TCR rearrangement, all the three LGL populations were found monoclonally rearranged. As expected, we found that patient's LGLs displayed low level of STAT3 activation and very high level of STAT5b activation as compared to indolent T-LGLL cases, leading to an increased transcription of Bcl-xl.
Summary and Conclusions: In this report we demonstrated for the first time the presence of intraclonal heterogeneity in a patient with aggressive STAT5b mutated LGL leukemia, characterized by one immunodominant STAT5b mutated clone coexisting with additional smaller LGL expansions showing different pattern of STAT5b mutation. The tight link between STAT5b mutations and the aggressive form of T-LGLL supports the importance of STAT5b mutations in the pathogenesis of this disease and indicates a new potential target for the therapy of the aggressive T-LGLL.

E1360

B-CELL CLONALITY IN BONE MARROW IS PREDICTOR OF OUTCOME IN DIFFUSE LARGE B-CELL LYMPHOMA PATIENTS TREATED WITH HIGH-DOSE CHEMOTHERAPY

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Background: Bone marrow involvement (BM) is a factor of poor prognosis for diffuse large B-cell lymphoma (DLBCL). Bone marrow involvement is a poor prognostic factor for patients treated with CHOP-like and with high-dose chemotherapy. Bone marrow involvement in DLBCL by histology is detected in 10-30% patients. The importance of B-cell clonality examination in bone marrow as a prognostic and staging factor has not been yet described in the literature.
Aims: To evaluate the significance of the clonal immunoglobulin heavy chain gene rearrangement analysis performed by PCR for identification of the bone marrow involvement frequency and its value for staging and prognosis in patients with d*e novo* DLBCL treated with high-dose chemotherapy (HDC).
Methods: We performed a retrospective analysis, including 113 adult patients (median age 45 years, range 17-69) with newly diagnosed DLBCL who were enrolled in HDC protocol (mNHL-BFM-90 program or scheme R-DA-EPOCH/R-HMA) since June 2007 till January 2015. 54 of the 113 patients had a GCB and 59-non-GCB phenotype; 23 pts (20%) had low IPI risk, 26 pts (23%) had low-intermediate, 25pts (22%) had high-intermediate, 39 pts (35%) had high risk. B-cell clonality was evaluated using PCR amplification by IGH (FR1, FR2, FR3) and IGK (Vκ-Jκ, Vκ/intron-Kde) gene rearrangements with multiplex BIOMED-2 primer sets and subsequent fragment analysis using ABI PRISM 3130 Genetic Analyzer (Applied Biosystems).
Results: In 28 out of 113 patients, bone marrow involvement (25%) was revealed: in 15 patients bone marrow involvement was identified by histological examination and in 13 patients only by clonality of bone marrow. 13 patients with only B-cell clonality in bone marrow were classified by IPI: 3 (23%) patients had high IPI, 7 (54%) patients-high-intermediate IPI and low-intermediate IPI-3 (23%) patients. So, in 9% patients had to change the risk group according to bone marrow involvement. In group of patients with only B-cell clonality in bone marrow (13 patients), we performed immunohistochemical examination of bone marrow trephine biopsy. In 7 (54%) of 13 cases we detected small number of CD20+ tumor cells in bone marrow, in other 6 cases we didn't prove bone marrow involvement by this examination. Univariate analysis of the entire cohort (n=113) revealed that age, IPI, extranodal involvement and phenotype were not of prognostic significance (p >0.05), but b-cell clonality in bone marrow was (p=0.05). In multivariate analysis, detection of B-cell clonality in bone marrow was an independent predictor of OS and relapse-free survival (RFS) (p ≤0.05). (Figure1A,B). OS, RFS in patients with histological detection bone marrow involvement and with only B-cell clonality in the bone marrow did not differ.

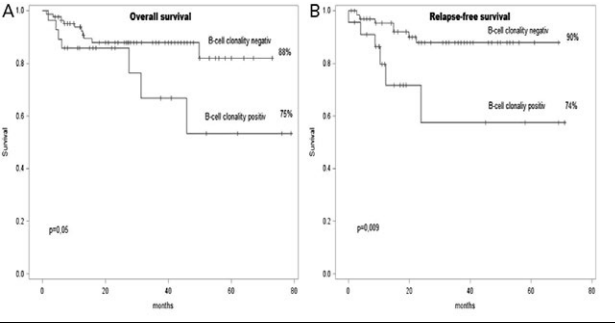

Figure 1.

Summary and Conclusions: Detection of B-cell clonality in the bone marrow seems to be an independent predictor of outcome in *de novo* DLBCL pts, treated with high-dose chemotherapy, while IPI and phenotype DLBCL cannot be considered as risk factors for this group of patients. It seems reasonable to include the detection of bone marrow B-cell clonality at primary diagnostics of the DLBCL for staging and predicting response in HDC.

E1361

SILENCING PROGRESSION OF TUMOR SUPPRESSIVE MICRORNAS ESSENTIALLY CONTRIBUTES TO DEVELOP AGGRESSIVE T-CELL LYMPHOMA

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Background: Multistep genetic alterations including microRNA(miRNA/miR) are occurring during developing aggressive cancer. We have shown that some tumor suppressive miRNAs including miR-150 and miR-16 were diminished in metastatic cutaneous T-cell lymphoma (CTCL), and that miR-150 could inhibit invasion and metastasis of CTCL cells by targeting a chemokine receptor CCR6 (Blood 2014). Furthermore, we recently found that when examined expression of these miRNAs against early and advanced CTCL, miR-150 and miR-16 were decreased during disease progression. These findings suggest that dysregulation of these miRNAs is deeply associated with the pathogenesis of early to advanced CTCL.
Aims: The aim of this study was to determine the possible role of miR-16 and miR-150 in the CTCL progression.
Methods: CTCL cell lines (My-La, HH, MJ, and Hut78) were employed for this experiment. We established a mouse model which died after day 30-35 after transplantation of CTCL cells into NOD/Shi-scid IL-2γnul(NOG) mouse due to tumor cell invasion and metastasis, namely "CTCL mouse". For *in vivo* administration of miRNA, miR-150 and/or miR-16 plus atelocollagen was injected into tail vein of CTCL mice after day14 from CTCL cell transplantation. Injection was conducted by every 5 days.
Results: Firstly we examined *in vivo* transplantation of miR-16, miR-150 stably transduced CTCL cells into NOG mice, and found that not only miR-150 but also miR-16 transduced CTCL cells injected NOG mice showed significant survival extentions than control mice. Interestingly, miR-16 transduced showed stronger tumor inhibition capability than miR-150 transduced, despite of lacking migration-inhibition capability. This result suggests that miR-16 might possess the other tumor suppressive function. Therefore we conducted functional analysis of miR-16, and found that miR-16 could lead to strong cellular senescence against CTCL cells. In miR-16 transduced CTCL cells, p21, which is a key mediator of senescence, was strongly up-regulated in a p53 dependent manner. Because it has been known that Bmi-1 negatively regulates not only p16 but also p21, we examined Bmi-1 expression against miR-16 transduced CTCL cells and found its down-regulation. This result suggested that miR-16 directly regulate Bmi-1 against CTCL cells leading to p21 up-regulation. To examine whether Bmi-1 could directly interact promoter region of *CDKN1A/p21* gene, we conducted cross-linking/chromatin immunoprecipitation (ChIP) assay against CTCL cells, and demonstrated the direct interaction. To examine whether miR-150 and/or miR-16 could lead tumor inhibition *in vivo*, we conducted intravenous administration of these miRNAs against CTCL mice. Though administration of either miR-16 or miR-150 extended survival of CTCL mice, use of both miR-150 and miR-16 yielded to the most survival extension because miR-150 and miR-16 cooperatively can inhibit distinct tumorigenic cascades of CTCL. Finally, we found that a histone deacetylase inhibitor, vorinostat/SAHA, led CTCL cells to induce senescence and migration inhibition with restoration of both miR-150 and miR-16, and their target proteins.
Summary and Conclusions: miR-150 and miR-16 collaborate to inhibit tumorigenic potential via down-regulating distinct tumor-associated cascades. Our findings suggest that miR-150 and miR-16 could be, at least in part, epigenetically silenced during disease progression and their restoration as well as vorinostat treatment could be key therapeutic strategy against CTCL.

E1362

AKT GENE EXPRESSION IS REGULATED BY THE AP-1 TRANSCRIPTION FACTORS JUNB AND CJUN IN ALK+ ANAPLASTIC LARGE CELL LYMPHOMA (ALCL): A NOVEL CROSSTALK MECHANISM

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Background: Anaplastic lymphoma kinase-positive (ALK+) anaplastic large cell lymphoma (ALCL) is an aggressive T-cell non-Hodgkin lymphoma type characterized by the t(2;5) resulting in overexpression and activation of the NPM-ALK oncogenic kinase. The NPM-ALK is known to activate multiple signaling pathways including the phosphatidylinositol-3-kinase (PI3K)/AKT/mTOR and the JAK/STAT3 pathways resulting in cell cycle and apoptosis deregulation. ALK+ ALCL is also characterized by strong AP-1 activity and overexpression of two AP-1 transcription factors, CJUN and JUNB, which are upregulated by NPM-ALK, and further contribute to ALCL oncogenesis.
Aims: We hypothesized that a biologic link between AP-1 activity and AKT kinase may exist, which operates in ALK+ ALCL. Therefore, the purpose of this study was to identify specific interactions of two AP-1 transcription factors, JunB and cJun, with AKT1 gene promoter in ALK+ ALCL and in non cancerous cell systems. In addition the biologic effects of AP-1 transcriptional control on AKT gene were analyzed.
Methods: Three ALK+ ALCL cell lines (Karpas 299, SR-786 and SUP-M2), as well as Jurkat and HEK293T cells were used in the study. AKT1 gene promoter was analyzed using chromatin immunoprecipitation (ChIP), site-directed mutagenesis, gene reporter assays and transient transfections. JunB and cJun genes were knocked down using specific siRNA constructs. Quantitative real-time RT-PCR and Western blotting were used to assess RNA and protein levels. Cell proliferation, cell viability and colony formation assays were also applied following gene silencing or pharmacologic inhibition studies.
Results: Using ChIP, reporter assays and site-directed mutagenesis, we show that JUNB and cJUN bind directly to the *AKT1* promoter at specific AP-1 binding sites, thus inducing *AKT1* transcription in ALK+ ALCL. Knockdown of JUNB and CJUN in ALK+ ALCL cell lines downregulated *AKT1* mRNA and promoter activity and was associated with lower AKT1 protein expression and activation. Of note, JUNB and CJUN seem to cooperate for the transcriptional regulation of AKT1 gene. We also provide evidence that this is a transcriptional control mechanism shared by other cell types even though it may operate in a way that is cell context specific. In addition, STAT3-induced control of *AKT1* transcription was functional in ALK+ ALCL and blocking of STAT3 and AP-1 signaling synergistically affected cell proliferation and colony formation.
Summary and Conclusions: Our findings uncover a novel transcriptional crosstalk mechanism that links AP-1 and AKT kinase, which coordinate uncontrolled cell proliferation and survival in ALK+ ALCL. These mechanisms may confer resistance to chemotherapy.

E1363

IDENTIFICATION OF LEUKEMIC STEM CELL CANDIDATES IN AN ATL MOUSE MODEL OF HBZ TRANSGENIC MICE

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Background: Adult T-cell leukemia (ATL) is a malignant disease caused by infection with human T-lymphotropic virus type 1 (HTLV-1). Only about 2-5% of HTLV-1-infected patients will develop ATL, and the response to chemotherapy is extremely poor because of intrinsic or acquired drug resistance. Thus, we hypothesized the existence of chemotherapy resistant leukemic stem cells (LSCs) in ATL. Recently, LSCs with characteristics of self-renewal, tumorigenicity, multipotency, asymmetric division, and drug- resistance were identified and characterized in various types of solid tumors and leukemias. We have previously identified ATL stem cell candidates in an ATL model using Tax transgenic (Tax-Tg) mice (Yamazaki *et al.*, Blood 2009). It has been shown that the HTLV Tax protein is crucial to initiate malignant transformation and spreading of ATL. Recently, HTLV-1 bZIP factor (HBZ) was also identified as an important factor in the development of ATL. HBZ transgenic (HBZ-Tg) mice also show ATL-like lymphoma/ leukemia and are thought to be an ATL model mouse (Satou *et al.*, PLoS Pathog, 2011).
Aims: We aimed to identify and characterize ATL stem cells in the HBZ-Tg mouse and compare their properties with ATL stem cells in the Tax-Tg mouse to clarify the clonal evolution of ATL stem cells and molecular mechanisms underlying ATL development and drug resistance.
Methods: We isolated and characterized splenic lymphomatous cells (named Ht48) derived from HBZ-Tg mice, transplanted 1×10^7 Ht48 cells intraperitoneally into C57BL/6 mice to assess tumorigenicity. Then, we performed forescence activated cell sorting (FACS) of Ht48 cells, and tested proliferative activity both *in vivo* and *in vitro* analyseslu.
Results: The transplanted mice showed massive splenomegaly and died within 25 days. The ATL cells had infiltrated into multiple tissues such as spleen, liver, and ovary. To evaluate serial transplantability of Ht48 cells, we performed nine consecutive serial transplantations. Ht48 cells regenerated the ATL-like disease and regained the original phenotypes ($CD4^-CD8^-/CD4^-CD8^+/CD4^+CD8^+$) after each transplantation. Next, we performed side population (SP) analyses to identify drug resistance. SP cells with high drug

resistance existed among Ht48 cells. Interestedly, more than 40% of c-kit+ cells were enriched with SP cells, while less than 5% of c-kit+ cells compared the other population. As expected, both SP and c-kit+ cells showed a high proliferative activity *in vitro* and *in vivo*, and these cells could differentiate into all lineages of ATL cells *in vivo*. These results suggest that c-kit expression is important to maintain ATL stem cell functions such as drug resistance and high tumorigenicity in Ht48 cells. To characterize ATL stem cells in detail, we divided c-kit+ cell fractions into four subpopulations and cultured the cells for six days to clarify which population of c-kit+ cells are LSCs. We found c-kit+/CD4-/CD8- cells possessed higher proliferative activity than any other subpopulation. In addition, a neutralizing antibody against c-kit ligand (SCF) inhibited ATL stem cell proliferation *in vitro*. ATL stem cell candidates gave rise to all cell types in Ht48 cells *in vivo*. These data indicated that ATL stem cells require an extrinsic signal from the niche to differentiate into all types of ATL cells. Finally, we performed comprehensive gene expression analyses of ATL stem cells in both HBZ- and Tax-Tg mice and identified some candidate genes that can trigger and maintain LSC development.
Summary and Conclusions: We identified ATL stem cell candidates in HBZ-Tg mice, and c-kit plays a key role in maintenance of LSCs and tumor development in a mouse model of ATL.

E1364

A NEW MOUSE MODEL FOR MATURE B-CELL LYMPHOMA REVEALING REQUIREMENT FOR FAS DOWNREGULATION IN LYMPHOMAGENESIS
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Background: Non-Hodgkin's lymphoma (NHL) mostly includes mature B-cell lymphomas, such as Burkitt lymphoma (BL), diffuse large cell lymphoma (DLBCL), and follicular lymphomas, which are all derived from germinal-center B (GCB) cells. Despite recent advances in the drug development for lymphoma, there are still patients with refractory and relapsed lymphoma. To analyze the detailed molecular mechanisms during lymphomagenesis, we require appropriate mouse models, with short latency, in which monitoring the whole process from the cell of origin to full-blown lymphoma is easy.
Aims: To analyze the factors involved in mature B-cell lymphomagenesis, we attempted to develop a new mouse model for lymphoma, originating from GCB cells.
Methods: Naive B-cells derived from C57BL/6 wild type (WT) or *Ink4a/Arf-/-* mice were cultured *ex vivo* and stimulated with IL-4 and anti-CD40 antibody to differentiate into GCB cells. GCB cells were retrovirally transduced with *c-Myc*, which is highly expressed in most human lymphomas, and then transplanted into sublethally irradiated syngeneic mice. Primary cultured lymphoma cells were treated with the reagents described below. Human NHL cell lines including 4 Burkitt lymphoma (Daudi, Ramos, Raji, and Namalwa) and 9 DLBCL (DB, Toledo, OCI-Ly19, WSU-DLCL2, SU-DHL6, NU-DHL1, NU-DUL1, OCI-Ly3, and OCI-Ly10) were used.
Results: Approximately 60% of mice transplanted with *Ink4a/Arf-/-* derived *c-Myc*-GCB cells, via intraperitoneal injection, exhibited splenomegaly and enlarged lymph nodes in the axilla, inguinal, and intestinal regions, and eventually died. Mice transplanted with WT derived *c-Myc*-GCB cells did not show any signs of lymphoma. Tissues from these mice showed mature B-cell immunophenotype (B220+IgM+IgD+) and were histopathologically similar to human BL. Remarkably, lymphoma cells from all mice did not show Fas expression at protein and mRNA levels in contrast to the original GCB cells that showed high Fas expression. Furthermore, lymphoma cells, which spontaneously developed in all λ-Myc mice (a transgenic mouse carrying *c-Myc* under the control of the Igλ light chain enhancer), showed no Fas expression. Fas is a cell death receptor which has an important role in negative selection of GCB cells, with self-reactive antibody and low-affinity antibody. To analyze the role of Fas downregulation in lymphoma formation, *Ink4a/Arf-/-* GCB cells were transduced with *c-Myc* and shRNA for Fas and then transplanted. All mice transplanted with shFas-transduced GCB cells developed lymphoma and died faster than mice transplanted with shControl-transduced GCB cells. To further analyze Fas regulation in lymphoma cells, we carried out primary culture of lymphoma cells. Fas expression was restored after CD40 ligand or anti-CD40 antibody treatments, suggesting that reduced CD40 signaling may be one of the reasons for Fas downregulation. Further, we found that 11 out of 13 human lymphoma cell lines, including 3 BL and 8 DLBCL, exhibited low expression of Fas whereas 2 cell lines exhibited high expression of Fas that induced apoptosis upon Fas ligand treatment. Among those Fas-downregulated cells, Fas expression was restored not only after CD40 ligand treatment, but also after a DNA methyltransferase inhibitor 5-Aza-dC treatment or an HDAC-inhibitor TSA treatment, indicating that Fas expression is also regulated by epigenetic mechanisms in human lymphoma cells.
Summary and Conclusions: The findings in this study suggest that Fas downregulation is a crucial event for mature B-cell lymphomagenesis to escape from immune surveillance and that restoration of Fas expression, such as anti-CD40 antibody treatment or epigenetic inhibitors, may be a promising strategy for NHL treatment.

E1365

MOLECULAR DIAGNOSIS BY GENE EXPRESSION PROFILING IN FORMALIN FIXED PARAFFIN EMBEDDED TISSUE-BURKITT LYMPHOMAS WITH EXPRESSION OF BCL2
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Background: Mitochondrial membrane protein BCL2 is widely expressed in many lymphoma entities but rarely in Burkitt lymphoma (BL). Expression of BCL2 can be considered as an indicator of diffuse large B cell lymphoma (DLBCL) where morphology is unclear. Co-expression of BCL2 and MYC has been reported to be an indicator of poor prognosis in DLBCL. Occasionally BCL2 expression occurs in BL and in lymphomas with features intermediate between BL and DLBCL (intermediates). Gene expression profiling (GEP) has helped to define the molecular features of BL (mBL). However, only few BCL2-positive BL have been assessed by GEP in order to elucidate if their molecular pattern shifts to more intermediate or DLBCL profiles.
Aims: To evaluate applicability of our recently developed BL and DLBCL molecular classifier using formalin fixed and paraffin embedded (FFPE) tissue on diagnostically ambiguous cases. To understand to what extent BCL2 expression is compatible with the diagnosis mBL. To assess the prognostic value of BCL2 expression in BL.
Methods: A cohort of 154 BL diagnosed by morphology, immunophenotype and fluorescence *in situ* hybridization (FISH) for *MYC* and t(8;14)/*MYC-IGH* translocations was analyzed for BCL2 expression by immunohistochemistry using two different antibodies. These constructed a tissue microarray (TMA).
A group of 17 lymphomas (age range 3-24, median 11 years of age) with the morphological an immunophenotypical features of BL but BCL2 expression (5% >90% in lesion) were additionally labelled by GEP using RNA from FFPE material. In 5 cases BCL2-positive and BCL2-negative areas of the biopsy block were macrodissected and analyzed separately. To assess if BCL2 expression in mBL correlated with poorer prognosis 43 further BL cases registered within the NHL-BFM database were compared to the initial BCL2-positive mBL cohort for clinical variables including outcome.
Results: In the TMA cohort BCL2 expression was detected in 21,3% (33/150) of the cases. No differences in clinical features and outcome were detected between BCL2-positive and BCL2-negative BL in 43 patients treated according to paediatric NHL-BFM protocols. Using GEP, 13/17 (76,5%) cases were classified as mBL and 4/17 (23,5%) as intermediates. The 5 lymphomas with spatial differences in BCL2 expression classified as mBL in both, BCL2-positive and BCL2-negative areas. In this group of predominantly paediatric patients, none of the 4 intermediate cases relapsed according to clinical follow-up.
Summary and Conclusions: Molecular classification indicates that lymphomas with the clinical and pathological features of BL but expression of BCL2 display molecular profiles compatible with mBL. BCL2 expression is not associated with inferior survival in pediatric BL. Diagnostically ambiguous lymphomas by histopathology and FISH can be reliably labelled by our GEP-based aggressive lymphoma classifier in FFPE specimens.

E1366

PROTEIN KINASE CK2 IN DIFFUSE LARGE B-CELL LYMPHOMA: DEFINING ITS ROLE TO SHAPE NEW THERAPIES
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Background: CK2 is a serine-threonine protein kinase formed by the assembly in a tetramer of two catalytic (a) and two regulatory (b) subunits. This enzyme regulates a wide variety of cellular processes, including cell proliferation, differentiation and survival. It is overexpressed and overactive in several solid tumors and hematologic malignancies and promotes a "non-oncogene addiction" phenotype in cancer cells. Our and other groups have shown that B cell tumors, such as chronic lymphocytic leukemia, mantle cell lymphoma and multiple myeloma, rely on high activity of CK2 and are extremely sensitive to the cytotoxic effect of CK2 inhibition induced either by gene silencing or with the clinical grade compound CX-4945 (Silmitasertib). It is well known that the strength of the B cell receptor (BCR) signaling influences mature B cell commitment and survival, and is a powerful determinant of B cell derived lymphoma cell growth. CK2, through a "lateral" regulation of BCR-dependent signaling cascades, could be involved in normal and malignant BCR-elicited signaling. Indeed, B cell specific CK2b knockout mice, generated in our laboratory, display a marked reduction of follicular B cells (FoB), suggesting a critical role of this kinase in FoB cell development and maintenance.
Aims: We culled to investigate CK2 levels and activity in diffuse large B-cell lymphoma (DLBCL), an aggressive germinal center derived B cell tumor. Further-

more, we intended to study the potential of combining CK2 inhibition with the BCR blockade as a therapeutic option for this neoplasia.
Methods: Immunohistochemistry was performed on 4 µm-thick formalin-fixed and paraffin-embedded sections. To inhibit CK2 we employed CX-4945, a selective ATP-competitive compound currently under scrutiny in phase I clinical trials. To evaluate protein changes after treatments, cells were lysed and Western Blot experiments carried out. To assess cell survival, MTT tests and AV/PI staining were performed. Combination Index (CI) was calculated in accordance with the Chou and Talalay method. To stimulate the BCR aIgM or aIgG antibodies were used. Acquisition was done with FACS Canto and analysis with FlowJo software.
Results: CK2 was found to be over expressed in a series of primary samples of activated B-cell-like (ABC) and germinal center B-cell-like (GCB) DLBCL and in ABC- and GCB-DLBCL cell lines as compared to normal counterparts. CK2 inhibition with CX-4945 caused apoptosis of both types of DLBCL cell lines in a dose dependent fashion even at low concentrations not toxic to normal B lymphocytes. Moreover, CX-4945 boosted the effects of Fostamatinib, an effective BCR signaling inhibitor, increasing malignant B cell death. Also, the downregulation of CK2 catalytic activity led to a reduction in Ca^{++} release from endoplasmic reticulum (ER) stores and impaired AKT activation after BCR stimulation.
Summary and Conclusions: Our study shows that CK2 levels and activity are abnormally high in DLBCL, and its inhibition causes strong apoptosis of lymphoma cells. CK2 is likely to lie downstream from chronic active and tonic BCR signaling, presumably controlling AKT activation and Ca^{++} release from ER stores, two critical events regulating B cell activation, survival and expansion. These findings suggest that CK2 inhibition could target multiple sites of the BCR-dependent pro-survival cascade, and could pave the way for the inclusion of CX-4945 in rational drug combination therapies with BCR pathway inhibitors such as Ibrutinib and Fostamatinib that are currently under clinical trials in DLBCL patients.

E1367

INHIBITORS OF MTOR/PI3K/AKT PATHWAY EXTEND THERAPEUTIC OPPORTUNITY IN WALDENSTRÖMS MACROGLOBULINEMIA CELLS THAT DISPLAY RESISTANCE TO BOTH ABT-199 AND IBRUTINIB.
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Background: Recent investigations support a role for constitutively active B-cell receptor (BCR), and Toll-like receptor (TLR) signaling in the pathogenesis of Waldenström macroglobulinemia (WM), a rare and indolent B cell disorder. Clinically, mitigation of BCR signaling with the first in class BTK inhibitor, ibrutinib, has shown promising results. However, clinical responses to ibrutinib alone are less than optimal and development of ibrutinib-resistant disease is being recognized as an important and impending dilemma. It has also been shown that Bcl-2 family of anti-apoptotic proteins supports BCR/TLR induced proliferation and survival of WM cells by prevention of apoptosis. This defective apoptotic signaling is associated with aggressive clinical behavior, chemoresistance, and a poor prognosis in many B-cell cancers, including WM. We have previously shown the utility of disrupting pro-apoptotic Bcl-2 protein interactions in WM, chronic lymphocytic leukemia and multiple myeloma and that this functional interference results in re-sensitization (or heightened sensitivity) of tumor cells towards chemoimmunotherapy. As such, we examined the effects of ABT-199, a Bcl-2 specific small molecule inhibitor; in preclinical models of WM and found these cells to be sensitive to ABT-199; an observation, which echoes early clinical efficacy noted in WM patients part of a phase 1 clinical trial. As resistance to ibrutinib is being noted in patients and early reports on ABT-199 suggest that resistance to its activity is imminent, we established 1.) WM models resistant to ibrutinib, 2.) WM models resistant to ABT-199 and 3.) WM models resistant to both ibrutinib and ABT-199. These models were then used to delineate common survival pathways that may lend therapeutic cross-sensitivity in cells resistant to ibrutinib and/or ABT-199.
Aims: To evaluate the effect of mTOR/PI3K/AKT inhibitors on WM cells resistant to Ibrutinib and ABT-199.
Materials and Methods: WM cell lines (BCWM.1, MWCL.1 and RPCI-WM1; WT-WM cells) including their ibrutinib resistant (IR-WM), ABT-199 resistant (ABTR-WM) or dual-ibrutinib/ABT-199 resistant (dual resistant WM) clones were developed in the laboratory and used in this study.
Results: A comparison of expression pattern of Bcl-2 family members, Bcl-2, Mcl-1, BCL-xL, A1 (anti-apoptotic members), Noxa, Puma, Bim (pro-apoptotic initiators) and Bak and Bax (pro-apoptotic effectors), revealed that IR-WM cells and ABTR-WM cells showed differential expression of Bcl-2 family members. Cell growth (MTS) assays using ABT-199 indicated that IR-WM cells remain sensitive to ABT-199 with an IC_{50} ranging between 2-4uM. ABT-199 induced a dose dependent increase in MOMP with concomitant induction of apoptotic cell death in IR-WM cells, the latter confirmed by observation of PARP-1 cleavage. ABT-199 induced intrinsic apoptosis with the activation of caspase 9 and caspase 3 in all the cells tested. This data indicated the Bcl-2 pathway as being relevant and essential in IR-WM cell lines. Further, combined addition of ABT-199 with ibrutinib showed synergistic cell death (as calculated using CalcuSyn software, Biosoft UK) in IR-WM cells. However, prolonged and incremental exposure to ABT-199 in 1.) WT-WM cell lines and 2.) in IR-WM cell lines resulted in outgrowth of tumor clones that displayed resistance to both ibrutinib and ABT-199. Interestingly, these drug resistant (IR-WM, ABTR-WM and dual-resistant) WM clones showed constitutive activation of AKT. As such, we tested multiple inhibitors of the mTOR/PI3K/AKT pathway (everolimus, LY294002 or MK2206) and observed a dose and time dependent cytotoxicity in the aforementioned drug-resistant WM models.
Summary and Conclusions: Drug resistance in WM as in other B-cell cancers is inevitable despite combination therapy and can result in development of tumor clones that are cross resistant to multiple drugs. However, even in this complex biological dynamic, these drug-resistant clones fortuitously may shift survival signaling towards an alternative (but common) pathway, lending therapeutic cross-sensitivity. Data from our ibrutinib, ABT-199 or dual-resistant preclinical models affirms this notion and suggests these cells common preference for AKT-mediated survival signaling when continual stress from ibrutinib and/or ABT-199 is applied.

E1368

HIGH THROUGHPUT *IN VITRO* COMBINATION SENSITIVITY SCREEN IN HEMATOLOGIC MALIGNANCIES WITH DUVELISIB, A DUAL PI3K-DELTA,GAMMA INHIBITOR
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Background: Phosphoinositide-3 kinases (PI3Ks) are key cellular signaling proteins that act as a central node for relaying signals from cell-surface receptors to downstream mediators. PI3K-δ and PI3K-γ isoforms are expressed in normal leukocytes, as well as many hematological malignancies such as indolent non-Hodgkin lymphoma (iNHL) and chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL). Given the key role for PI3K-δ and PI3K-γ in immune cell function, targeting these isoforms may provide opportunities to develop differentiated therapies for the treatment of hematologic malignancies. Duvelisib (IPI-145) is an oral dual inhibitor of PI3K-δ and PI3K-γ currently in clinical development in hematologic malignancies.
Aims: In order to gain mechanistic insights into the cellular response to duvelisib and identify novel combination pairings for duvelisib in a broad spectrum of hematologic malignancies, a high-throughput pharmacology screen was conducted. *In vivo* combination validation studies were also performed.
Methods: Duvelisib was evaluated alone and in combination with 35 compounds comprising a diverse panel of standard-of-care agents and emerging drugs in development for hematologic malignancies. Target and pathway inhibitor redundancy was included to observe similar patterns of activity across the cell line panel. These compounds were tested in 20 cell lines including diffuse large B-cell (DLBCL), follicular, T-cell, and mantle cell lymphomas, and multiple myeloma. Growth inhibition (GI) was measured by ATPLite (Perkin Elmer) in a 6x6 or 9x9 dose combination matrix.
Results: Single agent activity was seen in 14 cell lines treated with duvelisib, with a median GI50 of 0.59 µM. A scalar measure of the strength of synergistic drug interactions (Synergy Score) was devised and filtering on scores exceeding the mean self-cross plus twice the standard deviation revealed a synergy hit rate of 19.3% across the matrix of drug combinations and cell lines. Synergy was most prominent in DLBCL and follicular lymphoma cell lines and seen with approved and emerging drugs used to treat hematologic malignancies, including, but not limited to, dexamethasone, inhibitors of the B-cell receptor signaling pathway, such as ibrutinib, and the BCL-2 inhibitor venetoclax (ABT-199). Synergy patterns were similar among agents that target the same molecules or pathways helping to validate activities seen in combination with duvelisib. In select cell line GI studies and *in vivo* DoHH2 lymphoma murinexenograft models, the combination of selective PI3K-δ and PI3K-γ inhibitors showed enhanced effects compared to either inhibitor alone. Combination effects with select drugs and duvelisib were also seen in DoHH2 murinexenografts.
Summary and Conclusions: These studies support enhanced activity of combined PI3K-δ and PI3K-γ inhibition in lymphoma models and identified synergistic pairings with the PI3K-δ and PI3K-γ inhibitor, duvelisib. These results provide a rationale for exploring the combination of duvelisib and other therapeutic agents in clinical studies.

E1369

Abstract withdrawn

E1370

ARTISAN PCR: HIGHLY RELIABLE IDENTIFICATION OF FULL-LENGTH B-CELL RECEPTOR SEQUENCES
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Background: Full-length, error-free B-cell receptor (BCR) sequences are

instrumental to study B-cell function in health, autoimmunity, and lymphoma. Current amplification strategies are usually based on multiplex primers annealing to conserved upstream V segment regions. These primers obscure mutations at their binding site and are prone to amplification bias or even complete amplification failure (Koning *et al.*, 2014). We designed an alternative assay employing Anchoring Reverse Transcription of Immunoreceptor Sequences and Amplification by Nested PCR (ARTISAN PCR).

Aims: To demonstrate improved success rate of full-length BCR identification by ARTISAN PCR compared to multiplex strategies.

Methods: 39 EBV-transformed lymphoblastoid cell lines (LCL) were selected for clonality by flow cytometry. In addition, biopsy material (peripheral blood, bone marrow, lymph node, spleen, pleura) from 24 patients diagnosed with either chronic lymphocytic leukemia, monoclonal B-cell lymphocytosis, Waldenström's macroglobulinemia, multiple myeloma, follicular lymphoma, mantle cell lymphoma, marginal zone lymphoma or Burkitt's lymphoma (3 each) was selected. Poly(A)-RNA was isolated (Dynabeads; Invitrogen) and subjected to 5'RACE cDNA synthesis (Smartscribe, Clontech) with gene-specific primers that anneal to the Ig constant region of interest. Anchor-tagged cDNA was PCR-amplified with an anchor-specific forward primer and nested constant region-specific reverse primers. Nested barcoding primers further increased target specificity and enabled the combination of numerous PCR products in one sequencing run. For comparison, cDNA was amplified by conventional multiplex PCR (IGH FR1, IGK A, and IGL Clonality Assays, Invivoscribe) utilizing generic primers designed to anneal to V and J regions. EBV LCL amplicon libraries were sequenced on the PacBio platform (Pacific Biosciences) at a minimum of 8 cycles. Lymphoma biopsy amplicons were cloned and sequenced by Sanger sequencing.

Results: ARTISAN PCR on the 39 LCL yielded 52 clonal VDJ, 22 VJ-κ, and 39 VJ-λ sequences. 10 VJ amplicons were unproductive. In total, functional complete BCRs were readily identified for all LCL, of which 27 were identified as monoclonal and 12 as biclonal. By the Invivoscribe protocol, 6 VDJ, 1 VJ-κ, and 10 VJ-λ were missed, corresponding to success rates of 88%, 95%, and 74%, respectively, compared to ARTISAN PCR. Complete BCRs were identified for 38 of 51 (75%) LCL B-cell clones by Invivoscribe. ARTISAN PCR identified a complete functional clonal BCR in all 24 lymphoma biopsies (7 IgM-κ, 13 IgM-λ, 2 IgA-λ 2 IgG-κ). With the Invivoscribe approach, a complete functional clonal BCR was identified in 13 cases (54%). In 4 cases, no VDJ could be identified, and in 8 cases, no functional VJ. Neither in the LCL nor in the lymphoma biopsies was a clonal amplicon found by the Invivoscribe assays that was missed by ARTISAN PCR. Sequence analysis of the BCR obtained by ARTISAN PCR revealed individual reasons for each instance of Invivoscribe assay failure. In 19 instances, mutations in V or J primer binding sites led to deleterious mismatches. In 9 cases, lack of a primer for the particular lambda V allele family prevented VJ identification. In one case, a truncation obliterated the FR1 primer binding site. The cumulative error rate (cDNA synthesis, PCR, sequencing) of ARTISAN PCR was $1{,}26 \times 10^{-4}$ in the LCL panel at a minimum of 8 cycles of PacBio sequencing.

Summary and Conclusions: ARTISAN PCR permits reliable high-throughput identification of full-length BCR transcripts and proves vastly superior to a commercial assay based on multiplex primers.

E1371

NFKBIE MUTATIONS OCCUR IN 15% OF GCB DLBCL AND IN VARIOUS OTHER LYMPHOID MALIGNANCIES

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Background: Massively parallel sequencing technology in chronic lymphocytic leukemia (CLL) has recently identified acquired recurrent somatic mutations in the gene *NF-κB inhibitor I kappa B epsilon* (*NFKBIE*), a retrocontrol of NF-κB signaling. NF-κB activity is a hallmark of various haematological malignancies, and activating mutations in NF-κB transcription factors or upstream signaling components such as CD79b, CARD11, or TNFAIP3/A20 are common findings in lymphoid neoplasms. However, little is known regarding the prevalence of *NFKBIE* mutations in lymphoid malignancies other than CLL.

Aims: Since gene mutations are rarely specific of a given tumor entity and those identified in a given lymphoid malignancy are frequently found in other diseases, we aimed to assess the presence and frequency of *NFKBIE* mutations in a larger cohort of patients with lymphoid malignancies and to evaluate the range of mutational burden.

Methods: We investigated the mutational hotspot of *NFKBIE* in a series of 623 patients with lymphoid neoplasms. 113 chronic lymphocytic leukemias (CLL), 170 diffuse large B-cell lymphomas (DLBCL), 179 follicular lymphomas (FL), 57 mantle cell lymphomas (MCL), 94 T-cell acute lymphoblastic leukemias, 9 small lymphocytic lymphomas (SLL), and 1 mucosa-associated lymphoid tissue (MALT) lymphoma were analyzed by Sanger sequencing or targeted deep sequencing.

Results: Together, frameshift mutations in *NFKBIE* were detected in 24 out of 623 (3.9%) investigated patients. While *NFKBIE* mutations were rare in FL (3/179=1.7%), MCL (1/57=1.8%), and T-ALL (1/94=1.1%) patients, a higher frequency was detected in the CLL (6/113=5.3%), SLL (1/9=11.1%), and DLBCL cohorts (11/170=6.5%). Furthermore, the only patient with a MALT lymphoma in this series harbored a *NFKBIE* mutation. For 118 DLBCL patients, information was available on the cell-of-origin subtype derived from the immunohistochemistry-based Hans classification. We found a high frequency of *NFKBIE* mutations in the subgroup with germinal center B-cell-like (GCB) DLBCL (9/59=15.3%), whereas only one of the 59 Non-GCB DLBCLs showed a *NFKBIE* mutation (1/59=1.7%; *P*=0.017 GCB *vs* Non-GCB in Fisher's exact test). Next, we evaluated the mutation burden of 12 *NFKBIE* mutated lymphoma patients (6 CLL and 6 DLBCL based on DNA availability) by deep sequencing. The mutation burden varied from 8% to 47% (mean coverage of 1260 reads per patient sample). These data indicate that *NFKBIE* mutations may be part of both major and subclone variations.

Summary and Conclusions: In summary, we show a 3.9% prevalence of *NFKBIE* mutations in a large set of 623 patients with lymphoid malignancies. *NFKBIE* mutations were most frequently found in CLL, SLL and DLBCL patients. We further identified GCB DLBCL to harbor the highest prevalence of *NFKBIE* mutations, reaching a frequency of 15.4%.

E1372

NK CELLS CYTOTOXICITY IS AFFECTED BY VARIOUS BTK INHIBITORS

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Background: Targeting Bruton's tyrosine kinase, due to a huge therapeutic potential of ibrutinib, is becoming an attractive therapeutic approach in the treatment of hematological malignancies derived from B-cells. However, we have demonstrated that NK cell cytotoxicity, degranulation and cytokine secretion are significantly impaired upon ibrutinib treatment (Bojarczuk *et al.*, Leukemia 2014). Since NK cells are effectors in antibody dependent cell-mediated cytotoxicity (ADCC), which constitute one of the major mechanisms of anti-CD20 monoclonal antibodies widely used in hematooncology, in our ongoing studies we are focused to determine in details the influence of various BTK inhibitors on NK cells cytotoxicity, degranulation, cytokine expression and expression of activatory NK cells receptors.

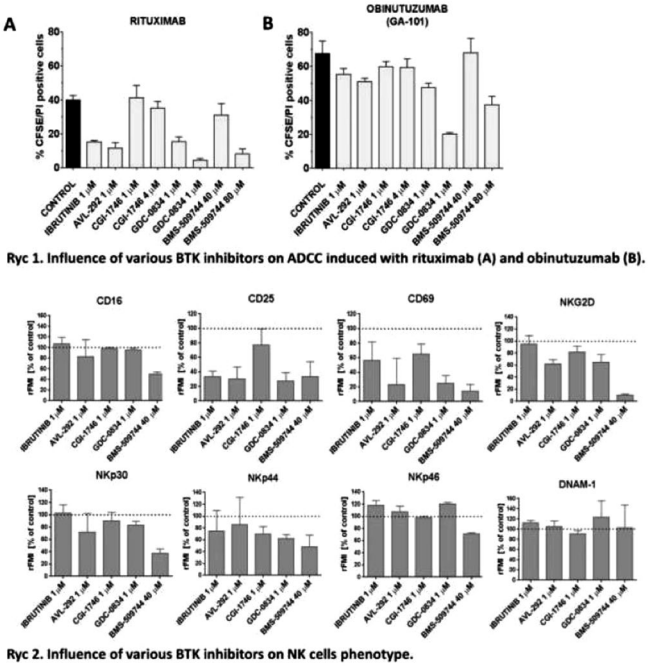

Figure 1.

Aims: The aim of the studies is to elucidate the influence of various BTK inhibitors on the antitumor activity and phenotype of primary NK cells isolated from blood samples of healthy volunteers as well as patients with leukemia and lymphomas.
Methods: All experiments were performed fully *in vitro* using human primary NK cells isolated from PBMC of healthy donors and NK cells from patients with B-cell derived tumors. ADCC was determined using anti-CD20 monoclonal antibodies (rituximab, ofatumumab, obinutuzumab). To determine cytolytic activity of NK cells we used CFSE/PI flow cytometry assay and/or CD19 and PI staining to determine absolute counts of B cells. Cytokine secretion and NK cells degranulation evaluated as the expression of CD107a on the surface of NK cells were determined with flow cytometry upon incubation with target cells for 4 h. Phenotype of NK cells pre-incubated with tested compounds was determined with flow cytometry using antibodies conjugated with fluorochromes.
Results: The initial results of the studies show that various BTK inhibitors differentially regulate NK cells antitumor activity and affect phenotype of NK cells. Interestingly, obinutuzumab-induced ADCC is not inhibited in the presence of BTK inhibitors in contrast to type I anti-CD20 monoclonal antibodies (Figure 1).
Summary and Conclusions: Our studies are focused on better understanding how newly approved inhibitors influence NK cells antitumor functions. This knowledge is especially important in light of combination therapies when monoclonal antibodies are used with small molecule inhibitors. On the basis of our observations we conclude that combining obinutuzumab with BTK inhibitors is a better therapeutic strategy than simultaneous combination of type I anti-CD20 antibodies and BTK inhibitors.

E1373

VDJH USAGE IN TRANSFORMED FOLLICULAR LYMPHOMA

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Background: Follicular lymphoma (FL) is the second most frequent non-Hodgkin lymphoma in western countries. Transformation into aggressive lymphoma continues being one of the worst events in the disease history. There is a growing interest in connecting new approaches into the biological basis of transformation and clinical application. IgH gene rearrangements in B lymphoproliferative disorders have identified the preferential use of certain VDJH gene segments, correlated in some cases with clinical prognosis. In this aspect, transformed follicular lymphoma (tFL) has not been yet studied.
Aims: To study the IGHV gene usage and clinical features in patients diagnosed of tFL and to compare with FLs that do not undergo transformation, as well as with diffuse large B-cell lymphoma (DLBCL), classified as GCB and non-GCB by immunostaining according to Hans algorithm.
Methods: A total of 213 non-Hodgkin lymphoma (NHL) patients from a single centre were included in the study, distributed in three groups: i) tFL patients (n=32), most of them with paired samples at diagnosis and transformation (n=25, 78%); ii) FL with no documented transformation at a median follow up of 66 months (n=74); and iii) DLBCL patients (n=107; 44% GCB, and 56% non-GCB). Clonal IGH rearrangements were amplified according to the BIOMED-2 protocol and PCR products were sequenced. Germline IGH genes were identified using IMGT/V-QUEST database. Statistical comparisons were performed using SPSS 20.0.
Results: Complete VDJH rearrangements were identified in 31 out of 32 tFL patients (97%). In addition, other case was discarded due to the identification of a different clone, and it was recorded as secondary NHL. A bias in the IGHV gene usage was observed in tFL patients, with IGHV3-23 (20%), IGHV3-48 (17%), and IGHV4-34 (17%) genes accounting for 54% of the cohort. These genes are common in both FL and DLBCL. However, and despite differences were no statistical significant, a higher frequency of IGHV3-48 was shown in tFL than in DLBCL (22% *vs* 6%), while IGHV4-34 showed higher frequency in tFL than in FL (19% *vs* 8%). Moreover, IGHV4-34 was found only in the non-GCB DLBCL subgroup. Regarding the clinical outcome, the average time to transformation of tFL is 54 months (range 4-222 months). Interestingly, we found a longer time to transformation in patients with IGHV4-34 as compared with patients using other IGHV (99 *vs* 44 months, p=0.023). Finally, and interestingly, only two patients have <2% SHM and both cases were composite of FL+DLBCL at diagnosis.
Summary and Conclusions: Clonality analysis is necessary to discriminate secondary NHL rather than tFL. IGHV in tFL gene usage is biased, similar to those of DLBCL and FL, as expected. Patients using IGHV4-34 showed longer time to transformation than other IGHV. This study should be considered as preliminary, requiring larger and homogeneous cohorts.
Financial support: Health Research Program (RD12/0036/0069, PS0901382), Health Council of Castilla y León (BIO/SA56/13, GRS265/A/08).

E1374

GENOME-WIDE DNA COPY NUMBER IMBALANCES ASSOCIATED WITH HIV POSITIVE PLASMABLASTIC LYMPHOMAS

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Background: Plasmablastic lymphoma (PBL) is a rare B-cell lymphoma preferentially occurring in immunosuppressed patients. Over the past few years this highly aggressive lymphoma has significantly increased in incidence in HIV-infected individuals from Southern Africa. Despite this increasing incidence and highly aggressive nature, very few studies have addressed the molecular pathogenesis of this lymphoma. These studies were limited to single case reports and candidate-gene approaches, with only one study investigating the presence of copy number aberrations by an unbiased, though low- resolution (1 Mb) BAC array approach. Thus, the molecular basis of HIV associated PBL remains mostly unknown. We have previously characterized a cohort of 45 patients with PBL of the oral cavity (31 with a documented HIV positive status) for immunophenotypic features and well-known chromosomal translocations. New to our study, was the finding of 7 cases (16%) with rearrangements of both immunoglobulin heavy chain alleles. These double-hit lymphomas underscore the aggressive nature of this disease in HIV infected individuals, stressing the need to elucidate its molecular pathology to inform better treatment strategies.
Aims: To investigate genome-wide DNA copy number imbalances associated with HIV related PBL using high-resolution molecular inversion probe (MIP) arrays.
Methods: We used whole-genome molecular inversion probe arrays, (OncoScan FFPE Express (MIP) platform, Affymetrix, Santa Clara, CA), which generates high quality copy number data from formalin-fixed paraffin-embedded (FFPE) samples, to investigate the genetic content of HIV associated PBL on 30 FFPE specimens. MIP data were analyzed with the Nexus 7 Copy number software (Biodiscovery, Hawthorne, CA) using the SNP-FASST2 segmentation algorithm and the Nexus default settings for significance. Copy-number threshold was 2,5 for gain and 1,5 for losses.
Results: We observed a considerable variability in the number of aberrations from one patient to the other ranging from 1 to over 100 aberrations per sample. The sum of aberrations revealed that gains were more frequent than losses and preferentially involved chromosomes regions 1q12qter, 6p22p21, 11q14qter, 12p, and chromosome 7. Analysis with the Genomic Identification of Significant Targets in Cancer (GISTIC) software also identified multiple significant regions of gains and losses. The most significant focal gain on 11p13, included the two genes CD44 and PDHX. The interleukin IL10 and MAPKAPK2 genes, both involved in stress and inflammation processes, were also amongst the most significant gains on chromosome 1q32.1. These gains were confirmed by fluorescence *in situ* hybridization using BACs RP11-595K11 and RP11-343H5 that span these loci respectively. MAPKAPK2 encodes for a kinase regulated through direct phosphorylation by p38 MAP kinase, as such it is involved in CD40-induced B-cell proliferation and its up-regulation may favor B-cell proliferation. Losses were less frequent. Of interest, we observed 2 significant focal losses at the common fragile sites FRA-16D and FRA-3B hosting the WWOX and FHIT genes respectively. Deletions at WWOX and FHIT are the commonest aberrations (85%) reported in primary effusion lymphoma, a disease closely related to PBL (Figure 1).

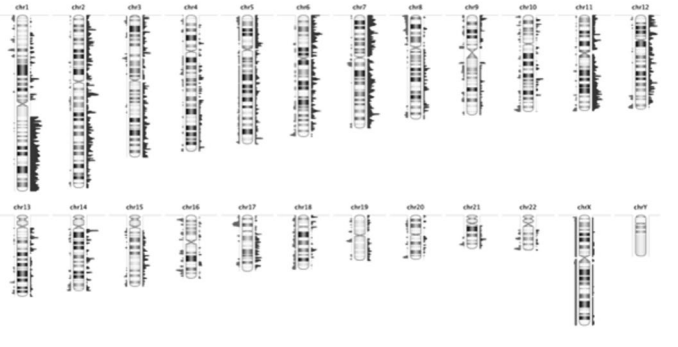

Figure 1. Summary of copy number aberrations in 30 PBL samples.

Summary and Conclusions: Genome-wide DNA copy number imbalances in HIV associated PBL specimens are heterogeneous in number from patient to patient. Significant recurrent focal gains at 11p13 and 1q32.1 warrant further studies aimed at determining their role in the pathogenesis of the disease.

E1375

EFFICACY OF THE PAN PI3K-INHIBITOR COPANLISIB COMPARED TO SELECTIVE PI3K-A, -B, -Δ INHIBITORS IN MANTLE CELL LYMPHOMA (MCL)

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Background: Immuno-chemotherapy is the current therapeutic standard in MCL. However, new therapeutic approaches are urgently warranted in relapsed and refractory disease.
Aims: Inhibitors of the different isoforms of the Phosphatidylinositol 3-kinase (PI3K) show promising results in clinical trials and have been recently registered for follicular lymphoma. We here investigate different PI3K inhibitors in mono and combination treatment.
Methods: Established MCL cell lines (Granta519, Jeko1, Jurkat, Rec1) were cultivated under standard conditions in RPMI1640 supplemented with fetal bovine serum. Cells were exposed to A66 (PI3K-α inhibitor) (5μM), TGX-221 (PI3K-β inhibitor) (5μM), Idelalisib (PI3K-δ inhibitor) (5μM), Copanlisib (5μM) and combinations of A66+Idelalisib and TGX-221+Idelalisib. Viable cells were counted after 24h, 48h and 72h, using a cell viability analyser based on trypan blue exclusion test. Additionally metabolic activity was tested after 48h and 72h using a TTC-assay. Western Blot-experiments were performed in Granta519 and Jeko1 cell lines exposed to the inhibitor-combinations and Copanlisib monotherapy). Similarly, cell viability of 4 patient samples was analysed after 72h exposure to A66, TGX-221, Idelalisib and Copanlisib (multiple concentrations between 30 nM and 10 μM) using a luminescent cell viability assay based on quantitation of ATP. Each experiment were performed in triplicates.
Results: Copanlisib significantly reduced cell growth in all cell lines (Granta519: 32%; Jeko1: 8%; Jurkat 16%; Rec1: 22%). Monotherapy with A66 (Granta519: 93%; Jeko1: 83%; Jurkat 89%; Rec1: 78%), TGX-221 (Granta519: 101%; Jeko1: 86%; Jurkat 64%; Rec1: 71%) and Idelalisib (Granta519: 103%; Jeko1: 69%; Jurkat 63%; Rec1: 75%) showed much only moderate effects. Unexpected, the combination of TGX-221+Idelalisib (Granta519: 101%; Jeko1: 68%; Jurkat 54%; Rec1: 77%) was not more effective than Idelalisib monotherapy, additive effects were only observed in the control cell line Jurkat. In contrast, the combination of A66+Idelalisib (Granta519: 95%; Jeko1: 35%; Jurkat 46%; Rec1: 46%) showed synergistic effects in 3 of 4 cell lines and additive effects in Granta519. TTC-assayshowed comparable results. In Western Blot analysis, treatment with Copanlisib strongly influenced AKT(S) and GSK3α/β as well as 4E-BP1(S) and Rictor phosphorylation. In contrast, combination of Idelalisib and A66 downregulated p42/44 and p38 MAPK. Finally, in patient samples Copanlisib significantly reduced cell growth in all samples investigated so far (0,625 μM-Pat1: 32%; Pat2: 17%; 0,5 μM-Pat4: 45%). In contrast monotherapy with A66 (0,625 μM-Pat1: 80%; Pat2: 71%; Pat3: 83% Pat4: 109%), TGX-221 (0,625 μM-Pat1: 56%) and Idelalisib (0,625 μM-Pat1: 48%; Pat2: 72%; Pat3: 40%; Pat4: 98%) were significantly less effective.
Summary and Conclusions: Copanlisib is highly effective in both cell lines and patient samples. Especiallythe combination of a and d inhibitor showed strong synergism comparable but not achieving the activity of Copanlisib. These results are due to different phosphorylation of PI3K downstream kinases which may explain the observed high clinical activity of less selective PI3K inhibitors.

E1376

WALDENSTROM MACROGLOBULINEMIA CELLS WITH PLASMACYTIC-PREDOMINANT FEATURES ARE INSENSITIVE TO DISRUPTION OF B-CELL RECEPTOR SIGNALING BUT RETAIN HEIGHTENED SENSITIVITY TO PROTEOTOXIC STRESS

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Background: In contrast to other B-cell malignancies, Waldenströms macroglobulinemia (WM) is a unique clinicopathologic entity in which the tumor compartment is comprised of a heterogeneous mixture of monotypic B-lymphocytes, lymphoplasmacytic cells and plasma cells. As such, anti-WM treatment strategies to date have leveraged experience from other B-cell cancers (comprising alkylating agents, anti-CD20 monoclonal antibodies) as well plasma cell disease (proteasome inhibitors). Recently, the BTK-inhibitor, ibrutinib, became the first drug to secure FDA-approval for treatment of WM. Ibrutinib has shown remarkable clinical activity in patients with chronic lymphocytic leukemia (CLL), a cancer of small mature B-cells but has been less effective in patients with plasma cell disease such as multiple myeloma (MM) (*Vij R et al. ASH 2014*). This observation carries important clinical and biological implications as the WM tumor compartment consists of both B-cells and plasma cells. It is now increasingly being recognized that the phenotypic profile of the WM tumor compartment can drastically shift after treatment with drugs that predominantly eradicate the B-lymphocyte population, resulting in a WM tumor population enriched with plasma/plasmacytoid cells (*Barakat et al., Am J Clin Pathol 2011; Morice et al. Mod Pathol 2009*). To evaluate if plasmacytic-WM cells are sensitive to ibrutinib as well as the underlying molecular basis of response, we utilized a molecularly validated WM model (RPCI-WM1) that displays plasmacytoid features ($CD19^{lo}/CD20^{lo}/CD138^{high}$), is $MYD88_{L265P}$+, and was established from a WM patient who had received multiple lines of chemotherapy (comparing against a WM cell line [BCWM.1] with B-lymphocytic-predominant features- $CD19^{hi}/CD20^{hi}/CD138^{low}$).
Aims: To evaluate if plasmacytic-WM cells are sensitive to ibrutinib as well as the underlying molecular basis of response.
Methods: Flow cytometry, western blot, mitochondrial membrane permeability (MOMP) assay, RNA-Seq (Illumina Hi-Seq), micro-RNA profiling (Exiqon qPCR array, real-time qPCR), methylation profiling (Infinium HumanMethylation 450K array), whole exome sequencing (WES) and mate-pair sequencing.
Results: Cell viability and apoptosis assays confirmed our initial hypothesis, revealing RPCI-WM1 cells as insensitive to ibrutinib (5uM) and contrastingly demonstrated their sensitivity to a first in class 19S proteasome inhibitor, VLX1570 (250nM), the latter causing immense proteotoxic stress and mitochondrial damage. Immunophenotype analysis demonstrated upregulation of several antigens on RPCI-WM1, which are typically restricted to plasma cells. Whole transcriptome profiling by RNA-Seq and gene pathway enrichment analysis in RPCI-WM1 cells, showed differential changes where upregulated genes were significantly enriched within the Protein Transport ($p<1.8E-98$) and Response to Unfolded Protein Gene Ontology groups ($p<5.9E-20$); downregulated genes were significantly localized within the B-cell receptor-signaling pathway (Broad MSigDB) ($p<6.1E-14$). As BCR signaling has been linked to dysregulated expression of several micro-RNAs (miRs), we examined miR expression in RPCI-WM1 cells and observed a decrease in several miRs, which target and regulate components involved in BCR activation (notably miR-155). To examine further epigenetic changes that may account for decreased BCR signaling, we examined the global methylation profile of RPCI-WM1 and BCWM.1 cells and noted hypermethylation of the promoter sites (200 base pairs upstream) for a number of transcription factors involved in the BCR pathway. Ongoing WES and mate-pair sequencing data is anticipated to reveal additional molecular alterations that account for the divergent responses to ibrutinib in WM cells that display predominantly plasmacytic features.
Summary and Conclusions: We have conducted a comprehensive immunophenotype and genomic/epigenomic analysis of plasmacytic-WM cells *vs* B-lymphocytic WM cells, using validated preclinical models representative of the phenotypic heterogeneity present in the human WM tumor compartment. Overall our data suggests that significant architectural differences between the two tumor cell types account for their differential sensitivity to the BCR signaling disruptor, ibrutinib or the novel 19S proteasome inhibitor, VLX1570. These findings will be validated in primary patients-derived WM cells. Further, these observations provide rationale for optimizing (or sequencing) ibrutinib therapy in distinct subgroups of WM patients as based on the predominance of plasma/plasmacytic WM cells, present in their individual tumor compartments.

E1377

EVALUATION OF GENOMIC IMBALANCES AND MIRNA EXPRESSION IN PATIENTS WITH MYCOSIS FUNGOIDES

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Background: Mycosis fungoides (MF) is a clonally derived lymphoproliferative disorder that preferentially involves the skin, which etiology and pathogenesis remains elusive. Morphological and clinical features are not always accurate enough to predict the disease outcome. Comparative genomic hybridization and microarrays have shown imbalances of *CDKN2A* (p16) (9p21) and *C-MYC* (8q24) genes, with probable prognostic value in this pathology. In addition, miR-155 overexpression was observed in several hematological and solid tumors, promoting genomic instability, proliferation, and survival of malignant cells.
Aims: In this study, we have evaluated *CDKN2A* losses and *C-MYC* gains and their association with miR-155 expression, in patients with diagnosis of MF. Results were correlated with clinicopathologic features of patients.
Methods: The study was performed on formalin-fixed and paraffin-embedded biopsies from 32 patients with MF (22 males; median age 62.3 years, range: 31-81 years): 28 cases showed tumor stage MF (T-MF), 13 of them in histologic transformation to a large T-cell lymphoma (TR-MF) and 4 cases with folliculotropic MF (F-MF) variant. FISH analysis using OTS9P21.3 (*CDKN2A*) and OTS8Q24 (*C-MYC*) probes (LiVE-Lexel, Argentina), was performed. Gene expression was quantified by real time PCR using TaqMan Gene Expression Assays. Ten control samples from benign skin diseases were also evaluated.

The study was approved by the local Ethics Committee. All individuals provided their informed written consent.
Results: FISH study was performed in 26 patients, 19 (73%) showed genomic alterations (GA): 11 (42.31%) cases had *CDKN2A* deletion, 8 (30.77%) showed *C-MYC* gains and 3 (11.54%) exhibited both anomalies. *CDKN2A* deletion was observed in 7/13 (54%) TR-MF, 3/4 (75%) F-MF and 1/9 (11%) T-MF meanwhile *C-MYC* gain was detected in 6/13 (46%) TR-MF and 2/4 (50%) F-MF. Thus, GA rate was: TR-MF 92%, F-MF 75% and T-MF 11% (p=0.006). These aberrations were more frequent in trunk and lower limbs (75%) in contrast with head, neck and upper limbs (25%) (p=0.012). Patients with GA showed higher CD30+ by immunohistochemistry (56.25%) and progression to histological transformation (75%) than cases with no alterations (NA) (10% and 20%, respectively) (p<0.03). Clinical response to treatment was absent in 8/16 (50%) of cases with GA *vs* 1/10 (10%) patients with NA (p=0.03). Although no significant differences were reached, mean LDH (532.6 UI) and Beta 2 microglobulin levels (3.4 mg/L), median proliferation index (Ki-67) (62.86%) and extracutaneous relapse (25%) with respect to those with NA. In addition, the group with GA showed shorter overall survival (96 months) compared to cases with NA, that not reached the median survival (Log-rank p=0.04), indicating their association with poor outcome. Gen expression analysis showed miR-155 upregulation in 27% TR-MF and 50% F-MF and in only 8% T-MF. No miR-155 expression was observed in controls. The correlation with FISH results found miR-155 overexpression in 33% of patients with GA and 12.5% of cases with NA.
Summary and Conclusions: Our results showed higher proportion of 9p21 losses than 8q24 gains in MF patients. TR-MF exhibited the highest frequency of GA, supporting a role in the process of neoplastic transformation. Although the number of F-MF is reduced in our series, this morphological variant would seem to be associated to an increased frequency of genomic imbalances. Moreover, our findings on miR-155 expression support its relation with genomic instability and tumor development in this pathology.

E1378

MULTIPLE INJECTIONS OF ANTIBODY-RADIONUCLIDE-CONJUTAGES TARGETING CD37 INCREASES TOLERABILITY IN NUDE MICE BEARING NON-HODGKIN LYMPHOMAxENOGRAFTS

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Background: CD37 is an internalizing transmembrane glycoprotein predominantly expressed by normal B cells and also B-cell malignancies, thus an interesting target for targeted therapies. The novel anti-CD37 antibody-radionuclide-conjugate (ARC) ^{177}Lu-DOTA-HH1 (Betalutin^{TM}), containing the short-ranged beta-emitter lutetium-177, is currently being tested as single injection therapy in a clinical phase I/II trial for non-Hodgkin B-cell lymphomas.
Aims: The present work is the first to explore the anti-tumor efficacy and tolerability of multiple dosing of ^{177}Lu-DOTA-HH1 in mice bearing non-Hodgkin B-cell lymphomaxenografts.
Methods: Ten million Ramos human lymphoma cells were injected subcutaneously on flanks of in-house bread mice from the strain Hsd:Athymic Nude-*Foxn1^{nu}*. Tumor-bearing mice and mice without tumor for tolerability control were given 2-4 weekly injections of 300 MBq/kg ^{177}Lu-DOTA-HH1 i.v (n=16-18 tumors in 10-11 mice, 3-4 non-tumor bearing mice). Control mice were given 4 injections of 0.9% NaCl (n=19 tumors in 10 mice, 3 non-tumor bearing mice). Tumor size, body weight and hematology were monitored regularly after administration.
Results: A tumor growth delay was observed in all groups given multiple injections. The delay was increased in the group given 3 injections compared to the group given 2 injections due to the increased radioactive dose. Durable complete regression >100 days was observed in 56% of the tumors in mice given 3 injections, compared to 28% in mice given 2 injections. It was found that 4 weekly injections of 300 MBq/kg ^{177}Lu-DOTA-HH1 were above the maximum tolerable dose, as 5 of 12 mice had to be euthanized at day 37 after initial injection due to radiation toxicity recognized by rapid weight loss and severely reduced health condition in combination with low hematology values. None of the 14 mice given 3 weekly injections of 300 MBq/kg ^{177}Lu-DOTA-HH1 needed to be euthanized due to radiation toxicity. An average decrease in body weight of 5% was seen for this therapy group during injections, with continued increase of body weight observed one week after last injection. Hematology analysis showed transient reduction in white blood cells (WBC) and platelet counts in all mice receiving treatment with ^{177}Lu-DOTA-HH1, with NADIR values (WBC: 9-25% of baseline values; platelets: 22-44% of baseline values) occurring 1-2 weeks after last injection. Platelet counts returned to baseline levels within 4-6 weeks and WBC within 7-9 weeks after last injection. A total injected activity of 900 MBq/kg was found tolerable in this multiple injection regime, compared to previously published single injection data showing a maximum tolerated dose of 400 MBq/kg and 50% toxicity-related deaths within 25 days after injection in nude mice given 800 MBq/kg (Repetto-Llamazares *et al.,* PLoS ONE 2014).
Summary and Conclusions: Weekly injections increase tolerability of ^{177}Lu-DOTA-HH1 compared to high dosage single injection in nude mice, allowing a higher total activity to be administered.

E1379

OFATUMUMAB OVERCOMES CD59-DEPENDENT RESISTANCE TO COMPLEMENT-DEPENDENT CYTOTOXICITY IN HUMAN B-CELL LYMPHOMA MODEL

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Background: Anti-CD20 monoclonal antibodies (mAbs) have revolutionized the treatment of CD20-positive lymphoid malignancies. Unfortunately, anti-CD20 immunotherapy is not without its obstacles. It turns out that nearly 50% of patients do not respond to treatment or the resistance appears in the further course of therapy. Accumulating evidence indicates that that resistance is related to the expression of membrane complement inhibitors, *i.e.* CD59 on the surface of target cells.
Aims: The aim of this study was to evaluate the efficacy of the rituximab-or ofatumumab-triggered complement-dependent cytotoxicity (R-CDC or O-CDC) against CD59-overexpressing lymphoma cells and to determine the amount of CD59 and CD20 which allows anti-CD20 mAbs to effectively trigger CDC.
Methods: Raji, human Burkitt's lymphoma cell line, found to be CD59-negative, was chosen to create stable CD59-expressing cell line using pLVX-IRES-Puro-CD59 lentiviral construct. Cell cloning by serial dilution was performed to establish different populations of transductants with various CD59 expression and invariable CD20 expression. All experiments evaluating expression of membrane antigens was performed with flow cytometry. The effectiveness of anti-CD20 mAbs was assessed in a series of distinct clones using MTT-reduction assay.
Results: The results show that CD59 expression, independently of CD20 level, has a critical influence on R-CDC and negatively correlates with the capability of rituximab to trigger the complement cascade (Figure 1A-D). To determine if this inhibitory effect of CD59 can be surmounted, using a panel of different clones we performed CDC assay with ofatumumab-a fully human anti-CD20 antibody. Interestingly, we observed that ofatumumab overcomes CD59-dependent resistance of B-cell lymphoma cells (Figure 1E).

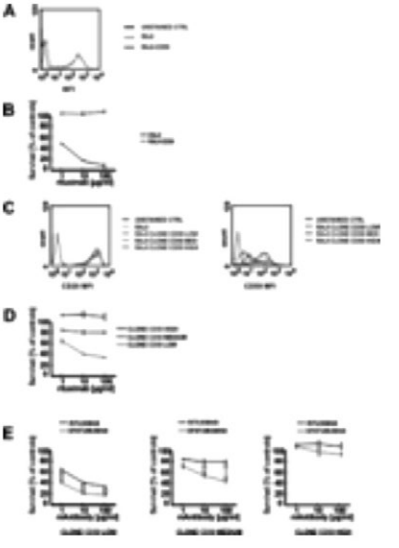

Figure 1.

Summary and Conclusions: Our study clearly shows that CD59 expression level affects the efficacy of anti-CD20 monoclonal antibodies and this observation seems to be clinically relevant. We suggest that evaluation of CD59 expression should be included in the diagnostic panels for leukemia/lymphoma immunophenotyping by flow cytometry. Moreover, determination of CD59 levels can have far-reaching implications for the planning of clinical therapeutic regimens. Since ofatumumab is capable to overcome a negative effect of CD59, it is substantiated to include ofatumumab in therapeutic regimens for patients with high CD59 expression.

E1380

THE EXPRESSION OF FOXO4 REGULATES LYMPHOMA STEM CELL-LIKE CHARACTERISTICS AND RESISTANCE TO TREATMENT IN B-CELL NON-HODGKIN LYMPHOMA

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Background: Although lymphoma cells are relatively sensitive to chemotherapeutic agents, a subset of cells remains as being viable even during treatment. This subset of lymphoma cells might have stem cell-like properties including tumor initiating ability, and result in resistance to chemotherapy such as doxorubicin. However, there is limited data about the presence of lymphoma stem cells and its association with treatment resistance. In this study, we used a group of lymphoma cells that maintained the viability after the exposure to antitumor agents including doxorubicin and phenylbutyrate as a model of cancer stem cells because doxorubicin is the most commonly used chemotherapeutic agent and phenylbutyrate, a histone deacetylase inhibitor is reported to induce stem cell characteristics in cancer cell lines.
Aims: We analyzed gene expression profiles and biological characteristics of viable lymphoma cells during the treatment with high concentration of doxorubicin and phenylbutyrate, and explored a potential marker for lymphoma stem cells that are associated with treatment resistance.
Methods: Human lymphoma cell lines including BJAB, Toledo (diffuse large B-cell lymphoma), Daudi, and Raji (Burkitt's lymphoma) were incubated with the IC90 concentration of doxorubicin (300nM) or phenylbutyrate (8mM) for 48 hours. After incubation, viable cells were sorted, and analyzed for their resistance to treatment. Gene expression profiles of those viable cells were evaluated with cDNA microarray, and biological characteristics including stem cell-like properties were also analyzed. Membrane proteomics was performed to explore surface markers for lymphoma stem cells, and immunohistochemistry for candidate markers were performed in the paraffin tissue of patients with non-Hodgkin B-cell lymphoma.
Results: Lymphoma cells could maintain their viability after they were exposed to the IC90 concentration of doxorubicin and phenylbutyrate for 48 hours in the experiment with four types of B-cell lymphoma cell lines. These viable lymphoma cells sorted after their exposure to treatment showed resistance to treatment and significantly better ability of colony formation and sphere formation than control cells ($P < 0.05$). The expression of stem cell-related genes including OCT4, NANOG, and SOX2 was also increased in viable cells after treatment. The comparison of gene expression profiles between survived cells after treatment and control cells showed that 42 gene were up-regulated in both conditions (doxorubicin and phenylbutyrate treatment). Among them, FOXO4 was significantly overexpressed in viable cells. The expression of FOXO4 was confirmed with primary lymphoma cells derived from malignant effusion of three patients with B-cell non-Hodgkin lymphoma. The amplification of FOXO4 expression in lymphoma cell lines also increased colony formation whereas the knockdown of FOXO4 decreased the expression of OCT4, NANOG and SOX2, and colony forming ability in lymphoma cells. The analysis of membrane proteins in these lymphoma cells survived after phenylbutyrate treatment showed increased expression of ENO1. The immunohistochemistry with paraffin tissue of patients with B-cell non-Hodgkin lymphoma showed nuclear expression of FOXO4 and membranous expression of ENO1.
Summary and Conclusions: A small subset of lymphoma cells resistant to treatment might have stem cell-like characteristics, and the expression of FOXO4 might play a crucial role in their maintenance. The membranous expression of ENO1 in this small subset of lymphoma cells might suggest the probability of ENO1 as a stem cell marker in B-cell non-Hodgkin lymphoma.

E1381

GLYCOLYTIC INHIBITOR 2-DEOXY-GLUCOSE SUPPRESSES CELL PROLIFERATION IN NON-HODGKIN LYMPHOMA CELLS THROUGH DOWN-REGULATION OF HIF-1 AND C-MYC

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Background: Metabolic reprogramming is linked to tumourigenesis, disease progression, clinical outcome and resistance to chemotherapy. However, the significance of glycolytic metabolism in non-Hodgkin's lymphoma (NHL) remains unclear.
Aims: To investigate the role of glycolysis in the pathogenesis of lymphoma and the potential target for therapy.
Methods: Cell proliferation and cytotoxicity were detected using Cell Counting Kit-8. Glucose consumption was determined via glucose uptake assay. Lactic acid generation was estimated using a lactic acid detection kit. Apoptosis and cell cycle analysis were measured by flow cytometry. Real-time PCR was performed to determine the levels of glycolysis-related genes. Cleavage of caspase-3 and PARP-1 as well as expressions of HIF-1α and c-MYC were demonstrated via western blot.
Results: Activation of glycolysis-related genes was seen in malignant tissues from patients with NHL, and a panel of NHL cell lines as well. Consistent with the gene expression levels, multiple NHL cell lines exhibited the glycolytic metabolic phenotype, suggesting a role of glycolysis in the pathogenesis of NHL. The glycolytic inhibitor 2-DG significantly suppressed the abilities of glucose consumption and lactic acid production in NHL cell lines. In consert with suppression of the metabolic phenotype, proliferation of NHL cell lines were significantly inhibited by 2-DG in a time- and dose-dependent manner. In contrast, no difference was observed in normal lymphocytes after 2-DG treatment. The Burkitt lymphoma cell line Raji was less sensitive to 2-DG than other cell lines *e.g.* SU-DHL-4, DB, Namalwa, Jeko-1. However, when cultured under hypoxia, the IC50 value of 2-DG for hypoxic Raji cells was significantly lower compared with its normoxic counterpart, accompanied by a more marked inhibition of cell proliferation, glucose cosumption and lactic acid generation. Meanwhile, hypoxic Raji cells displayed apoptosis induction after 2-DG treatment. Furthermore, down-regulation of HIF-1α and c-MYC were observed only in hypoxic Raji cells (Figure 1).

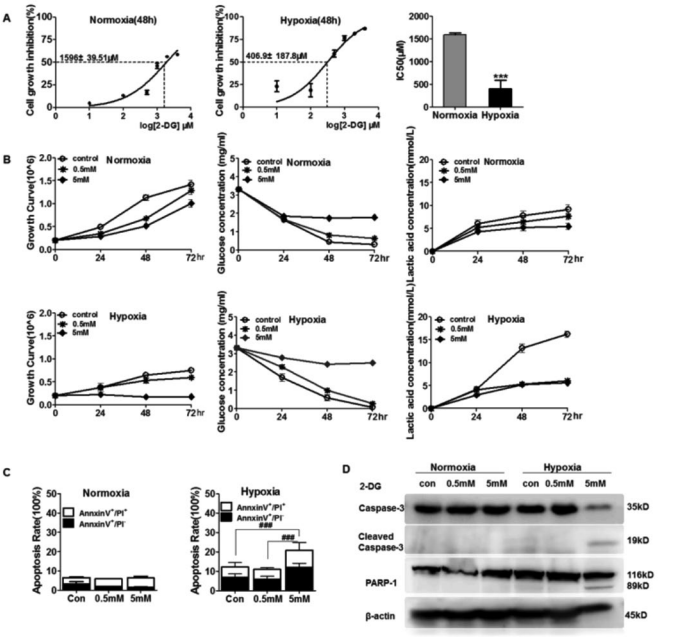

Figure 1.

Summary and Conclusions: In conclusion, these results present novel insight into critical roles of glycolytic pathway activation in NHL progression. Inhibition of glycolytic pathway may provide a new therapeutic strategy for the treatment of NHL.

E1382

IMMUNOPHENOTYPIC CHARACTERIZATION AND CLINICAL BEHAVIOUR IN CD19+CD5+ (NON-CLL) LYMPHOPROLIFERATIVE DISORDERS MUTATED AND UNMUTATED WITH AND WITHOUT T(11,14)

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Background: Mantle cell lymphoma (MCL), characterized by the presence of t(11;14)(q13;q32), shows a typical immunophenotype CD19+CD5+ that can be identified by flow cytometry (FC) and is easily distinguished from CLL. However, cases with typical MCL-immunophenotype lacking t(11;14) are frequently detected in the clinical routine. Further, clinical presentation could be variable and may show a different IGHV mutational status.
Aims: Characterize FC CD19+CD5+ (non-CLL) lymphoproliferative disorders (LPD), trying to identify immunophenotypic profiles that may predict the presence of t(11;14) and to find molecular differences associated to clinical behaviour.
Methods: We retrospectively reviewed a total of 98 patients with CD19+CD5+ (non-CLL) LPD detected by FC in the clinical laboratory of the University Hospital of Salamanca (Spain), in which information of immunophenotype, cytogenetics, and molecular biology (MB) (bcl-1 and IGHV mutational status) was available. The clonal B-cell population was detected in peripheral blood (n=50), bone marrow (n=29) and/or lymph node (n=19). Erythrocyte-lysed samples were stained with large panels of monoclonal antibodies, using 4-color direct immunofluorescence technique according to previously described methods, aimed to identify and characterize B neoplastic cells; the complete diagnosis was performed with ancillary techniques as MB (bcl-1 and IGHV

mutational status), karyotype and fluorescent *in situ* hybridization (FISH) for t(11;14).
Results: Out of the 98 samples with CD19+CD5+ immunophenotype (no CLL), 52% were t(11;14) positive (most of them studied by the tree techniques: 9% Karyotype(Kt)+FISH+BM+; 11% Kt+FISH+BM-; 22% Kt-FISH+BM+; 47% Kt-FISH+BM- and 11% Kt-FISH-BM+). FISH was the most useful technique, being positive in 88% of the positive cases. 84% of the t(11;14) positive cases showed typical CD22+/CD23- immunophenotype and bright expression of CD20+ and FCM7+, CD38 expression was negative in 53% of the cases. Negative cases for t(11;14) showed CD22+/CD23- in a slightly lower frequency (62%) being more heterogeneous, but with similar expression of CD20; CD38 was positive in a 33% of these cases. Concerning clinical behaviour and IGHV mutational status, the percentage of mutated cases was higher among leukemic cases (88%) than among nodal cases (35%), both with and without t(11;14). Despite no differences in OS, unmutated cases could be associated with more aggressive behaviour due to the need for earlier treatment (TTT median 15 days *vs* 29 months; p<0.001) and a higher progression risk (SLP median 52 months *vs* NR; p<0.001). Among the 98 CD19+CD5+ (non-CLL) cases, 8 were non-classifiable chronic LPD, 4 were classified as SMZL, 4 as SLL, 2 as DLBCL, 1 as FL and 79 as MCL. Overall, 75% of cases received therapy (of which 73% of them had nodal behaviour; 27% leukemic behaviour). The time to treatment was longer in leukemic than in nodal cases (median 82 months *vs* 15 days; p<0.001).
Summary and Conclusions: Although typical MCL's-immunophenotype cases are frequently detected, t(11;14) is present only in half of the cases and there is no clear difference in immunophenotype between cases t(11;14) positive and t(11;14) negative. Despite no differences in OS, and taking into account that patients were not treated uniformly, unmutated cases could be linked to more aggressive behaviour due to the need for earlier treatment and to an increased risk of progression, while mutated forms are generally presented as a less aggressive disease.

E1383

ANALYSIS OF THE IGVH SOMATIC MUTATIONS IN SPLENIC MARGINAL ZONE LYMPHOMA
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Background: Splenic marginal zone lymphoma (SMZL), a specific type of small B-cell lymphoma and characterized by a peculiar morphology with micronodular pattern of infiltration, biphasic cytology, and the almost constant presence of marginal zone differentiation.
Aims: The aim of our study to determine the immunoglobulin variable heavy chain (IgVH) gene usage and somatic mutation patterns in a series of SMZL patients and to correlate these findings with the clinical features and outcome.
Methods: Among a total of 125 SMZL patients diagnosed and followed up in our Center during the last 14 years, 24 were included in the present study, based on the availability of tissue specimens or frozen cell material. Diagnosis was based on standard WHO criteria. In all patients, the diagnosis was based on peripheral blood and BM findings. The baseline clinical and laboratory features as well as follow-up and outcome were recorded for every patient. Rearranged IgVH genes were amplified essentially in reactions that contained only one of the 5' leader region primers for the indicated 6 VH families and a 3'J primer. All PCR reactions were performed using appropriate positive and negative controls.
Results: The studied population included 15 women and 9 men with a median age at diagnosis of 60 years (range, 32 to 77 years). All patients had splenomegaly, with a median spleen size of 10 cm below the left costal margin. None of them had lymph node enlargement, except for the splenic hilar lymph nodes. BM infiltration was evident in all patients, either by conventional morphology and immunohistochemistry or by flow cytometry. B-symptoms were present in all patients. Anemia (hemoglobin<12 g/dl) and thrombocytopenia (platelets <100×109/l) were recorded in 14 and 7 patients respectively. Autoimmune hemolytic anemia was present in 3 patients, while paraproteinemia was detected in 10 patients, being of the IgM isotype in all cases except one. Serum lactate dehydrogenase (LDH) was elevated in 17, serology for hepatitis C was negative in all patients. All patients underwent splenectomy as first-line therapy. The rearranged VH genes identified for each case seemed to represent functional rearrangements because no stop codons or crippling mutations were identified. A comparison of the VH genes to reported germline sequences revealed that 6 cases used the VH3 family VH gene segments, 2 the VH4 family, 16 the VH1 family segments. The VH1 family genes V1-2 were used in 16 cases. In 4 out of 24 cases (16.67%), IgVH genes were in germline or near germline configuration, whereas in 20 cases (83.33%), IgVH genes were somatically mutated. We have shown no differences in clinical and laboratory characteristics, immunophenotype, outcome or overall survival were found between the mutated and unmutated cases. There was a trend for worse survival in unmutated *versus* mutated SMZL cases (median survival 70 *vs.*109 months), but the difference did not reach statistical significance.
Summary and Conclusions: Our analysis also showed the selective use of VH1 family genes in a high proportion of SMZL cases (66.67%), while VH4 and VH3 family genes were represented at a lower frequency (8.33% and 25%, respectively). The present study revealed that a significant fraction of SMZL cases derive from naive B-cells with unmutated IgVH genes, thus indicating that this is a heterogeneous group of disease with respect to its cellular origin. A prognostic significance of mutation status was not observed in this study.

E1384

FLOW CYTOMETRY IMMUNOPHENOTYPING AND CYTOLOGICAL ANALYSES OF CSF: A COMPARISON OF PERFORMANCES FOR LYMPHOMATOUS CELL DETECTION
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Background: Leptomeningeal involvement of lymphomas is a rare and aggressive condition, mainly occurring in large B-cell lymphomas (DLBCL) and Burkitt lymphomas. It is defined by the presence of malignant cells in the cerebrospinal fluid (CSF) and can be prevented by intrathecal chemotherapy. The prevalence of this affection is uncertain and may be explained by the various existing techniques. Cytomorphological (CM) examination is considered to be the "gold standard" but remains insufficiently sensitive due to the frequent sample paucity. Provided these limits, a multimodal investigation of CSF is required. Flow cytometry (FCM) techniques tend to be more sensitive and specific. The approach consisting of drawing the CSF sample on a Transfix™ tube is currently largely used for FCM and prevents early cell mortality.
Aims: The aim of our study was to assess the value of FCM in detecting CSF disease in a large cohort of patients with aggressive lymphomas.
Methods: This retrospective study was conducted on all CSF samples referred to our laboratory and coming from patients with primary cerebral lymphoma or other non-Hodgkin lymphomas (NHL) between January 2013 and September 2014. We matched FCM and CM results obtained from the analysis of 204 CSF drawn (107 patients). In this purpose, 2 samples were collected: 1 ml for CM analysis (with albumen added during cytospin and May Grünwald Giemsa staining) and 1 ml on a Transfix-containing tube (Cytomark). A cytological sample was classified as negative or positive by CM evaluating lymphocyte features defining lymphoid cells as typical or atypical. Immunophenotyping was performed on a FACSCanto II (BD Biosciences) using an 8-colour panel (anti-CD19, kappa, lambda, CD5, CD3, CD4, CD8 and CD45). A CSF sample was considered as positive by FCM when a cluster of at least 20 events with immunophenotypic features of neoplastic cells were detected. CM and CSF results were treated in a double-blind manner.
Results: We confirm that Transfix-treated CSF prevent early cell mortality and improves the quality of FCM tests. Identifying and analyzing cells by FCM remains possible even if the total cell count is ≤1 leucocyte/µl in CM count (n=70). Overall, 178 (87%) samples showed similar results by CM and FCM with presence (n=25) or absence (n=153) of lymphomatous cells. Seven percent of samples were classified as suspicious by one technique. Among them, 9 samples were positive by FCM but negative by CM. This lack of accuracy is due to poor cellularity in the CSF in patients with low-volume disease or to innocent aspect of neoplastic cells. False negative cases occur because of low sensitivity of CM compared with FCM. In contrast, three samples were positive by CM but negative by FCM. False positive results by CM can be generated by the presence of confounding atypical-like reactive lymphocytes. On the other hand, false negative results by FCM can be explained by the cell size (inherent cells parameter) particularly large lymphocytes outside the scope of our analysis or by the loss of one gating marker.
Summary and Conclusions: To date the debate pertaining FCM diagnostic accuracy as well as prognostic impact when compared to CM examination is still open. CSF is a biological sanctuary extremely accessible to FCM studies. Our results suggest that FCM increases the sensitivity and specificity of leptomeningeal disease detection. FCM is a well-reproducible quantitative method suited for the identification of small cell populations. In conclusion, both methods should be applied concurrently for complementary diagnosis assessment in patients with lymphomas.

E1385

EXPRESSION OF SOXC CLUSTER AND MIR-17-92 POLYCISTRON IN MANTLE CELL LYMPHOMA PATIENTS
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Background: Mantle cell lymphoma (MCL) is an aggressive B-cell lymphoid malignancy that accounts for approximately 6% of all non-Hodgkin´s lym-

phomas. *SOX4*, *SOX11* and *SOX12* genes, constitute the SOXC family of transcription factors involved in embryonic neurogenesis and tissue remodeling. Among them, *SOX11* shows aberrant expression in MCL, being considered a new molecular marker of adverse prognosis in this pathology; meanwhile recently it has shown that *SOX4* can bind and regulate the promoter of Dicer, a microRNA biogenesis factor. Furthermore, several studies have demonstrated the oncogenic role of miR-17-92 cluster in hematological malignancies, being scarce the information about the association between this cluster and SOXC expression levels in MCL.

Aims: In this study, we have performed a Gene Expression Analysis (GEA) of SOXC cluster and their correlation with the expression of miR-17, miR18a, miR19b and miR92a, members of the polycistronic oncomiR-17-92 in MCL patients.

Methods: mRNA samples of 45 MCL patients (15 males; mean age: 56.7 years, range: 34-71 years) and 12 normal controls were analyzed. Gene expression was quantified by real time PCR using TaqMan Gene Expression Assays. *GUSB* and *RNU6B* were used as housekeeping genes to normalize the expression of SOXC and miR17-92 clusters, respectively. The proliferation index was evaluated by immunohistochemistry with the Ki67 antibody. The analysis of mRNA expression data was done using the two-tailed Mann-Withney test. To calculate the expression cut-off values with the highest sensitivity and specificity, receiver operating characteristics curves were used. The study was approved by the Ethics Committee of our Institution. All individuals gave their informed consent.

Results: The analysis of SOXC members showed upregulation, of SOX11 and SOX12 in 60% and 71% patients, respectively, while SOX4 was downregulated in 45% of cases, compared to normal controls. GEA of SOX11 and SOX12 showed higher expression levels (2.1±0.24 and 3.1±0.16, respectively), with respect to SOX4 (1.2±0.21) (p=0,006 and p<0.0001, respectively). Moreover, an inverse relationship between the expression of SOX11 and SOX4 was detected (p=0.0017), being the last one undetectable in patients with high expression of SOX11. Regarding to the members of the miR17-92 cluster, miR17 and miR18a were downregulated in 86% and 84.4% of our cohort, respectively; meanwhile, miR19b and miR92a were upregulated in 95% of cases each, compared to controls. GEA revealed that miR19a (3.24±0.1) and miR92a (2.59±0.1) exhibited increased expression levels than miR17 (-2.7±0.15) and miR18a (-2.8±0.19) (p<0.0001). A positive correlation between miR92a with miR17 (p=0.0001), miR18a (p=0.0013), and miR19b (p<0.0001) mRNA levels, was observed. Moreover, we found a positive correlation between SOXC members and miR18a expression (p<0,012). In addition, patients overexpressing *SOX11*, *SOX12* and miR92a showed a higher percentage of Ki67 positive cells (37-41%), compared to cases without expression, but without reaching significant differences.

Summary and Conclusions: Our findings in MCL patients show for the first time an inverse correlation in the expression profiles of *SOX11* and *SOX4* genes. Additionally, and to the best of our knowledge, we found new evidence linking the differential expression pattern between SOXC genes and miR18a, suggesting an interaction that could add new insights in the biologic characterization of this pathology.

E1386

THE IMPACT OF MUTATION STATUS OF SOCS1 IN PATIENTS WITH HIGH-GRADE DIFFUSE LARGE B-CELL LYMPHOMA.

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Background: Suppressor of cytokine signaling 1 (SOCS1) is a negative regulator of JAK/STAT pathway. Nowadays there are studies that demonstrated high frequency of SOCS1 mutations in patients with diffuse large B-cell lymphomas. There is only one publication about the prognostic value of SOCS1 mutations in DLBCL patients with CHOP-like therapy.

Aims: To evaluate the frequency and prognostic impact of mutation status of SOCS1 in patients with *de novo* high-grade DLBCL treated with high-dose chemotherapy (HDC).

Methods: We performed a trial, including 22 adult patients (median age 40 years, range 20-66) with newly diagnosed DLBCL who were enrolled in HDC protocol (mNHL-BFM-90 program) since July 2007 till January 2014. Their clinical characteristics: m:f=9:13, extranodal involvement>1 in 13 (59%) patients, bone marrow involvement in 5 (23%) patients, high proliferative activity Ki-67>80% in 17 (77%) patients, GCB: non-GCB type=13:9, IPI risk>2 in 17 (77%) patients. Mutations of SOCS1 were detected using sequencing PCR products, which were received by single-round PCR. DNA for PCR was extracted from sections of frozen tumor samples. Amplification of VH gene rearrangements to control the tumor cells was performed by BIOMED-2 multiplex polymerase chain reaction protocol.

Results: In 8 (36%) of 22 patients the SOCS1 mutations were detected. By sequence analysis, we identified 18 (7 deletions, 11 single nucleotide substitutions) SOCS1 mutations. We didn't verify hotspots. Mutation status of SOCS1 gene didn't correlat with clinical characteristics of patients.

The median follow-up time in 22 patients was 46 months (range: 5-65 month). Four patients had died of the disease (~18%) and 18 patients were either alive. Univariate analysis of the entire cohort (n=22) revealed that age, IPI, extranodal involvement, Ki-67 and phenotype were not of prognostic significance (p >0.05). In a multivariate analysis, detection of SOCS1 mutations in patients with high grade DLBCL was an independent predictor for overall survival (OS) (p <0.05). OS in DLBCL patients with SOCS1 mutations and without was 62,5% and 90%, respectively. Event-free survival (EFS) was 62,5% and 71,4%, respectively. (Figure1A-B). There was no difference in overall survival and event-free survival in subgroups between SOCS1 deletions and SOCS1 single nucleotide substitutions, between SOCS1 single mutation and SOCS1 multiple mutations.

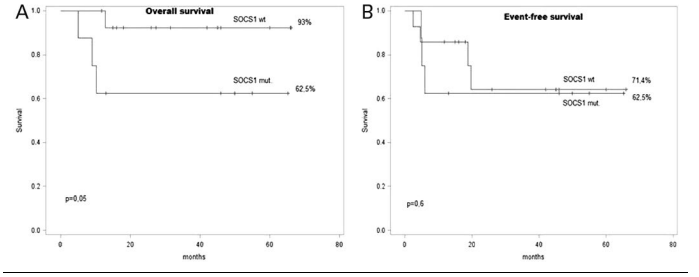

Figure 1.

Summary and Conclusions: Though a group of analyzed patients is small, however the detection of SOCS1 mutations seems to be an independent predictor of outcome in high grade DLBCL patients, treated with high-dose chemotherapy, while IPI and phenotype DLBCL cannot be considered as risk factors for this group of patients. It seems to be reasonable to continue a study of mutation status of SOCS1 gene in high-grade DLBCL patients, because it's may be a single gene prognostic biomarker in DLBCL. If it will be confirmed, this biomarker could determine an indication for autologous transplantation in this group of patients as first-line therapy.

LB2092

CARDIAC GLYCOSIDES SELECTIVELY INDUCE HEMATOLOGICAL MALIGNANCY CELLS APOPTOSIS THROUGH NKA/IP3R SIGNALING PATHWAY

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Background: Cardiac glycosides have been used to treat heart diseases for centuries because of their inhibition effects on Na^+/K^+-ATPase (NKA). Recently, more and more studies indicate that low doses of cardiac glycosides can inhibit cancer cells proliferation and induce apoptosis, but there is no evidence suggests which types of cancer cells are more sensitive to cardiac glycosides and until now the anti-apoptotic mechanism is still not quite clear yet. In our previous work, we found that low doses of cardiac glycosides without inhibition of NKA can trigger intracellular signaling pathway (NKA/IP_3R-Ca^{2+} oscillation-NF-kB) which regulates cells proliferation and apoptosis. Here we found that Burkitt's lymphoma cells are the most sensitive cells to cardiac glycosides and the mechanism is that Burkitt's lymphoma cells overexpress c-myc which is regulated by cardiac glycosides triggered signaling pathway (NKA/IP_3R-Ca^{2+}oscillation-NF-kB).

Aims: We attempt to elaborate on the following points: 1) The suppose that cardiac glycosides regulate the activity of NF-κB and c-myc and induce Raji cells apoptosis through NKA/IP_3R-Ca^{2+} oscillation-NF-kB signaling pathway will be certificated. 2) We set up Burkitt's lymphoma animal model to investigate cardiac glycosides anti-tumor activity *in vivo* study.

Methods: *In vitro* study: 1) Cells: 11 types of human malignant hematological cell lines and 7 types of primary culture of human malignant hematological cells were used. 2) Cardiac glycosides treatment: digoxin, digitoxin and ouabain were used. 3) Cells Proliferation rate was measured by Cell Counting Kit-8. 4) Cells apoptosis rate was detected and quantificated by Annexin-V-FLOUS Staining Kit followed by flow cytometry. 5) NF-kB activity was measured by the TransAM NF-κB p65 Kit. 6) NKAa1, IP_3R and c-myc protein level were measured by western-blot, mRNA level were measured by realtime-PCR. *In vivo* study: BALB/c-nu mice models of human Burkitt's lymphoma were established by injecting Raji cells subcutaneously. To study the effect of digoxin onxenograft engraftment rate, digoxin was injected into the abdominal cavity daily 3 days before or 1 day after tumor cells implantated into mice. Mice weight and volume ofxenograft tumors were measured daily. Western Blot, immunohistochemical staining and immunofluorescence staining were used to analyze c-myc protein level ofxenograft tumors.

Results: 1) Low doses of cardiac glycosides can inhibit human malignant hematological cells proliferation and induce apoptosis, Burkitt's lymphoma cells including primary culture cells and Raji cells are the most sensitive cells. 2) Burkitt's lymphoma cells overexpress c-myc not only protein level but also mRNA level, NF-kB is over activated in Burkitt's lymphoma cells. Cardiac glycosides can decrease the c-myc expression level and inhibit NF-kB activity. 3) Cardiac glycosides can increase Burkitt's lymphoma cells NKAa1 expression.

By using siRNA to knock down NKAa1, NKAa1 expression is decreased and this abolish the anti-apoptotic effect of cardiac glycosides and also abolish the effect of cardiac glycosides on c-myc and NF-kB activity. 4) *In vivo* study, by using naked mice with hypodermic injection Raji cells to set up Burkitt's lymphoma animal model. Digoxin reducedxenograft engraftment rate of Burkitt's lymphoma significantly if mice were treated with digoxin before tumor cell implantation. Digoxin suppressed the growth of subcutaneous tumors at the fifth day when mice were treated with digoxin after tumor established. This effect lasted until the endpoint of the observation. Digoxin reduced c-myc protein expression of tumor cells.

Summary and Conclusions: Compare to other human malignant hematological cells, Burkitt's lymphoma cells are the most sensitive cells to cardiac glycosides, this maybe because these cells overexpress c-myc which is regulated by cardiac glycosides triggered signaling pathway (NKA/IP_3R-Ca^{2+} oscillation-NF-kB). Cardiac glycosides also have anti-tumor *in vivo* study. Burkitt's lymphoma is a high grade malignancy, until now, there is no effective therapy method. Our finding suggests a positive new medicine for the therapy of Burkitt's lymphoma which may increase the survival time of Burkitt's lymphoma patients.

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Non-malignant hematopoietic disorders

E1387

FLOW CYTOMETRIC CHARACTERIZATION OF LEUKOCYTE SUBPOPULATIONS IN BONE MARROW FROM UNTREATED PATIENTS WITH GAUCHER DISEASE TYPE 1: PRONOUNCED T-CELL RESPONSE AND STEM CELL DEFECT?

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Background: Gaucher disease (GD) is a lysosomal storage disease, with deficient activity of glucocerebrosidase due to autosomal recessive mutations in *GBA1* gene. The defect causes ubiquitous macrophage infiltration in parenchymatous organs and bone marrow in all clinically distinguished phenotypes (GD 1-3), with additional nervous system involvement in type 2 and 3. Patients with GD demonstrate a variety of abnormal immunological responses and tendency to develop lymphoproliferative diseases. As the role of chronic inflammation in GD-affected organs was postulated, the role of respective immunological cell types is being discussed. Up till now studies focused exclusively on leukocyte subpopulations and cytokine profiles in peripheral blood.

Aims: The study aimed to characterize leukocyte subsets in bone marrow aspirates from treatment-naïve patients with Gaucher disease type 1 by multiparameter flow cytometry (FC), in comparison with data from healthy controls.

Methods: Twelve patients with GD1, previously untreated, followed at the Karolinska University Hospital Hematology Center were enrolled (4 females, 8 males). Bone marrow aspirates were taken between 2008 and 2013, as a part of routine clinical follow-up, and analyzed by flow cytometry. For comparison, bone marrow flow cytometry data on 12 healthy subjects, age and sex matched to GD-patients, were retrieved from the database of Department of Clinical Pathology and Cytology at the same institution. Samples were routinely processed, stained with monoclonal antibodies (Beckton Dickinson, DAKO), acquired using 4 color Canto A or 8 color Canto II cytometers (BD), and analyzed using BD FACSDIVA™ software. Analysis included cells present in mononuclear cell gate in each analyzed tube. The following cell populations were highlighted in each subject: CD3+ T-cells and fractions (CD4+, CD8+, CD4+/8+, CD4-/8-, T/NK-cells), CD56+/CD3- NK-cells, CD19+ B-cells at different stages of maturation. Besides, CD34+ stem cells, CD117+ precursors, CD14+ monocytes and CD33+ mononuclear immature myeloid cells were analyzed. For statistical analysis, Shapiro-Wilk test was used for verification of value distribution, and t-test and Mann Whitney U test for comparisons between groups.

Results: Median age was 55.5 years (mean 58.8±18.8) in GD patients and 57.5 yrs (57.6±17.3) in controls. Four GD patients were previously splenectomized. Bone marrow aspirates from GD1 patients contained more T-cells and respective fractions but the CD4/CD8 ratio was lower as compared to healthy controls; the differences were however not significant. Patients with GD1 had minimally fewer NK-cells, B-cells and plasma cells, with the exception of the CD19+/CD5+ B-cell fraction. They had also significantly fewer CD34+ stem cells, with almost the same proportion of CD33+ or CD14+ cells as controls. Splenectomized GD patients had significantly fewer precursor cells (CD34+, CD117+) but more NK-cells as compared to non-splenectomized ones. There were also non-significant trends for higher amounts of T-cell, B-cell and monocytes in splenectomized patients (Table 1).

Table 1. Leukocyte subsets in bone marrow in flow cytometry analysis.

Cell subsets	Gaucher disease (%total; mean±SD)	Controls (%total; mean±SD)	p-value
CD34+ stem cells	0.9±0.39	1.36±0.49	0.02
CD117+ precursors	1.13±0.47	1.46±0.53	0.12
CD33+ immature myeloid cells	5.02±1.67	5.01±1.14	0.4
CD14+ monocytes	3.29±1.52	3.29±1.09	0.64
CD3+ T-cells	9.73±5.39	7.98±4.46	0.49
CD4+	4.47±3.52	4.27±3.03	0.84
CD8+	4.63±2.02	3.26±2.03	0.11
CD4/CD8 ratio	1.03±0.68	1.55±1.26	0.26
CD4+CD8+ double positive T-cells (DPT)	0.18±0.16	0.09±0.09	0.14
CD4-CD8- double negative T-cells (DNT)	1.03±0.84	0.63±0.51	0.3
CD3+CD56+ natural killer/T-cells	1.1±0.72	0.76±0.6	0.1
CD3-CD56+ NK-cells	2.41±3.51	2.5±2.06	0.14
CD19+ B-cells	2.16±1.82	2.18±0.95	0.3
CD20+ mature B cells	1.61±1.55	1.6±0.85	0.3
CD10+ immature B-cells	0.38±0.48	0.72±0.77	0.3
CD5+ B-cells	0.44±0.54	0.35±0.37	0.73
CD38++ Plasma cells	0.27±0.19	0.31±0.19	0.49

Summary and Conclusions: Presented findings provide for the first time an overview of leukocyte subpopulations in GD1 patients who did not receive any eznyme replacement or substrate reduction therapy. On the contrary to some reports concerning lymphocyte subsets in peripheral blood from GD patients, our data suggest a more pronounced T-cell response and possibly a stem cell defect in the bone marrow of GD1 patients, where the actual Gaucher cells

infiltrates are present. This trend was even more pronounced in splenectomized patients, in whom the natural course of the disease varies from persons with preserved spleen.

E1388

FREQUENCY OF AUTOIMMUNE LYMPHOPROLIFERATIVE SYNDROME IN CHILDREN WITH CHRONIC IMMUNE CYTOPENIAS AND NEWLY DIAGNOSED LYMPHOMA

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Background: Autoimmune lymphoproliferative syndrome (ALPS) is caused by defective lymphocyte homeostasis resulting from mutations in FAS-mediated apoptotic pathway. It is characterized by chronic non-malign organomegaly and/or immune cytopenias and/or lifelong increased risk for lymphoma. Although there are only 300 families that has been described all over the world, the actual incidence is probably much higher than that.
Aims: The aim of the present study was to investigate ALPS in children with newly diagnosed lymphoma, chronic immune cytopenias with/without nonmalign organomegaly.
Methods: We studied a total of 34 children, including newly diagnosed lymphoma (n:13), non-malign organomegaly in association with immune cytopenias (n:6), chronic immune thrombocytopenic purpura (cITP) (n:10) and autoimmune hemolytic anemia (AIHA) (n:5) which meet diagnostic criteria of proven and probable ALPS. Their CD3+CD4-CD8- double negative T cells were isolated from peripheral blood mononuclear cells and then analysed by flow cytometry using anti-CD3, anti-CD4 and anti-CD8 coated magnetic beads. T cell apoptosis was assessed in peripheral blood using flow cytometry and staining with Annexin V. Soluble Fas ligand (sFasL) and interleukin (IL-10) 10 levels were measured using quantitative ELISA method. Other hematological and biochemical parameters were recorded from the patients clinical files.
Results: Eighteen (53%) of the 34 cases met the diagnostic criteria of proven or probable ALPS. Frequencies of proven and probable ALPS were 38% and 15%, respectively. The distribution of underlying condition was lymphoma (proven n:7) (21%), non-malign organomegaly (proven n:4, probable n:1) (16%), cITP (proven n:1, probable n:3) (10%) and AIHA (proven n:1, probable n:1) (6%). Elevated double negative T lymphocyte (>5%) and increased lymphocyte apoptosis ranged between 50-80% of all patients, but high sFasL (>200pg/ml) and IL 10 (>20pg/ml) levels were found in only 10-30% of them. Double negative T lymphocyte and lymphocyte apoptosis were significantly higher in patients with lymphoma and non-malign organomegaly compared to patients with cITP or AIHA ($p<0.05$). Ninety percent of lymphoma patients had anemia and/or thrombocytopenia in association with hypergammaglobulinemia which are secondary diagnostic criteria of ALPS.
Summary and Conclusions: Our data indicate that investigation of ALPS is warranted in children with lymphoma presenting with hematologic abnormalities at diagnosis, chronic non-malign organomegaly with immune cytopenias and probably in cITP and AIHA developing organomegaly on follow up.

E1389

PLASMA LEVELS OF PRESEPSIN (SOLUBLE CD14-SUBTYPE) AS A NOVEL PROGNOSTIC MARKER FOR HEMOPHAGOCYTIC SYNDROME

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Background: It is well known that hemophagocytic syndrome (HPS) is a rare, potentially fatal cytokine-related disorder, caused by genetic factors or triggered by infections, malignancies, autoimmune diseases and transplantation. Previous studies reported that soluble interleukin-2 receptor (sIL-2R), ferritin and cytokines such as interferon-γ (IFN-γ), tumor necrosis factors-α (TNF-α), interleukin (IL)-6 and IL-10 may be associated with the prognosis of patients developing HPS. Recently, presepsin (soluble CD14 subtype), a fragment of CD14 highly expressed on the membrane surface of monocytes/macrophages, has been reported as a new diagnostic marker for sepsis (Endo S, *et al.* 2012). The release mechanism is thought to be associated with phagocytosis of microorganisms based on the animal data (Shirakawa K, *et al*. 2010). A phenomenon wherein activated macrophages eat blood cells is commonly observed in patients with HPS. We therefore hypothesized that presepsin may be a more useful prognostic marker for HPS than previously reported markers.
Aims: Our purpose was to examine 1) whether presepsin was a superior prognostic marker for HPS compared with sIL-2R, ferritin, IFN-γ, TNF-α, IL-6 and IL-10, and 2) which combination of presepsin with one of the other markers was a more useful predictor of survival when compared to any of the other markers on its own.
Methods: We retrospectively analyzed consecutive patients with HPS, from whom blood samples were obtained at our institution between April 2006 and August 2014. We diagnosed HPS using the modified HLH-2004 criteria. Plasma levels of presepsin were measured by the PATHFAST® Presepsin kit (LSI Medience Corporation, Japan) and serum levels of cytokines were determined by the Bio-Plex Pro Cytokine Assay® system (Bio-Rad Laboratories, CA). We used Cox models to investigate the association between the levels of these markers at onset of HPS and day-90 mortality. In composite model analysis, using Cox models, we compared the group of patients with a higher median value of presepsin and higher median values of each of the other markers, with the others who did not have higher values for these markers.
Results: A total of 14 patients aged 22-65 years (median 46.5) were enrolled, and 12 were evaluable, and of these, nine underwent allogeneic hematopoietic cell transplantation. The median follow-up time in all patients and in survivors (n=4) was 250 days (range, 11-1185) and 900 days (343-1185), respectively. In addition, the course of seven patients was complicated by the following infections at the onset of HPS: bacteria (n=2), fungus (n=3), virus (n=1) and virus plus fungus (n=1). In univariable Cox models, there were no significant variables: presepsin (Hazard ratio per 1 SD 1.3, P=0.53), sIL-2R (1.4, P=0.28), ferritin (1.6, P=0.23), IFN-γ (1.4, P=0.30), TNF-α (1.1, P=0.81), IL-6 (0.5, P=0.48) and IL-10 (1.5, P=0.32). However, only the combination of presepsin (≥1,936 pg/ml) and sIL-2R (≥4,586 U/ml) was significantly associated with day-90 mortality (Hazard ratio 14.5, P=0.02), whereas no other combination was significant (1.0, P=0.98 for ferritin-included model; 1.9, P=0.53 for IFN-γ; 2.7, P=0.33 for TNF-α; 1.0, P=0.98 for IL-6; and 5.3, P=0.10 for IL-10, respectively).
Summary and Conclusions: Our results suggest that the composite model of presepsin and sIL-2R levels at the onset of HPS, possibly a reflection of the activation of macrophages and T cells respectively, may be a novel and powerful predictor of the prognosis for HPS.

E1390

INFECTIONS IN CHILDREN WITH CHRONIC NEUTROPENIA-A 13-YEAR EXPERIENCE IN A TERTIARY CARE CENTER

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Background: Patients with severe congenital(SCN), autoimmune(AN) and idiopathic neutropenia(IN) have frequent bacterial infections resulting in high morbidity in early childhood. Few data on specific morbidity and mortality are available in the literature for developing countries.
Aims: To describe incidence and type of infections requiring hospitalization and antibiotic treatment in children with chronic neutropenia diagnosed in our Department during a 13-year period.
Methods: Children less than 18 years of age presenting neutropenia (ANC<1.000/μL in infants and ANC<1.500/μL in children) of >6 months duration were evaluated. We reviewed the charts of all such patients seen at our center from January 2002 to December 2014.Exclusion criteria were pancytopenia, thrombocytopenia of less than 100.000/ μL, known hematologic malignancy, previous chemotherapy.
Results: A total of 34 patients with chronic neutropenia were identified(15 females and 19 males).Six patients had severe congenital neutropenia(17%), 18 patients had AN(52%) and 10 patients had IN(33%). The median overall follow-up was 5,2 years(range, 1-11,6 years). G-CSF was used only on demand(febrile infections) in 15/34 patients, in all patients(100%) with SCN,8/18(44%) patients with AN and 1/10(10%) patients with IN. All children did not receive antibiotic prophylaxis. One patient with SCN underwent unrelated donor hematopoietic stem cell transplantation.In 7 of 18 children with AN, neutropenia disappeared after a median of 18 months(range 10 to 54 months). From birth to the last follow-up, a total of 120 infections requiring hospitalization occurred in 21/34 patients.92/120 episodes were concentrated in 6/6 patients with SCN, 19 episodes in 9/18(50%) patients with AN and 9 episodes in 6/10(60%) patients with IN. Microbiological characterization was possible in 23/120 episodes and showed Gram positive bacteria in 10 cases and Gram negative bacteria in 13 cases. The most frequently occurring bacteria was *Staphylococcus aureus*(9 cases),*Escherichia coli*(5 cases), *Klebsiella pneumonia*(2 cases) and *Pseudomonas aeruginosa*(2 cases*), Enterococcus*(2 cases).Mouth infections(17%), pneumonia(16%), otitis(15%) and skin/subcutaneous infections(15%) were the most frequent localizations. During follow-up, 11 life-threatening infectious episodes occurred in 5 patients,10 of these episodes occurred in patients with severe congenital neutropenia. The median length of hospitalization was 14,3 days for the patients with SCN, 7,1days for the patients with AN and 5,7 days for the patients with IN. No septic deaths occurred. The median ANC before G-CSF was found 377/μl in patients with SCN, 487/μl in patients with AN and 980/μl in patients with IN.
Summary and Conclusions: The majority of patients presented a benign clinical course. The largest number of infectious episodes was in patients with congenital neutropenia. Oral cavity was the main involved site. Despite the fact that antibiotic and G-CSF were used only on demand and not as prophylaxis, no septic deaths were observed.

E1391

OUTCOME OF LANGERHANS CELL HISTIOCYTOSIS IN CHILDREN UNDER THE AGE OF 24 MONTHS- LCH-III PROTOCOL

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Background: Langerhans cell histiocytosis (LCH) is a rare childhood disease with annual pediatric incidence 2-5 cases/1000000/year. It is most common between 1 to 3 years of age. Prognosis is dependent upon the number of organs involved, and the age of the patient at the onset of the disease. Patients under the age of 24 months usually present with multisystem LCH and have a potentially fatal outcome. Gastrointestinal tract involvement (GTI) in LCH is a rare condition, typically noted in children less than two years of age; 86% of reported patients presented GTI under one year of age; 59% of reported patients have died within 18 months of diagnosis, suggesting a poor prognosis.
Aims: The spectrum of clinical presentations and outcome of LCH in children younger than 24 months of age was the aim of our study.
Methods: Eleven children, who were diagnosed with LCH in the first two years of age and treated in our Unit, were enrolled in this study. The age ranged from 26 days to 24 months (mean: 10 months). All patients were treated according to the LCH-III protocol and completed the chemotherapy regiment at least 2-7 years ago. All patients remain in follow-up.
Results: Five cases presented with multisystem disease in the first 6 months of life, including intestine involvement, 3 cases had multiple bone lytic lesions in rare sites, one case had periorbital mass and 2 cases had juvenilexanthogranuloma in the first month of life. All cases received chemotherapy according to the LCH-III protocol. Four cases (36%) with multisystem disease had reactivation within 8-12 months from the end of the chemotherapy and achieved second remission. All the cases still remain in remission.
Summary and Conclusions: 1) Although children with LCH under the age of 24 months have a poor outcome, the new treatment protocols achieve a higher survival rate for this patient group. 2) Reactivation of the disease is still high (36%) and occurred in the first 12 months from the end of therapy. The cases with reactivate disease had a second chance for stable remission. 3) In two cases, there was a rapid progression of the disease from the single organ involvement, intestine and single bone lesion, respectively to the multiple organs involvement. 4) The rate of dissemination of the disease may denote differences in the pathophysiology of the disease.

E1392

HYPERTRYPTASEMIA OF UNCERTAIN SIGNIFICANCE: A NOVEL CONDITION DETECTED IN A SCREEN OF 1330 UNSELECTED CASES IN A HEMATOLOGY CENTER

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Background: In systemic mastocytosis (SM) and other myeloid neoplasms, patients often have a slightly elevated serum tryptase (15-30 ng/ml). However, an increased tryptase may also be found in allergic patients and sometimes even in healthy individuals.
Aims: In this study, we explored the relative distribution of cases with elevated tryptase in various neoplasms and non-neoplastic states.
Methods: Tryptase-levels were measured in 1330 cases, including 914 patients with hematological neoplasms (myeloproliferative neoplasms, n=156; myelodysplastic syndromes, MDS, n=241; acute myeloid leukaemia, AML, n=317; SM, n=81; lymphomas, n=59; acute lymphoblastic leukaemia, n=26), 136 with non-neoplastic hematological disorders, 102 with non-hematological conditions, and 178 healthy subjects.
Results: In healthy individuals, the median serum tryptase-level amounted to 5.2 ng/ml. Elevated tryptase-levels were primarily found in myeloid neoplasm (SM: 90% of patients; AML: 38%; MDS: 25%). We also identified 15 cases (1.2%) in whom tryptase-levels were slightly elevated, but neither an allergic disease nor a hematologic disease could be identified. The median age of these 15 individuals was 44 years (range: 24-72 years) and the female-to-male ratio was 2:1. The median tryptase-level at first presentation was 20.6 ng/ml (range: 14.5-35.5 ng/ml). In all cases, tryptase-levels remained stable in the follow-up. Bone marrow examinations did not reveal any signs of a myeloid neoplasm, and in none of the cases, an abnormal karyotype or a *KIT* mutation at codon 816 were detected. Blood counts and differential counts were normal in all cases, and no molecular aberrations were detected by multiplex-PCR.
Summary and Conclusions: We have identified a cohort of apparently healthy individuals with a persistently elevated tryptase-level. We propose to call this condition hypertryptasemia of unknown/uncertain significance.

E1393

AUTOIMMUNE LYMPHOPROLIFERATIVE SYNDROME IN A FAMILY WITH PYRUVATE KINASE DEFICIENCY

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Background: Autoimmune lymphoproliferative syndrome (ALPS) is a rare congenital disease caused by defective mediated FAS lymphocyte apoptosis and characterized by non-malignant lymphoproliferation, hepatosplenomegaly, autoimmune manifestations and increased risk for lymphoma.
Aims: To describe the presence of ALPS in a family with pyruvate kinase deficiency.
Methods: We report on a family with three boys and a girl with known pyruvate kinase deficiency, followed at our outpatient clinic. The family was studied after initial diagnosis of pyruvate kinase deficiency in the family's first child. The boy had an additional history of secondary to infection autoimmune haemolytic anemia. His latest laboratory profile was compatible with a known mild haemolytic anemia and a recently diagnosed hypergammaglobulinemia. Both of his brothers presented splenomegaly and markers of mild haemolytic anemia. In addition, however, both had a direct Coombs test and in one of them extreme hypergammaglobulinemia was detected. Proband's sister had only haematological parameters of mild haemolytic anemia attributed to pyruvate kinase deficiency, as did the father. Proband's mother had a history of splenectomy, autoimmune haemolytic anemia and immune thrombocytopenia, also presenting with hypergammaglobulinemia.
Results: The family's history of splenomegaly could be attributed to pyruvate kinase deficiency, however, hypergammaglobulinemia and autoimmune manifestations raised suspicion of ALPS. Peripheral blood lymphocytes analysis by flow cytometry for double negative T cells revealed elevated circulating TCRαβ$^+$ cells in the proband, his mother and both of his brothers. In addition, elevated levels of serum vitamin B12 were found. These findings reinforced our suspicion for ALPS and blood sample was analysed for mutation in gene coding for FAS for definitive ALPS diagnosis, which secured diangosis.
Summary and Conclusions: ALPS should be suspected in patients with persistent splenomegaly and autoimmune manifestations as to achieve prompt diagnosis and establish appropriate management.

E1394

THE RESEARCH PARAMETER NEUT-X IN VITAMIN B12 DEFICIENCY

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Background: The SysmexxE-5000 analyzer (using fluorescence flow cytometry) provides a new parameter representing the structure of the neutrophils, NEUT-X. High values of NEUT-X reflect the presence of hypersegmented giant neutrophils.
Aims: The aim of this study is to investigate the NEUT-X parameter in non anemic patients with vitamin B12 deficiency.
Methods: The patients enrolled in this study were recruited from a larger cohort of patients who were referred to our laboratory for determination of vitamin B12, folate or ferritin levels without anaemia (haemoglobin levels within the reference intervals for age and gender). Exclusion criteria were pregnancy, C-reactive protein levels >5 mg/L, white blood cells count >10,00x 10^3/μL and absolute neutrophil cells count >6,00x 10^3/μL. 97 patients with vitamin B12 deficiency were included in the study (patient group). 149 healthy individuals (matched in age and gender) were randomly selected as a control group. Full blood count was performed with SysmexxE-5000 analyzer, vitamin B12, ferritin and folate acid levels were determined by Roche Elecsys Systems (electro chemiluminescence technology). Patients with vitamin B12 deficiency have had a blood film reviewed. Statistical analysis: chi-square test, Student's t-test and Mann-Whitney U test were applied. Values of P <0.05 were considered to indicate statistical significance.
Results: The two groups did not present a statistically significant difference in haemoglobin levels (P=0,065), white blood cells count (P=0,429), absolute neutrophil cells count (P=0,251), (Table 1), ferritin levels (P=0.623) and folate acid levels (P=0,135), (data no shown). Patient group found to have significantly higher NEUT-X values when compared with control group (P=0,013) (Table 1).

Table 1. Comparison between the two groups.

Group	N (male / female)	Hb, gr/dL	Vitamin B12, pg/mL	WBC, x10³/μL	Neut#, x10³/μL	NEUT-X, ch
Patients	97 (37 / 49)	13,6 ± 1,1	113 ± 70	7,11 ± 2,65	4,28 ± 2,49	138,8 ± 3,2 ¶
Healthy individuals	149 (49 / 100)	13,8 ± 0,9	293 ± 126	6,75 ± 1,55	3,83 ± 1,19	137,8 ± 2,7

Hb: haemoglobin, WBC: white blood cells, Neut#: absolute neutrophil cells
Data are presented as mean±SD. Patient group versus *control group: P=0,013*

Summary and Conclusions: The increased NEUT-X value found in non anemic patients with vitamin B12 deficiency reflects the hypersegmentation of the neutrophils. NEUT-X parameter could be considered for the early diagnosis of vitamin B12 deficiency, as the alteration of the morphological features of the neutrophils (represented by high NEUT-X) is observed in a non anemic population, with normal white blood cells count.

E1395

CHRONIC NEUTROPENIA IN CHILDHOOD-EXPERIENCE FROM A SINGLE CENTER

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Background: Neutropenia is defined as the reduction in the absolute number of neutrophils (ANC) in the blood circulation. Neutropenia is a relatively frequent and often a secondary finding in childhood. It can be classified as acute or chronic depending on its duration, lower or higher than 3 months. Acute neutropenia is most commonly associated with viral infections, is often well tolerated and normalizes rapidly, whereas chronic neutropenia (CN) has more complex clinical manifestations. CN can be due to a heterogeneous group of diseases in children. The differential diagnosis of CN often requires much time and a lot of investigations.
Aims: The aim of the present study is evaluated the reasons and clinical manifestations of the CN in childhood in a single children's hospital.
Methods: Between October 2004 and April 2014, values of patients with CN were evaluated retrospectively at the Dr. Behcet Uz Children Hospital. Patients with chemotherapy-induced neutropenia, those secondary to lympho-myeloproliferative disorders, systemic autoimmune diseases, myelodysplastic syndromes, and common variable immunodeficiency were excluded. Thirty-one patients were diagnosed as CN.
Results: All patients were followed up to maximum 114 months or until neutropenia was recovered. The median duration of following was 29 months. The median age of diagnosis was 21 months. Thirteen of the patients (41.9%) were before the age of 12 months at initial diagnosis. Seven of 13 patients with ≤12 months at initial diagnosis (53.8%) were identified as congenital neutropenia (CoN). There was no difference between the age >12 months and ≤12 months at onset according to ANC at diagnosis. Twenty-two of all cases (70.9%) were detected during acute infections. Hospitalization due to recurrent infections was determined in 11 patients (35.5%). Most of the recurrent infections site was lung (81.8%). CoN was identified in 14 patients (45.1%). Eight of 14 patients (57.1%) required GCSF treatment and no advers effect of GCSF was seen in any of these patients. Fifteen patients (48.3%) was considered as idiopathic neutropenia. Interaction between idiopathic and congenital neutropenia groups was evaluated. Whereas there was no difference for age, ANC or application with infection at diagnosis, there was an difference for sex and spontane recovery of neutropenia. Neutropenia was spontaneously recovered in 10 of all patients (32.2%) during a follow-up of 7-52 months.
Summary and Conclusions: In current study, we found a higher congenital neutropenia ratio in CN than other reports, it may be due to higher consanguineous marriages in our country. However, CN is a finding that required several laboratory investigations, prolonged follow-up, and advanced molecular analysis, it's etiology can be remain idiopathic.

E1396

STUDY OF ALPHA HEMOGLOBIN STABILIZING PROTEIN EXPRESSION IN PATIENTS WITH B THALASSEMIA AND SICKLE CELL ANEMIA AND ITS IMPACT ON CLINICAL SEVERITY

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Background: The α hemoglobin stabilizing protein (AHSP) is an erythroid protein that specifically binds α-Hb and prevents its precipitation suggesting that it may function to limit free α-Hb toxicities. Our aim was to study the level of AHSP level in β thalassemia syndromes in relation to their clinical severity and to compare it with its level in sickle cell anemia.
Aims: We compared patients with β-thalassemia (n=37), divided into β-thalassemia major (BTM)(n=19) and β-thalassemia intermedia (BTI) (n=18) with 12 patients with sickle cell anemia.
Methods: Comparison as regards clinical severity, age at presentation, transfusion dependency, initial hemoglobin electrophoresis, mean pre-transfusion hemoglobin level, use of hydroxyurea and AHSP expression by real time quantitative PCR.
Results: Median AHSP was significantly higher in patients with sickle cell anemia 2275 (3898) compared to patients with thalassemia 283 (718), P=0.001, with no significant difference in AHSP between BTM and BTI (P=0.346). It was significantly higher in non-transfusion dependent patients with β thalassemia (NTDT) compared to transfusion dependent ones (P=0.019), and in patients on hydroxyurea therapy (P<0.001). However, there was no significant difference in its level according to clinical severity score (P=0.946) or splenectomy status (P=0.145).
Summary and Conclusions: AHSP Expression was higher in sickle cell anemia *versus* thalassemia, with no significant difference between BTM and BTI. Expression was higher in patients with NTDT and on hydroxyurea therapy.

E1397

HEMOPHAGOCYTIC SYNDROMES IN SAUDI CHILDREN: SINGLE CENTER EXPERIENCE

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Background: Hemophagocytic lymphohistiocytosis (HLH) is a rare aggressive and life-threatening syndrome of excessive immune activation due to cytokine overproduction from excessively activated lymphocytes and macrophages. It most frequently affects infants from birth to 18 months of age, but the disease is also observed in children and adults of all ages. Chemo-immunotherapy-based treatments have improved the survival of patients with HLH, but outcomes of the patients are still unsatisfactory.
Aims: To evaluate patients' characteristics, laboratory and molecular findings and treatment outcome.
Methods: We retrospectively analyzed data of 11 HLH patients who were admitted to the Pediatric Hematology Department of Prince Sultan Military Medical City, Riyadh, Saudi Arabia between 2005 and 2014. HLH was diagnosed according to the HLH-2004 diagnostic guidelines. We defined primary HLH as Patients who were found to have a genetic abnormality and/or early-onset diseases (age 0-2 years) with family history were considered as having familial HLH.
Results: Patient characteristics are summarized in Table1.The median age at onset of HLH is 12 months. There was female predominance (8/12). Consanguinity rates significantly were high (75%) and positive family history in 5 cases. Of 12 patients, 9 were defined as primary, and 3 were secondary HLH (cases 4, 5 and 8). All patients met the criteria of HLH; at least five out of a total of eight criteria. (Figure 1) To dates, 6 of the 12 patients are still alive (50%), 4 of the patients died before HSCT, whereas one patient died of relapse after matched unrelated donors and one lost follow up.

Table 1. Patient characteristics.

#	Age	Sex	Consanguinity	Family history	Associated Infection	HLH criteria	Molecular Mutation	Treatment	HSCT	Outcome
1	4 months	M	+	+	-	6/8	PRF1	HLH 2004	Haplotype	Alive
2	4 months	M	+	+	EBV	7/8	STX-11	HLH 2004	No	Died
3	13 months	F	+	+	Candida	5/8	STX-11	HLH 2004	No	Died
4	3 years	M	-	-	Dengue Fever	5/8	Negative	HLH 2004	No	Alive
5	8 years	F	-	-	EBV	7/8	Not done	HLH 2004	No	Died
6	2 months	F	+	-	-	6/8	Negative	HLH 2004	Haplotype	Alive
7	11 months	F	+	+	-	6/8	STX-11	HLH 2004	Cord SCT	Alive
8	4 years	F	+	-	Dengue Fever	5/8	Negative	HLH 2004	No	Lost follow up
9	7 years	F	+	-	-	5/8	Not done	Refused treatment	No	Died
10	3 months	F	+	-	-	5/8	PRF1	HLH 2004	URD HSCT	Died
11	6 months	M	+	+	Gram +ve cocci	6/8	STX-11	HLH 2004	MRD HSCT	Alive
12	7 years	F	-	-	HAV	6/8	Pending	HLH 2004 Anakinra	On therapy	Alive

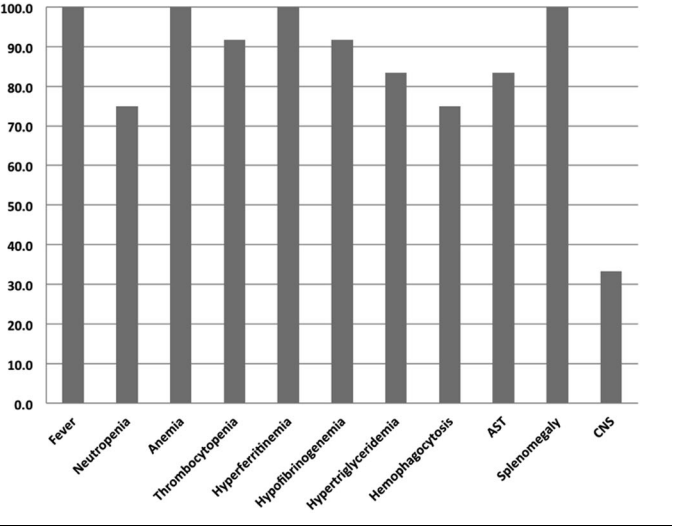

Figure 1. Patient clinical and laboratory parameters.

Summary and Conclusions: Hemophagocytic lymphohistiocytos is a serious and potentially life threatening disorder in children and rarely in adults. Familial HLH is the most common encountered type of syndrome. Chemotherapy regimen is not ideal yet to control the disease. Bone marrow transplantation is the only available cure. Awareness of the clinical symptoms and of the diagnostic criteria of HLH is important as correct diagnosis with adequate treatment with immunosuppressive /immunomodulatory agents in time can be life saving in these patients. Research to uncover the gene responsible for the disease by collaborative group is recommended.

E1398

LONG TERM TREATMENT WITH CONTINUOUS LOW DOSE LENALIDOMIDE RESULTS IN PET/CT DOCUMENTED EXCELLENT PARTIAL REMISSION IN A PATIENT WITH SEVERE MULTI-SYSTEMIC LANGERHANS CELL HISTIOCYTOSIS

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Background: Langerhans cell histiocytosis (LCH) is a non malignant orphan disease of clonal dentritic cells with a possible affection of any organ. Diagnosis is often difficult due to a wide clinical spectrum of presentation, ranging from a single lesion right up to a multi-systemic disorder. Treatment options are consisting of radiotherapy for bone lesions till conventional chemotherapy e. g. with cytarabin, etoposide and vinblastin. In multi-systemic organ involvement cladribine (2-CdA) is often used. Treatment with Thalidomide as could be a different option. In 2013 we already described a female patient (pat) achieving a complete remission of localized LCH of the vulva with Lenalidomide (Len) treatment. Positron emission tomography (PET), more specifically PET/CT has been well validated for staging and treatment response monitoring in oncology patients (pts). The use of PET/CT in LCH has been reported, but its use in adult pts is not well established. Especially in adult pts with severe comorbidities like renal insufficiency it is a very useful tool. In addition, other information like the metabolic activity defined as mean standardized uptake values (SUV) of LCH lesions can be very helpful.
Aims: Here we present the first pat with a multifocal aggressive and repeatedly relapsing LCH with an outstanding response to continuous long term treatment with low dose Len (10 mg every other day).
Methods: Case report.
Results: A 69 year old man with severe comorbidities (diabetes, renal insufficiency, coronary heart disease) was diagnosed in Jun 2011 with multi-systemic LCH. In between Aug 2011 and Dec 2011 he received 4 cycles of 2-CdA (0.14 mg/kg/BW) and obtained only a partial remission (PR). In May 2012 he presented with a multi-systemic relapse/reactivation in different lymph nodes and involvement of other organs like parotid gland, muscle, bone and testes. After histological confirmation and PET/CT based staging in Jul 2012, first cycle of Len 10 mg/d for 21 days q 4 weeks was started. A few days later a substantial benefit with decreasing cervical lymph nodes was noticed. Due to moderate leukocytopenia weekly G-CSF was used. PET/CT in Dec 2012 showed again PR and due to a cardiac intervention Len was stopped for 6 weeks. Due to a mild anemia, retreatment with Len 5 mg/d was initiated in Feb 2013. Because of a slight lymph node decrease only, Len dose was escalated up to 10 mg every other day as a continuous treatment option. PET/CT in Sept 2013 showed a mixed response, in May 2014 PET/CT showed an acceptable remission with a SUV decrease in the LCH lesions. Current PET/CT in Feb 2015 shows an excellent PR with only small residues submandibular and in left testicle (Table 1).

Table 1.

	PET/CT Jul 2012	PET/CT Sept 2013	PET/CT Feb 2015
Lymph node right neck [SUV]	15.1	14.8	2.8
Testicle right [SUV]	12.0	6.7	0
Testicle left [SUV]	0	8.5	4.9

Summary and Conclusions: We present a case of a highly effective treatment with continuous low dose treatment of Len in a pat with multi-systemic LCH. Long term treatment of Len is well tolerated without severe side effects. A dose of 10 mg every other day appears to be very effective with manageable toxicities. PET/CT based surveillance provides additional information of metabolic activity in the LCH lesions and is a very helpful tool, especially in pts with comorbidities like renal impairment.

E1399

SUSTAINED INFLAMATORY CYTOKINES IN NAÏVE GAUCHER DISEASE: A LINK WITH MULTIPLE MYELOMA?

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Background: Gaucher disease (GD), the most common lysosomal storage disorders, (~1/100,000 habitants outside Ashkenazi population), is secondary to the deficit of B-glucocerobrosidase activity leading to an accumulation of glycil-ceramide in monocyte-macrophagic system, it characterized by a latent chronic inflammation and expressed by hyperferritinemia, hypergammaglobulinemia, altered calcium homeostasis, metabolic syndrome and an imbalanced pro-inflammatory cytokine expression. The incidence of Monoclonal Gammopathy of Uncertain Significance (MGUS) and multiple myeloma (MM) and other B-cell disorders is increased in type 1 GD (GD1) patients (3.5-12.5 folds). Previous reports have identified osteoclastogenesis induced by TNFa and others cytokines like IL-6 in GD1 mouse models also some cytokines like MIP1a and TNF have gained importance in the pathogenesis of MM-bone marrow microenvironment induced changes. Based on that we hypothesize that deregulation of cytokines resulting from glucocerebroside accumulation may influence the development of hematologic malignancies in GD1.
Aims: To analyze and compare a panel of proinflammatory cytokines profile among MGUS, MM and GD1patients and correlate with outcomes.
Methods: A protocol was conducted with the Aragon biobank for select 4 cohorts of frozen stored plasma collected at least 5 years ago and with available follow-up data: 36 MGUS (58.3% women, mean age: 75 years, range 53-89), 11 IgGk; 6 IgGL; 5 IgAk; 8 IgAL; 3 IgMk; 3 doble IgG+IgA. Forty-nine MM (42% women, mean age 68 years, range 39-89), 20 IgGk; 5 IgGL; 8 IgAk; 5 IgAL, 8 ligth chain disease and 3 IgG+IgA. Concerning ISS classification 44.0% MM patients had a score 1, and 31.7% score 3; thirty-eight naïve GD1 patients (36.8% women, mean age 39.2 years, range: 17-79) and 71 healthy controls. A panel of eight cytokines (IL4, IL6, IL7, IL10, IL13, MIP1α, MIP1β and TNF) was tested (Luminex®100 platform, Millipore cytokine kits). Clinical and analytical data were obtained from the records of the Hematology Department and from the Spanish Registry of GD (FEETEG). Non-parametric tests Mann-Whitney-U and Kruskall-Wallis-H were used. Period of study: February-Sept 2014.
Results: After 5 years 21 MM and 8 MGUS patients had died (42.8%, 22.2%), 4 MGUS developed a MM (11.1%); 7 MM and 8 MGUS developed a second tumour (14.2%, 22.2%). One GD1 patients developed a MM and 2 a solid tumour. Cytokine analysis: significant difference ($p<0.05$) were observed in IL4; MIP-1α; MIP-1β and TNFα values between controls, MGUS, MM and GD1 patients; Also for IL13 in MM and GD1. MM and GD1 not showed differences in the median values of IL4 and IL13. These groups showed significant differences in MIP-1α, MIP-1β and TNFα values. In MM there are significant differences in IL4 and MIP-1α between ISS 1 and ISS 3. In addition there are significant differences in MIP-1α between IgGk *vs* IgAL and in MGUS patients who progressed to MM. Inside the most expressed cytokines MM showed the highest MIP-1α values, but GD naïve patients showed the highest inflammatory cytokines MIP-1β and TNFα respect all cohorts (Table 1). There were not significant differences in cytokines profile between patients that develop a second neoplasia.

Table 1. General overview (medians).

	Controls	GD1	MGUS*	MM*
IL4	•	Δ	↑	↑
IL6	•	•	•	•
IL7	•	•	•	•
IL-10	•	•	•	•
IL-13	•	↑	•	↑
MIP-1α	•	↑✓	↑	↑✓
MIP-1β	•	↑✓	↑	↓✓
TNF-α	•	↑✓	↑	↑✓

•: not-significant difference respect to controls. Δ: increased values, no significance. ↑: increased values significance difference ($p<0.05$). *: For MIP-1α, significant difference between MM IgGk vs. IgAL, and between MGUS vs. MGUS progressed to MM were registered. ✓ : Significant difference was found between GD1 vs MM. ✓ difference, but lowest value respect to GD or MM.

In MM there were significant differences between ISS-1 and ISS-3 for IL-4 and MIP1a

Summary and Conclusions: Cytokines produced in response to glucocerebroside accumulation, could be a critical factor in the pathogenesis of MGUS and MM in GD, both patients presents alterations on cytokines profiles, specially in MIP-1β, TNFα and MIP-1α that could be a good biomarker to predict transformation to MM.
This work was partially sponsored by FIS PS12/01219 and Fundación Española para el Estudio y Terapéutica de la Enfermedad de Gaucher (FEETEG) y otras lisosomales and Fundación para el Estudio de la Hematología y Hemoterapia en Aragón (FEHHA).

E1400

AUTOIMMUNE HEMOLYTIC ANEMIA: DESCRIPTIVE STUDY OF 100 CASES

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Background: Autoimmune haemolytic anemia (AIHA) is caused by autoanti-

body-induced hemolysis (the premature destruction of circulating red blood cells). Usually idiopathic, it is also associated with infection, lymphoproliferative disorders, autoimmune diseases, and some drugs.
Aims: Study the epidemiological, clinical, biological, etiological and therapeutic aspects of AIHA.
Methods: This is a retrospective and analytic study about 100 cases of AIHA observed in the hematology and internal medicine departments of Sousse, over a period of 14 years. In this work, we have tried to describe the clinical aspects of these AIHA and evaluate the contribution of different diagnosis exploration and therapeutic means used.
Results: There were 40 men and 60 women (sex ratio=0.66) with a median age of 51 years [14-87]. Regarding the medical history, 19 patients were hypertensive, of whom 8 were receiving Methyldopa, 9 patients were diabetic, 7 had thyroid dysfunction and 18 had a history of autoimmune disease. The circumstances of discovery were an anemic syndrome in 80 of patients, mainly due to paleness and asthenia. Physical examination revealed icterus in 42 cases, splenomegaly in 41 cases, hepatomegaly in 8 cases, lymph node in 16 cases and fever in 21 cases. Concerning biology, regenerative anemia was normocytic in 43 cases and macrocytic in 48 cases, thrombocytopenia below 100000/mm^3 was observed in 13 patients. There were also biological signs of hemolysis: hyperbilirubinemia in 70 patients, high LDH rate in 68 patients. Direct Coombs test was positive for IgG in 55 cases, C in 12cases, Ig G+C in 20cases, IgA in 1 case, IgG+C +IgM in 4 cases and cold agglutinins search returned positive in 8 cases. There was Evans syndrome in 14 patients. AIHA was idiopathic in 45 cases including 3 cases of pregnancy. In the other cases, it was secondary to lymphoproliferative desorders in 20 cases, autoimmune disorders in 25 cases, drug taking (Methyldopa) in 8 cases, associated with a myelodysplastic syndrome Methyldopa in 1case and CMV infection in 1 case. The therapeutic consisted of transfusion in 37 cases and all patients received corticosteroid treatment in addition to folic acid therapy and etiological treatment in the non idiopathic cases. A complete remission was obtained in 55 cases. In severe cases of chronicity or relapse, immunosuppressive therapy was prescribed in 14 patients, anti-CD20 monoclonalantibody were prescribed in 8 patients and splenectomy was performed in 3 patients.
Summary and Conclusions: Glucocorticoids and/or intravenous immunoglobulins are the mainstay of the treatment in the majority of patients with warm AIHA. When these treatments fail, patients often require cytotoxic drugs or splenectomy. The current research in many other autoimmune diseases that can sometimes be associated with AIHA should still allow a better understanding of the mechanisms involved in the occurrence of these diseases and to refine treatments whose essential aim is to improve the effectiveness of both new and already available treatments (including rituximab) in order to limit the use of corticosteroids.

E1401

PROSPECTIVE STUDY OF PLASMA BIOMARKERS ASSOCIATED WITH THE INFLAMMATORY RESPONSE IN TYPE 1 GAUCHER DISEASE PATIENTS TREATED DURING ONE YEAR WITH VELAGLUCERASE ALFA

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Background: Background: It is wide accepted that Gaucher patients have an impairment in their immune system, clinically reflected by a infections tendency and biologically by a chronic inflammatory state. The role of cytokines in this inflammatory state is partially known, and the modifications in this profile in GD patients under enzymatic replacement therapy (ERT) are under investigation. Our group has reported some changes on the cytokine profile in patients with severe bone involvement[1] and some inflammatory biomarkers of macrophage activation related to the iron profile[2].
The progressive infiltration by engorged macrophages led to a production of proinflammatory cytokines into bone marrow microenvironment, altering of the bone turnover, producing imbalance in cellular function, angiogenesis and patchy infiltration by Gaucher cells.
Aims: Aims: To explore the changes in the biomarkers of immune response in a cohort of Spanish type 1 Gaucher disease patients (GD1) after one year on Velaglucerase alfa therapy.
Methods: Patients and Methods: A total of 17 GD1 patients from 15 centers, were included in a prospective protocol following these criteria: symptomatic patients of both sexes, aged older than 4 years, naïve or previously treated but without ERT at least one month previous to be included. The study included blood counts, liver and spleen volume by MRI and bone marrow MRI evaluation following S-MRI protocol, and determine bone mineral density by Quantiative Ultrasound expressed by Z-score, and biomarkers: Chitotriosidase, CCL18/PARC, ferritin, immunoproteins and the following cytokine profile: IL-10, IL-13, IL-4, IL-6, IL-7, Mip1a, Mip1b,TNFa, performed at baseline and 12 months after therapy. The study was approval by ethical committees and designed according Helsinki declaration rules; every one patient signed the informed consent and commitment to use a safe contraceptive method during the study period and 3 months after completion of treatment. Non-parametric tests Mann-Whitney-U and Kruskall-Wallis-H were used. Period of study: November 2011- April 2014.
Results: Results: General characteristics: 9 males, 8 females, mean age: 37.5 years (9-72). 3 splenectomized patients (17.6%); genotype: 3 N370S homozygous one heterozygous for N370S/L444P and the rest heterozygous for N370S/other. Seven patients (41.2%) have previous history of bone disease complications. All patients received velaglucerase alfa 30U/kg iv every two weeks for 1 year in every day clinical practice. Patients achieved an objective response on disease goals, after normalization and/or stabilization in blood counts and visceral volumes no significant variation were observed. Nevertheless a significant increase in the Z-score was observed after one year on Velaglucerase alfa therapy. Respect to biomarkers, a reduction or stabilization of CT activity and CCL/18 concentration were observed, also ferritine concentration and serum free light chains do not show significant variation. The cytokine profile showed a decrease in all inflammatory cytokines tested, however for Mip1a (p=0.027) and TNFa (p=0.023), a significant reduction were registered. Table1. No infusion reactions were reported, neither antibodies against velaglucerase alfa.

Table 1. Comparative reduction of cytokines between baseline and 12 months.

	IL10	IL13	IL4	IL6	IL7	Mip 1a	Mip 1b	TNF a
Chi X^2	3.415	0.000	2.668	1.606	1.545	8.159	3.308	4.263
grade	2	2	2	2	2	2	2	2
significance	0.181	1.000	0.263	0.448	0.462	0.017	0.191	0.023

Summary and Conclusions: Velaglucerase alfa is a well-tolerated therapy in every day clinical practice. In our cohort we observed as part of the response to therapy a significant decrease on the inflammatory state reflected through the cytokine reduction.
Acknowledgements: This work was partially supported by a grant from Shire and FIS: PS12/01219.

Reference
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E1402

A CASE OF INDOLENT SYSTEMIC MASTOCYTOSIS IN A GIRL TREATED WITH INTERFERON

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Background: Mastocytosis refers to a group of myeloproliferative disorders characterized by excessive proliferation and accumulation of mast cells in tissues. It is rare in both adults and children and occurs in less than 0.01% of the general population.
Aims: A 5-year old girl who was diagnosed as cutaneous mastocytosis by our dermatology department when she was four months of age, was admitted to our pediatric hematology department by hepatosplenomegaly. In physical examination, widespread maculopapuler and itchy lesions were determined.
Methods: The assessment of bone marrow aspiration was found to be consistent with mast cells (rate >10%). Flow cytometry on bone marrow sample revealed that there was CD117/CD25 positivity of 18.4% and CD117/CD2 positivity of 0.7%. Diagnosis of SM was verified by one major and one minor WHO criteria: presence of multifocal, dense aggregates of mast cells in bone marrow (major criteria) confirmed by expression of CD2, CD25, and CD117 in bone marrow (minor criteria). Also serum tryptase level was 356 ng/ml (>20 ng/ml).
Results: We were diagnosed smouldering SM (a part of indolent SM) because of high serum tryptase level, organomegaly, and infiltration of mast cells in the bone marrow. During follow up, systemic anaphylaxis was determined and treated for three times. We recommended the self-administration of epinephrine on demand for anaphlactoid episodes. Treatment with montelucast, interferon alfa-2a and methylprednisolon produced a marked and sustained reduction in her symptoms, cutaneous lesion, and tryptase level (Figure 1A-B).

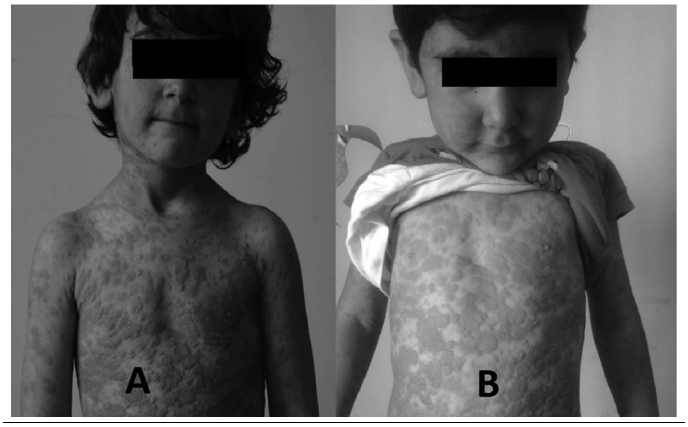

Figure 3.

Summary and Conclusions: Consequently, we report on the first case of indolent SM that was successfully treated with montelucast, interferon alfa-2a and methylprednisolon in children.

E1403

USEFULLNESS OF SERUM FERRITIN LEVEL >10,000 NG/ML TO DIAGNOSIS HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS (HLH) IN ADULTS.

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Background: Hemophagocytic lymphohistiocytosis (HLH) is a rare pathology characterized for clinical and laboratory alterations secondary to an uncontrolled immune activation that lead a severe inflammation systemic status. HLH can be congenital or acquired, idiopathic or secondary to infections, neoplasms (specially hematologic neoplasms) or rheumatic diseases. The diagnosis of HLH is based in the criteria determined by the Histiocyte Society, hyperferritinemia is a key criteria, it is specific for HLH in pediatric population; although some reports had outstand the value of serum ferritin (SF) over 10,000 ng/mL as the first suspicion clue for HLH. It is remarkable that HLH carry-on a mortality rate around 90% and diagnosis in adults is difficult and the value of SF is not fully established. Based on that, we perform a review to assess this aspect in hospitalized adults patients with SF >10,000 ng/mL in our institution.
Aims: To identified adults patients with SF >10,000 ng/mL, assess the HLH diagnostic criteria and describe the possible causes for high SF values and the usefulness of this cut-off for HLH diagnosis.
Methods: A chart review was performed. All hospitalized-adults with a SF level >10,000 ng/mL were selected to lead the retrospective assessment of the HLH criteria using the clinical and laboratory data available in the electronic medical registry. At the same time, selected patients were crossed with the transfusion service to check transfusion records; also patients with family history of congenic HLH were excluded. For HLH diagnosis the presence of 5-6/6 following criteria: >10,000 ng/dL SF, fever, splenomegaly, 2-3 cytopenias, hypertriglyceridemia (>252 mg/dL) and bone marrow hemophagocytosis (BMH), (sCD25 and NK functionality studies were not available in our center). Period of study: Jun-2009 to Jan-2015.
Results: A total of 78 patients were identified in the first screening for SF >10,000 ng/mL; twenty-three patients full flied HLH criteria assessment. Mean age: 53.91 y.o. (16-82). Male/female ratio: 12/11. According to criteria revision: 6 patients full flied 6/6 criteria and 18 patients 5/6 criteria. According to each criteria all patients presented fever and hyperferritinemia; mean SF level: 28,938 ng/mL (12,606.9-61,557.0), Cytopenias: 22/23 patients presented it, Mean values: Hb: 7.1 (2.3-12.5) g/dL, Absolute neutrophils count (17 patients <1.00x10^3/mL): 0.19x10^3/mL (0.00-10.4), Platelets: 18 (1-90)x10^3/mL. Mean serum triglycerides: 559 (252-1011) mg/dL, BMH: 13/17 patients with bone marrow aspiration performed show BMH. Splenomegaly: 18/23 patients. At the same time others biochemistry alterations (liver enzymes, LDH, bilirubin, coagulopathy) were registered in all patients. Neurological, pulmonary and skin alterations were recorded in 8, 9 and 3 patients. Possible etiologies of secondary HLH: infections: 7 cases, neoplasms: 12, both: 3 cases and other 1 case. Mortality: the median time duration of the hospitalization were 23 days (3-60), with the exception of 2 cases, one male with leishmaniosis and a female with Evan's syndrome and CMV infection are alive, the rest of the patients (91.3%) died during the hospitalization period.
Summary and Conclusions: HLH is a rare multisystemic syndrome with higher mortality rate, probably related with the difficulties during the diagnosis process. In our cohort the value of ferritin levels >10,000 ng/mL was a good biomarker to start the suspicion of the illness. More accuracy is necessary to identified and treats this severe pathology. In case of acceptance a complete review of all screened patients will be presented.

LB2087

DUSP4-MEDIATED ACCELERATED T-CELL SENESCENCE IN IDIOPATHIC CD4 LYMPHOPENIA

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New Information: This work has been prepublished online as Blood First Edition paper, March 2, 2015; DOI 10.1182/blood-2014-08-598565.
Background: Idiopathic CD4 lymphopenia (ICL) is a rare heterogeneous immunological syndrome of unclear etiology. ICL predisposes patients to severe opportunistic infections and frequently leads to poor vaccination effectiveness.
Aims: Chronic immune activation, expansion of memory T cells and impaired TCR signaling has been reported in ICL, but the mechanistic and causative links remain unclear.
Methods: To gain insight into the functional pathways implicated in ICL pathophysiology, we compared the gene expression profiles of $CD4^+$ T cells sorted from the blood of ICL patients and control groups of age-matched healthy subjects and patients with $CD4^+$ T-cell lymphopenia secondary to sarcoidosis. These arrays enabled us to identify an ICL-related genetic signature with predominant involvement of two gene groups, one related to TCR response and the other strikingly associated with T-cell aging. Among the first group, expression of the dual-specific phosphatase 4 (DUSP4), which negatively regulates MAPK activation, was increased similar in this respect to $CD4^+$ T cells from elderly subjects. Functional studies further linked this overexpression to accelerated T-cell senescence in ICL.
Results: We showed that late-differentiated T cells in 20 ICL patients displayed defective TCR responses and aging markers similar to those found in T cells from elderly subjects. Intrinsic T-cell defects were caused by increased expression of DUSP4. Normalization of *DUSP4* expression using a specific siRNA improved $CD4^+$ T-cell activity in ICL, as this restored TCR-induced ERK activation and increased the expression of the co-stimulatory molecules CD27 and CD40L. Conversely, repeated TCR stimulation led to defective signaling and *DUSP4* overexpression in control $CD4^+$ T cells. This was associated with gradual acquisition of a memory phenotype and curtailed by *DUSP4* silencing.
Summary and Conclusions: These findings identify a premature T-cell senescence in ICL that might be caused by chronic T-cell activation and a consequential DUSP4-dependent dampening of TCR signaling.
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LB2094

FINALLY A METHOD FOR ROUTINE DIAGNOSIS OF SOUTH-EAST ASIAN OVALOCYTOSIS

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Background: South East-Asian Ovalocytosis (SAO), is a dominantly inherited red cell membrane defect caused by a small deletion in exon 11 of *SLC4A1,* the gene encoding band 3. The deletion is commonly accompanied by a missense mutation in exon 4 (p.Lys56Glu). The 9 amino acid-long deletion eventually results in a markedly increased rigidity of the red cell membrane. Nevertheless, individuals affected are usually clinically asymptomatic and only in children slight haemolysis has been observed. Although, the homozygous state of the mutation was believed to be incompatible with life, recently a homozygous SAO child with severe dyserythropoietic anemia and distal renal tubular acidosis has been described. The hall mark of the disease is the presence of macro-elliptocytes, some with a transverse ridge across the central clearing, on the blood smear (Figure 1C). This is currently the only way to reliable diagnose the disease. The condition is wide-spread in certain ethnic groups as the condition protects against invasion of several malaria strains. However de precise Figure and incidence is unknown, as the investigation of the blood smear is not part of daily routine laboratory practice.
Aims: To establish a highly specific method to diagnose SAO using the CELL-DYN Sapphire automated haematology analyser.
Methods: EDTA samples of regular primary-care patients at our laboratory were examined on the CELL-DYN Sapphire Haematology Analyser using the complete blood cell count mode (CBC). Positive identification of SAO was followed by measurements using the Reticulocyte mode (CBC+RETC) and blood-smear analysis. To confirm our findings, anonymised EDTA samples were subjected to osmotic gradient ektocytometry on the LoRRca MaXis and DNA analysis of exons 4 and 11 of *SCL4A1* and for typical cellular behavioural examina-

tion by the automated Rheoscope Cell Analyser (ARCA). All cases sent were positively confirmed as being a SAO-carrier, and the physician was subsequently notified on these results.

Results: Analysis of scatterplots generated by our routine blood cell analysers revealed a small cluster of red cells in the 7/90° optical platelet scatterplot in conjunction with a slightly lowered MCV (Figure 1A). Examination of these red blood cells on a smear demonstrated typically SAO associated Macro-Elliptocytes (Figure 1C). Analysis of the CBC+RETC mode showed a typical first small peak close to the Y-axis followed by a broad asymmetric peak, displaying the heterogenic distribution of the Cellular Heamoglobin Concentration (CHC; Figure 1B). Using these two parameters we identified 32 SAO-carriers in our population. SAO was confirmed by morphology, DNA, ektocytometric (Figure 1D) and Rheoscopic analysis. Comparison with other Haematology Analysers revealed that this feature is probably due to the M.A.P.S.S. technique algorithm which is unique for the CELL-DYN Sapphire.

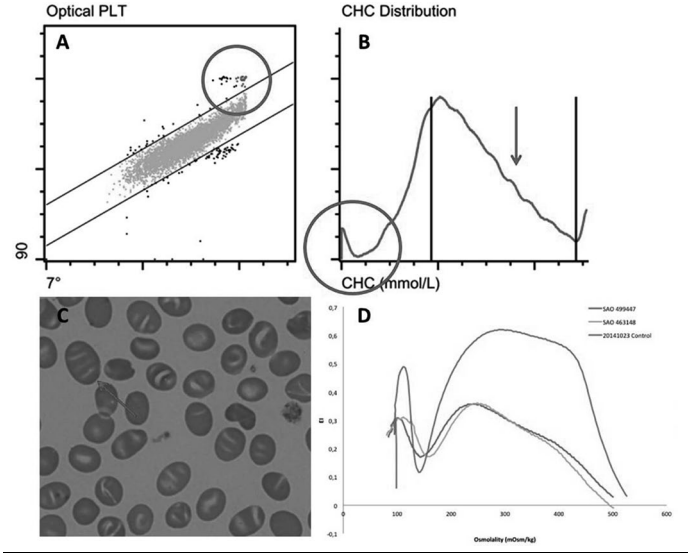

Figure 1.

Summary and Conclusions: South East Asian ovalocytosis is a rare disease in Europe and it is therefore difficult to study. We established a method by which SAO can be easily and reliably diagnosed using routine laboratory haematology parameters. When applied to a subpopulation in the Nothern part of the Netherlands, a considerable and higher than expected population of SAO carriers was detected. This method can be used to prevent embryonic lethality or the severe clinical presentation that accompanies homozygosity for SAO.

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Platelets disorders

E1404

EFFICACY OF HIGH-DOSE METHYLPREDNISOLONE AS A FIRST-LINE THERAPY IN ADULT PATIENTS WITH IDIOPATHIC THROMBOCYTOPENIC PURPURA

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Background: Guidelines for the emergency treatment of clinically important bleeding or prophylactic treatment before surgery/invasive procedures in ITP patients recommend several approaches either as high-dose methylprednisolone (HDP) or intravenous Ig (IVIG) and sometimes anti-D to provide a safe platelet count. There are various reports suggesting that IVIG treatment may result in a more rapid response thus more effective in these indications. The recommendations are usually based on the results of few number of controlled studies and more on individual experience.

Aims: In this study we tried to compare the efficacy of high-dose methylprednisolone with IVIG and conventional prednisolone (CDP) therapy as a firstline treatment in adult patients with ITP.

Methods: This retrospective study included 140 adults with either previously untreated acute ITP (51) or persistent/chronic ITP (22/67) patients with an acute attack. Patients with a platelet count <30x10^9/L or <50x10^9/L with a clinically significant bleeding were treated either by CDP therapy (1 mg/kg/day until response) or HDP (20 mg/kg/day for 3 days) or IVIG (1g/kg/day for 2 days). All the patients in three groups continued treatment with oral prednisolone (1 mg/kg/d) until stable platelet count was reached. Steroids were then tapered off in 4-6 weeks. Responses after the treatment and clinical course of the patients were evaluated.

Results: Of 140 patients enrolled initial platelet counts were similar in all treatment arms. HDP was given to 92 (65,7%), IVIG to 32 (22,8%) and CS to 16 (11,4%) of patients. The median follow up patients was 21.5, 13.1 and 9.3 months in HDP, IVIG and CDP groups respectively (P<0.05). HDP received patients showed the first response sooner (platelet counts ³30x10^9/L) compared to IVIG and CDP patients which was 6, 10 and 12 days respectively (P<0.05). Median platelet count at first response was similar between the three groups. Long-term complete response (>6 months) was higher in HDP group (57.6%) compared to IVIG (37.5%) and CS (25%) of patients (P <0.05). The initial treatment choice did not effect the response type. Relapse rate after achieving stable response was 62.8%, 81.3% and 88.9 % in HDP, IVIG and CDP group patients but the differences were statistically insignificant (P >0.05). Overall a negative correlation was observed between the response rates and age of the patients in all of the three groups (R=-0.172, P=0.04). Long-term remission rates also decreased as the patients become older in all the groups (R=-0,22, P=0,02).

Summary and Conclusions: Our results support that HDP can be more comfortably proposed as firstline treatment in adult patients with ITP. Due to its effectiveness, low cost, and convenience of use, high-dose methyl prednisolone still seems to be the best choice as initial treatment in ITP.

E1405

ELTROMBOPAG MAY BE USEFUL IN SECONDARY ITP PATIENTS IN CLINICAL PRACTICE

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Background: Eltrombopag is a thrombopoietin receptor agonist (TPO-RA) approved for treating chronic primary ITP patients. Nevertheless, due to the non-existence of clinical trials there are no clear efficacy and safety data of eltrombopag in secondary ITP.

Aims: To evaluate the efficacy and safety results using eltrombopag for treating secondary ITP patients in routine clinical practice in Spain.

Methods: 33 secondary ITP patients from 23 Spanish centers who had been treated with eltrombopag and included in the Spanish Eltrombopag ITP Registry were retrospectively evaluated. However, 5 patients were excluded from the final analysis because three of them were aplastic anaemias, one was an amegakaryocytic thrombocytopenia and another one was a acute myeloid leukemia related thrombocytopenia.

Results: Our secondary ITP case series included nine hepatitis C virus-ITP, five lymphoproliferative disorders, four systemic lupus erythematosus (SLE), three HIV-ITP, two synchronous HCV-HIV-ITP, two psoriatic arthritis, one Evans Syndrome, one common variable immunodeficiency and one Sjögren syndrome. The median age of our cohort was 54 (IQR, 35-66) years. There were 17 women and 11 men. 25% of patients had a Charlson Comorbidity Index score of 2 or more at diagnosis. The median time from secondary ITP diagnosis to eltrombopag initiation was 36 (IQR, 1-76) months. The median number of therapies before starting eltrombopag was 2 (IQR, 1-4), including rituximab (28%), romiplostim (17%) and splenectomy (10%). At the time of treatment start, 15 of 28 patients (53%) patients were receiving concomitant treatment for secondary ITP, mainly including corticosteroids (31%) or immunoglobulins (21%). 7 of 28 (25%) patients had bleeding symptomatology during the month preceeding the starting eltrombopag. At eltrombopag initiation the median platelet count was 9x 10^9/L (IQR, 6-15x10^9/L). 25 of 28 (89%) patients responded to eltrombopag treatment. 23 patients (82%) achieved a complete response (CR; platelet count >100x10^9/L). To point out that 2 patients needed concomitant treatment with low prednisone doses to achieve or maintain the response. It was a slight difference between men and women regarding the response and complete response rates obtained: men achieved 82% and 73% respectively meanwhile women achieved 94% and 83%. The proportion of patients achieving platelet response was quite similar regardless the other studied parameters: age (87% and 92% for patients <65 years-old and ≥65 years-old, respectively), use of concomitant ITP medication at baseline (87% and 93% for patients with and without concomitant baseline ITP medication use) and bleeding at starting eltrombopag (100% and 87% for patients with and without bleeding, respectively). Three patients achieved complete response after only one month of tretament without relapsing afterwards. In five patients splenectomy was made few months after eltrombopag treatment (3 patients were in CR). Only two patients failed to achieve response with eltrombopag treatment: common variable immunodeficiency and one HIV patients. After a 9 months median follow-up, 15 patients maintain the response. Only 3 patients relapsed from their disease. Only two adverse events were reported: a grade 2-3 cephalea in a SLE patient and a death caused by respiratory insufficiency in a HIV patient with a CR ITP at that moment.

Summary and Conclusions: Our case series describe the great efficacy and safety results observed with the use of eltrombopag in our secondary ITP patients. Our data suggest some diseases may not benefit from the use of eltrombopag. However more studies are needed to confirm the possible usefulness of TPO-RAs in this variety of secondary ITP cases.

E1406

ITP PATIENTS IN THE ASIA PACIFIC: ARE THEY DIFFERENT?

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Background: Immune thrombocytopenia (ITP) is an autoimmune disorder characterized by auto-antibody induced platelet (PLT) destruction and reduced PLT production, leading to a low PLT count. The availability of robust epidemiological and clinical data on ITP in regions outside Europe and the United States is limited. The International ITP Registry is a prospective cohort study which seeks to collect epidemiological and clinical data of recently diagnosed primary ITP adult patients, predominantly from the Asia-Pacific region. The International Registry has been established for >4 years.

Aims: To contrast and compare the International ITP Registry population with the Intercontinental Cooperative Immune Thrombocytopaenia Study Group Pediatric and Adult Intercontinental Registry on Chronic ITP (PARC-ITP Registry) adult population. The PARC-ITP Registry was selected as it represents a prospective cohort including newly diagnosed ITP patients, with a comparably sized adult cohort which is well established (>4 years) across the regions in which epidemiological data is already available[1].

Methods: Clinical and laboratory data from the International ITP Registry were compared with clinical and laboratory data reported for adults enrolled in the PARC-ITP registry.

Results: At the time of this comparison 306 patients were enrolled across 10 countries in the Asia-Pacific, Middle East and Latin America regions in the International ITP Registry with data available from 299 patients. The PARC Registry had 340 adult patients enrolled in 31 countries across Europe, Canada, United States, Latin America, the Middle East, and Asia. Patient characteristics are presented in Table 1. The International ITP Registry has a clear predominance of patients of Asian origin (68%) whereas the PARC-ITP Registry population is principally Caucasian (63%). A female predominance is seen in both cohorts. Comorbidities were reported more often in the International Registry group (67.5% *vs* 30%). Hypertension is the most often reported comorbidity in both groups. Of the comorbidities related to ITP diagnosis that are available for comparison, diabetes occurs more often in the International Registry group and thyroid disease is reported at similar rates in both groups (Table 1). Mean PLT count at baseline was 29.0x 10^9/L (range 1-96x 10^9/L) in the International Registry group and 25.4x 10^9/L (range 0-133x 10^9/L) in the PARC Registry adults. Corticosteroids were the most frequently used first-line treatment in both groups-68% in the International Registry and 59% in the PARC-ITP Registry adults. IVIg is used in both populations at much lower rates-18% in the International Registry and 6% in the PARC-ITP Registry adults. Splenectomy is uncommon, with 13 patients in our group having splenectomy, splenectomy is not reported on in the PARC-ITP Registry publication. TPO-receptor agonist use is reported in the International Registry group (n=23) but not reported on in the PARC-ITP Registry group. Anti-D is reported both groups at rates <5%, and both groups have similar rates of not treated patients (Table 1). Clinical signs of bleeding were assessed by location in both Registries. The International Registry also records severity of bleeding. Bleeding at any site was seen in 34% of patients in the International Registry whereas PARC -ITP Registry reports manifestations of bleeding in 69% of adults.

Table 1. Patient characteristics.

	PARC ITP		International ITP	
Number males n (%)	110 (32)		125 (41.8)	
Number of females n (%)	230 (68)		174 (58.2)	
Mean age in years (range)	39.0 (16.1- 85.8)		48.6 (18 - 96)	
Ethnicity n (%)				
Caucasian	213 (63)		61 (20.4)	
Asian	55 (16)		204 (68.2)	
Hispanic/Latino	52 (15)		16 (5.4)	
Middle Eastern	Not reported		16 (5.4)	
Pacific Islander/Aboriginal/ Maori	Not reported		1 (0.3)	
Africa	6 (2)		0 (0)	
Other	NA		1 (0.3)	
Not reported	14 (4)		0 (0)	
Comorbidities n (%)	102 (30)		202 (67.5)	
Cancer	1 (0.3)		15 (5.0)	
Cardiovascular disease	3 (0.9)		8 (2.6)	
Diabetes	4 (1.2)		19 (9.4)	
Gastrointestinal disease	12 (3.5)		18 (6.0)	
Arterial hypertension	23(6.8)		33 (16.3)	
Splenomegaly	1 (0.3)		5 (1.7)	
Thyroid disease	19 (5.6)		9 (4.4)	
Hypercholesterolaemia	Not reported		11(5.4)	
Treatments %				
Corticosteroids (all)	59		68	
IVIg	6		18	
Anti-D	2		3	
TPO agonists	Not reported		7	
Splenectomy	Not reported		5	
No treatment	29^		33^	
Platelet count at diagnosis (x10 ^{9}/L)				
Mean	25.4		29.0	
Range	0-133*		1-96#	
Bleeding (any site) n (%)	236 (69%)		103 (34%)	
Investigations n (%)	Negative	Positive	Negative	Positive
Antiphospholipid abs.	123 (94)	8 (6)	49 (89)	6 (11)
Antinuclear abs.	193 (90)	22 (10)	117 (65)	63 (35)
Platelet associated abs.	38 (53)	34 (47)	NA	NA
HIV	216 (99)	2 (1)	166 (99)	1 (1)
Hepatitis C	213 (97)	7 (3)	190 (99)	2 (1)
Helicobacter pylori	48 (69)	22 (31)	27 (59)	19 (41)

* PARC ITP used PLT count < 150 x 10^9/L for diagnosis

International ITP Registry used PLT count < 100 x 10^9/L for diagnosis

^ The PARC-ITP Registry reports no treatment as initial management. The ITP Registry reports no treatment recorded overall

Summary and Conclusions: Comparative data showed similarities in gender distribution, presenting platelet counts, diagnostic testing and first line therapy, whereas differences occurred in co-morbidity, bleeding, and second line therapies.

Reference

1. Kühne T, *et al.* on behalf of the Intercontinental Cooperative ITP Study Group. Newly diagnosed immune thrombocytopenia in children and adults: a comparative prospective observational registry of the Intercontinental Cooperative Immune Thrombocytopenia Study Group. Haematologica 2011;96(12): 1831-1837. doi:10.3324/haematol.2011.050799

E1407

CLINICAL FEATURES OF PATIENT WITH SEVERE ACQUIRED ADAMTS13 DEFICIENCY IN THROMBOTIC THROMBOCYTOPENIC PURPURA

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Background: Diagnostic and prognostic value of ADAMTS13 activity is controversial. We previously reported the characteristics of severe ADAMTS13 deficiency in thrombotic thrombocytopenic purpura (TTP) and patients with severe ADAMTS13 deficiency (Jang MJ *et al*., Int J Hematol 2011;93:163-9).
Aims: In this study we updated it by adding more patients to know the clinical characteristics of patients with severe ADAMTS13 deficiency at presentation.
Methods: One-hundred sixty nine patients with TTP from January 2005 to June 2014 were analyzed. ADAMTS13 activity and inhibitors were measured by immunoblotting of degraded von Willebrand factor. Clinical information was retrospectively collected and analyzed.
Results: Patients with severe ADAMTS13 deficiency at presentation had lower serum creatinine levels (P<0.0001), lower platelet counts (P<0.0001), and higher total bilirubin levels (P=0.0001) than patients with non-severe ADAMTS13 deficiency. Treatment outcomes did not differ significantly between two groups in response, remission, and mortality rate. After adjusting for clinical and laboratory features, multivariate analysis revealed age over 60 year old is an independent risk factor for TTP-associated mortality (P=0.0249).
Summary and Conclusions: TTP with severe ADAMTS13 deficiency is a unique subgroup characterized by lower platelet count and relatively good renal function. The severity of ADAMTS13 deficiency at presentation does not have prognostic significance.

E1408

A PERSONALIZED REFERENCE INTERVAL FOR PLATELET COUNT REDUCES THE PREVALENCE OF UNEXPLAINED THROMBOCYTOPENIA AND INCREASE THAT OF REACTIVE THROMBOCYTOSIS IN ELDERLY PEOPLE

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Background: The reference interval for platelet count currently in use in most Western countries is 150-450 or 150-400x10^9 platelets/L. However, many studies indicated that women have more platelets than men and platelet count progressively decreases with ageing. Moreover, ethnicity-related differences have been identified and the related genetic factors have begun to be unveiled.
Aims: To evaluate the outcome of a personalized reference range recently identified by the study of 40,978 Italian healthy subjects and which takes into account age and sex: 165-473x10^9 platelets/L, regardless of gender, under 15 years of age; 136-436 and 120-369x10^9/L in women and men, respectively, between 15 and 64 years; and 119-396 and 112-361x10^9/L in women and men, respectively, over 64 years (PLoS One 2013;81:e54289).
Methods: The new personalized reference range was applied retrospectively, in parallel to that presently in use of 150-450x10^9/L, to a series of 925 consecutive Italian patients admitted to a department of Internal Medicine. The investigated population was mainly composed of elderly subjects (mean age 74±14.2 years). Clinical records were used to identify the potential causes of thrombocytopenia and thrombocytosis in each patient. The institutional review board of the hospital approved the protocol, and all patients gave written informed consent in accordance with the Declaration of Helsinki.
Results: The prevalence of thrombocytopenia was 21.3% with the traditional reference range and 16.9% with the personalized one. Switching from thrombocytopenic to non-thrombocytopenic affected in most cases subjects without any apparent cause of reduced platelet count, whose number dropped from 67 to 37. So, the personalized range reduced by 44.8% the number of subjects with unexpectedly low platelet counts. Also a few patients with chronic liver disorders turned from thrombocytopenic to non-thrombocytopenic. The prevalence of thrombocytosis increased from 9.1% with the traditional range to 12.2% with the personalized range, and switching always occurred in patients with potential causes of high platelet count, the majority classified as affected by inflammatory disorders.
Summary and Conclusions: The most relevant effect of personalized reference intervals for platelet count in elderly medical patients was the reduction by nearly half in the proportion of subjects with an unexplained form of thrombocytopenia. Introducing the new range into the clinical practice is therefore expected to prevent many subjects to receive a series of unnecessary and expensive tests, this benefiting both involved people and the health system.

E1409

ROLE OF ASHWELL-MORELL RECEPTOR MEDIATED HEPATIC CLEARANCE OF PLATELET IN IMMUNE THROMBOCYTOPENIA (ITP): PLATELET KINETIC STUDIES AND AUTOANTIBODY TYPE DATA IN CLINICAL PRACTICE

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Background: Detection of antibodies (Abs) against platelet GPIIb-IIIa and/or GPIb-IX is a characteristic feature of immune thrombocytopenia (ITP) and Fc-mediated clearance of platelet (plt) by splenic macrophages has so far been addressed as the main mechanism of plt destruction. Recently, an Fc-independent mechanism has been proposed which involves mainly anti-GPIb Abs (1) which were shown to induce plt activation leading to GPIb desialyation. In turn, plt lacking sialic acid (desialylated plt) are removed by the hepatic Ashwell-Morell receptor (AMR or asialoglycoprotein receptor).
Aims: In order to test the clinical relevance of this novel mechanism of plt clearance, plt kinetic studies and Ab type data were retrospectively reviewed in a group of ITP patients (pts).
Methods: Charts of ITP pts who had data on both plt kinetic studies and Ab testing were reviewed. Pts are enrolled in a local Italian database (REL-ITP database), and informed consent to clinical data use is given at enrollment
Results: A total of 53 pts (M 24) were identified: 41 already splenectomized between year 2000 and 2014, 12 evaluated for splenectomy. All pts underwent or are candidate to splenectomy for steroid-dependent ITP; 1 pt only was primarily refractory to steroids and IVIG treatment. All but 2 pts achieved a complete response to splenectomy. 31/53 (58.4%) of pts tested positive to one or more than one antiGP Abs. Results of platelet kinetic studies and Ab testing are summarized in Table 1. Extra splenic clearance doesn't differ significantly (Fisher's test p=0.531) between Ab negative or positive pts.

Table 1.

Ab pattern	n(%)	site of plt clearance (111-Indium labeled plt)		
		splenic	hepatic	mixed
All patients	53 (100)			
Ab negative	22 (41.6)	17 (77.3%)	0 (0%)*	5 (22.7%)*
Ab positive	31 (58.4)	16 (51.6%)	4 (13%)*	11 (35.4%)*
one positive Ab	11/31 (35.5)			
GpIIb-IIIa	7	4	2	1
GpIb-IX	4	2	1	1
two positive Abs	7/31 (22,5%)			
GpIIb-IIIa & GpIb-IX	2	1	0	1
GpIIb-IIIa & GpIa-IIa	5	3	0	2
three positive Abs	13/31 (42%)			
GpIIb-IIIa & GpIb-IX & GpIa-IIa	13	6	1	6

*Fisher's exact test: p=0.531

Summary and Conclusions: Recent data have proposed that Ab type may determine plt fate in ITP by activating either Fc- or non Fc-mediated mechanisms of plt clearance resulting in splenic or hepatic uptake respectively. However, our data on plt kinetic and Ab type do not support such a mechanism being operative in ITP pts: no correlation was found between Ab type, number (single *vs* multiple antiGP) of Ab positive test and site of plt clearance. On the other hand, it is well known that plt desialylate as they circulate, thereby becoming the primary AMR ligand. Desialylation also occurs when plt are activated by a number of physiological stimuli (2). It may well be that AMR-mediated hepatic plt clearance rather represents a physiological mechanism involved in clearance of activated plt-a role for the AMR has been proposed in attenuating the coagulopathy associated with sepsis (3-4)-and in the regulation of TPO production by hepatocytes (5).

E1410

DIAGNOSIS AND MANAGEMENT OF THROMBOTIC THROMBOCYTOPENIC PURPURA (TTP) IN AUSTRALIA-FINDINGS FROM THE FIRST FIVE YEARS OF THE AUSTRALIAN TTP REGISTRY (2009-2014)

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Background: Thrombotic thrombocytopenic purpura (TTP) is a rare, life-threatening thrombotic microangiopathy. In 2009, the Australian TTP registry was established and began collecting data on patients presenting with TTP throughout Australia.
Aims: To summarise findings of the first five years (2009-2014) of TTP diagnosis and management from the Australian TTP registry.
Methods: Registry data from June 2009 to October 2014 were reviewed.
Results: 57 patients were identified with TTP (ADAMTS13 <10%) accounting for 72 clinical episodes. ADAMTS13 inhibitor testing was performed in 9/57 (16%) patients reflecting the limited availability of accredited testing facilities. 67/72 episodes were treated with therapeutic plasma exchange (PEX) with cryodepleted plasma (40% episodes), fresh frozen plasma (36%) or a mixture (22%). Exposure to plasma products was significant with a median exposure of 55.9L per episode. PEX was not commenced until 2 or more days after date of diagnosis in 15% of episodes. Adverse reactions to PEX were common with documented allergic reactions (including life-threatening) in 21% of episodes. Adjunctive immunosuppression was documented in 76% episodes (corticosteroid 71%, rituximab 39%). Platelets were transfused in 15% of episodes with no documented adverse reactions.
Summary and Conclusions: Australian TTP registry data reveal a heterogenous approach to the diagnosis & management of TTP in Australia from 2009-2014. Identified areas for potential practice improvement include a standardised approach to TTP diagnostic testing, improving access to timely PEX and a more uniform approach to adjunctive immunosuppression and supportive care.

E1411

RECENT TIME TRENDS IN THE UPTAKE OF SPLENECTOMY IN ADULTS DIAGNOSED WITH CHRONIC IMMUNE THROMBOCYTOPENIA: A NATIONWIDE HISTORICAL COHORT STUDY IN DENMARK, 1996-2012

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Background: Immune thrombocytopenia (ITP) is an autoimmune disorder characterized by isolated thrombocytopenia leading to an increased risk of bleeding. Low platelet counts in ITP result from both increased antibody-mediated platelet destruction and decreased platelet production. Because the spleen often removes circulating antibody-coated platelets and can be the site of antibody production, splenectomy can be an effective treatment option. Although it is generally viewed as a standard second-line treatment in adults with ITP, splenectomy has its risks (perioperative and long-term), and there are no clear clinical criteria (*e.g.*, previous response to steroids) that predict response. Additionally, spontaneous remissions are possible, and newer nonsurgical second-line therapies are available. Some clinicians and patients are therefore opting to avoid or defer splenectomy. We sought to investigate recent trends in the uptake of splenectomy in adults with chronic ITP (cITP) in Denmark.
Aims: To compare the rate of splenectomy in three time periods (1996-2001; 2002-2007; 2008-2012) in a cohort of adults diagnosed with cITP.
Methods: Using the Danish National Registry of Patients (DNRP), we included all adults (≥18 years) diagnosed with cITP (≥2 ITP diagnoses made ≥180 days apart) in Denmark from 1996 to 2012. The date of cITP diagnosis was defined as the date of the first ITP diagnosis occurring ≥180 days after the initial diagnosis and after the patient's 18th birthday. The DNRP was also used to identify if and when patients had undergone splenectomy and the presence of comorbidities. We computed the cumulative incidence of splenectomy (treating death as a competing risk) after a diagnosis of cITP by time period of diagnosis. Cox proportional hazards regression analyses were used to assess whether the hazard (rate) of splenectomy after a cITP diagnosis varied by time period of diagnosis, adjusting for gender, age, and presence of comorbidities. Patients were followed from the date of cITP diagnosis until 10 years post-cITP, death, emigration, or end of follow-up in 2012.
Results: We identified 1,993 non-splenectomized adults diagnosed with cITP. Women accounted for nearly 58% of the cohort; and 31%, 35%, and 34% of patients were aged 18-40, 41-65, and ≥66 years, respectively, at the time of cITP diagnosis. During a median follow-up of 3.9 years [range: <0.1-10.0], approximately 9% (n=177) underwent splenectomy. Among these patients, the median time to splenectomy following the cITP diagnosis was 0.5 years (range: <0.1-9.9). The 1- and 3-year cumulative incidence of splenectomy after the diagnosis of cITP was lower in more recent time periods of diagnosis (Table 1). After adjusting for gender, age, and comorbidities, patients diagnosed with cITP in 2008-2012 were less than half as likely to undergo splenectomy compared with patients diagnosed in 1996-2001 (adjusted hazard ratio: 0.4 [95% CI: 0.3-0.7]).

Table 1. Cumulative incidence of splenectomy in adults following a diagnosis of chronic ITP by time period of chronic ITP diagnosis.

Time period of chronic ITP diagnosis	Cumulative incidence of splenectomy (95% confidence interval)	
	1-year	3-year
1996-2001	10.1% (7.6-12.9)	12.9% (10.1-16.0)
2002-2007	5.9% (4.3-7.8)	8.7% (6.8-11.0)
2008-2012	3.4% (2.3-4.9)	5.1% (3.6-6.9)

Summary and Conclusions: Less than 10% of nearly 2,000 adults diagnosed with cITP in Denmark from 1996 to 2012 underwent splenectomy during a median follow-up of 3.9 years. The incidence of splenectomy after a diagnosis of cITP was lower for patients diagnosed in more recent years.

E1412

EFFICACY AND PROGNOSIS OF SHORT TERM AND VERY LOW-DOSE IVIG THERAPY (200 MG/KG/D) FOR NEWLY DIAGNOSED ACUTE IMMUNE THROMBOCYTOPENIC PURPURA IN CHILDREN

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Background: High-dose intravenous immunoglobulin G (IVIG) is the first therapy for childhood acute immune thrombocytopenic purpura (aITP). Individual low-dose method has been applied in the Department of Pediatrics, Kyungpook National University Hospital since 1984. We have reduced the daily dose from 400 to 200 mg/kg daily until platelet count (PLT) over 50,000/uL since 2000.
Aims: This study evaluated the efficacy of short term and very low dose IVIG (VLD-IVIG) therapy according to individual clinical response.
Methods: Forty-six childhood ITP Patients who's PLT less than 20,000/uL were evaluated from January 2007 to December 2013. We treated them with VLD-IVIG (200 mg/kg/day for 1~5 days) initially until rising PLT over 50,000/uL and then any patients without the response were treated additional IVIG up to 2g/kg totally.
Results: The mean age of 22 male and 24 female ITP was 26 months. The mean PLT was 8,300/uL at the time of diagnosis. Thirty nine patients responded with VLD-IVIG only. Four patients needed only one day, 11 patients 2 days, 9 patients 3 days, 10 patients 4 days, and 5 patients 5 days of IVIG (200 mg/kg) respectively. Six of 7 patients who did not raise PLT above 50,000 with VLD-IVIG had response after full dose (total 2 g/kg). Only 1 patient did not response with IVIG and needed corticosteroids and 1 patient recurred within 2 weeks after therapy. The mean numbers of IVIG injection to 50,000/uL was 3 times (621 mg/kg of dose). The mean PLT after therapy was 108,692/uL. The mean duration to recover PLT more than 150,000/uL was 2 months. No patient was diagnosed to chronic ITP. The mean follow-up duration was 24 months and mean PLT was 336,410/uL at last follow-up date.
Summary and Conclusions: The short term VLD-IVIG therapy according to individual clinical response is effective and is recommended for acute ITP to reduce admission days and cost of high dose IVIG therapy.

E1413

THE ROLE OF REGULATORY T CELLS IN IMMUNE THROMBOCYTOPENIA OF CHILDHOOD

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Background: Immune thrombocytopenia (ITP) is one of the most common blood diseases and the commonest acquired bleeding disorder in childhood. Although the development of autoantibodies against platelet glycoproteins by B cells remains central in the pathophysiology of ITP, dysfunctional cellular immunity has also great importance. $CD4^+CD25^+Foxp3^+$ T cells (T_{reg} cells) take part in the regulation of immune responses and maintenance of self-tolerance. It has been also shown that these cells play a key role in the pathogenesis of autoimmune diseases.
Aims: The aim of this study was the determination of the sub-populations of CD3+CD8+CD25+, CD3+CD4+CD25+, CD4+$CD25^{high}$ and CD4+$CD25^{high}$-Foxp3+ T cells in children with immune thrombocytopenia and the correlation with the levels of platelets' number and the disease phase.
Methods: Peripheral blood of 21 children, 12 with acute ITP (age range 5

months-11 years) and 9 children (age range 8-16 years) with chronic immune thrombocytopenia was studied. The percentage of regulatory T lymhpocytes' subpopulations was estimated with flow cytometry. The median platelets' number was 11900/µl in the group of acute ITP and 46600//µl for the patients in the chronic phase of the disease.
Results: According to our findings between acute and chronic phase of immune thrombocytopenia was not observed statistically significant difference in the levels of CD3+CD8+CD25+, CD4+CD25^{high} and CD4+CD25^{high}Foxp3+ T cells subpopulations. The levels of the CD3+CD4+CD25+ T cells sub-population were statistical significantly reduced in children with acute immune thrombocytopenia in contrast with children with chronic immune thrombocytopenia (2.94±0.68 *vs* 5.10±0.16, p=0.029). No statistically significant difference was observed in the levels of regulatory T cells between diagnosis of immune thrombocytopenia and the phase of recovery of number of platelets in normal values.
Summary and Conclusions: Between acute and chronic childhood ITP the number of $CD3^+CD4^+CD25^+$ T_{reg} cells was estimated with significant difference. This reduction of the $CD3^+CD4^+CD25^+$ T_{reg} cells in acute ITP might be one of the mechanisms implicated in the pathophysiology of the disease in children.

E1414

INCIDENCE OF MALIGNANCY IN ADULT PATIENTS WITH AUTOIMMUNE THROMBOCYTOPENIC PURPURA: A 12 YEAR SINGLE CENTER EXPERIENCE

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Background: Autoimmune thrombocytopenia (ITP) is an autoimmune disorder characterized by immune-mediated platelet destruction and suppressed platelet production. Depending on the presence or absence of associated disease, ITP is divided into primary and secondary types. Few studies have addressed the association between ITP in adult patients and malignant neoplasms.
Aims: To estimate the incidence of malignant neoplasms in adult patients with ITP.
Methods: All patients diagnosed with ITP and with a platelet count <100×10^9/L, between September 2002 and January 2015 were included in the study. Patients were retrospectively reviewed for diagnosis of malignancy, and inclusion criteria included concurrent ITP or ITP preceding the diagnosis of a malignant neoplasm within 1 year.
Results: A total of 86 patients (62 females and 24 males) were diagnosed between 2002 and 2015. Their ages ranged from 16 to 91 years with a mean (SD) of 35.7 (15.3) years. Among these patients, 13 (15.1%) developed malignancy during the study period. The incidence of malignancy was significantly higher in males ≥30 years old than in males ≤30 years old. It was 6.9% among patients aged 31-50 years and 58.8% among patients aged ≥50 years. Hodgkin's and marginal lymphoma were the most common cancers detected in this group of patients.
Summary and Conclusions: ITP had a strong association with malignant neoplasms, especially in male patients over 50 years of age. Hodgkin's and marginal zone lymphoma were among the cancers strongly associated with ITP. The results suggest that patients over 50 years of age with ITP should be examined for an underlying malignant condition. ITP may occur concurrently or precede the occurrence of malignant disease, which would have great diagnostic significance. ITP may also be the first early sign of the disease. Thus, prompt determination of the cause of ITP could be of great importance for the diagnosis, monitoring and therapy of malignant neoplasms.

E1415

AN OBSERVATIONAL CLINICAL PRACTICE STUDY OF ROMIPLOSTIM IN PATIENTS WITH CHRONIC IMMUNE THROMBOCYTOPENIC PURPURA (ITP)-PLATON INTERIM RESULTS

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Background: The thrombopoietin-receptor agonist romiplostim is indicated for adult splenectomised patients (pts) with ITP who are refractory to other treatments and may be considered as second line treatment for adult non-splenectomised pts where surgery is contra-indicated.
Aims: The aim of this ongoing study is to assess the use of romiplostim in clinical practice in Bulgaria, Czech Republic, Slovenia, Slovakia, Romania and Russia.
Methods: This international single-arm, non-interventional, multicenter, retrospective and prospective study is enrolling adult pts with ITP, who have received at least one application of romiplostim according to the licensed indication. A period of 6 months prior to romiplostim initiation was documented retrospectively from pt files; the prospective observation period started at the time of romiplostim initiation. The total planned observation period is 2 years. All pts provided written informed consent. Assessed parameters included pt demographics, romiplostim application, dosage, adverse drug reactions (ADRs) and reasons of discontinuations, concomitant ITP therapies, clinically relevant bleeding events, and consumption of resources. Data from a protocol-defined interim analysis (IA) at 1 year of prospective observation are reported here.
Results: 66 pts were analyzed for the IA (47% male, median age at romiplostim initiation 48 [range 19-82] years, splenectomized n=27, non-splenectomized n=39). Of these, 58 (88%) completed 1 year of study, 8 (12%) discontinued from the study (adverse drug reactions n=1, administrative decision n=2, lost to follow-up n=5). The median time from ITP diagnosis to romiplostim initiation was 2.89 (0-42) years. 34 pts (51.5%) had received ≥3 prior ITP therapies. During the 6 months before romiplostim initiation the event rate per 100 pt years was 111 (95% CI 75.1, 156.8) for bleeding and 92 (95% CI 73.2, 113.0) for ITP-related hospitalizations. The median platelet count at romiplostim start was 19.0 (range 1-411)x 10^9/L, increasing to 52.0 (range 2-361)x 10^9/L after 1 week and remaining above 50x 10^9/L for the period until IA data cut-off at 1 year (Figure 1). During the 1-year observation period for IA, pts received a median romiplostim dose of 2.13 (range 0.1-9.1) µg/kg/week and a median of 31 (range 2-53) injections. The event rate per 100 pt years during the 1-year romiplostim treatment period was 58 (95% CI 39.0, 83.6) for bleeding (no grade 3/4 events) and 94 (95% CI 69.3, 125.4) for ITP-related hospitalizations. 4 pts (6.1%) experienced a total of 11 ADRs, with 2 pts experiencing 2 serious ADRs each (thrombosis, dysphagia, lymphocytosis, leucocytosis). 51 pts (77.3%) were hospitalized for 77 ITP-related events during the 1 year observation period for IA for the following reasons: platelet transfusion (n=5, 9.8%), ITP treatment administration (n=24, 47.1%), bleeding event (n=14, 27.5%), infection-related (n=5, 9.8%), splenectomy (n=7, 13.7%), ADR (n=1, 2.0%), other (n=21, 41.2%).

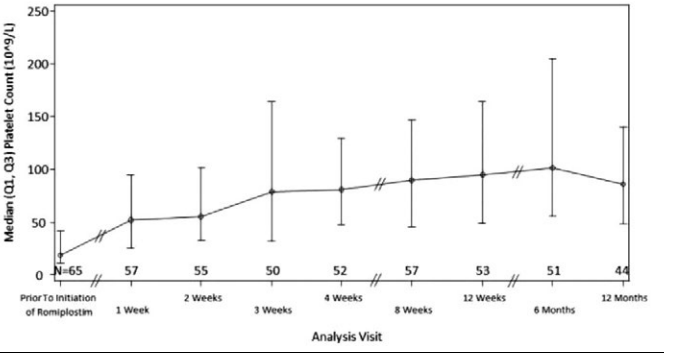

Figure 1.

Summary and Conclusions: These interim data show that platelet counts in ITP patients treated with romiplostim in clinical practice increased and were maintained within the desired range of 50-250x 10^9/L. The bleeding event rate was lower during romiplostim treatment. Romiplostim was generally well tolerated.

E1416

MULTI-CENTER, PROSPECTIVE STUDY TO EVALUATE THE EFFICACY OF "BIWEEKLY" ROMIPLOSTIM IN ADULT IMMUNE THROMBOCYTOPENIA (ITP); PROLONGED DOSE INTERVAL ≥2 WEEKS IS NOT RECOMMENDABLE

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Background: Romiplostim is recommended to administer weekly in adult immune thrombocytopenia (ITP) patients. However, the optimal dose interval has rarely been studied.
Aims: This is a multi-center, prospective study to evaluate the efficacy of "biweekly" romiplostim in maintaining platelet count ≥30x 10^9/L during 4 or more weeks.
Methods: Treatment was started with weekly injection (starting dose, 1mcg/kg), and the dose was escalated until achieving titrated dose that maintains platelet count 50-200x10^9/L for 4 consecutive weeks. Then, patients moved to biweekly schedule, and if platelet count became <30x 10^9/L, patients returned back to weekly schedule. The process could be repeated twice. But platelet count <30x

10^9/L on 2nd biweekly schedule, the study was finished after 4 weekly doses.
Results: Between Jul 2013 and Dec 2014, a total of 18 patients were enrolled (median platelet count, 14x10^9/L). After the 1st weekly schedule, 10 of 18 (55.6%) attained titrated dose of median 3mcg/kg and proceeded to 1st biweekly schedule. However, all of them failed in maintaining platelet count ≥30x 10^9/L for at least 4 weeks, and returned back to 2nd weekly schedule. 8 of 10 were titrated (median, 5mcg/kg) and moved to 2nd biweekly romiplostim; 3 of 8 (37.5%) showed platelet counts ≥30x 10^9/L during 4, 8, and 10 weeks. However, all 8 patients eventually experienced drop of platelet and entered onto the last stage; 2 (25%) almost did not respond to romiplostim at this time (Figure 1).

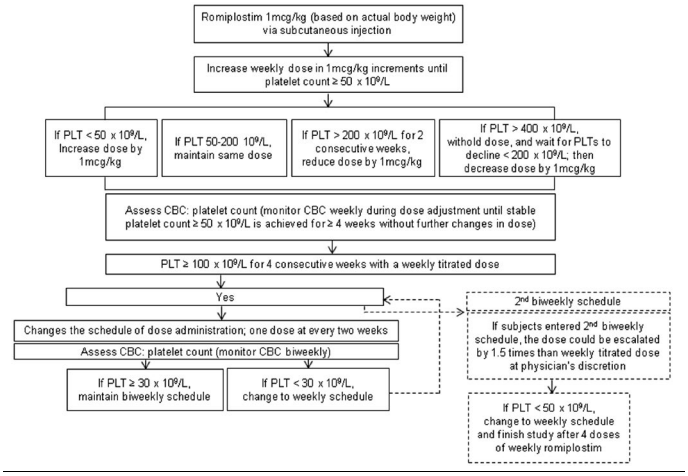

Figure 1. Overall treatment algorithm.

Summary and Conclusions: Lengthening the dose interval of romiplostim more than a week is not desirable for stably maintaining the platelet count and associated with fluctuating platelet response.

E1417

EFFECT OF THROMBOPOIETIN RECEPTOR AGONISTS ON COAGULATION AND PLATELET ACTIVATION IN PATIENTS WITH IMMUNE THROMBOCYTOPENIA

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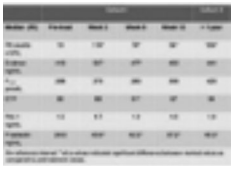
Background: Thrombopoietin receptor agonists (TPO) are effective treatment in immune thrombocytopenia (ITP). Thrombotic events were reported in up to 6% of patients in TPO-agent trials in raising concerns of coagulation and platelet activation.
Aims: To evaluate the effect of TPO-agents on coagulation and platelet activation.
Methods: The study comprised 2 ITP cohorts. Cohort 1 (n=26) with sequential blood samples at 0 (pretreatment) and 2, 6 and 12 weeks on treatment with TPO. Cohort 2 (n=18) patients on long-term treatment with TPO-agents (>1 year) in whom blood samples were collected only once. Markers of coagulation and fibrinolysis including endogenous thrombin potential (ETP) by calibrated automated thrombography (CAT) assay, prothrombin fragments 1+2 (F_{1+2}), plasminogen activator inhibitor-1 (PAI-1) activity, and D-dimer as well as soluble P-selectin as a marker of platelet activation were measured in all samples. Healthy controls (n=20) were analyzed for P-selectin only.
Results: Mean age was 55 years (32% males). Nineteen patients were treated with romiplostim, 22 with eltrombopag and 3 with avatrombopag. Median values of D-dimer, PAI-1, ETP (normalized against pooled normal plasma), P-selectin and platelet counts are shown in the Table 1. Platelet counts increased significantly after initiation of TPO treatment. No difference was found between pretreatment and on treatment (both cohorts) values of D-dimer, F_{1+2} or PAI-1. Median ETP at 6 weeks higher than pretreatment values (p=0.042); remaining levels at 2 and 12 weeks and cohort 2 did not differ from pretreatment. A statistically significant increase in P-selectin was found shortly after initiation of TPO-agents (2 weeks)(p=0.002) and through 6 (p=0.0001) and 12 weeks (p=0.003) into treatment. A higher level of P-selectin was also found in cohort 2 compared to pretreatment levels of cohort1. Pretreatment levels of P-selectin did not differ from healthy controls (mean 22.5 ng/mL) (p=0.4), however, all on treatment levels of P-selectin in cohort 1 (p=0.002, 0.001, 0.008 respectively) and cohort 2 (p=0.006) were significantly higher than healthy controls.

Table 1.



Summary and Conclusions: Conclusion: Evaluation of the coagulation and fibrinolytic systmes shows that thrombopoietic agents cause no activation of these systems confirming a previous study performed on eltrombopag. However, the finding of increasing soluble P-selectin during treatment with TPO may indicate degranulation of alpha-granules and hence platelet activation.

E1418

SPLENECTOMY IN THE TPO MIMETIC ERA-STILL REASONABLE APPROACH?

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Background: Immune thrombocytopenia (ITP) is an acquired, immunologically determined disease characterized by a decrease in platelet count and the associated increased risk of bleeding. The spleen plays a major role in the pathogenesis of ITP, it is the site of antibody production and often the major site of antibody-mediated platelet destruction. Splenectomy was the first method for ITP therapy and has long been the standard second-line treatment for adults with ITP who do not respond to corticosteroids. Recent advances in the understanding of etiopathogenetic mechanisms of ITP led to the development of novel therapies and changed the role of splenectomy in the management of ITP.
Aims: Describe the population indicated for splenectomy (before and after new guidelines). Evaluated efficacy and safety of splenectomy and therapeutic possibilities for refractory patients.
Methods: We retrospectively analyzed the data of 98 patients with primary ITP from four Czech hematological centers who underwent splenectomy between years 1995 and 2014. The response was evaluated according to the ITP International Working Group guidelines.
Results: In total, 98 patients (66% females) underwent splenectomy for ITP between 1995 and 2014 in four Czech hematological centers. The median age at diagnosis was 36 years (range 6-72) and median age at splenectomy was 38 years (range 17-73). 56% patients who were splenectomized before year 2010 had chronic ITP while 72% patients had chronic ITP if the splenectomy was performed after year 2010. Minority of patients had newly diagnosed ITP, in a subgroup of patients splenectomized before year 2010 it was 19% and in a subgroup of patients splenectomized after year 2010 were none with newly diagnosed ITP's. While the median interval between diagnosis and splenectomy in patients who were splenectomized before 2010 was 13 months, the median interval in patients who were splenectomized after 2010 was longer (32 months). After 2010, splenectomy was used most often as a third-line treatment. 25 patients in our group underwent labelled platelet scanning before splenectomy, majority of them had splenic uptake. The median platelet count before splenectomy was 65x 10^9/l (range 1-330x 10^9/l). 77 patients (79%) were prophylactic vaccinated against encapsulated bacterial pathogens. 61% of patients underwent splenectomy with an open technique and 39% with a laparoscopic technique and the rate of laparoscopic splenectomies increased over time. Of the 98 patients, 10 (11%) achieved a response (R) and 83 (84%) a complete response (CR), the overall response rate was 95%. 28 (29%) patients relapsed after a median time 6 months (range 1-125). 8 patients (8%) experienced peri-operative and 3 patients (3%) post-splenectomy complications. In 33 patients (34%) splenectomy was not successful because of the relapse or lack of response, but new treatment options especially TPO mimetics improved the outcome of these refractory patients.
Summary and Conclusions: Splenectomy is an effective treatment for ITP with two thirds of patients achieving long-term response and still has a place in the management of ITP. Recent trend is to postpone splenectomy until the chronic phase of the disease.

E1419

ASSOCIATION OF SINGLE NUCLEOTIDE POLYMORPHISMS IN SOME IMMUNOREGULATORY GENES WITH IMMUNE THROMBOCYTOPENIA

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Background: Immune thrombocytopenia (ITP) is an autoimmune disease characterized by thrombocytopenia due to platelet autoantibodies, causing an accelerated clearance of opsonized platelets by phagocytes. The etiology of ITP remains unclear, but both genetic and environmental factors can have a role in the development of ITP.
Aims: The aim of our study was to investigate a possible association of some single nucleotide polymorphisms (SNP) in genes for interleukin beta IL1β(-511C/T), tumor necrosis factor beta TNFβ(+252G/A), tumor necrosis factor alpha TNFα(-308G/A), cytotoxic T lymphocyte antigen 4 CTLA(+49 A/G), Fc gamma receptor FCGR2A-H/R131 and FCGR3A-V/F158 with ITP.
Methods: We analyzed 125 adult ITP patients (35 men, 90 women) with median age of 47(range 14-83) and 120 healthy matched controls. The median follow up was 44 months (12-384). All 125 patients were initially treated with corticosteroids, 38 were splenectomized. Forty two (34%) patients had refractory or unresponsive form of disease, according to the definition of the International Working group for ITP. Genotyping was performed by using polymerase chain reaction and restriction fragment length polymorphism methods. The distribution of genotypes and allele frequencies were compared with a chi-squared.
Results: Our results demonstrated significantly different distribution of the TNFβ(+252 G/A) genotypes and allele frequencies in patients with ITP (G/G=3, A/G=38, A/A=84) comparing with controls (G/G=16, A/G=35, A/A=69), p=0.005 and p=0.009. We didn't found significant differences in the genotype distribution or allele frequencies for TNFα(-308G/A) and ILβ (-511C/T), p=0.363 and p=0.845. There was significantly different genotype distribution and allele frequencies for TNFα(-308G/A) between patients with unresponsive ITP (n=83; G/G=75, A/G=8, A/A=0) and responsive ITP (n=42; G/G=30, A/G=11, A/A=1), p=0.016 and p=0.009. There was no significant difference in genotype distribution and allele frequencies for TNFβ(+252G/A) and ILβ(-511C/T) between these two groups of patients. Our results demonstrated significantly higher frequency of high affinity FCGR3A-158V allele in ITP patients comparing with controls (47.2% *versus* 37.5%; p=0.037). We did not found significant differences in the allele frequencies for FCGR2A-131H/R, p=0.478. In the group of patients with unresponsive and responsive ITP we found significantly different genotype distribution and allele frequencies for FCGR3A, p=0.036 and p=0.008 respectively. Our results confirmed that, the combination of high affinity FCGR2A-131H and FCGR3A-158V allele was more common in patients with ITP than in control subjects (55% *versus* 40%; p=0.024). Analysis of CTLA-4 polymorphisms did not confirmed significant differences in genotype distribution between ITP patients and controls, p=0.43.
Summary and Conclusions: Results of this study, suggest possible role of FCGR3A, TNF-β and TNF-α polymorphism in the etiology, development and clinical form of immune thrombocytopenia.

E1420

SWITCHING THROMBOPOIETIN RECEPTOR AGONIST (TPO-RA) IN ADULT IMMUNE THROMBOCYTOPENIA (ITP) PATIENTS: A RETROSPECTIVE CASE SERIES

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Background: Availability of TPO-RA offers a new opportunity of treatment with high response rates for ITP patients (pts). However, a small fraction of pts do not respond or lose response-*i.e.* desired platelet (plt) count achieved but not sustained over time-at follow-up or experience wide fluctuations in plt counts with either romiplostim (R) or eltrombopag (E). Albeit rare, untoward effects may lead to treatment discontinuation. Finally, pts preference may be an important issue considering the different route and timing of administration of the two drugs and the alimentary restrictions needed for proper E absorption. Availability of two TPO-RAs with different molecular structure and site of binding within the TPO receptor, makes it appealing to try switching with the aim of overcoming treatment limitations of either agent (1-3).
Aims: Data on TPO-RA switch in everyday clinical practice are presented.
Methods: Charts of ITP pts receiving TPO-RAs at two collaborating Centers were reviewed. All pts are enrolled in a local data-base and informed consent to clinical data use is given at enrollment.
Results: A total of 59 pts received either R or E between Jan 2009 and Dec 2014. 14/59 (24%) underwent TPO-RA switch (Table 1). All pts had received maximum product dose as per prescribing information prior to switch. Of the 5 pts switching because of lack of response to first TPO-RA, only 1 (#3) responded when switch from E to R, but developed neutralizing antibodies to R. Four of 6 pts who lost response on first TPO-RA responded to the second TPO-RA; the 2 non responders were splenectomized pts who had received multiple lines of therapy prior to TPO-RA treatment. Both pts switched for plt count fluctuations responded but one pt only achieved stable plt counts. Pts's preference was the reason for switching in one pt only who was receiving R; she did not respond to E and was switched back to R regaining a response.

Table 1.

N	REASON FOR SWITCHING	PREVIOUS THERAPY	TPO-RA SEQUENCE	OUTCOME after switching	COMMENTS
	1 st TPO-RA FAILURE sex, age				
1	M, 82 yrs	Dex, IVIG	R --> E	NR	NR to RTX; not elegible to spl
2	M, 47 yr	PDN, RTX	E --> R	NR	NR to CyA
3	F, 38 yr	PDN, IVIG	E --> R	CR->loss of response: Ab to R	Spl performed after Ab developm-> CR
4	F, 30 yr	PDN, IVIG	R --> E	NR	Re to MMF
5	F, 44 yr	Dex, IVIG, CyA	R --> E	NR	NR to cyclophospamide; not eligible to spl
	LOSS OF RESPONSE sex, age				
6	M, 23 yr	PDN	E --> R	Re	Spl performed for pt's preference->CR
7	F, 58 yr	PDN + IVIG, spl	R --> E	CR	platelet count fluctuation
8	M, 43 yr	PDN, spl, Dex	R --> E	NR	off therapy for pt's preference
9	M, 61 yr	PDN, IVIG, spl, VCR, RTX	R --> E	NR	off therapy for pt's preference
10	M, 19 yr	DEX, IVIG	R --> E	CR	Ab to R; E as bridge to spl -> CR
11	F, 56 yr	PDN, IVIG	R --> E	CR	E discontinuation for CR; off therapy
	PLT COUNT FLUCTUATION sex, age				
12	F, 64 yr	PDN,spl	R --> E	CR	persistent platelet count fluctuation
13	F, 21 yr	PDN, IVIG	E --> R	CR	resolved platelet count fluctuation
	PATIENT'S PREFERENCE sex, age				
14	F, 68 yr	PDN, spl	R --> E	NR	switched back to R; Re regained

R: Romiplostim; E: Eltrombopag
IVIG: intravenous immunoglobulin; DEX: dexamethason; RTX: rituximab; Spl: splenectomy; PDN: prednisone; VCR: vincristine; Cya: Cyclosporine; MMF: mycophenolate mofetil: Ab: antibodies; CR: complete response; Re: response; NR: no response;

Summary and Conclusions: Availability of two TPO-RAs offers a safe and often effective treatment alternative in pts not achieving the desired plt response on either R or E. However, differently from published case series (1-3) among our pts it seems that lack of response to either one of the two available TPO-RA identifies a subgroup of pts least likely to respond when switched to the second available TPO-RA. Similarly to reported series (1-3), plt count fluctuation stabilize in approximately 50% of cases upon switching. Finally, re-exposure to R (pt #14) was not associated with response loss, which confirms our published observation (4) of absence of tachyphylaxis.

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E1421

A STUDY OF HUMAN KILLER CELL IMMUNOGLOBULIN-LIKE RECEPTOR(KIR) AND MULTI-DRUG RESISTANCE (MDR)GENE POLYMORPHISMS IN CHILDREN WITH IMMUNE THROMBOCYTOPENIA

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Background: Childhood immune thrombocytopenia (ITP) is a common pediatric hematologic disorder characterized by increased destruction of antibody-sensitized platelets with normal to increased megakaryocytes in the bone marrow, as well as the presence of thrombocytopenia with otherwise normal red cells and leukocytes, absence of splenomegaly and the absence of other causes of thrombocytopenia. Although the etiology of ITP remains unclear, it is generally accepted that both environmental and genetic factors play an important role in the development of the disease.
Aims: To analyze the allelic and genotypic frequencies of MDR and KIR genes polymorphisms in healthy and ITP Egyptian subjects. In addition we assessed the potential role of these polymorphisms in relation to types of ITP, progression of disease and response to different treatment modalities.
Methods: A total of 48 pediatric ITP patients (24 newly diagnosed, 24 chronic) and 35 healthy controls were investigated via PCR-RFLP analysis for MDR1 and KIR2 genes.
Results: The frequency of MDR1 gene in patients and control was not significant (P=0.090). CT genotype was the highest distribution among all ITP cases(62.50% (n=30) and control (48.60%(n=17). There was a significant difference in age at diagnosis of MDR_1 gene with the CC genotype had the eldest age and lowest initial platelets count (P=0.029 and P=0.004). The distribution of KIR2 gene among all ITP patients and controls was significant

(P=0.026)with (KIRDL2-/KIRDS2-) genotype was the most prevalent among patients.
Summary and Conclusions: The frequency of MDR1 polymorphisms were not associated with susceptibility to the development and clinical progression of the disease. However, KIR2 gene polymorphisms were independently associated with childhood ITP in Egyptian patients with highest prevalence among (KIRDL2-/KIRDS2-) genotypes.

E1422

ROMIPLOSTIM AS A PREPARATION TREATMENT BEFORE SPLENECTOMY IN PATIENTS WITH IMMUNE THROMBOCYTOPENIA

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Background: Splenectomy is used as a second line therapy for immune thrombocytopenia (ITP) in patients with corticosteroid resistance. The operation may be risky for patients with a platelet count less than 30-40x 10(9/L) leading to severe hemorrhagic complications. Massive platelet transfusion often leads to immune complications. Moreover, it can be difficult to find retaining platelets in enough supply to be able to provide to the patient.
Aims: In the following trial romiplostim was used to reduce platelet transfusion requirement in splenectomized patients over pre- and postoperative periods.
Methods: Over a 3-week period, 12 patients with ITP (7 females and 5 males at age 26-42 years old) with a median platelet count of 7,3-7,5x10^9/L were treated with romiplostim once weekly.
Results: All that patients had resistance to corticosteroid therapy and bleeding episodes such as epistaxis, gum bleeding or skin hemorrhages. Immune globulin G therapy failed in hemorrhagic syndrome and did not cause the increase in platelet count. After 3 injections of romiplostim all patients demonstrated decrease of hemorrhagic manifestations. A median platelet count were 148±52,9x10^9/L. All patients were splenectomized. Nobody of them needed platelet transfusion in pre- or postoperative periods. No peri- or postoperative complications have developed. In 10 patients a platelet count increased up to 421±62,7x10^9/L on the second week after splenectomy. Two patients demonstrated a platelet count of 1457±132x10^9/L in 10 days after operation. In 4 weeks of treatment with heparin and hydroxycarbamide, their platelet count achieved the level of 402±37,5x10^9/L. No thrombotic complications in all patients in postoperative period have developed. Over next 11+-4,5 months all splenectomized patients had a complete clinical and biochemical remission.
Summary and Conclusions: Thus, romiplostim could be recommended as a preparation treatment before splenectomy in patients whose ITP does not respond to corticosteroids, according to our material. However, a high risk of thrombotic complications in postoperative period should be considered

E1423

GLANZMANN THROMBASTHENIA IN CHILDREN : TWENTY FIVE YEARS FOLLOW-UP

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Background: Glanzmann Thrombasthenia is a rare bleeding disorder. The cause of the disease is the qualitative or quantitative abnormalities of platelet membrane glycoprotein GPIIb-IIIa complex. Severity and frequency of the bleeding episodes may change from patient to patient.
Aims: In this report, clinical and laboratory features of 33 patients that were followed between 1990-2015 were evaluated.
Methods: Information of patients were retrieved from patient files and from the records contained in the electronic information processing environment created after 2005.
Results: They were 16 males (48.5%) and 17 females (51.5%). The age of onset was between 1 month and 7 years (mean 2.8 years). Familial consaguity was present in 17 (51.5%) of the patients. Bleeding time was prolonged and thrombocyte function tests were correlated with Glanzmann disease. Flow cytometric tests were done in 27 patients. Type 1 disease was present in 5 (18.5%), type 2 in 10 (37%), type 3 in 12 (44.5%) of the patients. The bleeding types seen in our patients were as follows; oral mucosal bleedings 81.8%, superficial skin bleedings 72.7%, epistaxis 66.6%, menorrhagia 15.1%, heamaturia 6%, haemarthrosis 6% and intracranial bleeding 1.8%. Local therapies, antifibrinolytic agents, oral contraceptives and if severe bleeding is present thrombocyte suspensions were given. For five of our patients with six bleeding attacks rFVIIa was used. One of our patients died due to tuberculous pericarditis and dilated cardiomyopathy.Follow up duration was between 1 and 24 years (mean 11.4 years). There was not any documanted alloimmunization. Thirty two of our patients are still alive and being followed up.
Summary and Conclusions: In Turkey where consanguineous marriages are common, patients with mild bleeding may be underdiagnosed. It is also known that even diagnosed patients may seek help only when they have severe bleeding problems. As a result; we believe that in order to diagnose these patients as early as possible, to perform their follow-ups and scan their families and to provide adequate patient education and genetic counselling, a nationwide program should be organized.

E1424

DIAGNOSTIC VALUE OF TESTS USED AT IMMUNE THROMBOCYTOPENIA DIAGNOSIS TO DETECT ASSOCIATED DISEASES. A PROSPECTIVE MULTICENTER COHORT STUDY

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Background: The frequency of immune thrombocytopenia (ITP)-associated diseases is not well known. The diagnostic values of the tests performed at ITP onset in order to detect these diseases are discussed or unknown.
Aims: To compare the diagnostic value of the tests used at ITP diagnosis to detect ITP-associated diseases in the overall ITP population, in the presence of signs evocative of these diseases and in the absence of these signs.
Methods: Study population was the patients included between June 2013 and December 2014 in the CARMEN (*Cytopénies Auto-immunes : Registre Midi-PyréneEN*) registry. This multicenter registry is aimed at the prospective follow-up of all incident ITP adults in the French Midi-Pyrénées region (3 million inhabitants). Each investigator prospectively follows every patient newly diagnosed for ITP in routine visit or hospital stay, providing informed consent was received. ITP is defined in accordance with French guidelines: platelet count <150x 109/L and exclusion of other causes of thrombocytopenia. Investigations performed at ITP diagnosis are recorded with their results. We assessed their positivity rates in the entire cohort and depending on the clinical and biological context. These rates were expressed in frequencies and in number of patients needed to test (NNT) to observe one positive test.
Results: We included 113 patients. Median age was 65 years (range: 17-95). Half of the patients were female, 24 (21.2%) had a secondary ITP, 58 (51.3%) had bleeding signs, median platelet count was 16x109/L (range: 1-126) and 72 patients were treated for ITP within the month following the diagnosis. Bone marrow aspirate was performed in 87 patients. Four myelodysplastic syndromes (MDS) were found (2 refractory cytopenia with unilineage dysplasia and 2 with multilineage dysplasia); these 4 ITPs had platelet count <10x 109/L and responded to corticosteroids. The NNT to detect 1 MDS was 12 in case of anemia or neutropenia and 52 in case of isolated thrombocytopenia (among patients aged >60 years, NNTs were 10 and 25, respectively). The NNTs for antinuclear antibodies (ANAs, tested in 92 patients without known connective tissue disease, positive if titer≥1/160) were similar in case of clinical signs of unknown connective tissue disease (NNT=2) or not (NNT=3). Antiphospholipid antibodies were positive in only 1 lupus patient (36 tested). The NNT for positive direct antiglobulin test (tested in 47 patients) was 4 in case of anemia or low haptoglobin level, and 7 otherwise. The NNT for hypogammaglobulinemias (<5g/L) on serum protein electrophoresis (tested in 92 patients) was 23 in case of lymphopenia and 69 otherwise. No patient had a history of repeated or severe infections. HCV, HBV and HIV were tested in respectively 89, 97 and 86 patients, including 7 with elevated alanine aminotransferase. No new infection was detected. The NNT for Helicobacter pylori testing (n=11) was 2 in case of gastro-intestinal symptoms and 3 otherwise. Thyroid stimulating hormone (TSH) or thyroid autoantibodies were tested in 57 patients without history of thyroiditis. None had thyroiditis clinical signs. Isolated autoantibodies or mild TSH abnormalities were found in 9 patients but no specific treatment was started.
Summary and Conclusions: Except for ANAs (whose presence may discuss the use of hydroxychloroquine), NNTs were at least two-fold higher in case of evocative context, discussing the systematic use of these tests. HIV, HCV and HBV revealed by ITP seem very rare in France.

E1425

STUDY OF ADAMTS13 LEVELS IN PATIENTS WITH THROMBOTIC THROMBOCYTOPENIC PURPURA (TTP) DURING PREGNANCY

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Background: TTP is an acute, life threatening thrombotic microangiopathy associated to a congenital or acquired deficiency of the von Willebrand factor cleaving protease ADAMTS13. Limited data on utility of ADAMTS13 measurements during pregnancy are available.
Aims: Aim of this study was to evaluate the importance of closely monitoring ADAMTS13 levels on pregnancy outcome.
Methods: Four consecutive pregnant women with TTP (2 congenital, 1 acquired and 1 with probably congenital) were enrolled into the study. ADAMTS-13 activity (chromogenic assay), inhibitors (mixing studies), and anti-ADAMTS-13 antibodies (ELISA) were measured in plasma.
Results: Patient 1, a 35 y.o. woman with acquired TTP history not-related to pregnancy had a TTP relapse at 21th week gestation (first pregnancy). A complete ADAMTS13 activity deficiency associated to anti-ADAMTS13 auto-antibodies was detected. Oral steroids and weekly plasma exchange (PEX) were started. However, ADAMTS13 activity remained significantly low (<5%) and she prematurely delivered (25th week) an alive baby. At remission she had persistent ADAMTS13 activity deficiency associated to auto-antibodies. Patient 2, a 21 y.o. woman developed TTP during her first pregnancy (15th week). She had a complete ADAMTS13 activity deficiency and no auto-antibodies, was successfully treated with plasma infusion therapy plus steroids, and delivered on term a healthy baby. At clinical remission, she had no ADAMTS13 activity and no auto-antibodies. A possible congenital TTP diagnosis was hypothesized. About two years later (May 2014) she was again pregnant. No ADAMTS13 activity or auto-antibodies were detected, and at molecular analysis a compound homozygosity for two novel ADAMTS13 mutations was found. She received prophylactic plasma infusions and delivered (38th week) a healthy baby without disease manifestations. Patient 3, a 28 y.o. woman with a HELLP syndrome history during her first pregnancy. She developed TTP during her second pregnancy, PEX treatment was started, however cesarean section was performed at the 32th week because of clinical worsening, with a successful outcome. She had a persistent low ADAMTS13 activity levels with no auto-antibodies, and at molecular analysis a compound heterozygosity for two novel ADAMTS13 mutations was found. Patient 4, a 30 y.o. woman developed TTP after her first pregnancy. After the delivery, she developed severe thrombocytopenia, and an evaluation of ADAMTS13 levels was performed. She had a complete ADAMTS13 activity deficiency and no auto-antibodies. The inherited nature of severe ADAMTS-13 deficiency in this patient was established by phenotype family analysis. The results of ADAMTS13 levels of parents showed a moderate reduction in ADAMTS13 activity in the mother (47%) and father (43%), associated with no antibodies. The genetic analysis is under investigation.
Summary and Conclusions: Our data endorse the utility of ADAMTS13 study for differential diagnosis between congenital and acquired TTP and subsequent type of treatment with either plasma infusion or plasma exchange. In addition, our data indicate that the measurement of ADAMTS13 can represent an effective means to monitor women at risk of TTP recurrence in pregnancy.

Quality of life, palliative care, ethics and health economics

E1426

SYMPTOMS, QUALITY OF LIFE AND HEALTH CARE UTILISATION IN MULTIPLE MYELOMA-A LONGITUDINAL STUDY OF PREDICTIVE DEMOGRAPHIC AND CLINICAL FACTORS

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Background: Multiple myeloma remains an incurable cancer with evidence that patients suffer more symptoms than in other haematological conditions. Palliative care services are rarely involved.
Aims: We aimed to determine how symptom prevalence, severity and quality of life as well as cost/health care utilisation change over time, and what demographic, clinical and social factors predict changes.
Methods: We recruited patients into a 14-site multicentre, longitudinal observational study, consenting patients at various stages of their illness (newly diagnosed, stable and progressive/relapsed disease). At baseline and then on up to 4 occasions over eight months, patients completed self reported demographic, clinical, symptom, palliative, quality of life and service use questionnaires. Clinical details were abstracted from medical records. We used myeloma-specific and generic, validated scales, including the Myeloma Patient Outcome Scale (MyPOS), a quality-of-life questionnaire specifically developed for multiple myeloma. We tested for predictors using multivariable analysis, adjusting for confounders.
Results: 257 patients with multiple myeloma with a median age of 69 years (range: 34-92) and on average 2.5 years post-diagnosis participated. 18.2% were newly diagnosed, 47.9% had stable disease and 32.7% had relapsed disease or were in the advanced, palliative phase of illness. Patients reported a mean of 5.1 (SD=2.7) symptoms. Over 70% had pain, 88.7% fatigue and 61.1% breathlessness. The most burdensome symptoms in the advanced stages were fatigue, poor mobility, pain, and tingling in the hand/feet. Over the eight months, patients showed distinct trajectories according to whether they were in an early or advanced treatment-interval or in an early or advanced treatment-free interval. Trajectories of physical functioning did not follow other domains of quality of life. The strongest predictors of higher levels of symptoms and health care service use at the end of follow-up were initial scores on MyPOS, type of myeloma, performance status and co-morbid conditions.
Summary and Conclusions: Burden of symptoms in multiple myeloma is high and symptoms are not resolved even during the treatment-free intervals. Those with a high symptom burden and with light chain disease are at increased risk for poor HRQOL, should be monitored and could potentially be considered for early referral to palliative care services.

E1427

NET SURVIVAL AND EXCESS MORTALITY AFTER A FOLLICULAR OR DIFFUSE LARGE B CELL LYMPHOMA: TREND BY EUROPEAN AREA.

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Background: Since the 1990's, Non Hodgkin Lymphoma (NHL) has been affected by profound changes with a better knowledge of the disease and new therapeutic tools. Follicular (FL) and Diffuse Large B-cell Lymphoma (DLBCL) were the first to benefit of these.

Aims: To evaluate changes on survival in the general population; to compare FL and DLBCL cancer mortality across European areas by studying the trend of net survival; and estimating the effect of age and year at diagnosis on the excess mortality hazard (EMR) in order to study the care management over time.
Methods: FL or DLBCL diagnosed between 01-01-1996 and 12-31-2004, and aged ≥15 years were selected from the EUROCARE-5 database. Vital status was at 12-31-2008 (French registries at 12-31-2007). Registries that covered the entire period were included and categorized into 5 areas (Northern, UK, Central, Eastern and Southern Europe). We estimated the 5-year age-standardized net survival (5-year NS), using the Pohar-Perme's estimator. To model effects of covariate on the EMR, the flexible model of Remontet *et al.* has been used in combination with a model-building strategy as proposed by Wynant and Abrahamowicz.
Results: 13,988 FL and 25,320 DLBCL have been studied. An improvement of the 5-year NS for the more recent year of diagnosis was observed. An area disparity was evident within the last period [2002-04] with an age-standardized 5-year NS varying from 61%[54-69] to 75%[69-80] for FL and from 46%[43-50] to 58%[54-62] for DLBCL between Eastern and Northern Europe. A significant effect of the year on the EMR was observed for all areas and ages, except in Eastern Europe for DLBCL. The dynamic of the EMR varied among areas and ages.
Summary and Conclusions: These results identify differences across Europe. There remained an area-related difference in FL and DLBCL survival especially in extreme ages (youngest and oldest patients) even if the gap has reduced in recent years of diagnosis.

E1428

IMPLEMENTATION OF "LEAN" WORKING PRACTICES AND AN INTEGRATED ECP SYSTEM RESULTED IN A 50% INCREASE IN PATIENT TREATMENTS WITH THE SAME NUMBER OF NURSES EMPLOYED IN THE UNIT

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Background: We previously predicted substantial efficiency and productivity opportunities through the implementation of lean working practices and use of a Therakos integrated photopheresis system ("Cellex") for the administration of extracorporeal photopheresis (ECP). The challenge faced in 2013/2014 was to substantially increase the number of patients receiving ECP with no increase in staff costs, together with a relocation of the ECP unit.
Aims: The aim of this study was to implement lean working practices and the use of integrated ECP systems to increase the number of patients who could be treated without increasing nursing staff.
Methods: We implemented lean working practices and changed the configuration of our instruments to two Therakos "XTS" photopheresis systems and four Therakos "Cellex" systems. This new process was implemented in the St. Thomas ECP unit a few weeks before moving to Guys Hospital. We then underwent a 5-day *kaizen* (rapid improvement) event shortly after moving into the new ECP unit at Guys Hospital. We implemented a standardized planning concept offering fixed appointment times depending on the type of machine required and whether the patient could tolerate double-needle or single-needle mode (Table 1). This standard approach enabled us to fill vacant slots by telephoning "reserve" patients 1-2 days in advance, ensuring that all slots were being filled and thus utilizing as much of our capacity as possible.

Table 1.

Location	Treatment	Mon 11:30-20:00	Tue 08:00-20:00	Wed 08:00-20:00	Thu 08:00-20:00	Fri 08:00-20:00	Sat 08:00-18:00	Capacity Needed	Capacity Given	Nurse	WTE
Guys Hospital ECP unit											
	"XTS" system	12:30	12:30	12:30	12:30	12:30	12:30	526	520	1	0.61
	15:30	15:30	15:30	15:30							
	"Cellex" system	12:30	12:30					329	312		0.24
	(single-needle)	15:30	15:30								
	"Cellex" system	12:30	09:00	09:00	09:00	09:00	09:00	2993	3016	2, 3, 4	2.63
	(double-needle)	15:30	12:30	12:30	12:30	12:30	12:30				
			15:30	15:30	15:30	15:30	15:30				

Results: The weekly average increased from 43 treatments per week in 2012 to 63 per week in 2014-a 50% increase. A trend analysis was performed for treatment numbers *versus* week number in the period May 2013 to May 2014 as illustrated in the plot below (Figure 1).
Summary and Conclusions: Implementation of these new practices has meant that we are able to treat significantly more patients in the unit with the same number of nurses. We continue to see an upward trend in treatment numbers. We were able to eliminate Sunday sessions and treat the extra patients in a 6-day week with the same number of nurses. The elimination of working on Sunday has released funding towards the cost for the extra treatments.

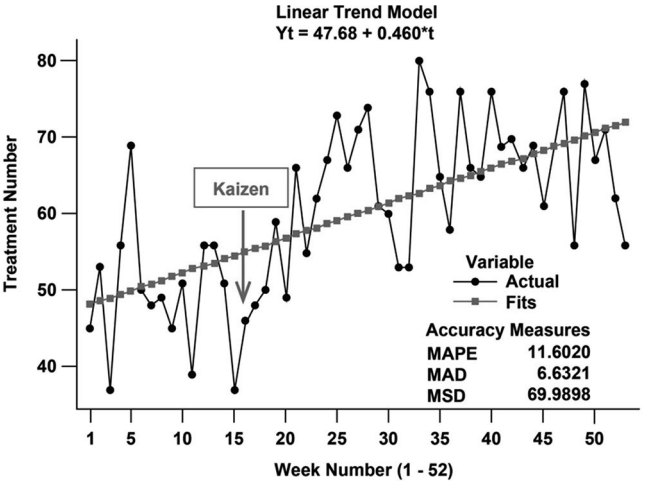

Figure 1. Trend analysis plot for ECP volumes at Guys Hospital May 2013-May 2014.

E1429

PROGNOSTIC VALUE OF NEURO-PSYCHOLOGICAL PARAMETERS IN CLINICALLY FIT OLDER PATIENTS WITH HEMATOLOGICAL MALIGNANCIES

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Background: A Comprehensive Geriatric Assessment (CGA) is currently recommended to detect vulnerable cancer patients for whom chemotherapy may lead to severe impairment on functionality, quality of life, or survival. Although CGA is useful for a better management of older patients with unsuspected problems, little is known about the reliability of CGA to optimize the therapeutic approach in patients with malignant hemopathies. Particularly, the prognostic value of neuro-psychological parameters such as cognition, in older patients with hematological malignancies admitted to receive chemotherapy, are poorly investigated.
Aims: To assess in "clinically fit" (go-go and slow-go) patients with malignant hemopathies, the reliability of different clinical parameters of CGA as a prognostic tool to predict one-year overall survival (OS) treatment-related toxicity and loss of autonomy after treatment.
Methods: Between October 2010 and June 2013, CGA was proposed to 107 consecutive older patients (65+-yrs) with hematological malignancies, admitted to the hospital and judged clinically fit, by their refereeing physicians, to receive chemotherapy. An initial full-dose or reduced-dose chemotherapy has been administrated to them according to a multidisciplinary team decision. Nutritional disorder was screened by MNA (<23.5). Psychological disorder was assessed by GDS-4 (>1) and HADS (>13) and cognitive impairment by MMS (<27) and MoCA (<26). Log-rank test and multivariate Cox proportional hazards model were used to predict one-year OS.
Results: Ninety patients -completely assessed by CGA- were evaluable for one year OS; Non Hodgkin's Lymphoma (53%), Multiple myeloma (16%), Acute Myeloid Leukemia and Myelodysplastic Syndrome (15%), Chronic Lymphocytic Leukemia (14%) and Myeloproliferative Neoplasms (2%). Median age is 74 (65-89) yrs. 56% are males. 80% were considered as "vulnerable" when evaluated with CGA (≥2 impairments). During the first year of follow-up, 28% (n=22) of older patients treated for hematological malignancies died. Although the leading cause of death (82%) was the disease progression, 18% died due to toxicity related to treatment. Retrospectively, the CGA total score (≥2 impairments) had no impact on the initial treatment choice and was not predictive for one-year OS. In univariate analysis, nutritional (p=0.946) and psychological disorders (p=0.884) were not associated with OS. In a multivariate analysis, age (p=0.015; HR=1.1) and diagnosis (p<0.001; HR=7.2), were major prognostic factors of OS. Regarding specific CGA measures, the multivariate analysis showed that mild cognitive impairment (evaluated with a MMS<27 and a MoCA<26) had the strongest prognostic value (p=0.03; HR=3.6) for OS. The loss of autonomy after treatment is still under-evaluation. In our "clinically fit" hematological patients, a poor

CGA score does not translate into a worse survival, more likely because of two reasons: the disease itself remains the major cause of death and malnutrition, a reversible impairment after effective treatments, strongly impacts CGA. The objective of an effective screening tool should thus be the identification of the population who could benefit from optimal treatment combined with a comprehensive management of unsuspected specific problems.
Summary and Conclusions: Our observations in "clinically fit" older hematological patients suggest that 1) the major cause of death is the disease itself and the priority should be to identify all patients susceptible to benefit from full dose chemotherapy 2) an abnormal CGA are not specific enough and does not translate into a worse one year OS 3) some specific measures factors in the CGA (such as cognitive impairment) are highly correlated with one-year OS 4) age remain a continuous variable with a poor prognostic factor. Prospective trials are needed to further determine whether the combination of optimal treatment and improved management of impaired functions, including cognition, could lead to a better OS.

E1430

NOA PROGRAM INFLUENCING SURVIVAL IN CML PATIENTS FROM RESOURCE CONSTRAINT SETTINGS

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Background: Patient assistance program are a huge boon in the resource constraint settings in aiding patients to combat malignant disorders from public perspective. The average daily income in these countries is much lower than the daily expenditure of the anticancer medication. The Novartis Oncology Access (NOA) is a patient-access program sponsored by Novartis Oncology and administered by The Max Foundation (MAX) that provides imatinib free of charge to patients of chronic myeloid leukemia (CML) in resource-restricted countries who are not able to afford this treatment. There is no study to-date from India on the efficacy of this program. Also most studies from other countries/MAX have no control population for comparison.
Aims: To assess the role of NOA program on survival of CML patients. Our objectives were to study OS in all CML patients enrolled either in NOA program or on self-purchased medication. Subset analysis to study the role in AYA, middle aged & elderly CML patients was also done.
Methods: It is a single centre observational study from North India. Patients of both sexes with diagnosed CML (ELN guidelines) aged more than 12 years were included. Total of 1677 case files were screened of which 1241 patients with complete records were analysed. This included 263 adolescents and young adults (AYA) and 168 elderly CML patients. The patients were divided into two arms-NOA arm (wherein patient was supplied with lifetime free medication) and self-purchased (SP) arm (patients procured medication on own expenses). Survival was analysed using telephonic calls, hospital records and OPD visits over the last 3 months. Statistical analysis was done using SPSS version 16.0.
Results: Amongst 1241 patients 67% of the patients were in the NOA arm. The two groups were not significantly different in mean age, median delay in diagnosis, median duration of hydrea therapy and median delay in starting imatinib (Table 1). The average monthly income was significantly (p-0.001) higher in SP arm (≈109€) than NOA arm (≈57€). The average cost for CML therapy in our settings is approximately 60€ per month. The mean duration of therapy was significantly (p-0.001) higher in NOA arm (1533.16+1055.35) than SP arm (1080.53+982.82). Frequency of change in dose/type TKI was significantly lower amongst patients under NOA (p<0.001). Cumulative Survival was much better in patients in NOA arm than SP arm, with the difference being statistically significant (p<0.001) with the effect being pronounced only in AYA-CML and adult CML, but not in elderly CML (Figure 1).

Table 1: Baseline characterstics.

Variable	NOA		Not on NOA/Self Purchased		P value
	Mean	SD	Mean	SD	
Age (years)	38.59	19.12	41.45	19.55	0.108
Income (INR)	4419.09	4721.14	8449.86	8508.875	0.001
Median Duration of Hydrea therapy (months)	7.0468	17.19	8.039	30.84	0.685
Delay in starting Imatinib from Diagnosis (days)	280.53	695.56	248.56	693.09	0.625
Delay in Diagnosis (days)	176.39	298.815	160.84	384.821	0.634
Mean Duration of Therapy (days)	1533.16	1055.35	1080.53	982.82	0.001
Mean OS (days)	1695.75	1204.21	1251.43	1113.186	0.001

Summary and Conclusions: Patient assistance programs being carried out in a systematic manner would help in improving the outcomes of CML patients. The retrospective nature of the study and uncertainty of exact compliance of the patients were the limitations of the study. This study emphasises the effectiveness of NOA program in improving the survival of CML patients.

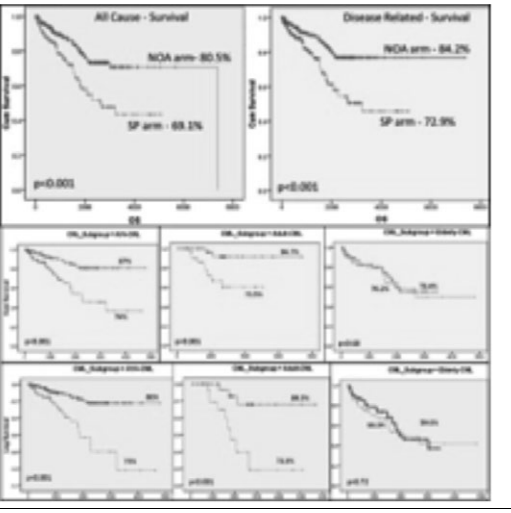

Figure 1.

E1431

A MULTI-CENTRE COST COMPARISON OF INTEGRATED *VERSUS* "OFF-LINE" SYSTEMS FOR PERFORMING EXTRACORPOREAL PHOTOPHERESIS PROCEDURES

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Background: A multicenter cost-analysis study of extracorporeal photopheresis (ECP) providers at four centers was carried out to compare "off-line" and integrated systems. Patients in these hospitals are treated with ECP for acute and chronic graft *versus* host disease (GvHD), cutaneous T-cell lymphoma (CTCL), solid organ transplantations, and various dermatological disorders. All four centers have the option of performing ECP using either an integrated system or by using a selection of off-line systems.
The integrated Therakos photopheresis system delivers ECP via a unique one-step process where the patient remains connected to the system for treatment. This eliminates the risk of improper infusion and also alleviates the need for a crossmatch of re-infused materials. The off-line system requires the removal and isolation of the collected cells from the patient to a regulated cell manipulation facility for further processing. According to European Guidelines for minimal cell manipulation, off-line procedures should be performed in a class A laminar airflow cabinet located in a class D laboratory. During off-line" procedures, cultures of the product for aerobic, anaerobic bacteria, and fungi should be done immediately before reinfusion into the patient.
Aims: The aim of this study was to compare the cost of performing ECP using the off-line systems *versus* the Therakos integrated system.
Methods: The additional costs of materials, overhead, and labor incurred by use of the off-line system were considered in this study. For a meaningful cost comparison it is important not to be misled by comparing the costs of off-line kits and Therakos system procedural kits alone.
Results: The average cost of performing ECP using the off-line systems was more expensive than the Therakos integrated system (€1,349.01 *vs* €1,285.81) (Table 1).

Table 1.

	Pitié-Sâlpétrière		Hautepierre Strasbourg			PTV Rome		EFS Nancy	
	Off-line (Macopharma)	Integrated (Therakos)	Off-line (UVA PIT)	Off-line (Macopharma)	Integrated (Therakos)	Off-line (UVA PIT)	Integrated (Therakos)	Off-line (Vilber Lourmat)	Integrated (Therakos)
Collection of leukocyte concentrate (PTC)	€173.75	€1,009.20	€215.00	€215.00	€1,074.00	€168.36	€991.86	€793.60	€781.52
EFS leukocyte procedural cost	—	—	—	—	—	—	—	—	€576.50
Biologic analysis (patient)	€59.00	€59.00	€59.00	€59.00	€59.00	€53.00	€53.00	€15.66	€15.66
Biologic analysis (cell collection)	€31.05	—	€29.00	€29.00	—	€32.00	—	€54.00	—
Transportation of cells to CT area	€7.76	—	€5.00	€5.00	—	€5.00	—	€6.54	—
Irradiation of leukocyte concentrate	€616.55	—	€605.00	€360.00	—	€450.00	—	€580.37	—
Biologic analysis (irradiated cells)	€76.00	—	€76.00	€76.00	—	€31.00	—	€43.20	—
Transportation of cells to ward	€7.76	—	€5.00	€5.00	—	€5.00	—	€6.54	—
Injection (triple access)	€12.00	—	€12.00	€12.00	—	€1.00	—	—	—
Personnel costs	€108.00	€40.50	€134.00	€134.00	€82.00	€180.00	€48.00	€161.43	€52.20
Bed retention cost/treatment (per hours used)	€337.50	€156.00	€325.77	€325.77	€144.79	—	—	€42.43	—
Total cost	€1,429.37	€1,264.70	€1,465.77	€1,220.77	€1,359.79	€925.36	€1,092.86	€1,703.77	€1,425.88

	Off-line	Integrated system
Average cost	€1,349.01	€1,285.81

Summary and Conclusions: The substantial reduction in patient treatment times offered by the Therakos integrated system improves patient quality of life, increases staff capacity to undertake additional treatments, and decreases bed retention time. In all four centers only one treatment per day can be carried out per bed using the off-line systems compared to three treatments per day per bed using the integrated system. The cost of performing ECP using the Therakos integrated system is comparable to the cost of treatments using the off-line systems.

E1432

HEALTH-RELATED QUALITY OF LIFE IN LEBANESE CHILDREN WITH SICKLE CELL DISEASE

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Background: Sickle Cell Disease (SCD) is a common genetic disorder associated with serious acute and chronic complications necessitating frequent hospitalizations and heavily impacting on quality of life (QoL) of affected patients. To date, there are no data on QoL of Lebanese patients with SCD.
Aims: To assess Health Related QoL (HRQoL) of children with SCD through self and proxy reporting of the PedsQL (4.0) questionnaire, compare it with that of age matched healthy controls, and identify socio-demographic and clinical factors that contribute to variation of QoL in SCD.
Methods: SCD children (age range 8-18 years) with no evidence of end organ damage, stroke or mental disease from 2 comprehensive SCD centers in Northern Lebanon and Beirut and healthy age sex-matched siblings (age range 8-16 years) were included in this study. PedsQL$^{4.0}$ was presented to the groups and general, physical and psychosocial scores were calculated as previously described.
Results: 47 SCD patients (66% SS, 32% SB0, 2% SD, 49% males, 51% females, mean age 12.3±3 years) and 30 sibling controls (53% males, 47% females, mean age 12.6±2.3 years) were given the PedsQL (4.0) questionnaire. All patients had received at least one transfusion. 93%, 74%, 59% of patients received folic acid, penicillin or hydroxyurea (HU), respectively. Mean general (54±20 *vs* 78±13), physical (49±25 *vs* 79±13) and psychosocial (57±19 *vs* 77±14) scores were significantly lower in patients than their age-matched siblings ($p<0.0001$ for all three). Patients receiving folic acid had significantly higher levels for general (56±18 *vs* 28±7), physical (51±24 *vs* 19±9) and psychosocial (59±19 *vs* 33±12) scores ($p<0.05$). More females than males had a general score below the median (median=55.4) ($p<0.05$). There was a positive correlation between patients' general scores and each of psychosocial score and number of siblings ($r=0.9$, $p<0.05$). Physical score was positively correlated with hemoglobin at presentation, age at first transfusion and the number of siblings of the patients ($r=0.9$, $p<0.05$). Psychosocial score was positively correlated with the age at first acute chest syndrome event ($r=0.9$, $p<0.05$). No significant impact of pain, hospitalization rate, joint necrosis, osteomyelitis, total number of transfusions, HU or penicillin intake on HRQoL scores could be detected.
Summary and Conclusions: Lebanese children with SCD have significantly decreased HRQoL compared to their siblings for all scores. The tendency for higher scores among males compared to females patients may be attributed to the more favorable nature of boys in Lebanese society. The strong association between FA treatment and higher scores underscore the beneficial impact of this inexpensive medication. The lack of impact of HU on HRQoL is surprising and may be related to the small sample size and the suboptimal compliance with this treatment in this patient group. Conclusively, the staggering difference among patients and siblings warrants a strong intervention to improve patients' QoL by advising caregivers and parents about the importance of addressing early the multiple psychosocial and physical challenges that patients with SCD face.

E1433

IMPACT OF CARFILZOMIB ON HEALTH-RELATED QUALITY OF LIFE: RESULTS FROM A PHASE 2 POST-HOC ANALYSIS OF SINGLE-AGENT CARFILZOMIB (PX-171-003-A1) IN PATIENTS WITH RELAPSED AND REFRACTORY MULTIPLE MYELOMA

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Background: In the single-arm phase 2 study PX-171-003-A1 (NCT00511238), heavily pretreated patients (pts; median 5 prior lines of therapy) with relapsed and refractory multiple myeloma (RRMM) who received single-agent carfilzomib achieved meaningful clinical benefit as evidenced by durable responses (Siegel *et al*., *Blood* 2012;120:2817-25). In addition, the tolerability profile of carfilzomib was found to be acceptable. In pts with RRMM who have active symptoms, treatment-related toxicities, and multiple comorbidities, it is important to preserve health-related quality of life (HRQL) while improving clinical outcomes.
Aims: To assess HRQL in pts treated with single-agent carfilzomib via the Functional Assessment of Cancer Therapy/Gynaecologic Oncology Group-Neurotoxicity (FACT/GOG-Ntx) questionnaire.
Methods: Pts with RRMM (N=266) previously treated with bortezomib and an immunomodulatory agent (thalidomide or lenalidomide) were enrolled in the single -arm phase 2 study PX-171-003-A1. Pts completed the FACT/GOG-Ntx at screening and on day 1 of cycles 3, 5, 7, 9, and 11 and at the end of treatment (EOT) visit. Minimally important difference (MID) thresholds for changes over time (2 points for physical wellbeing [PWB], social wellbeing [SWB], emotional wellbeing [EWB], and functional wellbeing [FWB]; 3 points for total FACT-G; 3.3 for the neurotoxicity [Ntx] subscale) were used to establish the proportion of pts who improved, were stable, or worsened at each assessment. Longitudinal effect sizes were calculated as change from baseline divided by the pooled standard deviation. Change from baseline to best response in HRQL was evaluated by response group.
Results: Median pt age was 63 years old (range, 37-87); 58% were male. Most pts (80%) had progressed during their most recent therapy or within 60 days of completing treatment. Pts who completed the FACT/GOG-NTX from screening to cycle 3 dropped from 84.2% to 47.0% overall; however, expected completion rate (pts alive and on treatment) was high throughout the study (≥72.3%). The majority (n=169, 64%) had a HRQL assessment completed at baseline and during at least 1 other assessment period. The majority of pts were considered stable or improved at each assessment point over the study period using the pre-specified MIDs. Specifically, the proportion who were stable or improved was greatest for SWB (83% to 89%), EWB (79% to 89%), and Ntx domains (80% to 86%). A smaller majority of pts were stable or improved on the PWB (70% to 79%) and FWB (64% to 73%) domains. Longitudinal effect sizes showed some small but clinically relevant improvements in EWB (0.26 to 0.39 from cycle 5) and SWB (0.13, 0.20, and 0.10 at cycles 3, 7, and EOT, respectively). PWB had declined moderately (-0.37) by the EOT visit, and there was a small reduction in FWB at cycle 3 (-0.14) and EOT (-0.26). Pts with a partial response (PR) or better had improvements in HRQL across all domains (Figure 1). Pts with stable disease (SD)/minimal response (MR) or progressive disease (PD) had declining HRQL on PWB and FWB domains. Pts with PD also demonstrated a decline on EWB domain; SWB was not correlated with response.

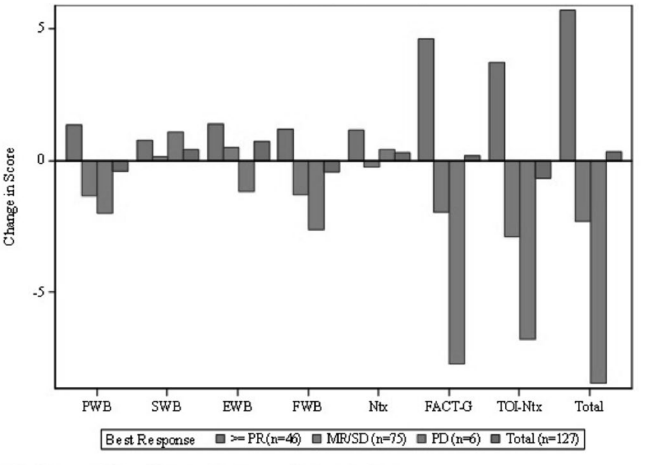

Figure 1. FACT/GOG-Ntx acores by best response.

Summary and Conclusions: For the majority of pts with available assessments, HRQL was maintained following treatment with carfilzomib. HRQL scores generally correlated with response to treatment. The effect of carfilzomib on HRQL is being assessed in randomised phase 3 trials.

E1434

PATIENTS' PERCEPTIONS OF RECEIVING A DIAGNOSIS OF A HAEMATOLOGICAL MALIGNANCY, FOLLOWING THE SPIKES PROTOCOL

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Background: Sharing potentially devastating news with a patient is often considered the most difficult task of a healthcare professional. The quality of the

delivery of the bad news can have a direct impact on patients' emotions and adjustment to the condition; therefore doctors have a great responsibility.
Aims: The main aim of this study was to learn the theory behind breaking bad news and discover patients' perceptions of receiving bad news in the haematology setting; including what they thought was done well and areas that could be improved. The study was designed following the SPIKES protocol because it comprises the basic principles and provides a structure. There is an ever increasing amount of literature available surrounding this topic. Yet, there is a noticeable lack of research concentrating on the experience from the patient's view.
Methods: A questionnaire was written based on the steps of the SPIKES model. The questions were divided into sections. The first section, titled 'patient demographics', aimed to elicit personal information without comprising anonymity. The next six sections represented the six steps of the SPIKES model. There were a mixture of qualitative responses and multiple choice answers; however the participants were always able to give qualitative responses if they felt there were no multiple choice answers that represented their views. The participants were asked the questions by interviewers, who documented their answers. The interviewers were also available to explain the questions to the participants. The questionnaire was attempted by 20 participants who were in/out patients at Arrowe Park Hospital. The inclusion criteria for participation comprised a diagnosis of a haematological malignant disease and treatment for the disease at this hospital. Additionally, it was essential the patients gave voluntary and informed verbal consent.
Results: Due to help from the interviewers, all of the 20 questionnaires attempted were completed and used in this study. The participants rated their experience: 55% said 'excellent'; 25% reported 'good'; 5% said 'satisfactory' and 15% stated 'poor'. 'Poor' was rated more commonly by women and participants aged 45-64. Those who received their diagnosis within the past few years tended to have a better consultation. The main differences between the 'excellent' and 'poor' consultations include the doctor's sensitivity and the patients having their understanding checked. Problem areas include only 35% of patients were asked their existing knowledge and 85% of consultations failed to discuss the impact of the diagnosis on daily life.
Summary and Conclusions: Overall, most patients were happy with their consultation. The areas patients particularly praised were the set-up of the consultation and how sensitively the news was delivered. The knowledge section was done well generally, however it showed the greatest difference between 'poor' and 'excellent' consultations; therefore should be improved to standardise the experience. Other areas patients felt needed improvement include the doctor determining the patient's existing knowledge and what they would like to know. With a poorer prognosis, doctors should work on reassurance and conveying hope. Most consultations could be improved by the doctor exploring the effect of the diagnosis on other areas of the patient's life. This study was limited by a small sample size and potential recall bias.

E1435

IMPACT OF BURDEN OF THALASSEMIA MAJOR ON HEALTH RELATED QUALITY OF LIFE IN OMANI CHILDREN

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Background: The availability of oral iron chelators and new techniques for detection of iron overload has markedly improved the prognosis and survival of patients with beta thalassemia major. The chronic nature of the disease however poses a significant burden affecting many aspects of patient's life such as physical activities, family adjustments and school performance. As chronically affected children are more vulnerable than adults and are less able to express their concern, health related quality of life (HRQoL) serves as an important domain for patients' perception of the disease on physical and psychosocial health functions. The impact of the disease on HRQoL in young Omani patients with thalassemia has not been evaluated earlier.
Aims: We aimed to evaluate the HRQoL in Omani children with thalassemia major by using Pediatric Quality of Life Inventory (PedsQL) 4.0 domain scores; assess the domain scores based on child and parent reporting and correlate the association between demographic-clinical characteristics and total PedsQL scores in thalassemic children to improve health care.
Methods: This cross-sectional study was done at the pediatric thalassemia day care center, Sultan Qaboos University Hospital Muscat, Oman from August 2013-February 2014. All patients with thalassemia major (5-18 years) on regular hypertransfusion/chelation were included. Demographic and clinical information were obtained from electronic patients' records. The 23-item PedsQL 4.0 Generic Core Scales with 4 Multidimensional Scales and 3 Summary Scores was used to assess the HRQoL based on parent and child reports. The 4 Multidimensional Scales included the essential core domains for pediatric HRQoL measurement: physical functioning (8 items), emotional functioning (5 items), social functioning (5 items) and school functioning (5 items). The 3 summary scores included the physical summary score, psychosocial health summary score and the total scale score. The physical summary score is the sum of all items of physical functioning and the psychosocial health summary score is the sum of all items of the emotional functioning, social functioning and school functioning. Appropriate forms in Arabic were distributed to children (aged 5-7, 8-12 and 13-18 years) and parents. Questionnaires were completed independently by children above 8 years and their parents. Children aged 5-7 years were interviewed by nurses.Statistical analysis was done using SPSS version 19.
Results: Patients' perception: The mean total summary (73.57±18.46) and the psychosocial health summary (74.55±18.25) scores were higher than the physical health summary score (72.26±22.34). School functioning score (68.05±21.3) was the lowest, followed by emotional (74.85±19.8) and social functioning (80.72±21.4) scores. Older patients were more likely to have higher HRQoL compared to their younger counterparts. Caregivers' perception: Similar to the patients perception, mean total summary (76.36±19.84) and psychosocial health summary (78.73±18.45) scores were higher than the physical health summary score (69.91±19.86). School functioning score was the lowest (69.71±18.96). In both groups gender had no significant association with HRQoL scores. Both groups revealed that patients with pre-transfusion Hb levels ≤9.5 and serum ferritin >1000 ng/ml had lower total summary score than those with Hb levels >9.5 g/dl serum ferritin ≤1000 ng/ml (Table 1).

Table 1. Clinical and demographic characteristics of Omani patients with thalassemia major.

Characteristics	n = 42
Age; median (range), years	11 (5-18)
Gender	n (%)
Male	23 (54.8)
5-7 year	11 (26.2)
8-12 year	10 (23.8)
13-18 year	2 (4.8)
Female	19 (45.2)
5-7 year	8 (14.3)
8-12 year	8 (19)
13-18 year	5 (11.9)
Age at diagnosis; median (range), months	6 (2- 31)
Age at initial transfusion; median (range), months	8.15 (2-31)
Age at onset of iron chelation; median (range), months	24.3 (16-46)
Duration of transfusion; median (range), years	9.15 (3.4-17.5)
Frequency of transfusion, weeks	
Mean ±SD	3.10 ± 0.44
Median (range)	3 (2-4)
</= 3	31
> 3	11
Pre-transfusion hemoglobin, g/dl	
Mean ±SD	9.48 ± 0.43
Median (range)	9.4 (8.7-10.7)
</= 9.5	25
> 9.5	17
Serum Ferritin, ng/ml	
Mean ±SD	1374 ± 534
Median (range)	1393.5 (463-3723)
</= 1000	10
>1000	32
Chelation, n	
Deferiprone	15
Deferasirox	16
Combination of Deferasirox and Deferiprone	11

Summary and Conclusions: Physical health function was more affected than psychosocial health function from both patients' and caregivers' perspective. School functioning domain was the most affected parameter of the psychosocial summary score in both child and parent reports. Overall, children with higher hemoglobin and lower serum ferritin levels scored better reflecting the significance of adequate blood transfusion and optimal chelation in these patients. Patients' ratings of HRQoL were lower for themselves, suggesting the need for emotional and social support. This study indicates that there is a need to address children's psychosocial and physical requirements in order to improve their quality of life. There is also a need to improve the ability of schools to adjust for the special needs of these children for better performance.

E1436

QUALITY OF LIFE IN PATIENTS WITH LOWER RISK MYELODYSPLASTIC SYNDROMES WITH SEVERE THROMBOCYTOPENIA TREATED WITH ELTROMBOPAG: INTERIM RESULTS OF A RANDOMIZED, PLACEBO-CONTROLLED PROSPECTIVE TRIAL

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Background: About 10% of low and Intermediate-1 risk (International Prognostic Scoring System, IPSS) patients with myelodysplastic syndromes (MDS) experience severe thrombocytopenia. Bleeding and the scarce efficacy of platelet (PLT) transfusions drive research in novel treatments. Eltrombopag is an oral agonist of the thrombopoetin-receptor (TPO-R) indicated for treating chronic immune thrombocytopenic purpura. Eltrombopag's potential in increasing platelet (PLT) counts in lower risk MDS has not been evaluated. We present interim results on the efficacy of eltrombpag in lower risk MDS with severe thrombocytopenia in a Phase II, multicentre, prospective, placebo-controlled, single-blind study (EQoL-MDS).
Aims: Primary endpoints are safety and efficacy of eltrombopag in low and intermediate-1 IPSS risk. Secondary endpoints include changes in quality of life (QoL), PLT transfusion requirement, incidence and severity of bleeding, and survival.
Methods: Inclusion criteria are adult age; PLT<30 Gi/L; ECOG performance status <4; ineligibility for, relapsed or refractory to other treatments; and naive to TPO-R agonists. Eltrombopag/placebo (2:1) is administered at a 50 mg daily starting dose with 50 mg increases every 2 weeks to maximum 300 mg to target PLT 100 Gi/L. Dose interruptions or reductions are required for PLT >200 Gi/L or adverse events. PLT response is defined as Response if: 1) baseline PLT>20 Gi/L: absence of bleeding and PLT≥50Gi/L; 2) baseline PLT<20 Gi/L: PLT>20 Gi/L and increase by at least 100%, not due to PLT transfusions; and Complete Response if PLT≥100 Gi/L and absence of bleeding. QoL scores are evaluated by the EORTC QLQ-C30 and by the MDS-specific instrument, QOL-E.
Results: Sixty-four patients (34 males) of mean age 67, SD±13 have been randomized at the time of the present report. Thirteen patients had comorbidities. ECOG performance status was 0 in 24 cases, 1 in 37 cases, 2 in 2 cases and 3 in one. According to the WHO 2008 classification, 19 patients had refractory cytopenia with unilineage dysplasia, 9 had refractory anemia with ringed sideroblasts, 29 had refractory cytopenia with multilineage dysplasia (of which 15 with ringed sideroblasts), 5 had refractory anemia with excess blasts-1 and 2 were unclassified. IPSS score was low in 21 cases. Mean baseline platelet (PLT) counts were 16 SD±9 Gi/L, mean hemoglobin levels 10.8 SD±2.6 g/dL and mean white blood cell count was 5 SD±4 Gi/L. Twenty-two patients were red blood cell transfusion-dependent. Thirty patients had a WHO bleeding scale of 1, 6 experienced mild blood loss and 2 gross blood loss; 13 patients had required PLT transfusions in the 8 weeks prior to randomization. Fifty-seven patients were evaluable for response at 3 weeks. Of the 29 patients receiving eltrombopag, 10 (32%) responded *versus* none in the placebo arm with a median PLT rise of 46±1 Gi/L (p=0.009). Poor QOL-E scores were registered in the physical (N=33), functional (N=30), social (N=40), and fatigue (N=20) domains. Significant improvements in fatigue were perceived by patients in the eltrombopag arm from median QOL-E baseline score 73, interquartile range (IQR) 56-82, to median 81, IQR 62-91, at 3 weeks (p=0.006) and confirmed by the EORTC QLQ-C30 scores (p=0.012). Though QOL-E social scores did not change in the eltrombopag arm, there was a significant worsening in the placebo arm, from median 50, IQR 6-69, to median 25, IQR 13-50 (p=0.041), not detected by the EORTC QLQ-C30.
Summary and Conclusions: Early after treatment initiation with eltrombopag, one third of lower risk MDS patients with severe thrombocytopenia experience significant improvements in PLT counts accompanied by improvements in fatigue.

E1437

MEDICAL AND SOCIO-ECONOMIC IMPACT OF A FREE HEALTHCARE POLICY ON MIGRANTS WITH ACUTE MYELOID OR LYMPHOID LEUKEMIA

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Background: Acute leukemia is a rare disease. However, it occurs all over the world and there is a wide disparity in access to treatment. The French National Health Insurance (NHI) is one of the most generous insurance systems in Europe in providing care for foreign citizens. Since 2000, the NHI has provided free health coverage for irregular aliens who have been in France longer than 3 months, called *Aide Medicale d'Etat* (AME). The AME assumes all healthcare costs with no charges. For foreigners who are in France for less than 3 months, there is also a critical care fund.
Aims: We have been interested in evaluating the outcome of patients who presented with acute leukemia while being in France with no health insurance from a medical, social and economic point of view.
Methods: From 2006 to 2013, 52 irregular aliens with acute leukemia (AL) were treated in the Saint-Louis Hospital in Paris (France). Medical and social datas were collected by reading medical files and evaluating the computerized medical records. To determine the treatment cost for each patient, the hospital financial unit used an approach based on a day's costs. In 2013, the cost of one day of hospitalization in our hematology intensive care unit was €2,816.89 (US $3,764.82). We used an exchange rate of: €1=US $1.34.
Results: Fifty-two patients have been included (31 with an acute myeloid leukemia (AML) and 21 with an acute lymphoïd leukemia (ALL)). Thirty-five of them (67%) came in France after a diagnosis of AL in their country and 18 have already been treated before. The other patients already lived in France illegally without a visa. Median age was 38,5 in the AML group and 31,2 in ALL group. We first observed that these patients received an appropriate therapy, based on intensive chemotherapy for the majority of them. For AML, the complete remission (CR) rate was 80.6%, 48% of patients were able to receive stem cell transplantation with a median time between CR and allogenic stem cell transplantation (ASCT) of 143 days (71-158). For ALL, CR was 95.2%; 52% of patients were able to receive ASCT with a median time of 142 days (91-681). Overall, the survival rate was 52% for AML and 50% for ALL, with a median follow-up of 36 months. All patients were supported socially during treatment and benefited all from the AME after three months in France. Nevertheless, one-third of the patients lived in precarious conditions during their treatment (No personal accommodation or socially isolation). Social situations during treatment did not affect the outcome in our cohort. At follow-up, only 8 patients had returned to their countries. Long-term social data were available only for 11/24 patients who stayed in France and in CR. They all had regularized administrative situations but half of them still lived in precarious conditions. We calculated the median total cost of care for the patients with AML to be €284,389 (US $381,081). The median total cost of care for the patients with ALL was €314,421 (US $421,325). The economic portion of the study revealed that 38% of the patients had a hospital debt of €2.16 (US $2.90) million in total.
Summary and Conclusions: Acute leukemia is a rare disease that requires expensive care. There is a wide disparity in access to care, which causes migration to countries where expensive and innovative agents are available. This study included 52 irregular aliens who benefited from the AME and were treated for acute leukemia in France. Their outcome was similar to that of other patients with health coverage. These foreign patients thus benefited from appropriate medical and social management, but this left a large financial impact on the hospital budget.

E1438

KNOWLEDGE ASSESSMENT AND EDUCATIONAL INTERVENTION AT A PATIENT CENTERED CANCER SYMPOSIUM

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Background: A diagnosis of cancer leads to significant information requirements for patients to make health care decisions. In this situation, many patients demonstrate a knowledge deficit that can translate to decreased quality of life, personal life disruptions and decreased compliance. Through analysis of patient centered cancer symposiums in 2013 and 2014, this deficit can be mitigated by patient education. This requires identifying knowledge deficits and desired information topics.
Aims: The aim of this study is to evaluate the impact of a patient centered cancer symposium on the knowledge level, patient reported symptom burden, and desired information from a broad population of cancer patients.
Methods: Surveys were distributed to the attendees of the "Living with and Surviving Cancer" patient symposium in January 2015. Surveys included demographic data, EORTC QLQ-C30, Linear Analogue Self Assessment (LASA), as well as a questionnaire evaluating disease comprehension, symptom burden, information sources and desired topics of improved understanding.
Results: 113 patients completed the pre-intervention survey to date. The average age of participants was 64.7 years with a female predominance (55%). Disease types included hematologic malignancies (N=50), breast cancer (N=25), prostate cancer (N=17), bladder/renal cancer (N=10) and other solid tumors (N=26). The majority of patients were greater than 3 years from cancer diagnosis (48%). Most patients reported a history of cancer treatment (91%) and half were undergoing cancer treatment at the time of the symposium (55%). Most respondents reported "quite a bit" or greater comprehension of their disease (81%), screening tests (74%), making end of life decisions (73%), disease monitoring tools (72%), nutrition (70%), disease symptoms (68%), common side effects of chemotherapy (81%) and radiation therapy (68%), relationship management (68%), and stress management (66%). The lowest percentage of understanding ("quite a bit"/"very much") was reported for financial considerations (50%), fatigue management (49%), symptoms associated with relapse (46%), pain management (38%), long term side effects of chemotherapy (33%), and legal issues regarding disease and treatment (14%). A large proportion of

participants indicated "quite a bit" or greater desire for increased understanding of their disease (79%), risk factors (78%), screening tests (81%), nutrition (84%), and symptom management (70%). To manage stress, participants reported utilizing exercise (65%), spirituality (58%) and anti-anxiety medication (21%). Similarly, to manage fatigue, participants reported utilizing exercise (65%), yoga (13%) and spirituality (43%). Participants reported highest level of stress directly following diagnosis (73%) while fatigue level was highest during treatment (56%). Mean overall quality of life on the LASA was 7.6 (SD 1.79). Reported mean overall quality of life in the past week based on the EORTC QLQ-C30 survey was 5.1 (SD 1.16).
Summary and Conclusions: A cancer diagnosis creates a knowledge deficit that can complicate the numerous decisions, directly or indirectly, related to cancer care. Individuals diagnosed with cancer pursue information to improve their disease understanding and management. A patient centered symposium can serve as a forum to assess desired information and provide education in hopes of easing the knowledge discrepancy experienced by patients and possibly improving their health care experience overall. Further results of cancer knowledge and the impact of this intervention are being assessed.

E1439

COST-EFFECTIVENESS OF IDELALISIB IN COMBINATION WITH RITUXIMAB FOR THE TREATMENT OF RELAPSED/REFRACTORY CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) IN PORTUGAL

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Background: CLL is the most common leukemia in the Western world and is clinically characterized by peripheral blood B-cell lymphocytosis as well as lymphadenopathy, organomegaly, cytopenias and systemic symptoms in advanced stages. All CLL patients eventually relapse and most require multiple treatment regimens. Idelalisib, a potent, oral, selective inhibitor of PI3Kδ has been approved, in combination with rituximab, for the treatment of adult patients with CLL who have received at least one prior therapy. The cost-effectiveness of this and other recently approved agents for common indolent lymphoproliferative disorders may become a socio-economical concern in a large number of countries.
Aims: To assess the cost-effectiveness of idelalisib in combination with rituximab compared to rituximab plus placebo in patients with relapsed/refractory CLL from a Portuguese societal perspective.
Methods: The international cost-effectiveness model was adapted to Portuguese health settings and is based around a partitioned survival approach, classifying patients by survival status (alive/dead), and for those alive by disease status (pre- or post-progression after prior therapy) (Figure 1). The model begins with 100% of individuals in the pre-progression state. During any given cycle, patients can be dead, alive in the pre-progression state or alive in the post-progression state. Those in the latter state remain there until death (there is no modelling of remission). Costs and benefits are estimated at fixed time points (cycle length is one week) for a fixed period of time (lifetime horizon). Costs and outcomes were discounted at a 5% annual rate, as recommended by Portuguese guidelines. One-way sensitivity analyses assessed the robustness of the model. Clinical efficacy and safety data were based on the published results from the phase 3 idelalisib plus rituximab clinical trial. Survival curves were extrapolated using a Weibull function. Utility data was taken from published evidence. The consumption pattern of outpatient resources (visits, medication, diagnostic exams, etc) was estimated based on a geographically representative national expert panel. The experts responded independently to a questionnaire sent by mail and the responses were analyzed using the simple average of the values of the responses and excluding any outliers, using the trimmed mean. The unit costs were taken from Portuguese legislation and NHS references. Diagnosis-Related Groups database (DRG) for 2013 was used to estimate inpatient costs with adverse events (AE). Inpatient episodes were identified using the appropriate code (ICD 9-CM).

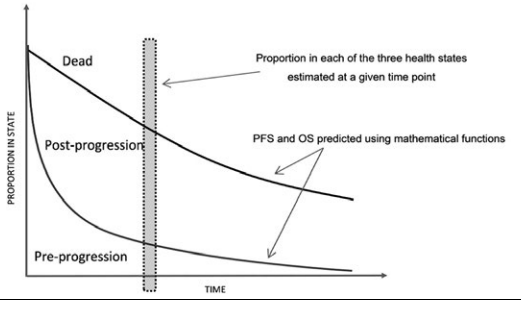

Figure 1.

Results: Survival gains (measured as life years [LYs] and quality-adjusted life years [QALYs]) were higher with idelalisib plus rituximab. Combination of idelalisib to rituximab, compared with placebo plus rituximab, led to increased direct costs (drug acquisition) but reduced the costs related to AEs and end-of-life costs. Treatment with idelalisib plus rituximab yielded a gain of 2.51 QALYs, 5.16 LYG and an increase of € 82,189 in total cost, resulting in an incremental cost-effectiveness ratio (ICER) of € 32,702 per QALY and € 15,935 per LY. Univariate analyses showed that ICER values were robust and ranged from € 31,288 to € 34,176 per QALY when different parameters were varied.
Summary and Conclusions: The use of idelalisib plus rituximab in the treatment of relapsed/refractory CLL, compared with rituximab plus placebo, is cost-effective in Portugal.

E1440

QUALITY OF LIFE OF ANTICOAGULATED PATIENTS: SURVEY SATISFACTION

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Background: Four years after the arrival of direct-acting anticoagulants onto the market the controversy over their place in preventing embolisms during non-valvular AF in relation to classical warfarin continues. A symptom of this situation is the major differences over penetration of these drugs in different European countries. A treatment that aimed to replace warfarin based on not needing periodic controls of the level of anticoagulation has not quite settled.
Aims: In these past years we have encountered several analyses both from the pharmaceutical industry and healthcare professionals from various disciplines and public health managers on the efficacy, safety, advantages, disadvantages, in short, on the efficiency of these drugs. However, this discussion has lacked the opinion of users on those issues incumbent upon them; comfort, the value of safety and their position on the cost of drugs both for them and for public health.
Methods: To bridge this gap we have undertaken a survey on the degree of comfort or discomfort of dicumarinics for 1244 anticoagulated patients with acenocumarol in the HUA and healthcare centres of this area. Four questions were put to them on comfort in regard to professional activity and another four related to family life; after these they were asked to classify their level of comfort on a scale from 1 to 10. They were also asked whether they would prefer a drug that does not require controls, whether they would prefer it to be more expensive for public health, more costly for them and finally, whether it was less safe in case of serious accidents.
Results: The results reveal that the average comfort level was 7.6 on a scale from 1, maximum discomfort to 10, total comfort. The level of comfort was less, 6.6, for the youngest patients and in relation to professional activity. A total of 61% of patients would prefer to use a drug that did not require periodic controls. This percentage would reduce to 47% in case it were more expensive for public health and 31% more expensive for the patient. Finally, only 3.9% would prefer this kind of drug if they were less safe in case of a serious accident. The results show us a highly acceptable level of comfort, and it coincides with the fact that only 61% would prefer a drug that does not require control. They also show us that this preference reduces in relation to both the public and private cost and a clear safety preference on comfort.
Summary and Conclusions: We believe that within the discussion on the introduction of new anticoagulants, in addition to considering their efficacy, efficiency, compliance and side effects, we would have to sound out the opinion of users in regard to relevant topics such as the comfort, cost and safety as to whether or not an antidote exists.

E1441

QUALITY OF LIFE EQ-5D RESULTS FROM THE AETHERA TRIAL: A PHASE 3 STUDY OF BRENTUXIMAB VEDOTIN CONSOLIDATION FOLLOWING AUTOLOGOUS STEM CELL TRANSPLANT FOR HL

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Background: The AETHERA trial demonstrated that early consolidation treatment with brentuximab vedotin (BV) post-ASCT significantly improved PFS in

patients with Hodgkin lymphoma (HL). Peripheral neuropathy (PN) was the most common adverse event.
Aims: Here, we report the results of the Quality of Life (QoL) component of the AETHERA trial.
Methods: After ASCT, 329 patients were randomized to BV 1.8 mg/kg q3wk or placebo for up to 16 cycles. The EQ-5D questionnaire, including the descriptive system and visual analog scale (VAS), was administered at each cycle, end of treatment (EOT), and q3 months during follow up until 24 months from Day 1. Utility index value scores were calculated using the time trade-off (TTO) method for US- and UK-based value sets. Differences between arms were compared to the lower bound of an estimated minimally important difference (MID) in cancer patients (Pickard *et al.* 2007).
Results: In both arms, EQ-5D scores declined from baseline to 24 months. Slightly lower scores were seen with BV *vs* placebo from months 9-18, but this resolved by end of follow up. In the analysis as randomized (US TTO), the difference between arms was <0.06 (MID) at all timepoints except for months 15 and 18. Scores by cycle were similar in the 2 arms; mean differences did not reach the MID threshold. In both arms, scores for patients with progressive disease (PD) per investigator were lower over time *vs* patients with no PD. In the BV arm, scores for patients reporting PN were similar to those who did not. Similar results were obtained with US- or UK-based value sets. EQ VAS scores did not show an important difference between arms at any timepoint (Table 1).

Table 1. US-indexed EQ-5D TTO scores.

	BV N=165 Mean	Placebo N=164 Mean	Mean Difference (BV-placebo) (95% CI)
Baseline	0.897	0.907	-0.010 (-0.035, 0.014)
3 mos	0.869	0.884	-0.014 (-0.048, 0.019)
6 mos	0.868	0.872	-0.004 (-0.041, 0.032)
9 mos	0.816	0.860	-0.043 (-0.087, 0.001)
12 mos	0.799	0.859	-0.059 (-0.109, -0.010)
15 mos	0.782	0.852	-0.071 (-0.126, -0.015)
18 mos	0.776	0.837	-0.061 (-0.121, 0.000)
21 mos	0.783	0.814	-0.029 (-0.094, 0.037)
24 mos	0.757	0.787	-0.030 (-0.102, 0.042)

Summary and Conclusions: As assessed using the EQ-5D questionnaire, treatment with BV did not have a sustained impact on QoL in HL patients. In both arms, decreased QoL was observed after progression.

E1442

ON DEATH AND DYING: CULTURAL END-OF-LIFE ISSUES IN MULTIPLE MYELOMA

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Background: Multiple Myeloma (MM) is a chronic, virtually incurable, neoplastic condition characterized by increasingly shorter periods of disease remission followed by relapse. This reality, taken together with the fact that bone pain is one of the clinical markers of disease, means that MM patients are frequent users of palliative and hospice care. Historically, patient deaths took place at home, within the family setting. However, in developed countries, patients are increasingly spending their end-of-life twilight in a Hospital ward, often at the cost of a marked decrease in quality of life. This loss of quality of life (QoL) is compounded by a loss of dignity when patients die in Emergency Rooms, which are often crowded and impersonal.
Aims: This study aims to analyze the end-of-life period in a cohort of MM patients in a tertiary referral Center in a middle-income country, to better characterize the problem and help develop palliative and hospice care solutions to improve the QoL and dignity of dying MM patients.
Methods: We analyzed a cohort of 372 MM patients diagnosed between 01-01-2001 and 12-31-2014, for whom end-of-life data was available.
Results: After a median follow-up of 84.1 months, the median overall survival for this cohort was 42.1 months. A total of 64.0% of patients died during the follow-up period. Decedents were, on average, half-a-decade older than survivors (73.4±0.7 *vs* 68.0±1.0, p<0.001). Among decedents, 29.8% died within the first 12 months of diagnosis, and 2.9% died 10 years (120 months) or more after diagnosis; there were no differences in the age at death for the two groups, or the intermediate (12-120 months) cohort (73.6±10.8, 74.9±9.2 and 70.5±12.3 years, respectively, p=NS). We found that 54.6% of patients died within our tertiary referral Hospital setting (72.9±10.4 years *vs* 73.9±12.4, p=NS), corresponding to 49.7% in a ward (Hematology or other) and 4.9% in the emergency room floor, less than 12 hours after presentation to the Hospital. Longer survivors were more likely to die outside our Center (OS=48.9±122.9 months), compared to patients dying *in situ* (30.7±27.9 months, p<0.001). Patients dying in the ER were over a decade older (79.4±3.8 *vs* 67.4±9.0 years, p=0.04) than patients dying in a ward; there was no difference in the length of disease between the two groups.
Summary and Conclusions: In our real life cohort of patients treated in a tertiary referral Center in a middle-income country, we observed a worrying trend, with nearly 55% of patients dying away from home, in our Hospital. Notably, nearly 5% of patients died in the Emergency Room, which is the least desirable outcome for end-of-life quality and dignity.
While longer experience with the disease (reflected in longer survival) was a predictor of death outside the Hospital, age failed to associate with in-Hospital *vs* out-of-Hospital death. Age did, however, predict for death in the Emergency Room floor, as opposed to death in the ward, perhaps reflecting a more restricted systemic reserve and a shorter exitus mortis.
Palliative and hospice care programs should address this reality, and develop adequate alternatives to serve the population and improve end-of-life in chronic, painful, neoplastic conditions.

E1443

UTILITY VALUES FOR PATIENTS WITH INDOLENT NON-HODGKIN LYMPHOMA (INHL) IN TOP FIVE EUROPEAN COUNTRIES (EU-5)

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Background: To our knowledge, there is no published data to inform quality of life of relapsed or refractory patients with iNHL. In oncology, cost-effectiveness models often include three health states: progression free survival (PFS), progressive disease (PD) and death, for which accurate utilities are needed.
Aims: In order to inform future HTA submissions, this study aims to review and generate utility estimates for patients with previously-treated iNHL.
Methods: First, a literature search was conducted in MEDLINE and on UK HTA websites. Thirty-two publications were screened and only one relevant study, published in 2006, was identified (Wild et al). Utilities were estimated at 0.805 (SE=0.018) for PFS and 0.618 (SE=0.056) for PD. This study supported a NICE STA, and was criticised for its small sample (33 patients had PD). As the retrieved information was not recent and criticised, additional data collection through a web-survey including socio-demographic and clinical questions, as well as the EQ-5D, was considered. Remission was defined as no sign of active disease and progression defined as a patient experiencing symptoms of the cancer. Given the burden of iNHL in Europe, the questionnaire was conducted in France, the United Kingdom, Germany, Italy and Spain. First, an analysis of utilities per health state (*i.e.* remission and progression) of patients recruited to our study was performed using the English tariff then, we combined the results of Wild *et al.* with those of our study using a Bayesian approach. To do so, means and standard errors reported by Wild *et al.* were used to specify prior distributions.
Results: The results of this web-survey show that the quality of life of a patient with iNHL is impacted by disease progression. 82 patients were included in the web-survey between the 25th of June 2014 and the 13th of August 2014 (*i.e.* 18 in Spain, 16 in France, 18 in the UK, 15 in Italy and 15 in Germany). All of these patients were previously treated, and several of them received several lines of therapy (*i.e.* 21 patients received 2 lines of therapy or more). The patients with the highest utility scores were those who had experienced a remission of their disease (N=55, u=0.713, SD=0.31). Those patients who had the lowest utility scores were those in progression (N=27, u=0.506, SD=0.35). The mean utility increment in patients in remission compared to patients in progression was 0.207. Compared to Wild *et al.*, this difference is wider in our analysis (*i.e.* 0.207 compared to 0.187 in Wild *et al.*) and patient' quality of life was lower in both states of remission and progression as shown by our analysis. Whilst in our study some patients were heavily treated, this information was not recorded by Wild *et al.*, which might explain the different results.
Summary and Conclusions: In the EU-5, the EQ-5D is a standardised and validated generic instrument which can be used to elicit utility in iNHL patients. This study confirmed that patient' quality of life is higher in disease remission than in progression. This study also shows how to use Bayesian statistics to refine estimates already available in the literature, which can be of interest for study questions related to small patient populations.

E1444

THE IMPACT OF PALLIATIVE RADIOTHERAPY ON QUALITY OF LIFE IN MULTIPLE MYELOMA PATIENTS WITH PAINFUL BONE DESTRUCTIONS

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Background: Multiple myeloma (MM) is still an incurable neoplasm of plasma cells that accounts for approximately 10% of all hematological malignancies. As a result, the most common clinical features of MM are diffuse osteopenia, osteolytic bone destructions, pathologic fractures, hypercalcemia and bone pain resulting from increased osteoclasts activity. Osteolytic lesions are detected in 70-80% of patients and increase the risk for pathological fractures, spinal cord compression and requirement for surgery or radiotherapy to bone destruc-

tions. Skeletal related events impair survival and undermine quality of life (QoL). Radiotherapy, surgery and analgesics are required in order to overcome pain, to evoke recalcification and to improve the QoL of MM patients.
Aims: To evaluate the impact of palliative radiotherapy on QoL in patients with MM and painful bone destructions.
Methods: 46 patients (27 women and 19 men, median age: 69 years) with MM and painful bone destructions were treated by palliative radiotherapy with 24 weeks follow-up. Patients completed QoL questionnaires including the EORTC QLQ-C30 version 3.0 and EORTC QLQ-MY20 before treatment and after 4 weeks. The EORTC QLQ-C30 consists of 30 items on five functional scales, nine symptom scales and a scale of global QoL. The EORTC QLQ-MY20 consists of 20 items on two functional scales and two symptoms scales. The patients' single items responses were linearly transformed to scores from 0 to 100 according to the EORTC scoring rules. High scores on the functional scales indicate a good functional status of the patient and high scores on global health status indicate a high QoL, while high scores on symptoms scale indicate poor health condition. We have analyzed the influence of clinical criteria (sex, age, clinical stage of disease, Karnofsky index, type of paraprotein, hemoglobin level, irradiated sites, performed surgery, concurrent chemotherapy, pain score at admission, type of pain medication) on QoL scores before and after radiotherapy.
Results: Before radiotherapy better functional scores (p=0,017) and lower symptoms scores (p=0,042) were observed in men, than in women. Significant higher symptom scores were observed in patients with bone destructions in the spinal column (p=0,038 and p=0,028) than in other affected sites. Better global health status and functional scale were found in patients with mild pain score at the admission (p=0.023 and p=0.031). Results showed that after radiotherapy emotional function improved in patients older than 65 years (p=0.04), female (p=0.005), receiving opioid analgesics (p=0.01), without surgery (p=0.008), in patients with higher level (>82 g/l) of hemoglobin (p=0.007), with Karnofsky index ≥60 (p=0.006), with concurrent chemotherapy (p=0.01), with severe pain at admission (p=0.03), with irradiated spinal vertebral (p=0.001), in III clinical stage of MM (p=0.005). Fatigue decreased in patients with severe pain at admission (p=0.01). Pain decreased in patients with higher level (>82 g/l) of hemoglobin (p=0.03), with Karnofsky index ≥60 (p=0.05), in III clinical stage of MM (p=0.05). Cognitive functioning improved in patients with higher level (>82 g/l) of hemoglobin (p=0.05), with Karnofsky index ≥60 (p=0.02), without concurrent chemotherapy (p=0.008), with severe pain at admission (p=0.05). Disease symptoms decreased in patients without concurrent chemotherapy (p=0.05), in III clinical stage of MM (p=0.05). Global health status improved in patients with IgM, IgD and light chains MM (p=0.04), with irradiated spinal vertebral (p=0.009).
Summary and Conclusions: Demographic and clinical criteria influence the QoL characteristics before radiotherapy. Palliative radiotherapy improves health-related quality of life with MM and painful bone destructions.

E1445

Abstract withdrawn

E1446

QUALITY OF LIFE IN MULTIPLE MYELOMA: VALIDATION OF THE TURKISH VERSION OF THE QLQ-MY20 INSTRUMENT

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Background: Health-related quality of life (HRQoL) has become an important outcome measurement in hematological malignancies. Different instruments are introduced to accurately define and scale various quality of life items. QLQ-MY20 is produced by EORTC to quantify HRQoL in multiple myeloma patients and validated in different patient populations.

	Mean (SD)	Median	Floor (%)	Ceiling (%)	Item own-scale correlations*	Item other-scale correlations*	Reliability (Cronbach's α)
QLQ-C30							
Global health/QoL	57.6 (27.3)	50	0.0 (4.9)	100.0 (12.2)	0.88	0.05-0.48	0.921
Functional scales							
Physical	54.1 (25.2)	53.3	0.0 (1.2)	100.0 (1.2)	0.42-0.67	0.06-0.57	0.842
Role	64.3 (35.7)	66.7	0.0 (7.1)	100.0 (37.6)	0.80	0.04-0.56	0.887
Emotional	67.2 (27.7)	75	0.0 (1.2)	100.0 (15.5)	0.61-0.75	0.15-0.56	0.906
Cognitive	72.7 (24.5)	83.3	0.0 (1.2)	100.0 (27.7)	0.37	0.04-0.41	0.480
Social	63.1 (31.3)	66.7	0.0 (8.4)	100.0 (20.5)	0.61	0.01-0.51	0.785
Symptom scale							
Fatigue	51.7 (26.6)	55.6	0.0 (3.5)	100.0 (8.2)	0.47-0.60	0.07-0.59	0.787
Nausea and vomiting	13.7 (25.1)	0.0	0.0 (67.5)	100.0 (3.6)	0.61	0.08-0.48	0.746
Pain	40.7 (30.5)	33.3	0.0 (19)	100.0 (6.0)	0.70	0.12-0.54	0.817
Dyspnea	15.5 (26.1)	0.0	0.0 (67.9)	100.0 (3.6)	-	-	-
Insomnia	36.1 (37.3)	33.3	0.0 (39.8)	100.0 (18.1)	-	-	-
Appetite loss	30.6 (35.9)	33.3	0.0 (48.8)	100.0 (13.1)	-	-	-
Constipation	30.4 (33.6)	33.3	0.0 (43.8)	100.0 (11.1)	-	-	-
Diarrhea	14.9 (28.2)	0.0	0.0 (72.3)	100.0 (6.0)	-	-	-
Financial difficulties	42.5 (36.4)	33.3	0.0 (31.3)	100.0 (6.0)	-	-	-
QLQ-MY20							
Functional scales							
Future perspective	59.8 (25.9)	66.7	0.0 (3.6)	100.0 (7.2)	0.39-0.57	0.0-0.52	0.730
Body image	74.8 (24.9)	66.7	0.0 (2.4)	100.0 (40.2)	-	-	-
Symptom scales							
Disease symptoms	31.3 (23.0)	27.8	0.0 (7.1)	100.0 (1.2)	0.35-0.58	0.05-0.48	0.827
Side effects of treatment	29.2 (19.1)	26.7	0.0 (2.4)	76.7 (1.2)	0.09-0.67	0.0-0.53	0.835

** Spearman correlation coefficients.*

Aims: Our aim was to validate the quality of life questionnaire (QLQ)-MY20 instrument in patients with multiple myeloma (MM) in Turkey.
Methods: The Turkish versions of QLQ-C30 v3 and QLQ-MY20 instruments were applied to patients who were diagnosed and treated at two tertiary centers in Izmir, Turkey. Validity and reliability tests were performed. Test-retest was carried out in a selected group of patients.
Results: 86 patients who were diagnosed with MM were included in the study. Questionnaire completion rates were high, and instrument was well accepted. Internal consistency tests demonstrated good convergent and divergent validity. Regarding reliability analysis, cronbach's α coefficients were all >0.7 for all scales of both QLQ-C30 and QLQ-MY20 except functional cognitive scale of QLQ-C30 questionnaire (Table 1).
Summary and Conclusions: Turkish version of QLQ-MY20 questionnaire is reliable and valid for the assessment of HRQoL in patients with MM and can be used in clinical trials in Turkish community.

E1447

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E1448

BRENTUXIMAB VEDOTIN IN PATIENTS AT INCREASED RISK OF HODGKIN LYMPHOMA PROGRESSION POST AUTOLOGOUS STEM CELL TRANSPLANT: EVALUATION OF HEALTHCARE RESOURCE UTILIZATION IN THE AETHERA TRIAL

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Background: Brentuximab vedotin received approval for the treatment of relapsed/refractory Hodgkin lymphoma (HL) after failure of autologous stem cell transplant (ASCT) or ≥2 prior therapies. AETHERA is a randomized, double-blind, Phase 3 study of brentuximab vedotin and best supportive care (BSC) *versus* placebo and BSC for patients at increased risk of HL relapse or progression following ASCT. Brentuximab vedotin consolidation therapy post-ASCT substantially improved progression-free survival (PFS) per independent review with a median PFS of 43 months in the brentuximab vedotin arm compared with 24 months in the placebo arm (HR=0.57, P=0.001). The most common treatment-emergent grade ≥3 adverse events (AEs) were neutropenia (29% brentuximab vedotin *vs* 10% placebo), peripheral sensory neuropathy (10% *vs* 1%), thrombocytopenia (4% *vs* 3%), peripheral motor neuropathy (6% *vs* 1%), and anemia (4% *vs* 2%). Treatment discontinuation due to AEs occurred in 33% *vs* 6% of patients, and 53 patients died on-study (17% *vs* 16%). High risk patients who relapse post-ASCT often report a high disease and treatment burden requiring healthcare intervention.
Aims: To compare the healthcare resource utilization (HRU) associated with brentuximab vedotin and placebo for the treatment of patients at risk of HL progression post-ASCT in the AETHERA trial.
Methods: HL patients aged ≥18 years at high risk for post-ASCT disease progression, defined as a history of refractory HL, relapse or progression <12 months after frontline therapy, or extranodal involvement at the time of pre-ASCT relapse, were eligible. Patients were randomized to receive brentuximab vedotin 1.8 mg/kg or placebo via IV infusion on day 1 of each 21-day cycle, for a maximum of 16 cycles or until disease progression. All medical care encounters that occurred from time of informed consent up to 24 months post first study treatment were collected for the intent-to-treat population. The total number of hospitalizations, outpatient visits, and the number of missed days of work/other activity for patients or caregivers, were summarized by treatment group.
Results: A total of 329 patients (median age 32 years [range 18-76]; 53% male) were randomized to receive brentuximab vedotin (n=165) or placebo (n=164) at 78 sites in the USA and Europe. There were 68 (41%) *vs* 61 (37%) patients with ≥1 hospitalization on the brentuximab vedotin *vs* placebo arms, respectively, with a total of 176 *vs* 198 hospitalizations. The hospitalization rate per patient-year was 0.58 (95% CI: 0.49, 0.67) *vs* 0.65 (95% CI: 0.56, 0.74). The median duration of stay was 16 *vs* 26 days per patient. The most common reasons for hospitalization were disease-related signs and symptoms (36% *vs* 40%) and AEs (29% *vs* 15%). There were 119 (72%) *vs* 133 (81%) patients with ≥1 outpatient visit, with a total of 2687 *vs* 3803 visits. The

outpatient visit rate per patient-year was 8.84 (95% CI: 8.51, 9.18) *vs* 12.43 (95% CI: 12.03, 12.82). The most common reasons for outpatient visits were disease-related signs and symptoms (36% *vs* 42%) and AEs (22% *vs* 14%). There were 85 (52%) *vs* 94 (57%) patients with ≥1 missed day of work/other activity, with a median number of 15 *vs* 26 missed days. There were 7 (4%) *vs* 24 (15%) caregivers with ≥1 missed day of work/other activity, with a median number of 7 *vs* 16 missed days.
Summary and Conclusions: Preliminary results suggest a trend toward lower HRU with brentuximab vedotin compared with placebo. These data prompt further investigation of the economic impact of early consolidation post-ASCT with brentuximab vedotin in HL.

E1449

COST-EFFECTIVENESS AND QUALITY ASSURANCE IN BONE MARROW ANALYSIS: AN AUDIT OF SAMPLING TECHNIQUE AND SPECIALISED HAEMATOLOGICAL TEST REQUESTING AT A HAEMATO-ONCOLOGY CENTRE

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Background: There is little published data on 'real-life' practice of bone marrow assessment in haematology, particularly in publicly funded healthcare systems where budgetary restraints and demonstrable cost-effectiveness must be applied to high-quality evidence-based practice. The British Committee on Standards in Haematology (BCSH) outlines standards for biopsy quality and additional specalised haematological testing (cytogenetics by fluorescent in-situ hybridisation (FISH) and G-banding, immunophenotyping by flow cytometry). As knowledge of the prognostic implications of specialised testing increases, there is greater tendency to order more costly tests (currently £200/€280 per immunophenotyping sample, £110/€150 for G-banding to £441/€605 for myeloma FISH panel) without influencing clinical management.
Aims: The objective of this audit was to assess current practice in our busy haemato-oncology unit against national guidelines and determine a pragmatic approach to bone marrow assessment which is equitable, evidence-based and deliverable within a restricted budget.
Methods: Bone marrow reports on consecutive procedures undertaken between January and October 2014 were examined (n=365) using the hospital's pathology database. Inadequacy of aspirate was defined by a report stating 'haemodilute and aparticulate'.
Results: Indications for bone marrow included 23% acute leukaemia (AL) diagnosis/response assessment, 21% cytopenias/suspected myelodysplasia (MDS), 16% suspected multiple myeloma (MM), 15% lymphoma staging and 6% myeloproliferative disease. 19% aspirates were unfit for morphological analysis. Trephine lengths were recorded in 82% of cases (range 1-38mm, median 16mm) with only 50% exceeding the BCSH minimum standard (16mm) and 11% deemed entirely inadequate for assessment. Approximately £65,000/€90,000 in total was spent on specialised testing: £15,000/€20,000 on immunophenotyping and £50,000/€70,000 on cytogenetics. 34% of cases had incorrect specialised tests sent. 20% and 13% of diagnostic AL samples were sent without immunophenotyping and cytogenetics respectively. Cytogenetics and immunophenotyping were sent on 76% and 21% of MDS requests and 63% and 14% of MM requests respectively: both recommended in BCSH guidelines but rarely contributing to clinical management. 15% of lymphoma staging requests were submitted for immunophenotyping and 9% for cytogenetics without additional contribution to diagnosis.
Summary and Conclusions: Our study suggests that special tests are frequently missed or inappropriately requested with clinical and cost implications: an analysis against which there is little published data to compare. Sample quality is often suboptimal and while often unavoidable highlights a need for continuous assessment of operator skill. Current national and international guidelines increasingly request more specialised tests against the backdrop of questionable clinical value and increasing financial restraints, while national and international bodies recommend we 'choose wisely'. This challenges practicing clinicians to rationalise evidence-based best care, consensual guidelines and budgetary contraints in an age of increasing scientific discovery but with spiralling costs and increased scrutiny of healthcare economics.

E1450

BLOOD AND PLATELET TRANSFUSIONS FOR HAEMATOLOGY PATIENTS REQUIRING PALLIATIVE CARE: WHAT CAN UK HOSPICES PROVIDE?

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Background: There is increasing evidence that haematology patients benefit from palliative care services. Often haematology patients require more intensive supportive treatment including IV antibiotics, blood and platelet transfusions, for symptom control and to maintain their quality of life, even near the end of life. Specialist palliative care services in the UK may not always provide these supportive treatments and this may be a barrier to haematology patients needing palliative care.
Aims: To identify what blood products UK hospices are able to provide for haematology patients
Methods: A mixed categorical and free text questionnaire was sent via email to all Medical directors of adult hospices in the UK and Ireland with inpatient services. 168 Questionnaires were sent.
Results: The response rate was 42% (n=70). Please see Table 1.

Table 1.

Provision of transfusion	Yes	Sometimes	No
RED CELL transfusion 9am-5pm, Monday-Friday	62 (94%)	4 (6%)	n/a
RED CELL transfusion out of hours	15 (23%)	30 (45%)	21 (32%)
PLATELET transfusions 9am-5pm, Monday-Friday	38 (58%)	4 (6%)	24 (36%)
PLATELET transfusions out of hours	11 (24%)	13 (28%)	22 (48%)

Summary and Conclusions: There was variation in what services are offered by the palliative care units. Medical Staff being non-resident had implications for whether transfusions could be done out of hours. Although the same training is required to administer red cells and platelets, more units were able to provide red cell transfusions. There were misconceptions regarding timing of platelet transfusions, storage and need for continuous agitation. With further education hospices that provide red cell transfusions should be able to provide platelet transfusions. Close working between haematologists and palliative care with regard to transfusion decisions is important to embed these supportive treatments in palliative care practice which ultimately can improve patient care.

E1451

COST-EFFECTIVE ANALYSIS OF PROPHYLAXIS WITH LAVIVUDINE FOR PREVENTION OF REACTIVATION IN OCCULT HEPATITIS B (OBI) IN PATIENTS WITH NON HODGKIN LYMPHOMA CD20+ UNDERGOING CHEMOTHERAPY

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Background: Occult HBV infection (OBI) is defined by the persistence of HBV in the liver without serum HBsAg and HBV-DNA. It represents a life threatening event during immunosuppressive chemotherapies. An OBI occurs in approximately 18% of HBcAb+ patients. International guidelines suggest surveillance for HBV markers in immunosuppressed patients, in particular monoclonal antibodies. In our study, the prevalence of OBI reactivation in Non-Hodgkin Lymphoma (NHL), in 498 NHL patients in our centre of Southern Italy, was 10.42% in HBcAb+ HBsAb- patients. In this work, we want to perform a cost-effectiveness analysis regarding the use of Lamivudine for the prevention of reactivation in OBI in patients with Non-Hodgkin Lymphoma undergoing chemotherapy with or without Rituximab. In fact, considering guidelines and literature, universal prophylaxis should have been applied to all HBcAb positive HBsAg negative patient. A cost-benefit issue arises : is it more cost-effective to treat all the HBcAb positive HBsAg negative patients with Lamivudine to prevent the OBI reactivation occurrence in a small quote of them, or may it be more effective a "wait and see" protocol?
Aims: Our idea was to perform a cost-effectiveness analysis, comparing the costs of prophylaxis of an eventual HBV reactivation and the "monitoring" approach that was used in our patient based on the international guidelines.
Methods: We calculated the cost of prophylaxis with Lamivudine in a time interval of twelve months, which encompasses the time of a standard Rituximab-containing chemotherapeutic protocol and a minimum time of follow-up. It has to be noticed that, very often, NHL patients need more than one chemotherapy cycle to obtain NHL remission, and, sometimes, if they do not obtain a complete remission, undergo to long-term "maintenance" treatments with Rituximab. These patients (HBcAb positive) are at high risk of HBV reactivations, due to long times of immunosuppression.

Table 1.

	Unitary Cost	n. patients	Total per patient	Duration [days]	Total
Cost of prophylaxis					
Lamivudine	€ 3,18	48	€ 152,64	360	€ 54.950,40
HBV DNA monitoring	€ 130,00	48	€ 6.240,00	6	€ 37.440,00
HBsAg monitoring	€ 17,00	48	€ 816,00	6	€ 4.896,00
AST/ALT monitoring	€ 5,74	48	€ 275,52	12	€ 3.306,24
Total	**€ 155,92**	**48**	**€ 7.484,16**		**€ 100.592,64**
Cost of HBV Reactivation					
HBV DNA monitoring	€ 130,00	48	€ 6.240,00	6	€ 37.440,00
AST/ALT monitoring	€ 5,74	48	€ 275,52	12	€ 3.306,24
Cost of DRG 205 [v24 Grouper]	€ 3.769,10	5	-	-	€ 18.845,50
Total	**€ 3.904,84**	-	-	-	**€ 40.746,24**

Drg 205: Liver disease except malignancies, cirrhosis, alcoholic hepatitis with cirrhosis.

Results: Nevertheless, even if our calculations underestimated the costs of prophylaxis, the "monitoring approach" resulted cost- effective. Moreover, even though in our series no serious events in terms of morbidity and/or mortality occurred, in other literature reports a monitoring approach did not guarantee patients survival. These detrimental results could be ascribed to the delayed start of Lamivudine treatment if the monitoring is not adequately strict. Also, it has been reported that performing only the transaminase monitoring should not be acceptable to prevent severe reactivations. Our monitoring approach resulted efficacious probably because of the monthly ALT assay was strictly observed Table 1.

Summary and Conclusions: We report an advantage in the "monitoring" approach even if, due to the retrospective nature of our study, we cannot draw any firm conclusion on which should be the best approach (universal prophylaxis *vs* monthly ALT monitoring). A randomized controlled trial might be needed to properly address this issue.

E1452

DEMAND MANAGEMENT OF ANALYTICAL REQUEST IN ERYTHROPATHOGY SECTION OF A TERTIARY HOSPITAL

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Background: The analytical requests to clinical laboratory have experienced in the latest years changes increasing the amount of them. During 2010-2012 a significant increasing of the number of requests for erythroid maturation factors and of the number of repetitions of these factors in the same patient during the same year were observed, coming from primary care centers (PCC). Given this, an intervention was performed for a more efficient management of demand; a corrective action was established from November 2011 on, to reduce the number of unnecessary requests.

Aims: 1) Reduce the number of unnecessary requests for erythroid maturation factors (ferritin, vitamin B12 and folate), without limiting the ability to request testing by physicians, so no analysis of any of the requests made was denied. 2) Encourage the ordering physician awareness of the usefulness of certain tests, the expenses incurred and diagnostic clinical advantage provided.

Table 1.

Requests	2010	2012	2013	2014
Ferritin	94876	126708	108045	107436
Folate	11685	32199	49278	47657
Cobalamin	11677	35856	51882	50069

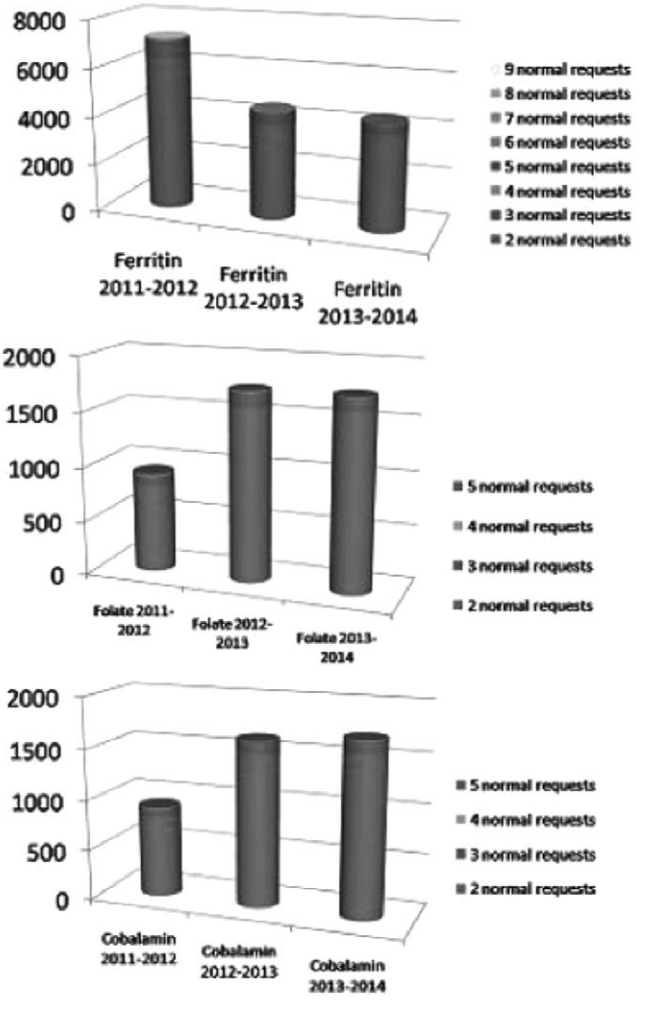

Figure 1.

Methods: A descriptive and analytical study was conducted within 2 years after developing corrective action (01/11/12-31/10/14). Requests of maturational factors have been analyzed in the population covered by our center (health area: 483 422 inhabitants), taking the total number of requests from PCC of each test and the number of repetitions of them on the same patient with a normal prior determination. This measure was taken to inform the physician that with a normal result, there is no need to repeat the determination of these factors unless there are clinical changes that warrant. To that end a comment was added next to the result when it was within normal limits: *"normal ferric profile (or cobalamin or folate). Repeat within one year only if there are alterations in blood count, the patient is treated with iron, folate or cobalamin, if clinical and/or laboratory changes or (in the case of ferric profile) family history and/or hemochromatosis or hyperferritinemia in the patient".* Also we have conducted briefings to primary care physicians about the measure and its results.

Results: After establishing the tool indicated at the end of 2011, a gradual reduction in the total number of requests was observed (Table 1), but especially in the number of determinations to patients who had normal previous result, especially if we consider the cut of 3 or more normal test. Note that no longer exist patients with more than 5 determinations made in one year, as it happened before (Figure 1). During the first year, a significant increase in the number of requests for folate and cobalamin was observed. This fact seems justified because in 2012 these tests were added to the computerised requests of the PCC. Previously the physician had to remember to include it and write it manually. Ferritin already existed in the prior computer application and therefore the same effect doesn't occur. Despite this increase, an effect of the intervention can be observed since the first year: the number of patients with 4 or more normal determinations decreased and afterwards the total number of requests remains stable and the number of repeats with normal previous result decreased as well.

Summary and Conclusions: The measure produced a greater decrease in the total number of requests and subsequent repeats. The decrease was more pronounced in the first year of its establishment. Subsequently seems

to remain effective in controlling demand and thus health spending, so we believe this tool should be maintained in the coming years to efficiently manage the requests for erythroid maturation factors.

E1453

MICRO-COSTING STUDY OF RITUXIMAB SUBCUTANEOUS INJECTION *VERSUS* INTRAVENOUS INFUSION IN DUTCH SETTING

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Background: Rituximab for subcutaneous (SC) administration has recently been approved for use in common forms of diffuse large B-cell lymphoma (DLBCL). This form of rituximab is supplied in ready-to-use vials that do not require individual dose adjustment. It is expected that SC-injection will shorten the treatment time per administration of rituximab in comparison with currently available intravenous (IV) infusion.
Aims: The goal of this study is to identify and compare all direct costs of IV and SC rituximab given to the DLBCL patients in the Netherlands.
Methods: Using a prospective, observational, bottom up, micro-costing study we collected primary data on the direct medical costs of the preparation, administration and acquisition of rituximab. Drug costs and spillage, labor costs, material costs and remaining daycare costs were identified using standardized forms, structured using guideline prices and compared for the IV and SC forms of rituximab.
Results: Measurements were done on 53 administrations (33 IV and 20 SC). The mean total costs of the IV infusion were €2174, and €1907 for the SC injection. The estimated difference of €267 per administration was mainly due to spillage costs and differences in chair time, related daycare costs and drug costs.
Summary and Conclusions: Rituximab administered in the form of SC injection is less costly than its IV form. Taking into account their equal effectiveness, favorable pharmacoeconomic profile of SC rituximab can result in significant savings when transferred to the total DLBCL population in the Netherlands.

E1454

BIOSIMILAR FILGRASTIM FOR THE PREVENTION OF FEBRILE NEUTROPENIA IN ELDERLY DLBCL PATIENTS HAS COST EFFECTIVENESS COMPEERED WITH ORIGINAL FILGRASTIM

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Background: According to American Society of Clinical Oncology guideline on the use of granulo- colony-stimulating factors (G-CSF), primary prophylaxis is recommended for the prevention of febrile neutropenia (FN) in patients who are at high risk based on age, medical history, disease characteristics, and myelotoxicity of the chemotherapy regimen. G-CSF use allows a modest to moderate increase in dose-density and dose-intensity of chemotherapy regimens. Prophylactic G-CSF for patients with diffuse aggressive lymphoma aged 65 years and older treated with R-CHOP should be given to reduce the incidence of FN. A similar biologic medicinal product, commonly referred to as biosimilar, is a copy version of an approved original biologic medicine whose data protection has expired. Among of them, biosimilar filgrastim have been approved and become available in Europe on the basis of comparable quality, safety and efficacy to the originator product. In Japan, biosimilar filgrastim approved in 2013. However, biosimilar filgrastim has not been used widely in Japan though it may have the reduction effect of costs. Here we investigated the efficacy and the cost saving effect in use of biosimilar filgrastim.
Aims: To evaluate the efficacy and the cost saving effect in use of biosimilar filgrastim.
Methods: From July 2013 to December 2014, our Institution used the biosimilar filgrastim (Filgrastim BS®; Mochida Industrial Products, Tokyo, Japan), at dosage of 75μg/day from day +10, for FN prophylaxis and hematological recovery in elderly diffuse large B cell lymphoma (DLBCL) patient who received R-CHOP therapy. These patients were retrospectively compared with same elderly patients who received original filgrastim (Gran®; Kyowa Hakko Kirin Co., Ltd, Tokyo, Japan), at dosage of 75μg/day from day +10 as FN prophylaxis after R-CHOP (from April 2010 to June 2013). Comparisons between qualitative variables were carried out using the χ^2 test. Statistical analyses were performed with the software package Stata version 11 (Stata Corp LP, College Station, TX, USA). In all analyses, P values were 2-tailed, and a P value less than 0.05 was considered to be significant.
Results: A total of 102 elderly patients with DLBCL were identified for this study, comprising 41 patients treated with Filgrastim BS® and 61 treated with Gran®. Comparisons of patient outcomes between groups are presented in Table 1. There was no statistically significant difference among the two patient cohorts. Mortality during therapy was similar in two group (p=0.647). Furthermore, number of injection during one cycle was significant less in Filgrastim BS® group (4.2 *vs* 5.0; p <0.001). In addition, mean medical costs during hospitalization were statistically lower in the Filgrastim BS® group than in the Gran® group (1,356.3±94.1 *vs* 2,357.2±113.4 Euro; p <0.001).

Table 1.

	Biosimilar filgrastim	Original filgrastim	*p* value
Age (year)	71 ± 13	69 ± 14	0.645
Sex (male / female)	20 / 21	32 / 29	0.842
Injection (/ cycle)	4.2 ± 1.7	5.0 ± 1.8	<0.001
FN (episode / total cycle)	7 / 218	12 / 327	0.672
Mortality (number)	1 / 41	2 / 61	0.647
Cost (Euro) (/ cycle)	256.1 ± 8.5	473.2 ± 29.5	<0.001
Cost (Euro) (/ patient)	1356.3 ± 94.1	2357.2 ± 113.4	<0.001

Summary and Conclusions: According to our study, it is revealed that biosimilar filgrastim (Filgrastim BS®) has similar efficacy and safety compared with original filgrastim in febrile neutropenia prophylaxis after R-CHOP therapy in elderly patients. This data confirm previous evidence that supports the non-inferiority of biosimilar filgrastim in this setting. Furthermore, mean medical costs during chemotherapy were statistically lower in the Filgrastim BS® group than in the Gran® group.

E1455

IS CLINICAL TRIAGE EFFECTIVE? AN AUDIT OF OUTPATIENT REFERRALS TO THE HAEMATOLOGY DEPARTMENT AT NORTHWICK PARK HOSPITAL, LONDON NORTHWEST NHS TRUST

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Background: The haematology service at Northwick Park Hospital is known to be busy, with a minimum of seven follow-up clinics and five new patient clinics running weekly. One Friday afternoon new patient session has been introduced last year as a measure to keep up with the new outpatient referrals that come to the department. In order to manage these referrals better, a local consultant-led triage system screens and inputs each one onto a common departmental database.
Aims: The aim is to increase the efficiency and safety of the outpatient service by assessing the necessity of each referral and making sure patients are seen within the right time frame by the appropriate clinician.
Methods: To assess this, an audit was carried out on all the new outpatients referred to the haematology department at Northwick Park Hospital over a 6 month period. Data was extracted and analysed using simple descriptive statistical tools available on Microsoft Excel.
Results: There were 1016 referrals recorded from July 2014 to December 2014, with most cases being triaged within 1 to 3 days on receipt of referral. 14% of these were rejected, either because they were deemed inappropriate (41%) or the patient would have benefitted from an alternative specialist review (11%). Out of all the accepted referrals 24% needed the red cell service, 29% required clotting advice and 34% of patients needed review at a white cell clinic. 14% of haematology referrals had no specified reason documented. 74% of referrals come from primary care, with 55% coming directly from the GP and another 19% via the *Choose and Book* patient scheme. The vast majority of rejected referrals came from primary care (96%) with most clinical questions requiring only simple advice, at times given over the phone. 71% were direct GP referrals and 25% were *Choose and Book* electronic referrals. Triaging cases to avoid unnecessary consultations is very likely to have positive financial implications, considering that costs of GP referrals to the NHS come to £15 billion per year. 156 patients (15%) needed to be seen urgently within a two week period, were the majority were referred due to a white cell disorder (69%). Such cases constitute an average of 40% of a typical new patient clinic list (2 slots out of 5 per clinic session), with an appreciable number of patients ending up requiring hospital admission for expedited work-up.

Summary and Conclusions: With the ever rising demand for specialty referral, rationalizing the limited human and fiscal resources in secondary/tertiary care is becoming important. Every hospital seeks to see the appropriate referrals first; preventing unnecessary consultations whilst picking up urgent cases early. This audit has shown that clinical triage and assessment has significant strengths conveying several advantages. Filtering out inappropriate referrals saves the GP money and prevents unnecessary waiting times. Also, referrals can be directed to the most appropriate setting, making patient care more efficient. With 2/3 of our urgent appointments being made up of white cell disorders, a good triage system helps fast-track diagnosis of possible cancer. Clinical triage, coupled with peer review and readily accessible consultant feedback to GPs through letters or phone, may prove to be both cost- and clinically-effective in today's busy practice.

E1456

PROGRESSION-FREE SURVIVAL AS THE PRIMARY ENDPOINT IN ONCOLOGY TRIALS: ITS VALUE AND CREDIBILITY FROM DIFFERENT STAKEHOLDERS' PERSPECTIVES

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Background: The primary and secondary endpoints selected for oncology trials have to meet the needs of diverse stakeholders: patient groups, clinicians, regulators, and health technology assessment (HTA) agencies, each of whom considers trial outcomes from a different perspective. Progression-free survival (PFS; time from randomization until progression or death from any cause) is becoming a more widely accepted measure of treatment efficacy, but there is tension between regulators and payors regarding its acceptability as a primary endpoint: payors still consider overall survival (OS; time from randomization until death from any cause) the 'gold standard' because of uncertainty about translating PFS gain to OS whereas regulators accept non-OS endpoints in order to drive faster approvals.
Aims: The aim of this paper is to present and discuss PFS as a credible endpoint among different stakeholders, considering the heterogeneity in stakeholder perspectives and preferences that needs to be accommodated in clinical trial design and endpoint selection in order to provide adequate information for all decision-makers.
Methods: We conducted a targeted review of the published and grey literature to identify regulatory and HTA guidance on PFS as an endpoint. We identified examples of decisions by both regulators and HTA agencies in which PFS data were presented as the primary outcome and compared the assessment of the products by regulators and different HTA agencies. Afatinib (A), erlotinib (E), and bevacizumab (B) were identified as examples of products for which decision-making appears to have been influenced by the use of PFS data.

Table 1.

Cancer	Drug	FDA	EMA	NICE (UK)	TC (France)	G-BA (Germany)
NSCLC	A	Yes	Yes	Yes + interim OS+PAS	ASMR 5	5 subgroups, benefit range: Indication of major benefit to added benefit not proven
	E	Yes	Yes	Yes + interim OS+PAS	ASMR 4	NA
Ovarian	B	Yes	Yes	No Too much uncertainty	ASMR 4	NA

Results: We identified several publications by regulatory and HTA agencies that report PFS to be a valid and credible endpoint in oncology trials; its suitability is determined by the type and stage of cancer. The magnitude of PFS gain and the associated uncertainty are considered by all stakeholders, but decision-making processes vary. Regulators assess the magnitude and certainty of survival gain in order to assess the risk-benefit profile. Clinicians and patients use PFS data to make decisions about individual treatment options. Payors use the data to assess the opportunity cost of recommending one drug rather than another. This variation in processes is demonstrated in the following decisions by regulators and HTA agencies for submissions based on PFS data (Table 1). ASMR 4, minor improvement over standard of care (SOC); ASMR 5, no improvement over SOC; NA, assessment not publicly available; PAS, patient access scheme.
Summary and Conclusions: PFS is a valid and credible endpoint, and measure of efficacy, in many oncology trials; by reducing trial length compared with OS, efficiency is also improved and fewer patients exposed to a clinical trial. Limitations associated with PFS are largely manageable. The value of PFS needs to be consistently recognized by HTA agencies, and approaches harmonized between HTA agencies and regulators.

E1457

IMBALANCES IN THE ANNUAL EHA CONGRESS. THE FUTURE OF HAEMATOLOGY

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Background: During the last EHA congress in Milan, our attention was brought to the existing imbalance between the time and space dedicated to oncohaematology in relation to other areas of haematology: red blood cell series, haemostasis and transfusion.
Aims: We have proposed reviewing whether this imbalance is timely or maintained over the last few years.
Methods: we reviewed the weight of the various areas of haematology in the last 10 EHA Congresses, based on book abstracts of communications that can be consulted on the European Haematology Association's official publication's website. We have classified communications into six sections: oncohaematology, red blood cell series, haemostasis, transfusion, transplant of haematopoietic cells and various. We analysed their percentage weight and time pattern.
Results: we detected that the participation of the RBC series varied between 4% and 6.9% with an average of 5.5%, haemostasis between 6.9% and 15.6% with an average of 9% and transfusion between 1% and 3.8% with an average of 2.1%. Oncohaematology occupied between 42% in 2005 and 67.4% in 2014, with an average of 60%. Of the 10,868 communications presented between simultaneous sessions and posters, 60% referred to Oncohaematology, 5.6% to RBC series, 9.1% to haemostasis, 2.1% to transfusion, 6.8% to transplant and 16.5% to various other issues: the taking part of patients, management, efficiency of treatments, granulocytic function and a long etc. of topics difficult to fit into the other five haematology areas. If we analyse the parallel sessions, those aimed at oncohaematology varied between 14 in 2005 to 27 in 2014 with an average of 20, which meant 64% of the total sessions. However, only one session in 3 of the 10 congresses was dedicated to transfusion (<1%). The study of the variation in the last 10 years revealed a gradual increase in the existence of oncohaematology in congresses mainly from 2009, maintenance of the RBC series and haemostasis in general below 10% and a trend towards a gradual disappearance of transfusion. We only need point out that in the last 10 years there have only been three parallel sessions which have tackled this field of our speciality.
Summary and Conclusions: We observed a gradual separation from Haematology as a speciality in favour of oncohaematology, with a gradual loss of the other three parts of the speciality, which could lead in the medium term to reconsidering the survival of the Haematology speciality as we know it today.

E1458

HEMATOLOGY AND SOCIAL MEDIA: COMMUNICATION AND EDUCATION IN THE MODERN ERA

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Background: Medical education, professional networking and communication are changing as social media becomes more integral to the lives of academics, doctors, health care professionals and patients. Social media (SoMe) is gaining popularity as a platform for informal, user-directed learning; the ease with which educational material and discussion can be accessed from multiple devices is key to this.
Aims: We aimed to develop a hematology-oriented presence within SoMe. We wanted to promote Hematology as a specialty willing to engage in international and truely multi-disciplinary dialogue via the most modern platforms. We also wanted to create an educational dialogue that would allow interested parties, from the smallest to largest institutions, to participate in teaching and the dissemination of knowledge.
Methods: We chose Twitter, a free, widely used SoMe microblogging website as it allows instant dissemination and discussion of educational material. Our Twitter profile (@TeamHaem) contains a short biography of who we are and our objectives. @TeamHaem established a Twitter presence by 'following' education and medical professionals, enabling us to see their status updates. We subsequently asked them to 'tweet' about us; therefore gaining our own followers. We created case-led discussions, which evolve over time and are

designed to be topical and relevant to our followers, targeting their various skill sets and levels of expertise. We used a blogging website to create the backbone of our case and Twitter to lead the discussion. Images, such as blood films, radiographs and marrow histology can be posted in addition to links from journal articles. Any follower can contribute or can simply watch the case developing. We react to followers' suggestions and encourage evidence-based discussion, closing each case with a summary. We moderate discussion and explicitly refrain from offering medical advice.
Results: @TeamHaem has been operational since 2012 and we currently have 1296 followers from 112 countries around the world, including doctors, nurses, students, pharmacists and biomedical scientists. We have published 32 interactive cases so far. We have also presented at medical education conferences and participated in the twitter coverage of various hematology conferences, including The Americal Society of Hematology 2014 congress which was the topic of over 20,000 tweets. We have encountereed numerous difficulties with this project, including technological literacy, compliance with confidentiality and SoMe guidelines, time management and promotion of the service to non-Twitter users. These obstacles have been overcome by following published guidelines, seeking advice from senior colleagues, presenting at local and national meetings and publishing in specialty specific newsletters.
Summary and Conclusions: Hematology is a specialty that is frequently scientifically ground-breaking. Many in the field are interested in methods of sharing knowledge and promoting the specialty, as well as learning from colleagues. @TeamHaem is an entirely novel programme which has succeeded in developing an international network for professionals and students working in Hematology. We have over 1000 followers from more than 100 countries and continue to curate high quality educational networks and debate for the Hematology community.

E1459

NARROWBAND ULTRAVIOLET-B PHOTOTHERAPY IN A PEDIATRIC PATIENT WITH CHRONIC GRAFT-*VERSUS*-HOST DISEASE

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Background: The advantage of narrowband UVB (NB UVB) phototherapy for inflammatory skin diseases in adults and children is unquestionable, but its use in graft *versus* host disease (GVHD) has been scarcely reported.
Aims: We present here a 15 years-old boy, in whom complete and durable remission was achieved with the addition of NB UVB.
Methods: A 15 years old male patient with monosomy 7 positive bilineage acute leukemia, underwent allogeneic peripheral blood stem cell derived hematopoietic stem cell transplantation (allo-HSCT), from his full-matched 50 years old mother. Conditioning consisted of total body irradiation (12 Gy/total), cyclophosphamide (120mg/kg) and etoposide (30 mg/kg). Methotrexate and cyclosporine were used for GVHD prophylaxis. 2.18x 10^6/kg CD34+ stem cells were transplanted. Full engraftment was achieved with neutrophil, platelet and erythrocyte recovery being 16 days, 22 days and 23 days respectively. He presented with overlap syndrome type GVHD on the 30th day of transplantation with a score of 3 in the skin and a final global assessment of severe GVHD. He was resistant to steroid, but remission was achieved with the combination of tacrolimus and mycophenolate mofetil. He was in hematological complete remission with good health condition and without immunosuppression for 2 months, until his second presentation with overlap syndrome type GVHD on the 128th day of transplantation with a score of 3 in the skin and a final global assessment of 'severe' GVHD. A partial remission was achieved with methylprednisolon, but there were not any response to treatment in his exfoliative skin lesions, especially which were localized on his upper extremity and palmar face of his hands (Figure 1). On the 132th day of transplantation NB UVB phototherapy was started as an adjuvant therapy to steroid, tacrolimus and mycophenolate mofetil. By means of a Waldmann UV-7002 K (Villingen-Schwenningen, Germany) cabin NB UVB phototherapy was administered. Treatments were given three times weekly and the starting dose was determined by the minimal erythema dose with increments of 0.02 mJ/cm^2 every two treatments. After the 10th treatment, complete remission was achieved and skin lesions were totaly resolved in the patient who received a total of 2.94 j/cm^2 therapy until that time. He is on the 165th day of transplantation at the time of this report, steroid therapy was discontinued. Tacrolimus and mycophenolate therapies planned to continue with the goal of reaching the 30th NB UVB treatment.
Results: Narrowband UVB is controlled administration of UVB component of sunlight. It is a method of treatment in which, UVB with 311-313 nm wavelength is used to treat a wide range of inflammatory skin diseases, especially psoriasis and vitiligo. In particular, phototherapy alters antigen presentation and T-cell responsiveness in skin, primarily via inactivation of Langerhans cell antigen-presenting capacity and secretion of immunosuppressive cytokines.

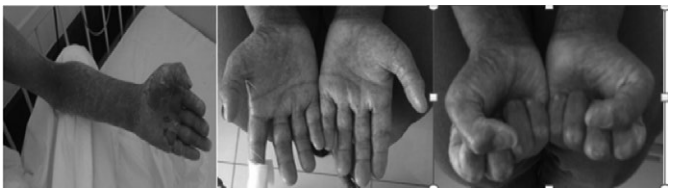

Figure 1.

Summary and Conclusions: NB-UVB phototherapy is a useful adjuvant therapeutic modality, effectiveness of which is proven in GVHD and can be indicated in the treatment of patients with cutaneous GVHD as a nonaggressive adjuvant therapy to immunosuppressive drugs.

Red blood cells and iron - Biology

E1460

HEMOGLOBINOPATHIES WITH HIGH OXYGEN AFFINITY

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Background: Hemoglobinopathies are the world's most frequently found monogenic disorders. Depending on the replaced amino acid nature and its location in the globin chain, changes will be observed in the hemoglobin molecules' stability, solubility and function that finally lead to clinical haemoglobinopathies. In the cases with high oxygen affinity, the decrease in the liberation of the oxygen determines a secondary erythrocytosis.
Aims: In this work, we present 18 unrelated families of Caucasian race and of Spanish origin, with eleven variants of hemoglobin or hemoglobinopathies with high oxygen affinity, which were diagnosed in our laboratory.
Methods: The haemoglobinopathies were characterized either through electrophoresis in an alkaline pH (8.6 pH) cellulose acetate, or an isoelectrofocussing (IEF) in polyacrylamide gel (5.5-8.5 pH), or agar citrate (pH 6.0) electrophoresis or reverse phase HPLC for globin chains or ionic exchange HPLC. Hemoglobin function was established through P50 in the oxygen balance curve plotted by a TCS Haemox-Analyzer (TCS Medical Products Co., Huntingdon Valley, PA, USA). The molecular analysis was completed by automatic sequencing of the α and/or β genes' PCR-amplified products using the ABI Prism TM dRhodamine Terminator Cycle Sequencing Ready reagents kit (PE Applied Biosystems, Foster City, AC) and the sequence was studied in an ABI Prism 310 Genetic Analyzer (PE Applied Biosystems).
Results: All the demographic data, hematrimetric results, P50 and chromatography findings are summarized in Table 1.

Table 1.

Fam	Nº patients (Age/sex)	Hb (g/dL)	Hto (%)	P50 (mmHg)	HPLC Hb	HPLC Chains	Molecular mutation
1*	1 (50/M)	20	58	13,2	N	β^; β^; α	Hb Olympia [β20(B2) Val>Met; GTG→ATG]
2*	1 (25/M)	21,3	63,7	11	HbX / HbA	β^; β^; α	Hb Syracuse [β143(H21)His→Pro; CAC→CCC]
3**	3 (54/M;26/F; 19/M)	13-17	44-52	21-22	N	β^; β^; α	Hb San Diego [β109(G11) Val→Met; GTG→ATG]
4*	1 (20/F)	18,6	60,2	20	N	β^; β^; α	Hb San Diego [β109(G11) Val→Met; GTG→ATG]
5*	1 (52/F)	16.7	48.2	14	N	β^; β^; α	Hb San Diego [β109(G11) Val→Met; GTG→ATG]
6*	1 (22/M)	18.4	55	15	N	β^; β^; α	Hb San Diego [β109(G11) Val→Met; GTG→ATG]
7**	2 (74/M; 47/F)	17-19	53-61	17-19	N	β^; β^; α	Hb Johnstown [β109(G11)Val→Leu; GTG→CTG]
8*	1 (32/F)	15.3	45.8	20	N	β^; β^; α	Hb Johnstown [β109(G11) Val→Leu; GTG→CTG]
9**	4 (41/F; 11/F; 9/F; 5/F)	14,2-17	46,6-50,2	16-19	N	β^; β^; α	Hb Johnstown [β109(G11) Val→Leu; GTG→CTG]
10**	4 (52/F; 26/F; 23/M; 21/M)	17,6-19,6	53-57,5	14-19	N	β^; β^; α	Hb Johnstown [β109(G11) Val→Leu; GTG→CTG]
11*	2 (30/M; 27/F)	18-19	60	13-15	N	β^; α. not β^	Hb Johnstown + β°Tal IVS-1-nt1 (G→A)
12**	2 (23/M; 53/M)	17,3	49,2	12,5	HbX / HbA	β^; β^; α	Hb Bethseda [β146(HC2) Tyr→His; TAT→CAT]
13***	3 (17/F; 35/F; 66/F)	17	48-49	22	HbX / HbA	β^; β^; α	Hb Badalona [β31(B13) Leu→Val; CTG→GTG]
14*	1 (66/M)	15,9	49,5	21	N	β^; β^; α	Hb Strasbourg [β23(B5) Val→Asp; GTT→GAT]
15*	1 (25/M)	18,4	55	17	HbX / HbA	β^; β^; α	Hb Malmö [β97(FG4) His→Glu; CAC→CAA]
16**	4 (66/F; 59/M; 35/M; 29/F)	16-18	50-55	16-21	N	β^; α^; α^	Hb Columbia-Missouri [α2 88(F9) Ala→Val; GCG→GTG]
17**	2 (22/M; 50/F)	17-20	50-60	16-17	N	β^; β^; α	Hb La Coruña [β38 (C4) Thr→Ile;ACC→ATC]
18*	1 (2/M)	17.8	54.6	18	HbX / HbF	β^; α; γ	Hb Andrew-Minneapolis β144(HC1) Lys→Asn; AAG→AAC + dβ°Tal Spanish

*One generation. **Two generations. ***Three generations. M: male. F: female

Summary and Conclusions: Of the eleven haemoglobinopathies, in four (the Hb San Diego, the Hb Johnstown, the Hb Malmö and the Hb Columbia-Missouri), the change of amino acid affects zones of the contact a^1b^2; in two variants (the Hb Strasbourg and the Hb Syracuse), it affects the unions with 2,3-DPG in the central cavity; in the other two (the Hb Badalona and the Hb La Coruña), the cavity of contact with the group heme is affected; in one (Hb Bethesda), it affects the zone of contact a^1b^1; in another one (Hb Andrew-Minneapolis) it affects the extreme C-terminal contacts of β chain and in the last one (Hb Olympia), the position 20 of the chain in the helix B in the surface of the protein is affected. In all cases, the change of amino acid, though of different form, facilitates that the quaternary structure of the hemoglobin becomes stable in its relaxed configuration so the transfer of oxygen and the P (50) value are decreased. All cases were sent to our laboratory because of shown erythrocytosis. In the majority of them, the diagnosis was done during an analysis of routine or for being relatives of the first ones. Although it is rarely diagnosed, the reported 18 families that show 11 different variants in the same geographic area lead us to think that the percentage of secondary erythrocytosis in hemoglobinopathies of this type could be even higher and, therefore, their systematic detection should be emphasized in erythrocytosis of unknown origin and, more so, when a family history is available.

E1461

TRANSFUSION-DEPENDENT HEREDITARY SPHEROCYTOSIS AND COMPLETE DISTAL RENAL ACIDOSIS: THE FIRST CASE WITH A HOMOZYGOUS EXON 12 C.1430C>A (P.SER477X) MUTATION OF THE SLC4A1 GENE

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Background: Mutations in the *SLC4A1* gene, which encodes a bicarbonate anionic exchange protein (AE1 or Band 3), cause a rare and unique combination of hereditary spherocytosis (HS) and distal renal acidosis (dRTA). The fast majority of patients reported so far are compound heterozygotes. So far only three types of homozygote mutations in this gene have been documented, namely one with a p.V488M in exon 13 (Ribeiro *et al.*, Blood 96:1602;2000), one with a p.S667F in exon 16 (Toye *et al.*, Blood 111:5380;2008) and another one p.Ala858Asp in exon 19 (Fawaz *et al.*, Europ J Haematol 88:350;2012).
Aims: To determine the genetic cause of complete dRTA in combination with HS.
Methods: A Turkish boy presented with a severe transfusion-dependent hemolytic anemia, complete dRTA and a psychomotor developmental delay since birth. Regular transfusions obstructed the establishment of a firm HS diagnosis with a flow cytometric assay that is based on the reduced binding of eosin-5-maleimide (EMA) to spherocytes. However, this test was abnormal in his consanguine parents and his two siblings, who all had subclinical signs of spherocytosis, despite normal RBC counts. These findings therefore prompted us to screen the family for causative mutations in the *SLC4A1* gene.
Results: Sequencing of the entire coding region revealed two homozygous sequence changes in the patient, namely a disease-relevant nonsense mutation c.1430C>A (p.Ser477X) in exon 12 and a disease-unrelated single nucleotide polymorphism c.2312-48T>G (rs13306780). The nonsense mutation creates a stop codon and results in a C-terminally truncated protein that only contains the cytoplasmic domain and the first three trans-membrane segments. In accordance with an autosomal recessive inheritance pattern of the disease, both parents as well as his siblings were heterozygous carriers of this gene-disrupting sequence variant.
Summary and Conclusions: The respective p.Ser477X mutation was previously found in only two heterozygous individuals with an incomplete form of dRTA. These cases were identified when families with HS were screened with an urine acidification test for this purpose (Rysava *et al.*, Nephrol Dial Transplant 12:1869;1997), whereas our patient is the first one who is affected by this mutation in a homozygous form. The recurrence of this identical mutation in an apparently unrelated family therefore may imply a common founder origin and proves that, if homozygote, it produces the complete clinical picture of a severe transfusion-dependent form of HS together with a complete dRTA. Whether the psychomotor development delay is directly related to the mutation or presents a preventable consequence of the hemolytic anemia and renal disturbance remains to be shown.

E1462

CHARACTERIZATION OF 6 HEMOGLOBINOPATHIES OCCURRING WITH CYANOSIS AND/OR LOWERING THE OXIGEN SATURATION

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Background: Structural hemoglobinopathies are the result of mutations in globin genes. In over 95% of cases the structural alteration corresponds to the substitution of a single amino acid in the sequence of the globin chains. Until now described over 1000 different variants being in most cases clinically and phenotypically silents. However in some instances the amino acid change may determine that the heme iron chain with the mutation this oxidized permanently or submit a decreased affinity for oxygen enrolled in these situations with cyanosis and/or decreased oxygen saturation.
Aims: In this work, we want to present the molecular characterization of six variants of hemoglobin that his suspicion was conducted by the existence of a decrease in oxygen saturation and/or cyanosis unexplained, which were diagnosed in our laboratory.
Methods: The haemoglobinopathies were characterized either through ionic

exchange HPLC or reverse phase HPLC for globin chains. Hemoglobin stability was determined using the isopropanol test, while the function was established through P50 in the oxygen balance curve plotted by a TCS Haemox-Analyzer (TCS Medical Products Co., Huntingdon Valley, PA, USA). The molecular analysis was completed by automatic sequencing of the α and/or β genes' PCR-amplified products using the ABI Prism TM dRhodamine Terminator Cycle Sequencing Ready reagents kit (PE Applied Biosystems, Foster City, AC) and the sequence was studied in an ABI Prism 310 Genetic Analyzer (PE Applied Biosystems).
Results: All the demographic data, hematrimetric results, P50 and chromatography findings are summarized in Table 1.

Table 1.

Fam	Nº patients (age/sex)	Hb (g/dL)	metHB (%)	isopropanol test	p50	HPLC Hb	HPLC Chains	Molecular mutation
1*	1 (35/M)	17.9	Not done	-	↑	HbA / HbX	β^A; α^A;α^X	Hb M-Boston [α²58(E7) His→Tyr; CAC>TAC]
2**	3 (35/F;32/F; 62/F)	10.6-12.1	10.5-12.3	+	N	HbA / HbX	β^S; β^A; α	Hb M-Hyde-Park [β92(F8) His→Tyr: CAC>TAC]
3*	1 (19/F)	11.36	9.5	+	N	HbA / HbX	β^S; β^A; α	Hb M-Saskatoon [β63(E7) His→Tyr; CAT>TAT]
4*	1 (34/M)	12.3	0.5	-	↑	HbA / HbX	β^A; α^A;α^X	Hb Titusville [α2 94(G1)Asp→Asn; CAC>TAC]
5*	1 (22/M)	15.6	18.5	+	Not done	HbA / HbX	β^S; β^A; α	Hb Higashitochigi [β25626(B667)Gly>0; del GGT]
6**	2 (5/M; 32/M	10.8-12.8	Not done	+	↑	N	β^S; β^A; α	Hb Arta [β45(CD4) Phe→Cys; TTT>TGT]

*one generation. **two generations. M: male; F: female.

Summary and Conclusions: In the Hb M-Boston and M-Saskatoon changing the distal histidine for tyrosine in α and β chains respectively and the Hb M-Hyde Park changing the proximal histidine for tyrosine in anchoring the β chain Heme group to both chains, determines the oxidation of iron the heme group permanently to yield an electron and thus can not capture oxygen, this being the cause of the decrease in oxygen saturation and cyanosis in these hemoglobinopathies. In Hb Titusville changing aspartic for asparagine at position G1 α chain involved in the contact α1β2 determines Hb stabilization conformationally deoxy T or so has a low affinity for oxygen being the reason the decrease in oxygen saturation and cyanosis. Hb Higashitochigi, the loss of a glycine in the helix B distorted indirectly heme pocket at the end distal level of the distal histidine conformocionales miss the relationships between helix B and helix E end which favors the oxidation of iron central. In the Hb Arta the mutation affects segment CD is critical in the transition from deoxy T or R configuration or oxy Hb, changing a phenylalanine for cysteine at this level configuration favors what deoxy T and so determines a lower affinity for oxygen chain. The possibility of the presence of structural hemoglobinopathies must be taken into account in cases of cyanosis and decreased oxygen saturation unexplained. Although no clinical impact a confirmation saves people carrying extensive and unnecessary studies.

E1463

IDENTIFICATION OF TWO NEW MISSENSE MUTATIONS AND A 5BP DELETION IN THE ERYTHROID-SPECIFIC PROMOTOR OF THE PKLR GENE IN TWO UNRELATED PATIENTS WITH NON-SPHEROCYTIC HEMOLYTIC ANEMIA

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Background: Pyruvate kinase (PK) deficiency is the most common form of non-spherocytic hemolytic anemia (NSHA), which concurs with significant comorbidities. As an autosomal recessive disease it results from a variety of functionally impairing mutations that are scattered over the entire coding region of the *PKLR* gene, although they show a somewhat ethnicity- and region-specific biased distribution. More than 200 different mutations have been documented so far, but only two of these, which were functionally characterized in detail, reside in the promotor region (Zanella *et al.* Brit J Haematol, 130:11, 2005).
Aims: To determine the genetic cause of a suspected PK deficiency.
Methods: We present two children from unrelated families, a five month-old girl and a three-year old boy, who both suffer from severe transfusion-dependent NSHA since birth. PK enzyme activity was not determined before initiation of transfusions. However, this test still revealed a moderate reduced activity in both the children's healthy parents, who all had normal RBC counts. In consideration of these clues, we Sanger sequenced the entire coding region of the PKLR gene in the two patients and subsequently verified the parental inheritance pattern of all identified sequence changes.
Results: Our mutation screening confirmed that both patients were compound heterozygote carriers of causal *PKLR* mutations. The girl had a previously recorded maternally-derived exon 9 (c.1174G>A; p.Ala392Thr) missense mutation and a novel paternally-derived 5bp deletion in the promoter region (c.-39-49_-39-45delTCTCT). The boy had two hitherto unknown missense mutations, namely a maternally-derived c.907-908delCC (p.Pro303GlyfsX12) in exon 7 and a paternally-derived c.1381g>A (p.Glu461Lys) in exon 10. The ensuing shift in the reading frame of the former prematurely interrupts amino acid synthesis after AS 313 and truncates the protein, whereas the other one converts a highly conserved amino acid from an acid to a basic one.
Summary and Conclusions: We succeeded to determine the genetic cause of a suspected PK deficiency in two patients and thereby uncovered three novel mutations in the *PKLR* gene, namely two missense ones in exons 7 and 10 and a 5bp deletion in its erythroid-specific promotor, which is so far only the third one of its kind. The identification of underlying mutations is an essential prerequisite not only for further functional studies that can provide important insights into the respective disease mechanism but it also provides the necessary basis for adequate counseling of the affected families.

E1464

HB PUERTA DEL SOL, HB VALDECILLA, HB GRAN VÍA, HB MACARENA, AND HB EL RETIRO: DESCRIPTION OF 5 NEW HEMOGLOBINOPATHIES

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Background: Structural hemoglobinopathies are due to mutations that cause the change of the amino acid sequence of the protein chain. Some of these variants have altered electrophoretic mobility. They do not have a clinical impact but can cause interference in the analytical determination of some parameters such as the glycated hemoglobin in diabetic patients. Thalassemias are the most common monogenic disorders worldwide and represent a serious health problem in areas where their incidence is high. The α-thalassemias are due to a deficiency or absence of synthesis of the α-chain of hemoglobin (Hb). The defects in the post-translational modifications produce hyper-unstable Hbs that are not detected by most of electrophoretic or chromatographic methods available so far.
Aims: We shown 5 new α-chain Hb variants. One is a structural variant having altered electrophoretic mobility and the other 4 are hyper-unstables and behave as α-thalassemias.
Methods: We studied 7 patients, belonging to 6 unrelated families. The first 2 families were studied because they showed a peak of abnormal Hb during routine analytical assays. The other 4 families were studied because they had microcytosis and hypochromia with normal Hb A2 and Hb F without iron deficiency. Haematological data were obtained on a haematology analyzer. HbA2 and F quantification and the separation of abnormal Hb were performed by ion-exchange HPLC. The abnormal Hb also was separated by capillary electrophoresis. The study of the globin chains was performed by reversed-phase HPLC. The most frequent mutations were ruled out by α-*globin StripAssay*. The molecular characterization was performed by specific sequencing.
Results: The haematological parameters and the genotype of the studied subjects are summarized in Table 1. The electrophoretic and chromatographic studies are recruited in Table 2.

Table 1. Haematological parameters and genotype of the studied subjects.

Family	Age (years)	Sex	Hb (g/dL)	MCV (fL)	MCH (pg)	HbA_2 (%)	Hb F (%)	Hb X (%, by HPLC-Variant)	Genotype
A	46	M	16	84.2	29.2	2.9	0.7	14.3	$\alpha\alpha^{PS}/\alpha\alpha$
B	4	M	10	78.9	26	3	0.3	17.5	$\alpha\alpha^{PS}/\alpha\alpha$
	1	M	12.6	78.7	25.9	3	0.2	17	$\alpha\alpha^{PS}/\alpha\alpha$
C	86	M	11.9	66.6	21.1	2.5	0.3	--	$\alpha^{V}\alpha/-\alpha^{3.7}$
D	38	M	11.3	83.4	27.1	2.7	0.4	--	$\alpha^{GV}\alpha/\alpha\alpha$
E	1	M	13	76.2	23.3	2.3	1.7	--	$\alpha^{M}\alpha/\alpha\alpha$
F		M	15.4	76.6	24.3	2.9	0.3	--	$\alpha^{ER}\alpha/\alpha\alpha$

M=Male; F=Female; α^{PS}=Puerta del Sol; α^{V}=Valdecilla; α^{GV}=Gran Vía; α^{M}=Macarena; α^{ER}=El Retiro

Table 2. Chromatographic and electrophoretic studies.

HEMOGLOBIN	IE-HPLC	CE	RP-HPLC	MUTATION
PUERTA DEL SOL	Yes, R.T. 4.36 min	Yes, FZ4	No, β^A, α^A	α_1 49 Ser>Arg
VALDECILLA	No	No	No	α_2 Initiation Met>Ile
GRAN VÍA	No	No	No	α_2 32 Met>Arg
MACARENA	No	No	No	α_2 119 Pro>Ser)
EL RETIRO	No	No	No	α_2CD120/121(+GTG)

Summary and Conclusions: Hb Puerta del Sol has the same mobility than HbS by IE-HPLC but a lower percentage. This variant has a normal blood count. In the Hb Valdecilla the initiation codon is affected and the carrier shows a thalassemia trait with anemia, microcytosis and hypochromia. This mutation is also associated in trans to an heterozygous α^+-thalassemia deletion. Within the non deletional α-thalassemia, a very important group are those due to the lack of stability of the α-globin chain, which causes the called hiper-unstables Hbs. The three new Hbs (Gran Vía, Macarena and El Retiro) described in this work are hiper-unstables although by different mechanisms. In Hb Gran Via, instability could be due to the replacement of a Met (apolar) by an Arg (polar positively charged) at position 13 of the B helix of the α_2 chain. This mutation alters the sequence of 6 residues (CD30-CD35) involved in the contact between α1/β1 subunits Hb tetramer. In Hb Macarena, residue 119 of the α helix H chain is affected, which has a key role interacting with the stabilizing protein alpha chain of hemoglobin (AHSP). For Hb Macarena, its hiperinstability is probably due to a decreased affinity for the AHSP. Most hemoglobinopathies are the result of a point mutation, while only a few such as Hb El Retiro originate as a result of small insertions or deletions. In Hb El Retiro a Val (apolar) is inserted at position 122 (H5), therefore displacing a polar positively charged amino acid (His) involved in α1/β1 contact. This could be the cause of instability of this hemoglobinopathie. All these variants, although uncommon and privatives to some families, reveal the complexity and variety of disorders that can be found in the genes coding for Hb, having over 1000 different Hb variants described to date. Some variants such as Hb Valdecilla, Hb Gran Vía, Hb Macarena and Hb El Retiro, with significant clinical impact if associated with other forms of α-thalassemia could lead to more serious forms of this group of pathologies as is the Hb H disease. Therefore, it is important to maintain an adequate program of screening of these diseases in countries where prevalence is high to prevent the occurrence of severe forms.

E1465

IDA AND ON THE TREATMENT OF IDA DNA DAMAGE: 8-HYDROXY-2-DEOXYGUANOSINE LEVEL

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Background: Iron deficiency anemia (IDA) is a common and an important health problem in developing countries. Ionized form is potentially dangerous while the iron components which are in the form of inorganic components have an important duty. Ionic iron causes damages at the cellular level by forming free radicals. Therefore, decreases and increases in the level of iron have clinic significances. Reactive Oxygen Species (ROS) lead to the formation of oxidative base damages in DNA. Among these forms the most common one and the one which has the best known mutagenity is 8-hydroxy-2'-deoxyguanosine (8-OHdG).
Aims: We aimed to determine IDA and its different forms of treatments' (p.o., i.m., i.v.); probable oxidative damage on DNA by looking at the level of 8-OHdG. In this way, we aimed to bring a new perspective to the treatment of IDA.
Methods: The total number of patients studied was 80 and 60 of them had IDA and the rest were healthy control group. The patients were divided into 4 subgroups: (First group: Oral treatment (p.o.) group with 20 patients]; Second group: Intramuscular treatment (i.m.) group with 20 patients]; Third group: Intravenous treatment (i.v.) group with 20 patients]; Fourth group: Healthy control group 20 patients]. Blood and urine samples were taken from all patients totally four times; just before the treatment, at the twenty-fourth hour of treatment, at the first week of treatment and at the third month of the treatment. 8-OHdG levels detected in blood and urine samples were compared with the control group.
Results: Especially at the twenty-fourth hour of treatment and also at each stage, statistical significance high was detected at the 8-hydroxy-2-deoxyguanosine blood and urine level in i.v. group compared to other groups ($p<0.05$).
Summary and Conclusions: IDA and the treatment of it affect the level of 8-OHdG. Oral iron therapy should be the top priority on children. If p.o. iron therapy cannot be applied, i.m. iron therapy can be preferable. Intravenous iron treatment might be chosen if the indications make a need.

LB2088

SEVERE NEONATAL JAUNDICE DUE TO A *DE NOVO* GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENT CLASS I MUTATION

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Background: G6PD deficiency is the most common human enzyme deficiency worldwide. Acute hemolysis induced by exposure to oxidative stress, as infection, drugs or fava beans ingestion, is the most frequent clinical presentation, however G6PD deficiency can be a major contributor to neonatal hyperbilirubinemia. To date different mutations causing chronic non spherocytic hemolytic anemia (CNSHA) have been identified. Most of these class I mutations lead to amino acids replacements close to the dimer interface and the "structural NADP+", suggesting that that the integrity of these regions is very important to form stable dimmers.
Aims: To report a case a of a newborn infant with severe G6PD deficiency associated with a *de novo* G6PD class I mutation.
Methods: The patient was born by caesarean section after a 39 weeks gestation. Parents of Spanish origin were non-consanguineous and no family members had a history of anemia or jaundice. A few minutes after birth the patient developed clinical respiratory distress accompanied by skin pallor and jaundice. Hb of 9 g/dL and data of extravascular hemolysis were evidenced. He required a blood transfusion and later blood exchange transfusion at 15 hours of life for hyperbilirubinemia (maximum of 17.4 mg/dL, mostly indirect). The episode was associated with mild thrombocytopenia requiring transfusion of a platelet unit. Biochemical analysis: All tests and evaluations were performed in accordance with the Declaration of Helsinki and after parents' informed consent given. G6PD enzyme activity assays in the red blood cell hemolysates were performed by quantitative spectrophotometer analysis according to the recommendations of the International Committee for Standardization in Hematology (ICSH). DNA analysis: Mutation screening of genes encoding erythrocyte enzymes, beta globin and membrane proteins was performed on a Next Generation Sequencing platform (Ion Torrent PGM, Life technologies, Carlsbad, CA, USA). The founded variants were confirmed by direct sequencing by dideoxy chain termination reaction using the ABI PRISM BigDye Terminator v1.1 Cycle Sequencing kit (Applied Biosystems, *Foster City,* USA). Investigation of the new mutation was assessed by bi-directional sequencing of G6PD gene in proband's and mother's independent DNA samples obtained from EDTA peripheral blood, buccal cells and hair. HLA-STR markers were analyzed in proband and relatives to confirm mother-child genetic relationships using Luminex (Gen-Probe Transplant Diagnostics, Stamford, CT, United States).
Results: Enzymatic study was performed in the reference laboratory, in which a G6PD deficiency was present (145 mU/10^9 RBC; normal value: 221-570 mU/10^9 RBC) and confirmed with a second quantification (<35 mU/10^9 RBC). The sequencing analysis on the male proband, revealed a previously described cytosine to timine transition at position c.827 on exon 8, resulting in the amino acid change proline to leucine at the residue position 276 (c.827C>T; p.Pro276Leu). The c.827C>T mutation was absent in the proband's mother genomic DNA, suggesting that this is a spontaneously occurring new genetic alteration *i.e.*, a *de novo* G6PD mutation.
Summary and Conclusions: We report a case of a severe G6PD deficient male with neonatal persistent jaundice accompanied by mild anemia and evidence of extravascular haemolysis, requiring a blood transfusion and later blood exchange transfusion at 15 hours of life for hyperbilirubinemia. NGS analysis is a powerfull tool that let to conduct a comprehensive screening for different diseases in a serious situation such as neonatal jaundice, where the enzyme activity assays and peripheral morphology cannot provide sufficient data to establish a diagnostic approach. In conclusion, this *de novo* p.Pro276Leu mutation underlying a CNSHA phenotype (class I variant) maps near the dimer interface of G6PD protein, and may severely compromise the formation of the dimmer affecting enzyme stability and the steady-state level of G6PD is so low, limiting for the survival of red cells even in the absence of any oxidant challenge.

Red blood cells and iron - Clinical

E1466

THE CLINICAL FEATURES AND TREATMENT OF IRON OVERLOAD IN PYRUVATE KINASE DEFICIENCY (PKD): DATA FROM THE PKD NATURAL HISTORY STUDY (NHS)

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Background: Both transfusion-associated and transfusion independent iron overload occur in individuals with congenital hemolytic anemias, particularly in those with ineffective erythropoiesis. The prevalence and monitoring practices differ by type of anemia, and these are not well described for PKD.
Aims: To describe the demographic features, co-inheritance of hereditary hemochromatosis, iron monitoring, and chelation practices in transfusion dependent and transfusion independent PKD patients (pts) with iron overload.
Methods: Between March 2014 and January 2015, 105 pts with PKD enrolled on the PKD NHS at 14 IRB approved sites. For this study, baseline and retrospective enrollment data were included. Log_{10} transformation was applied to liver iron concentration (LIC) by T2* MRI and ferritin values to improve normality. Analysis of variance was performed to test for associations of LIC and/or ferritin with categorical covariates of interest. General linear regression models were used to identify the association of LIC and ferritin with continuous variables. Analyses were performed in the overall cohort and repeated excluding the 46 Amish pts with a homozygous 1436G>A mutation. Significant results are reported for those that were identified in either of the analyses.
Results: Of the 105 PKD pts, iron screening data were available for 71 (68%) with ferritin [35/71 Amish] and 49 (47%) with LIC [40/49 Amish]. Ferritin was significantly higher in pts who had a prior splenectomy, even after controlling for transfusion history (p=0.01). Higher ferritin correlated with higher LIC (n=38; p<0.0001, Figure 1); however, LIC was not significantly higher in pts with prior splenectomy (p=0.23). In the 9 pts with available LIC measurements who had never been transfused or only transfused for acute stressors, 7 had a LIC >4 mg/g dry weight (DW) liver. Based on clinical screening, 4 (3.8%) pts had a homozygous mutation associated with hereditary hemochromatosis (HH), and 8 (7.6%) pts were heterozygotes. HH was not found to be significantly associated with an elevated ferritin (p=0.23) or LIC (p=0.80). Older age was significantly associated with higher baseline ferritin (p=0.005).
21 non-Amish pts (20%) had received chelation therapy starting at a median age of 11.4 years (range 1-44 years). Median ferritin and LIC at the start of chelation were 1131 ng/ml (range 182-5630) and 8.3 mg/g DW liver (4.3-46), respectively. Chelator types included: Deferoxamine (n=9), deferasirox (n=13), deferiprone (n=2), and combination chelation (n=2). Of the 21 who received chelation, 5 had complications: abdominal pain, ulcers, diarrhea, serum sickness, rash, kidney stone, hearing loss, and/or vision loss. Only 1 pt had a LIC measured both before and during/after chelation; thus, there is insufficient data to report efficacy by LIC. Two pts had phlebotomy for iron removal with no improvement in ferritin or LIC.

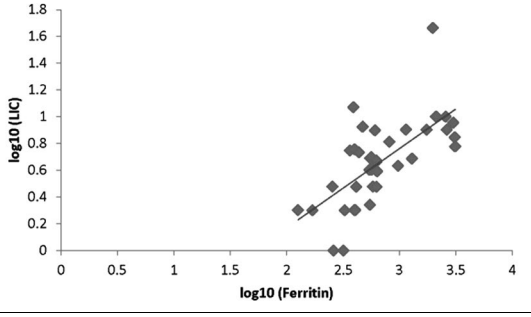

Figure 1. Correlation of ferritin (ng/ml) and liver iron concentration (mg/g dry weight liver) in the Pyruvate kinase deficiency natural history study (n=38).

Summary and Conclusions: Iron overload assessed by ferritin or LIC was common in all age groups regardless of transfusion history when monitored in this cohort of PKD pts, but monitoring had not been performed in 30% of this cohort. The efficacy of chelation in PKD is unknown, as most pts in this cohort were not adequately monitored. Since the cohort in the NHS with iron testing was mainly Amish, these results may not apply to PKD pts with other mutations. Ferritin correlates well with LIC in this cohort; thus, regular ferritin testing starting in early childhood could be considered to screen PKD pts for iron overload.

E1467

THE QUESTIONING OF THE NEED FOR MONTHLY MONITORING OF PROTENURIA AMONG PATIENTS WITH BETA THALASSEMIA MAJOR USING DEFERASIROX

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Background: The major cause of morbidity and mortality among patients with beta-thalassemia major (BTM) is transfusional iron accumulation in heart, liver and endocrine organs and iron chelation is the mainstay of the treatment in these patients. Deferasirox is an oral, once-daily active iron chelating agent and nephrotoxicity is a common toxicity among users. Nephrotoxicity of deferasirox is usually manifested with usually clinically significant rises in serum creatinin levels or rarely with renal failure especially among patients with concomitant health problems. Proteinuria is also one of the potential adverse effects of deferasirox and monthly follow-up for proteinuria is suggested by FDA and EMA.
Aims: In this study, we aimed to investigate the necessity for monthly monitoring for proteinuria among patients with BTM on deferasirox.
Methods: Thirthy-seven patients with BTM on deferasirox therapy were enrolled. Median age of the patients was 23 years (4-39); 22 being females. At the time of initiation of deferasirox all patients had normal serum creatinine, glomerular filtration rate and spot urine protein/creatinine ratios. All patients were monitored for proteinuria q3 or q4 weeks after initiation of deferasirox with serum creatinin and spot urine protein/creatinine ratios throughout the course of deferasirox treatment. The management of patients who developed spot urine protein/creatinine ratio ≥0.8 were recorded.
Results: The median follow-up time of the 37 patients was 44 months. Throughout this follow-up time 1490 times of testing was done for proteinuria detection. Although all patients were ordered urine test at each visit (1987 order), 1490 of the patients delivered their urine samples to laboratory; indicating a test compliance of 75%. Of the patients, 7 (18.9%) developed significant proteinuria (ratio ≥0.8). Of the 1490 measurements, 12 tests (0.8%) were proteinuric. The risk of proteinuria was higher at ages above a cut-off point of 23 years (p=0,019). Patients who were on deferasirox at doses above 29 mg/kg/day were found to have higher risk of proteinuria development (p=0.004). The patients who developed proteinuria had a concomitant rise in serum creatinine level more than 50% of the serum creatinine level measured at deferasirox initiation. Of the proteinuria occurences, three resolved after 1 week of drug cessation. The rest of the proteinura cases resolved spontaneously in 1 to 3 visits of time, without any intervention. None of the patients required hospitalization for proteinuria.
Summary and Conclusions: The compliance of the patients for monthly follow-up proteinuria is not satisfactory. Additionally, the proteinuria usually resolves without any complication or major intervention. Potentially more risky groups (age above 23 years-old and dose above 29 mg/kg/day) might be suggested to be followed monthly, other than monitoring all of the patients.

E1468

CAUSES OF NEWLY DIAGNOSED ANAEMIA IN GENERAL PRACTICE: THE RELEVANCE OF MCV

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Background: Anemia is a common finding amongst the elderly population and its prevalence rises from about 5.6% in the age group 50-64 years to about 23.1% in the age group 85+ years. In recent years it has been shown anemia is associated with increased mortality and morbidity and decreased quality of life. Despite anemia being such a frequently encountered clinical problem, surprisingly few data are available on the prevalence of the underlying causes. Several studies on causes of anemia in the general population have been published, but these generally included relatively small patient numbers and provided a limited analysis of underlying causes. A large cohort study of general practice patients with newly discovered anemia was set up to allow for an extensive analysis of the causes of anemia and to assess the validity of MCV-based algorithms used for the diagnosis of the underlying cause.
Aims: To analyse the causes of anemia in general practice and assess the validity of MCV-based algorithms.
Methods: Patients were included after presenting to one of the 63 participating general practitioners with a newly diagnosed anemia (*i.e.* no diagnosis of anemia in the preceding two years). Anemia was defined as hemoglobin level

below 13.7 g/dL (8.5 mmol/L, men) and below 12.1 g/dL (7.5 mmol/L, women). The inclusion period lasted from the 1st of February 2007 until the 1st of February 2013. The follow-up period ended on the 1st of September 2013. Both men and women were included when aged 50 years or older. A wide range of parameters was analyzed for each patients to aid diagnosis. Two experts independently reviewed the laboratory results of all patients and established the underlying cause of anemia. In case of discordance, the experts deliberated until a consensus was reached. The population was divided into age groups (50-64, 65-74. 75-84 and 85+), class of anemia (based on MCV) and type of anemia (based on hemoglobin). The differences between age groups, classes and types were analyzed.

Results: A total number of 2513 patients were included in the study, 1238 men (median age 72 years) and 1275 women (median age 77 years). A single cause of anemia was found in 2284 patients (90.9%). Anemia of chronic disease was found 823 times (29.8%), renal anemia 340 times (12.3%), haemoglobinopathy 17 times (0.6%), hemolysis 16 times (0.6%) and possible bone marrow disease 105 times (3.8%). Vitamin B12 deficiency was found 115 times (4.2%), iron deficiency 516 times (18.7%) and folic acid deficiency 23 times (0.8%). Other causes were found 106 times (3.8%). The cause remained unknown in 703 cases (25.4%). Iron deficiency and haemoglobinopathy were significantly associated with microcytic anemia, while anemia of chronic disease and unknown were significantly associated with normocytic anemia. Hemolysis, possible bone marrow disease, vitamin B12 and folic acid deficiency and other causes were significantly associated with macrocytic anemia. Apart from haemoglobinopathy, all causes were found in each class at least once. Other observations included a prevalence of renal anemia rising with age. In addition, anemia of chronic disease and unknown anemia were associated with mild anemia, while iron deficiency and hemolysis were associated with severe anemia.

Summary and Conclusions: Several diagnostic algorithms developed for determining the cause of anemia generally attribute a specific set of causes to microcytic, normocytic and macrocytic anemia. Here it was shown causes of anemia should not be excluded based solely on the MCV value, indicating these existing algorithms should be reconsidered.

E1469

USEFULNESS OF LASER ASSISTED OPTICAL ROTATIONAL CELL ANALYZER (LORRCA) IN THE DIAGNOSIS OF HEREDITARY HAEMOLYTIC ANAEMIAS

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Background: Hereditary hemolytic anemias (HAs) are heterogeneous disorders mainly due to defects of red cell (RBC) membrane and metabolism. Hereditary spherocytosis (HS), elliptocytosis, and more rarely stomatocytosis (dehydrated, DHSt and overhydrated), are caused by defects of cytoskeleton or membrane permeability. Among RBC enzyme defects pyruvate kinase (PK) deficiency is the most common, followed by glucose-6-phosphate isomerase (GPI), and pyrimidine 5'-nucleotidase (P5'N) deficiency. Because of the rarity and heterogeneity of these diseases, diagnosis may be often challenging, notwithstanding the availability of a battery of laboratory tests. Ektacytometry, and more recently, laser-assisted optical rotational cell analyser (LoRRca MaxSis, Mechatronics Instruments, NL), able to measure RBC deformability in osmotic gradient conditions (Osmoscan), is reported to be a useful tool in the detection of RBC membrane disorders and in particular for the differential diagnosis of HSt; few data are available in other haemolytic anemias.

Aims: To evaluate the diagnostic power of LoRRca MaxSis in the diagnosis of RBC membrane disorders and other haemolytic conditions in a cohort of 84 patients with a confirmed diagnosis of haemolytic anemia of different cause

Methods: We analysed the Osmoscan curves in 54 cases with RBC membrane disorders (47 HS, 7 DHSt), 17 cases with enzymopathies (11 PK, 4 GPI, 2 P5'N), 7 with congenital dyserythropoietic anemia type II (CDAII) and 6 patients with paroxysmal nocturnal hemoglobinuria (PNH). Among them 5 HS, 2 PK, 3 GPI, 2 P5'N and 5 CDAII patients were splenectomysed. Normal ektacytometry curves were obtained from 75 healthy control subjects. The evaluated parameters were: 1) Omin (osmolality at which the elongation index (EI) is minimal, coinciding with the 50% haemolysis in osmotic fragility assays), 2) EImax (maximal deformability), 3) Ohyper (osmolality at ½EImax, representing cellular hydration status). Moreover, patients' curves were compared with a normal control reference curve interval. All the analyses were performed on EDTA blood sample, stored at 4°C and within 48h from collection.

Results: All the 47 HS (27 spectrin, 16 band3 deficiency and 4 with undetectable defect at SDS-PAGE analysis) showed typical altered ektacytometry curve, consistently with the reduced area of the curve and regardless the biochemical membrane defect. (Figure 1a). The 7 DHSt patients displayed the typical left-shift of the curve (Figure 1b). All GPI cases showed an altered enlarged Osmoscan curve associated with significant increased Ohyper (Figure 1c). No alterations were found in not-splenectomised PK (n=9), CDAII (n=2) cases, and in all PNH cases (Figure 1d). However, osmoscan curves of splectomized PK (n=2), CDAII (n=5), and P5'N (n=2) patients fell in a defined atypical area, regardless the pathology (Figure 1e).

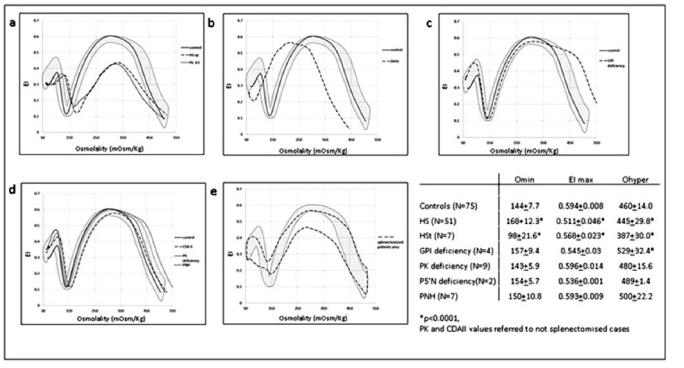

Figure 1.

Summary and Conclusions: All the HS and DHSt patients analysed showed diagnostic Osmoscan curves. Among the analysed parameters Omin and EImax were more significantly altered in HS patients, whereas Ohyper was significantly increased in GPI deficiency. For the first time abnormal Osmoscan curve was described in GPI deficiency, offering a new tool for the diagnosis of this rare enzyme defect. Splenectomy may interfere on the RBC deformability evaluation, thus suggesting caution in the interpretation of curves. Osmoscan analysis performed by LoRRca Maxsis represents a useful and feasible first step screening test in the diagnosis of RBC membrane disorders and other rare haemolytic anemias.

E1470

IDIOPATHIC AND CONGENITAL ERYTHROCYTOSIS: A STUDY OF A LARGE COHORT

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Background: Idiopathic Erythrocytosis (IE) is an absolute erythrocytosis (estimated prevalence 1.1 casesx 1000 persons) without an identified cause and its diagnosis is based on the exclusion of primary or secondary erythrocytosis. Familial erythrocytosis (FE) are rare diseases sometimes recessively transmitted and we and others have studied patients with congenital erythrocytosis (*i.e.* VHL mutation) while the relatives carrying the same heterozygous mutation show no signs of erythrocytosis. Therefore, FE may be diagnosed as IE, as well as 1% of patients with Polycythemia Vera (PV) who do not carry a *JAK2* mutation (*JAK2*-WT).

Aims: We tried to recognize, within a large cohort of patients with erythrocytosis, simple features able to discriminate IE, FE and PV.

Methods: Over the last 20 years, we followed 99 patients with *JAK2*-wild-type (JAK2-WT) erythrocytosis in whom a complete molecular study was available. The molecular study was performed during follow up in all the patients diagnosed before 2005. 78 patients were considered as IE after exclusion of secondary causes of erythrocytosis or a negative family history; 21 patients have been classified as FE in the presence of a familial pattern or the demonstration of *EPO-R*, *PHD2*, *VHL* or *HIF1-2-alpha* mutation. As controls we used 136 PV patients carrying *JAK2*V617F (allele burden 49±31%) or *JAK2* exon 12 mutations. The Ethical Committee of Padua Hospital approved the study. Nominal variables were compared with thex^2 test and continuous variables with the Mann-Whitney test. Logistic regression model was used for multivariate analysis.

Results: The clinical and laboratory data of our patients, collected at diagnosis, are summarized in the Table 1 (serum EPO [EPOs] in FE is extremely variable and not considered in the statistical analysis). FE patients were significantly younger in comparison with both IE and PV ($p < 0.001$). In multivariate analysis, low EPOs, high platelet count, thrombosis at or before diagnosis and splenomegaly were confirmed as independent features non-suggestive for IE when compared to PV. During follow-up no haematological progression to myelofibrosis (MF) or acute leukaemia (AL) was observed in IE and FE, while 9 PV patients evolved in MF and 3 in AL. None of FE nor IE patient out of the 28 (35.9%) with a follow-up longer than 5 years nor the 12 (8.3%) with a FU longer than 10 years progressed into an over PV.

Summary and Conclusions: This is a large cohort of IE and FE patients compared with PV patients followed in a single centre. Our data suggest that, in a patient with erythrocytosis associated with increased platelet count and/or splenomegaly and suppressed EPOs, PV as to be suspected. In contrast, normal spleen, normal platelets count and no cardiovascular complications argued

against early forms of PV. This study confirms that IE is a common and indolent form of erythrocytosis with a low risk of thrombosis. The role of phlebotomies and low-dose aspirin is still debated, but cytotoxic drugs seem to be avoided. We failed to identify simply clinical or laboratory data that can distinguish IE and FE even if the latest is found often in very young people. The observation of isolate erythrocytosis even, if sporadic, has to be evaluated as a possible genetic disease.

Table 1.

	IE	FE	PV (controls)	p IE vs PV	P FE vs PV
N° of patients	78	21	136	-	-
Male/Female	66/12	16/5	73/63	<0.001	0.05
Mean age (y)	52.8±16.4	24±20	60.5±15.3	0.001	<0.001
Mean WBC (x 10^9/L)	7.5±2.7	8.1±3.7	10.5±4.7	<0.001	0.003
Mean plts (x 10^9/L)	222±67	236±74	567±219	<0.001	<0.01
Mean serum EPO (UI/L)	12.8±7.7	-	6.3±5.3	<0.001	-
Splenomegaly yes/no (%)	15/63 (19)	5/16	81/55 (60)	<0.001	0.03
Thrombosis at/before diagnosis yes/no (%)	8/70 (10.3)	0/21	38/98 (30)	0.002	0.02

E1471

LEFT VENTRICULAR GLOBAL FUNCTION INDEX AND LEFT VENTRICULAR MASS VOLUME RATIO BY CMR: ASSOCIATION WITH HEART FAILURE AND ARRHYTHMIAS IN THALASSEMIA MAJOR PATIENTS

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Background: Recently two novels indicators of left ventricular (LV) performance assessed by Cardiovascular Magnetic Resonance (CMR) have been introduced: the LV global function index (LVGFI) and the LV mass/volume ratio (LVMVR). The LVGFI combines LV stroke volume, end-systolic and end diastolic volumes, as well as LV mass, integrating structural as well as mechanical behaviour. Elevated LVMVR is indicative of concentric remodelling. A LVGFI <37% and a LVMVR>1 were shown to be associated with the occurrence of cardiovascular events in no-thalassemic populations.
Aims: This retrospective cohort study aimed to systematically evaluate in a large historical cohort of thalassemia major (TM) in the CMR era whether the LVGFI and the LVMVR were associated with a higher risk of heart failure and arrhythmias.
Methods: We considered 812 TM patients (391 M, 30.4±8.6 years), consecutively enrolled in the Myocardial Iron Overload in Thalassemia (MIOT) network. LVGFI and LVMRI were quantitatively evaluated by SSFP cine images. The T2* value in all the 16 cardiac segments was evaluated and a global heart T2* value <20 ms was considered indicative of myocardial iron overload (MIO).
Results: Eighty (9.9%) patients had a LVGFI<37% and, compared to the patients with a normal LVGFI, they showed a significant higher frequency of heart failure (43.8% *vs* 4.2%; P<0.0001) and of arrhythmias (7.5% *vs* 3.0%; P=0.036). Patients with a LVGFI<37% had a significant higher risk of heart failure (odds-ratio-OR=17.59, 95%CI=9.95-21.09; P=<0.001). The risk remained significant also adjusting for the presence of MIO (OR=15.54, 95%CI=8.05-26.27; P=<0.001). Patients with a LVGFI<37% had a significant higher risk of arrhythmias (OR=2.62, 95%CI=1.03-6.66; P=<0.001). No adjustement was performed since arrhythmias were not associated to MIO. Thirty (3.7%) patients had a LVMVR≥1% and, compared to the patients with a normal LVMRI, they showed a significant higher frequency of heart failure (20.0% *vs* 7.7%; P=0.015) but not of arrhythmias. Patients with a LVMVR≥1% had a significant higher risk of heart failure (OR=3.01, 95%CI=1.18-7.64; P=0.021). The risk remained significant also adjusting for the presence of MIO (OR=3.44, 95%CI=1.31-9.01; P=0.012). In a multivariate model including LVGFI, LVMVR and heart iron, the significant predictors of heart failure were a LVGFI<37% (OR=14.05, 95%CI=7.66-25.77; P=<0.001) and a global heart T2*<20 ms (OR=1.94, 95%CI=1.08-3.47; P=0.026).
Summary and Conclusions: In TM patients a LVGFI<37% was strongly associated with an higher risk of heart failure, independent by the presence of MIO. Moreover, patients with a LVGFI<37% were more likely to have arrhythmias. A widespread program using CMR exploiting its multi-parametric potential can have considerable power for the early identification and treatment of patients at risk for heart failure.

E1472

SIGNIFICANT IMPROVEMENT OF SURVIVAL BY T2* MRI IN THALASSEMIA MAJOR

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Background: In 2004 seven Italian centers reported survival data for patients with thalassemia major (TM) and showed that heart disease due to iron overload was the most common cause of death (Borgna *et al.* Haematologica 2004). In the same years the accurate and noninvasive assessment of cardiac siderosis was made possible in Italy by the introduction of the T2* cardiovascular magnetic resonance (CMR).
Aims: We aimed to evaluate if the deployment of T2* CMR had an impact on the mortality rate.
Methods: Four centers contributed to the present study, updating the data of the enrolled patients until August 31, 2010. For the patients who died, the date of the death represented the end of the study. 577 patients (264 females and 313 males) were included.
Results: One-hundred and fifty-nine (27.6%) patients died, 124 of whom (77.9%) died before the year 2000. MRI was not performed in 406 patients (70.4%) and no patient had been scanned before his/her death. Among the survivors, MRI was not performed in the 59% of the cases (P<0.0001). The absence of an MRI scan was a significant univariate prognosticator for death (HR=43.25, 95%CI=11.32-165.33, P<0.0001). The study was restricted to the patients dead after 2004 (19/159=12%) or followed until August 2010 (N=357). In this subgroup of 376 patients, MRI was not performed in the 52.4% of the survivors and in all dead patients (P<0.0001). The absence of a MRI exam was reconfirmed as a strong predictive factor for death (HR=49.37, 95%CI=1.08-2263.24, P=0.046). The Kaplan-Meier curve is showed in Figure 1. The log-rank test revealed a significant difference in the curves (P<0.0001).

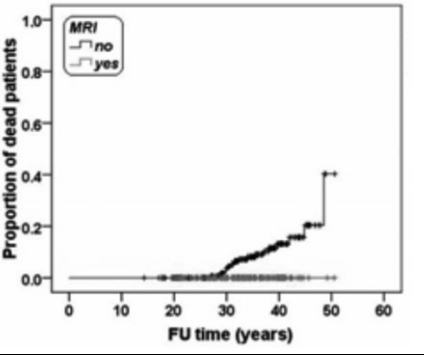

Figure 1.

Summary and Conclusions: Our data suggests that the use of T2* CMR, that enables individually tailored chelation regimes reducing the likelihood of developing decompensated cardiac failure, allowed the reduction of cardiac mortality in chronically transfused TM patients.

E1473

A NON-INTERVENTIONAL OBSERVATIONAL STUDY ASSESSING SAFETY OF DEFERASIROX IN PATIENTS WITH HEMOGLOBINOPATHIES AND TRANSFUSIONAL IRON OVERLOAD: RESULTS FROM THE "ENERGY" STUDY

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Background: A spectrum of large clinical programs has established the efficacy and safety of the oral iron chelator deferasirox (DFX) in transfusion-dependent patients with iron overload. We report the results from a multicenter, prospective, open-label, observational study reflecting the standard clinical practice in thalassemia centers in Greece.
Aims: To assess the safety profile of DFX in transfusion-dependent patients with iron overload.
Methods: Patients with hemoglobinopathies and transfusional iron overload, baseline serum ferritin levels <4000 ng/mL, and for whom the physician decided to initiate chelation with DFX under the local approved label and standard medical practice, were included in the study. Patients with myelodysplastic syndromes, severe cardiac siderosis (T2* <10 ms), left ventricular ejection fraction <56%, serum creatinine (SrCr) >upper limit of normal (ULN), alanine aminotransferase (ALT) ≥500 U/L, creatinine clearance (CrCl) <60 mL/min or significant proteinuria were not eligible.
Results: Of the 230 patients enrolled, 226 were evaluated (4 patients were excluded due to screen failures); a subset of the enrolled patients were pediatric (age <16 years, n=24). Mean age was 35.1±13.8 (pediatric, 8.7±4.2; adults, 38.3±10.9) years. The most common underlying conditions for transfusional iron overload were β-thalassemia major (66.8%, n=151), thalassemia intermedia (17.7%, n=40), and sickle cell anemia (9.3%, n=21). Prior chelation treatment was reported for 146 (64.6%) patients, most common being a combination of deferoxamine and deferiprone (19.0%; n=43). None of the patients had prior DFX exposure. Of the 226 evaluable patients, 179 completed the 12-month observation period, whereas 47 withdrew from the study prematurely. The 3 main reasons for discontinuation were adverse events (AEs; 7.1, n=16), lost to follow-up (4.4%, n=10), and consent withdrawal (2.2%, n=5). A total of 356 non-serious AEs were reported in 103 (45.6%) patients, of which 167 AEs observed in 65 patients (28.8%) were reported to be related to DFX. The most common non-serious AEs related to DFX were increase in SrCr above ULN (8.4%), upper abdominal pain (4.9%), and diarrhea (4.0%). Twenty-one (9.3%) patients experienced 56 serious AEs (SAEs), of which 13 SAEs in 3 patients (1.3%) were reported to be related to DFX, including gastrointestinal disorders (9 events), abnormal ALT, albumin above ULN, musculoskeletal pain and cholecystectomy (all 1 event each). The mean SrCr levels increased from 0.69 mg/dL at baseline to 0.74 mg/dL at 12 months, and the mean CrCL decreased from 127 mL/min at baseline to 123 mL/min (p<0.001 and p=0.015 for the mean differences, respectively). Transaminases (ALT and aspartate aminotransferase, [AST]) did not show an increase at any point in time. Notably, while AST showed a decreasing trend at 12 months (p=0.052), ALT decreased significantly at 12 months (p<0.001). Of the 15 patients who underwent ocular examination, an improvement was seen in 1 patient, while no changes were observed in the remaining patients. Of the 13 patients who underwent auditory examination, an improvement was noted in 1 patient, 1 patient showed deterioration, and 11 patients did not show any change.
Summary and Conclusions: This 1-year, multicenter, observational study conducted in Greece, under standard clinical practice, showed that the safety profile of DFX was consistent with previously published data.
Acknowledgement: We acknowledge the efforts of personnel from all sites, involved in this study. Authorship was based on study enrollment.

E1474

DESCRIPTIVE ANALYSES OF DEFERASIROX SAFETY AND EXPOSURE IN PATIENTS AGED >65 *VERSUS* 18 TO ≤65 YEARS

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Background: Patients discontinued from the EPIC study most frequently due to adverse events (AEs). Discontinuation rates due to AEs were notably higher in the MDS cohort (n=78/341, 22.9%; mean age 67.9 yrs) compared with the overall EPIC population (n=153/1744, 8.8%; mean age 30.6 yrs); most frequently due to gastrointestinal (GI)-related AEs (Gattermann *et al.* Leuk Res 2010). Altered drug exposure is known to be clinically relevant in elderly patients for different drugs. It is hypothesized that differences in deferasirox pharmacokinetics (PK) could contribute to observed differences in AEs.
Aims: 1. To determine whether there is a higher incidence of selected AEs in elderly adult (>65 yrs) *vs* younger adult patients (18-≤65 yrs); 2. To investigate whether variations in AE frequency were due to differences in deferasirox PK.
Methods: EPIC study design and inclusion/exclusion criteria have been previously described (Cappellini *et al. Haematologica* 2010). Patients initially received deferasirox 10-30 mg/kg/day, depending on blood transfusions frequency, with dose adjustments of 5-10 up to 40 mg/kg/day, based on 3-month serum ferritin trends and safety markers. For this safety analysis, frequency of selected AEs of clinical interest over 1 year (abdominal pain, diarrhea, nausea, vomiting, gastroenteritis, rash, renal-related) were summarized by age category. Relative risk and 95% confidence intervals (CI) were estimated based on a non-modeling approach. For PK analyses, steady state trough deferasirox concentration ($C_{trough,ss}$) and concentration measured 2 hours post-dose (C_{2hr}) – surrogates for drug exposure (AUC) and peak levels (C_{max}), respectively – were described by age category in all patients with available data, regardless of underlying disease, and in MDS patients only. A regression analysis of deferasirox dose-normalized concentration *versus* age was also performed at Weeks 12 and 28.
Results: In total, 1079 patients ≥18 yrs were enrolled: n=837 aged 18-≤65 and n=242 aged >65 yrs. Overall, 210/242 patients >65 yrs had MDS. The frequencies and relative risks of reported AEs of interest are summarized (Table 1). There was a significantly increased risk of diarrhea and renal AEs in patients aged >65 *vs* 18–≤65 yrs and a significantly reduced risk of rash. Distributions of deferasirox dose-normalized $C_{trough,ss}$ and C_{2hr} were comparable between patients aged >65 *vs* 18–≤65 yrs in the overall patient population with PK available (n=748) and within MDS patients only (n=232). There was no trend for altered total drug exposure or peak levels between the two age categories.

Table 1. Frequencies and relative risks of reported AEs on interest in younger adult (18-≤65 years) *versus* elderly adult (>65 years) patients in the EPIC study.

AE	Frequency in elderly adult patients, n (%)	Frequency in younger adult patients, n (%)	Relative risk	95% CIs
Renal AEs	15 (6.2)	20 (2.4)	2.59	1.35, 4.99
Diarrhea	112 (46.3)	244 (29.2)	1.59	1.34, 1.89
Vomiting	31 (12.8)	88 (10.5)	1.22	0.83, 1.79
Abdominal pain	71 (29.3)	203 (24.3)	1.21	0.96, 1.52
Nausea	52 (21.5)	150 (17.9)	1.20	0.91, 1.59
Rash	24 (9.9)	146 (17.4)	0.57	0.38, 0.85
Gastroenteritis	4 (1.7)	44 (5.3)	0.31	0.11, 0.87

Summary and Conclusions: There were differences in the deferasirox AE profile between patients aged >65 *vs* 18–≤65 yrs. Diarrhea was the only GI-related AE significantly more frequent in patients >65 yrs; others did not differ significantly. Renal AEs were also significantly more frequent in patients >65 yrs, possibly due to higher baseline serum creatinine levels, common in older patients. AE variations did not appear attributable to differences in deferasirox exposure as assessed by $C_{trough,ss}$ and C_{2hr}. Therefore, this analysis does not support the need for considering deferasirox dose adjustments based on differences in drug exposure in elderly patients. Since the majority of elderly patients had MDS, concomitant medications/underlying complications may have influenced the AE profile, and should, therefore, be considered during deferasirox therapy to anticipate and manage AEs.

E1475

SERUM CYSTATIN C IN SICKLE CELL DISEASE: RELATION TO CARDIOVASCULAR DYSFUNCTION AND SICKLE CELL NEPHROPATHY

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Background: Sickle cell disease (SCD) patients have ongoing hemolysis and inflammation secondary to the sickling phenomenon. Many mechanisms contribute to the complex pathophysiology of SCD, with dysfunction of the vascular endothelium as a unifying theme. Renal dysfunction is a leading cause of morbidity in sickle cell disease. Cystatin C has been recognized as a useful marker of renal dysfunction and a strong predictor for risk of cardiovascular events.
Aims: To determine the level of cystatin C in 53 children and adolescents with SCD compared with 35 age- and sex-matched healthy controls and to assess its relation to markers of hemolysis, iron overload, renal affection and endothelial dysfunction.

Methods: SCD patients in steady state were studied stressing on transfusion history, hydroxyurea therapy, hematological profile, serum ferritin and urinary albumin creatinine ratio (UACR). Cystatin C was measured by enzyme linked immunosorbent assay (ELISA). Ecchocardiograhy was performed and carotid intima media thickness (CIMT) of the common carotid artery was assessed using high resolution ultrasonography. Heart disease was defined by at systolic left ventricle (LV) dysfunction (LV shortening fraction <30% or LV ejection fraction <55%).
Results: Cystatin C levels were significantly higher in SCD patients compared with control group (584.3±232.8 ng/mL *versus* 100.4±40.6 ng/mL; p<0.001). SCD patients with nephropathy or heart disease had significantly higher levels than those without (p<0.001 and p=0.041, respectively). Patients who had history of frequent sickling crisis (>3 attacks/ year) had higher cystatin C levels than those who were in steady state (p=0.034). SCD patients on regular hydroxyurea therapy had lower cystatin C levels than untreated patients (p=0.039). CIMT was elevated among SCD patients than controls (0.64±0.12 mm *versus* 0.42±0.02 mm; p<0.001). Cystatin C was positively correlated with corrected WBCs count (r=0.328, p=0.016), UACR (r=0.605, p<0.001) and CIMT (r=0.588, p<0.001). The cutoff value of cystatin C at 580 ng/mL could differentiate SCD patients with and without nephropathy with 77.8% sensitivity and specificity of 84.6% while the cutoff value 650 ng/mL could differentiate SCD patients with and without heart disease with 100% sensitivity and specificity of 74.4%.
Summary and Conclusions: Cystatin C may be considered a biological marker for vascular dysfunction and subclinical atherosclerosis in SCD as reflected by its positive correlation with CIMT. Serum cystatin C correlates with the level of albuminuria and may be a reliable method to measure renal function in SCD. Elevated Cystatin C levels would help in early crisis prediction and to identify patients at risk of cardiac complications as well as monitoring the response to hydroxyurea therapy. Further longitudinal studies are needed to verify the practical utility of Cystatin C measurement and validate the cutoff values for detection of cardiac and renal complications in SCD.

E1476

DEFERASIROX IMPROVES LIVER FIBROSIS IN B THALASSAEMIA MAJOR PATIENTS. A 5 YEARS FOLLOW UP STUDY
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Background: β Thalassemia major (βTM) patients often develop liver fibrosis due to transfusion related liver iron overload and/or hepatitis virus C (HCV) infection. Transient elastography (TE) is a non-invasive method that accurately measures liver stiffness (LSM) and therefore could serve as a helpful tool for the fibrosis assessment.
Aims: To evaluate prospectively the liver fibrosis status in β TM patients being chelated with deferasirox only and explore the contribution of iron overload in liver fibrosis course.
Methods: We conducted a prospective 5 years (2008-2013) follow-up study in 22 βTM patients (9 males/13 females) with a median age of 32 (20-47) years. All patients were HCV PCR negative and on regular transfusion regime (2 RBC units every 15 days) retaining a pre-transfusion Hb level >9,5gr/dl, had no other comorbidities and were not on other medications of known effect on LSM. During the study period 7 female patients had to discontinue chelation therapy for 16(12-18) months due to successful pregnancies and restarted deferasirox 12 (8-16) months before the 2nd fibrosis evaluation. Patients were assessed with TE (LSM in kPa) at baseline-within 35(5-47) days after having been switched to 35(30-40) mg/Kg deferasirox-and reexamined with the same method by the same operator at 5 years. Readings of >8 kPa were considered indicators of severe liver fibrosis. Serum ferritin levels (ng/ml) and MRI T2* liver and cardiac imaging (using Anderson-Pennell protocol in ms) were concurrently run for the evaluation of iron overload.
Results: When we analysed the whole group of patients we found a significant positive correlation between ferritin changes and kPa fluctuation (p<0,004) and a modest negative correlation among kPa and MRI T2* changes (p<0.06) during the study period. Nevertheless there were no significant differences in mean kPa, ferritin levels or liver MRI T2* readings between baseline and 5 years follow up measurements (6.995 *vs* 6.573, 1133 *vs* 1035 and 8.21 *vs* 8.19 respectively). Interestingly enough, when we excluded patients that discontinued deferasirox from the analysis we found significant differences in the mean values of the examined parameters {kPa: 7.5 *vs* 6.093 (p<0.035), MRI T2*: 6.5 *vs* 8.2 (p<0.05), ferritin: 1125 *vs* 941, at baseline and 5 years follow up respectively}. In this subgroup there was also a consistent strong positive correlation between kPa and ferritin fluctuations (p<0.009) and negative correlation with liver MRI T2* changes, although not significant. In more details, 14 patients succeeded complete or partial reversal of the liver fibrosis, including 4 with severe disease at baseline, 1 remained stable and there was no case of progression.
Summary and Conclusions: Based on our data it seems that undisturbed iron chelation with deferasirox could offer significant improvement in liver fibrosis as assessed by means of TE. This is also in line with a better control in iron overload as shown by the decrease in ferritin levels together with the improvement in liver MRI T2* succeeded throughout the 5 years treatment period. Duration, compliance and other than the iron overload reduction possible modes of action are open research fields in order to establish the use of deferasirox in reversing or stabilizing liver fibrosis in βTM patients.

E1477

PREMATURE ATHEROSCLEROSIS IN CHILDREN WITH BETA- MAJOR THALASSEMIA: NEW DIAGNOSTIC MARKER
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Background: Early vascular alteration, atherosclerosis and coronary artery disease have emerged as important cardiovascular complications in beta-thalassemia major (BTM) patients due to oxidative stress of iron overload.
Aims: were to assess the prevalence of premature atherosclerosis among BTM patients, and to investigate the diagnostic value of serum osteoprotogrin (OPG) as marker for atherosclerosis
Methods: This prospective cross-sectional study included ninety-eight children; as sixty-five children with beta thalassemia major aged 5-18 years, on regular blood transfusion regimen represented the patient group. While thirty-three healthy children, with comparable age and gender, were assigned as control group. All participants were evaluated for laboratory investigations including; complete blood count, liver and kidney function tests, hepatitis B and C screening, C- reactive protein, lipid profile, serum ferritin and serum osteoprotogrin. Carotid artery intima media thickness (CAIMT) was performed by duplex ultrasound for patients and controls.
Results: Our BTM patients were transfusion-dependent for as long as 8.5±3.8 years with significantly higher serum ferritin levels (2490±1579 ng/dl *vs* 83±32 ng/dl, p=0.001), and C-reactive protein (5.7±5.7 *vs* 0.9±0.9 when compared to controls. Significantly higher serum triglyceride (128±20 *vs* 101±7 mg/dl, p=0.009) and atherogenic index of plasma (0.45±0.12 *vs* 0.22±0.04, p=0.001) were recorded in patients than comparisons. Carotid arteries intima media thickness (CAIMT) of both side; were significantly increased for patients (Rt 0.62±0.2 *vs* 0.29±0.07 mm, p=0.001 & Lt 0.66±0.17 *vs* 0.29±0.05 mm, p=0.001) when compared with healthy controls, and showed positive correlation with body mass index (BMI), serum triglyceride, atherogenic index of plasma, and serum osteoprotogrin levels. Assay of serum osteoprotogrin (OPG) revealed significantly higher levels for thalassemia patients than healthy peers (427±102 *vs* 324±126 pg/ml, p=0.02). Of particular interest is the obvious positive correlation between OPG levels and CAIMT of both sides (Rt r 0.54, p=0.001 &Lt r 0.479, p=0.001).
Summary and Conclusions: subclinical atherosclerosis started prematurely in children with beta- thalassemia. Carotid artery intima media thickness appeared to correlate well with serum osteoprotogrin. This finding highlighted the possible validity of OPG assay as an early predictor of atherosclerosis in thalassemia children

E1478

ASSESSMENT OF LIVER IRON CONCENTRATION IN PATIENTS WITH THALASSEMIA MAJOR USING MRI TECHNOLOGY: FINAL RESULTS OF A PROSPECTIVE COMPARATIVE STUDY BETWEEN T2* AND FERRISCAN
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Background: Iron overload is a significant complication of hemoglobinopathies. Iron accumulates mainly into the endocrine glands, the liver and the heart leading to potentially life-threatening events. The accurate monitoring of total body iron is extremely important in patients with thalassemia major. Magnetic resonance imaging (MRI) is the most accurate non-invasive technique for the evaluation of iron overload in thalassemia patients. The MRI gradient echo (T2*), the reciprocal of T2* (known as R2*) and the spin echo (T2) techniques have managed to quantify tissue iron in the liver and the heart with high sensitivity. Another MRI methodology, which is firstly described by St. Pierre *et al.* (Blood 2005;105:855-61) evaluates liver iron concentration (LIC) using R2 (where R2=1/T2) signal decay rates, commercially available as FerriScan. In that study there was a significant correlation between R2 values and biopsy results. The FerriScan analysis produces a map of liver iron and calculates a mean LIC measurement.
Aims: The aim of this prospective study was to compare the results of the LIC evaluation obtained by conventional MRI and by FerriScan in patients with thalassemia major.
Methods: We prospectively studied 21 patients (9M/12F, median age 45 years, range 38-61 years) with thalassemia major who are followed-up in our Center. All patients had MR examinations of the liver that were performed on a General

Electric 1.5T Signa HDxt scanner (GE Healthcare, Milwaukee, USA). The pulse sequence used to estimate the T2* relaxation time of the examined tissues is a multi-echo fast gradient echo which has the ability to acquire 3 to 16 echoes in a breath hold time of 12 to 18 sec. The post processing of the data is performed with use of relevant software on a GE Advantage Windows 4.6 workstation according to the relevant literature. On the same day serum ferritin was measured, while LIC was evaluated using the FerriScan methodology. More specifically, images were transmitted to the FerriScan Analysis Centre in DICOM format, according to the FerriScan protocol. Images were analyzed off-site, as previously described by St Pierre *et al.*, and average LIC results were returned electronically to our center.
Results: All patients were under oral chelation therapy at the time of evaluation. Their ferritin levels were (mean±SD) 712±648 ng/ml. LIC based on T2* and R2* values were 7.81±6.32 mg/g dry weight and 302±259 Hz, respectively. LIC values estimated by FerriScan were 131±133 mmol/kg. There was a strong correlation between R2* and FerriScan values (r=0.940, p<0.0001). Both R2* and FerriScan values strongly correlated with ferritin levels (r=0.707, p<0.001 and r=0.721, p<0.001 respectively). There was no superiority of one of the MRI techniques *versus* the other regarding its correlation with serum ferritin (p=0.467).
Summary and Conclusions: This study demonstrated a very strong positive correlation between R2* values and FerriScan-determined LIC. Furthermore, we did not find any difference between the correlations of R2* or FerriScan with serum ferritin. This may be the result of very effective chelation therapies, as all our patients were under chelation treatment. However, if this result is confirmed in a larger series of patients, it could potentially lead to more timely patient results and cost savings as R2* is cheaper and easier to be performed compared to FerriScan.

E1479

25-OH VITAMIN D DOWN-REGULATES *IN VITRO* PRODUCTION OF ANTI-ERITHROCYTE ANTIBODIES IN AUTOIMMUNE HAEMOLYTIC ANAEMIA
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Background: Vitamin D is a well known regulator of bone and calcium balance that has multiple immune-modulating activities. Reduced levels of 25-OH vitamin D have been found in various autoimmune diseases, such as systemic lupus systemic lupus erythematous and multiple sclerosis, and its levels and supplementation did correlate with disease clinical severity.
Aims: We evaluated a) the effect of vitamin D on the *in vitro* production of anti-RBC autoantibodies in patients with autoimmune haemolytic anaemia (AIHA); b) vitamin D levels, VDR expression and immunemodulatory cytokines.
Methods: Clinical and haematological parameters, serum samples and informed consent were collected at the time of enrolment from January 2013. 25-OH vitamin D levels, VDR and IL-6, IL-10, IL-17, TNF-alfa and IFN-gamma were evaluated in 40 patients and in 40 age and sex matched healthy controls, using ELISA kits. Heparinized blood samples from 7 AIHA patients were tested for anti-RBC production in unstimulated and PWM-stimulated 48h cultures with or without vitamin D at increasing concentrations (10, 20 and 40 ng/mL). The number of immunosuppressive therapy lines (steroids, immunosuppressors, rituximab, splenectomy) were retrospectively collected.
Results: Laboratory features of the patients (15 males and 25 females; mean age 58 years; 18 CAD, 18 WAIHA and 4 DAT negative AIHA) are shown in Figure 1a. Vitamin D levels were significantly reduced in patients *versus* controls, regardless sex, age nor season at sampling. VDR was increased in patients compared to controls; IL-6, IL-10, IL-17 and IFN-gamma serum levels were higher in AIHA patients *versus* controls, whereas TNF-alfa was significantly reduced. As shown in Figure 1b, vitamin D (at 10, 20, and 40 ng/mL) exerted a dose dependent inhibition on *in vitro* production of anti-RBC antibodies, with a delta% reduction of 6, 16 and 31 in unstimulated conditions, and 2, 1, 16 in PWM-stimulated conditions, respectively. As regards treatment at sampling, 24 patients were under low dose steroids (*e.g.* 0,2-0,5 mg/Kg/day prednisone); vitamin D status was comparable in treated and untreated cases. Retrospectively, 2 cases were therapy naïve, 13 cases had been treated with steroid only, 15 cases with both steroid and a second line treatment (9 rituximab and 4 cyclophosphamyde), and 10 with more than 2 lines of therapy (4 splenectomy, 10 rituximab and 6 cyclophosphamyde). Vitamin D levels were significantly lower in patients who had been treated with 2 or more lines of therapy (1,72±1 *versus* 2,78±2 ng/mL, p=0.04).
Summary and Conclusions: Vitamin D deficiency/insufficiency was observed in patients with AIHA, both therapy naive or previously treated, with concomitant alteration of immuno-modulatory cytokine levels. Moreover, we found increased VDR expression, possibly reflecting an up-regulation due to ligand deficiency or increased shedding because of reduced receptor recruitment. Vitamin D deficiency was more evident in relapsed/refractory patients, suggesting a more pronounced immune disregulation in these cases. *In vitro* studies demonstrated a dose dependent inhibitory effect of vitamin D on the production of anti-RBC auto-antibodies even at very low concentrations and suggested that vitamin D supplementation may be useful in AIHA.

a

	AIHA (N=40)	Controls (N=40)
Hb g/dL	11.6±2.3	Male NV 13.5-17.5 Female NV 12-16
Ret x10e3/mcL	145±75	NV 20-100
LDH U/L	245±98	NV 135-225
25-OH vitamin D (ng/mL)	2.4±1.6*	6.0±6.0
VDR (pg/mL)	23.9±7.4	20.9±6.3
IL-6 (pg/mL)	2.0±2.0*	1.2±1.0
IL-10 (pg/mL)	3.1±4.5*	0.6±0.6
IL-17 (pg/mL)	2.6±6.0	0.6±0.4
IFN-g amma (pg/mL)	1.3±3.0	0.6±0.8
TNF-alfa (pg/mL)	1.2±0.5‡	1.9±0.5

b

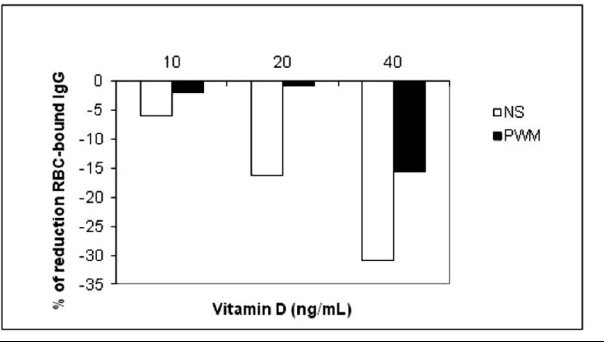

Figure 1.

E1480

A CROSS-SECTIONAL STUDY COMPARING DIFFERENT HEMATOLOGICAL AND BIOCHEMICAL DISEASE MARKERS FOR CLINICAL DIAGNOSIS OF IRON DEFICIENCY
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Background: Iron deficiency (ID) is a widespread condition throughout the world. It is considered to be one of the most prevalent forms of malnutrition. Heightened awareness of the adverse consequences of ID, such as changes in cognitive development, immune function, energy metabolism or temperature regulation, has renewed efforts to reduce the prevalence of this micronutrient insufficiency. Nevertheless, no full international consensus exists on disease markers to be used for assessing the human iron status. Furthermore, uncertainty about the optimal epidemiologic approach and the quantitative measurement of its severity still exists.
Aims: This study was conducted to compare performances of serum ferritin and transferrin saturation (TSAT) with the soluble transferrin receptor (sTfR)/log ferritin and reticulocyte hemoglobin content (CHr), also known as Thomas-plot, to serve as biomarkers in patients with ID. Furthermore, separate logistic regression models, for patients without (C-reactive protein [CRP-] value ≤0.5 mg/dl) and with acute-phase reaction (CRP-value >0.5 mg/dl) were used to predict functional ID.
Methods: This cross-sectional study was approved by the Ethical Committee of Upper Austria (Linz, Austria), and is in accordance with the current version of the Helsinki Declaration. A total of 445 hospitalized adult patients with suspected ID were investigated for complete blood cell count, ferritin, TSAT, sTfR, sTfR/log ferritin ratio and CHr (*i.e.* Thomas-plot). All in all, 31.69% (n=141) were male and 68.31% (n=304) were female. The median age was 70 years (range: 18-92). Patients below 18 years of age were excluded from the study. Descriptive statistics were computed for the categorical and metric variables and to compare different parameters and definitions of ID. Logistic regression models for the probability of functional ID (CHr <28 pg) were constructed for all 445 patients, for 225 patients without (C-reactive protein [CRP] ≤0.5 mg/dL) and 220 patients with acute-phase reaction (CRP >0.5 mg/dL).
Results: Based on the Thomas-plot analyses, 153/445 (34.38%) patients were identified with ID. When ID was diagnosed by means of serum ferritin levels <30 ng/mL and TSAT levels <20%, 105/445 (23.60%) and 215/445 (48.31%) patients were identified with ID, respectively. Based on the logistic regression models for the probability of functional ID (CHr <28 pg), the sTfR/log ferritin ratio showed the best positive predictive values (PPV) (62.50 and 64.41%) to indicate functional ID in patients without as well as with acute-phase reaction

compared to sTfR (58.14 and 61.67%), ferritin (32.50 and 32.86%) and TSAT (26.74 and 42.86%).
Summary and Conclusions: In conclusion, the prevalence of ID in the study population investigated here varied with marker selection and its definition. Ferritin and TSAT demonstrated weaknesses in the detection of functional ID (CHr <28 pg). Regarding the results of this work, for routine laboratory diagnosis, however, we suggest to use Thomas-plot analyses in combination with ferritin single-marker measurements to efficiently identify patients with ID.

E1481

CONGENITAL DYSERYTHROPOIETIC ANEMIA TYPE III AND PRIMARY HEMOCHROMATOSIS; COEXISTENCE OF MUTATIONS IN KIF23 AND HFE
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Background: Congenital dyserythropoietic anemia type III, CDA III, is a very rare hereditary disorder. The disease is caused by mutation c.2747C>G, p.P916R in *KIF23,* a protein with crucial role in cytokinesis, the final step of mitosis. In the Västerbotten CDA III family, 48 individuals in 6 generations have been diagnosed with CDA III. Secondary hemochromatosis, a clinical problem in CDA I and II, has not been reported in CDA III. Absence of iron accumulation in CDA III has been explained by intravascular hemolysis, leading to urinary loss of iron. However, we have recently seen that some individuals in the Västerbotten family, both CDA III positive and negative ones, have elevated ferritin levels. The prevalence of primary hemochromatosis caused by mutations in the *HFE* gene is high in northern Europe.
Aims: To study the coexistence and clinical appearance of mutations in *KIF23* c.2747C>G and *HFE* c.845G>A and c.187C>G in the Västerbotten family.
Methods: DNA from 37 CDA III positive individuals and 21 of their CDA III negative siblings, was examined by PCR-TaqMan genotyping. *HFE*-genotype was established concerning *HFE* c.845G>A and *HFE* c187C>G.
Results: Heterozygous mutation of *HFE c.845G>A* or *c.187C>G* was found in 18 CDA III positive individuals. Both mutations were found in six CDA III patients. Nine of the CDA III negative siblings carried heterozygous mutations, and two homozygous mutations of either of the examined loci of the *HFE* gene. Two were found to have heterozygous mutations of both loci. Treatment with phlebotomy to normalize ferritin and transferrin iron saturation (TSAT) was indicated in the CDA III negative person with homozygous mutation of *HFE* c.845G>A, in two CDA III positive individuals with single heterozygous mutations (ferritin 1084 ug/L and 987 ug/L, TSAT 80% and 100%) and in one CDA III positive patient with coexistence of *HFE* c.845G>A and c.187C>G (ferritin 700 and TSAT >55%). So far, phlebotomies have been performed without problems in spite of anemia. All patients were screened for diabetes. One CDA III positive male with heterozygous *HFE* c.187C>G (ferritin 1084 ug/L, TSAT 80%) was diagnosed with type II diabetes mellitus.
Summary and Conclusions: Mutations in one or two of the examined *HFE*-loci were found in 65% of CDA III positive cases and in 62% of their CDA III negative siblings. One CDA negative female with homozygous *HFE* c.845G>A needed treatment with phlebotomy. Three patients with CDA III, heterozygous *HFE*-mutations, and laboratory signs of iron overload have started treatment with phlebotomy. Heterozygous mutation of the *HFE*-gene seems to be sufficient to generate pathologic iron overload when occurring concomitantly with a haemolytic disorder such as CDA III. Screening for primary hemochromatosis should be performed in patients with congenital dyserythropoietic anemia.

E1482

ERYTHROCYTE-DERIVED MICROVESICLE RELEASE DURING *EX-VIVO* CARDIOPULMONARY BYPASS PROCEDURES
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Background: Cardiopulmonary bypass procedures (CPB) are commonly used during heart surgery. The stressful conditions that this procedure imposes on blood cells may cause damage leading to loss of cellular contents or vesiculation of the cells. In this study we examined the effects of CPB in both an ex-vivo and in-vivo set-up on erythrocyte behavior and erythrocyte-derived microvesicle release.
Aims: To study the effects of CPB on deformability and phosphatidylserine (PS) exposure of erythrocytes, the release of microvesicles (MVs) during this procedure, and their ability to induce thrombin generation.
Methods: We investigated MV formation, in particular erythrocyte-derived MVs, during ex-vivo CPB. In this set-up 150 ml of heparinized blood from 3 healthy volunteers was hemodiluted and pumped through a membrane oxygenator back to the venous blood reservoir. Osmotic gradient ektacytometry was performed to study the deformability of erythrocytes after ex-vivo CPB. After ex-vivo CPB M*Vs* were purified by differential centrifugation from platelet-free plasma. Nanoparticle Tracking Analysis (NTA), Western blot analysis, and hemoglobin measurements were carried out to confirm the presence of M*Vs* in whole blood from healthy volunteers in this ex-vivo CPB model. The isolated M*Vs* after ex-vivo CPB were added to an automated thrombin generation test to determine its effects on blood coagulation, whereas in this test the formed thrombin is able to split a substrate to a fluorescent product. Microvesicle formation after routine CPB with patients (n=30) samples was studied similarly as after ex-vivo CPB.
Results: The number of erythrocyte-derived M*Vs* after ex-vivo CPB increased concomitantly with both the number of microcytic erythrocytes and PS expression on erythrocytes as determined by flow cytometry. Despite this vesiculation, deformability of the erythrocytes as determined by osmotic gradient ektacytometry did not significantly alter during the ex-vivo CPB procedure. The formed M*Vs* were found to be able to stimulate thrombin formation, thereby highlighting the potential contribution of M*Vs* on side effects of CPB. Remarkably, except for one sample, patient blood samples obtained after CPB contained free hemoglobin but did not contain erythrocyte microvesicles (MVs) (Figure 1).

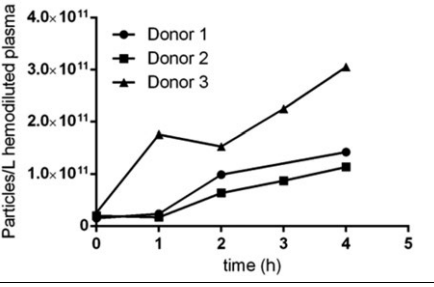

Figure 1.

Summary and Conclusions: In this study we show that hemoglobin containing erythrocyte-derived M*Vs* are generated during an ex-vivo CPB procedure. These nascent M*Vs* are able to induce thrombin generation, we therefore hypothesize that the reported diffuse inflammation after CPB could, at least partly, be mediated by vesicles formed in blood. Patients samples after CPB did not contain erythrocyte-derived MVs, possibly indicating high-clearance rates of M*Vs* in patient blood, suggesting that patients are at risk when clearance systems (*e.g.* macrophages) get saturated.

E1483

HS-TROPONINE T RELATES WITH MYOCARDIAL IRON OVERLOAD IN TRANSFUSION DEPENDENT THALASSEMIC PATIENTS
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Background: Myocardial iron overload (MIO) is the main cause of Heart failure (HF) in β-thalassemia major (β-TM) patients. The transfusion-related hemosiderosis leads to myocardial damage thus influencing the clinical outcome. Measurement of high sensitive-troponine-T (hsTnT) provides diagnostic and prognostic information in patients with coronary artery disease, heart failure and even in the general population. No data are available in literature about hs-ThT and MIO in transfusion dependent thalassemia (TDT) patients.
Aims: We aimed to evaluate the relationship between MIO quantified by Magnetic Resonance Imaging (MRI)T2* and hs-ThT serum levels in a consecutive cohort of 50 TDT patients enrolled into the MIOT network.
Methods: This pilot study recruited 50 consecutive TDT patients from June 2013 to January 2014 at Division of Haematology, Unit of Thalassemia, University of Catania, Italy. Male/Female ratio was 1:1; Median age was 32 years (range 16-48). All of them were suffering from β-TM, except 2 male patients suffering from Thalassemia Intermedia with Major-like phenotype. Myocardial iron overload (MIO) was quantified by a multislice multiecho T2* sequence (Meloni *et al.*, Int J Cardiol, 2014). Biventricular function parameters were evaluated by cine images (Marsella M *et al.* Haematologica 2011). HF was defined if clinically evident signs or symptoms were present. Raw data were expressed as mean±standard deviation. Comparison between groups was performed using the Mann-Withney test. Odds ratio were calculated with Chi^2 test. Correlation was performed using the Spearman correlation. Sensitivity and specificity were calculated with Receiver operating characteristic (ROC) analysis. P<0,05 was considered as statistically significant.
Results: Median value of hs-TnT was 3,925 ng/L, ranged between 3 and 60,3 ng/mL (normal range 0-14). Overall, 5 patients presented levels of hs-TnT greater than 14 ng/mL. 6 patients presented Heart Failure (HF), 1 with augmented levels of hs-TnT. In our population we confirmed that both the presence of a global Heart T2* <20 msec (Odds Ratio 15,6; 95%CI 2,25-108,12; p<0,0001) and the number of cardiac segments with T2*<20msec was related

with HF (Table 1). Presence of an HF related with higher levels of hs-TnT with a cutoff value between 4,23 ng/L (sensitivity: 100%, specificity: 65,12%) and 5,2 (sensitivity: 83,3%, specificity: 67,44%) (AUC:0,7; p<0,01) (Figure 1). A relationship was found between levels of hs-TnT and the number of myocardial segments with T2* <20 msec (p=0,011), but not with the global Heart T2* (p=0,85). However, clustering patients according to the presence of severe cardiac siderosis (global heart T2*<20 msec), patients with significant iron overload had levels of hs-TnT significantly greater than patients with global T2* ≥20 (5,74 ng/mL±9,15 *vs* 10,5±5 ng/mL; p<0,0001). 38 patients underwent biventricular function evaluation. Of these, 22 (57,9%) had a left, right or biventricular dysfunction. In these patients there was a trend toward higher levels of hs-TnT compared to patients without impairment (8,85±12,52 *vs* 4,49±4,19), without reaching the statistical significance (p=0,068).

Table 1. HF *vs* occurrence of cardiac siderosis (defined as T2*<20msec). Comparison between groups (Mann-Withney test).

	Patients with HF	Patients without HF	p
	(N=6)	(N=44)	
Global heart T2* (ms)	35.55±10.58	16.89±5.57	0.001
Number of segments with T2* <20 ms	2.48±4.47	11.17±4.22	0.001

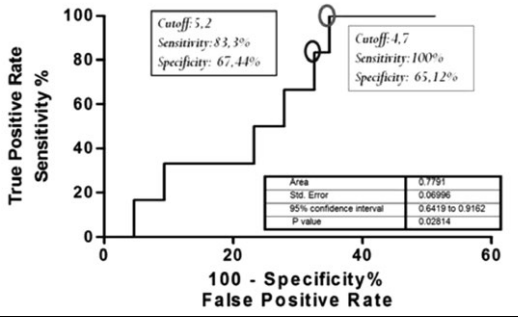

Figure 1.

Summary and Conclusions: Our findings suggest that in TDT patients, hs-TnT would be a sensitive index of myocardial impairment consequent to iron overload.

E1484

COMPARISON OF IRON CHELATION EFFECTS OF DEFEROXAMINE, DEFERASIROX, AND COMBINATION OF DEFEROXAMINE AND DEFERIPRONE ON LIVER AND CARDIAC T2* MRI IN THALASSEMIA MAIOR

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Background: Cardiac complications due to chronic transfusion-induced iron overload are the most common cause of death in patients with thalassemia major. Successful treatment with iron chelators is required to prevent iron deposition in various tissues.
Aims: The aim of this study is to compare iron chelation effects of deferoxamine, deferasirox, and combination of deferoxamine and deferiprone on cardiac iron load in these patients measured by T2* MRI.
Methods: 108 patients with thalassemia major aged over 10 years who had iron overload in cardiac T2* MRI assay, were studied in terms of iron chelators effectiveness and efficacy on reduction of myocardial siderosis. 104 completed the treatment. The first group received only DFO, the second group only DFX, and the third group a combination ofDFO and deferiprone. Myocardial iron was measured at baseline and 12 months after the beginning of treatment through T2* MRI technique.
Results: In the DFO group, myocardial T2* was increased from 12.0±4.1 ms at baseline to 13.5±8.4 ms at 12 months (p=0.10). Significant improvement was observed in myocardial T2* of theDFX group from 13.0±4.5 ms at baseline to 17.5±7.1 ms at 12 months (p<0.001). In the combined treatment group, myocardial T2* was increased from 11.6±3.8 ms at baseline to 16.8±9.9 ms at 12 months (p=0.001). These differences among the three groups were not significant at the 12 months (p=0.09). A significant improvement was observed in liver T2* at 12 months compared to baseline in the deferasirox group (p=0.01) and the combination group (p=0.005), while this improvement was not significant in the deferoxamine group (p=0.72). The most common reported adverse effect in all three groups was mild and transient gastrointestinal side effects.
Summary and Conclusions: In comparison to deferoxamine monotherapy, combination therapy with deferoxamine and deferiprone and deferasirox monotherapy have a significant impact on reducing iron overload and improvement of myocardial and liver T2*.

E1485

MRI PROSPECTIVE SURVEY ON CARDIAC AND HEPATIC IRON AND CARDIAC FUNCTION IN TRANSFUSION-DEPENDENT THALASSEMIA INTERMEDIA PATIENTS TREATED WITH DESFERRIOXAMINE, DEFERIPRONE AND DEFERASIROX

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Background: Few studies have evaluated the efficacy of iron chelation therapy in thalassemia intermedia (TI) patients.
Aims: Our study aimed to prospectively assess by quantitative Magnetic Resonance imaging (MRI) the efficacy of the three available chelators in monotherapy in transfusion dependent (TD) TI patients.
Methods: Among the 325 TI patients enrolled in the MIOT (Myocardial Iron Overload in Thalassemia) network, we selected 103 TI patients TD with an MRI follow-up (FU) study at 18±3 months who had been received one chelator alone between the two MRI scans. Iron overload was assessed by the T2* multiecho technique. Hepatic T2* values were converted into liver iron concentration (LIC) values. Biventricular function parameters were quantified by cine SSFP sequences.
Results: Three groups of patients were identified: 27 patients (13 females, mean age 40.12±10.31 years) treated with desferioxamine (DFO-mean dosage 37.52±8.69 mg/kg/die**),** 23 patients (14 females, mean age 34.73±10.67 years) treated with deferiprone (DFP-dosage 71.70±14.46 mg/kg/die) and 14 patients (9 females, mean age 36.63±10.92 years) treated with deferasirox (DFX-mean dosage 27.75±5.04 mg/kg/die). Excellent/good levels of compliance were similar in the DFO (92.6%), DFP (100%) and DFX (100%) groups (P=0.345). The mean starting age of regular transfusion was 14.73±15.89 years. At baseline in DFO group two patients (7.4%) showed a global heart T2*<20 ms and one of them showed no cardiac iron at the FU. At baseline in DFP group two patients (8.7%) showed a global heart T2*<20 ms and one of them showed no cardiac iron at the FU. All the 5 patients (35.7%) under DFX therapy with pathological global heart T2* at the baseline remained at the same status at the FU. The percentage of patients who maintained a normal global heart T2* value was comparable for DFO (100%), DFP (100%) and DFX (88.9%) groups (P=0.164). Among the 46 patients with hepatic iron at baseline (MRI LIC ≥3 mg/g/dw), the reduction in the MRI LIC values was significant only in the DFO group (DFO: -3.39±6.38 mg/g/dw P=0.041; DFP: -2.25±6.01 mg/g/dw P=0.136 and DFX: -0.36±5.56 mg/g/dw P=0.875). The decrease in MRI LIC values was comparable among the groups (P=0.336). The number of patients with a MRI LIC<3 mg/g/dw went up from 10 (37%) to 11 (40.7%) in the DFO group, from 6 (26.1%) to 8 (34.8%) in the DFP group and from 2 (14.3%) to 8 (57.1%) in the DFX group. The percentage of patients who maintained a normal MRI LIC value was comparable for DFO (90%) *vs* DFP (50%) and DFX (100%) groups (P=0.191). In no group there was a significant change in biventricular volumes, ejection fractions and left ventricular mass index (Figure 1).

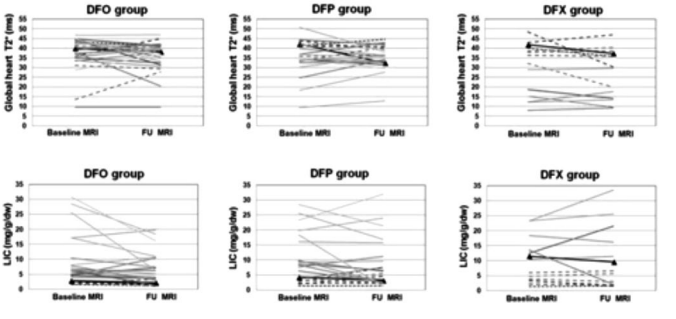

Figure 1.

Summary and Conclusions: Prospectively in transfusion-dependent TI patients at the dosages used in the clinical practice, DFO and DFP showed 100% efficacy in maintaining a normal global heart T2* value while DFX had 100% efficacy in maintaining a normal LIC value. Further prospective studies involving more patients with iron at the baseline are needed to establish which is the most effective drug in reducing iron levels.

E1486

TISSUE DOPPLER IMAGING IN CHILDREN WITH SICKLE CELL DISEASE: EVALUATION OF PULMONARY HYPERTENSION

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Background: The prevalence of Pulmonary Hypertension (PHT) in young children with sickle cell disease (SCD) is unclear. However, early detection and intervention of this potentially lethal problem may reverse the problem and prevent its serious consequence. For diagnosing PHT in SCD, an elevated tricuspid regurgitant velocity (TRV) ≥2.5 m/s appears to have limitations in specificity. However, new parameters characteristic of the right ventricular (RV) function in patients with PHT by means of Tissue Doppler Imaging (TDI) may increase the probability of a correct diagnosis of PHT.
Aims: Our aim was estimating the prevalence of PHT in children with SCD and to assess the clinical, laboratory and cardiac parameters for correct diagnosis of PHT.
Methods: fifty SCD children with mean age 11.28±7.48 years and fifty healthy control were enrolled. Various clinical and laboratory evidence of hemolysis and inflamation were assessed. Conventional echocardiography and Tissue Doppler Imaging were done for all patients and control. Pulmonary artery systolic pressure (PASP) was measured using TRV; the maximal transtricuspid gradient was calculated according to the simplified Bernoulli equation.
Results: The frequency of PHT was 18%, 9/50 patients had TRV ranged from 2.51 to 3.59 m/s. The TDI showed evidence of diastolic dysfunction at the right ventricle as evidenced by significant increase in Tricuspid Es, As and Es/As in 10 patients (20%). Nine out of these10 patients had TRV ranged from 2.51 to 3.59 m/s. Cases with PHT showed significant increase in aortic root diameter, LVEDD and PA diameter than cases without PHT, p-value=0.024, 0.000, 0.000 respectively. Cases with PHT showed significant increase in RVET, PAT/RVET, IVRT-LV, TRV and PASP than cases without PHT, p-value=0.005, 0.005, 0.001, 0.000, 0.000 respectively. The TDI showed significant increase in Tricuspid Es, As and Es/As in cases with PHT than cased without PHT, p-value=0.019, 0.004 and 0.001 respectively. Furthermore, cases with PHT showed significant high systolic blood pressure, high LDH level, high reticulocytic count, high builirubin level, low Hb and high platelet count.
Summary and Conclusions: Tissue doppler imaging combined with Echocardiography may be useful in diagnosing PHT.

E1487

TOWARD BETTER PATHOPHYSIOLOGICAL CHARACTERIZATION AND THERAPEUTIC SOLUTION IN BETA-THALASSEMIA TRAIT PATIENTS WITH IRON OVERLOAD. PRELIMINARY REPORTS FROM AN ONGOING STUDY

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Background: A certain degree of iron overload (IO) is sometimes seen in subjects with β-thalassemia trait (βTT), a mild form of non-transfusion-dependent β-thalassemia. The pathogenesis is unclear, but recently a population study in Sri Lankan children has shown that βTT is characterized by mild hepcidin suppression due to increased erythropoietic activity (Jones E, Blood 2015). In individual patients, this may be further aggravated by genetic (*i.e.* mutations in hemochromatosis genes) or acquired factors (*e.g.* alcohol abuse or non-alcoholic liver diseases). Treatment of IO in βTT is problematic, since "standard" large-volume phlebotomies are not feasible in mildly anemic subjects, as well as the lack of approval of oral iron chelators and of specific guidelines. Deferoxamine (DFO) is the only approved therapy, but it is poorly applicable because of inconvenient parenteral administration and side effects. Sporadic case reports have suggested the use of "mini-phlebotomies" in βTT patients with IO, but feasibility and efficacy of such approach has not been evaluated in patients' series.
Aims: This study has been designed to better characterize factors involved in the development of relevant IO in βTT patients, and to evaluate feasibility and efficacy of mini-phlebotomies to remove IO in this condition.
Methods: Up to now we have enrolled 17 consecutive βTT patients with IO (14 males and 3 females, mean age 58 years) addressed to our tertiary referral center for Iron Disorders in Verona (northern Italy). IO was documented through increased ferritin levels (in most cases >1000 ng/ml, in repeated assays), and either MRI or liver biopsy when indicated. All subjects gave written informed consent. The study plan includes collection of detailed information about clinical history through a structured questionnaire, measurement of serum hepcidin by in-house mass spectrometry-based method, and a comprehensive genetic analysis of the five hemochromatosis genes (HFE, HFE2, HAMP, TFR2, SLC40A1) through next-generation-sequencing (NGS).
Results: Mean Hb values were not uniform (9.5-13.5 g/dl), in agreement with the known phenotypic variability of the βTT. A substantial alcohol consumption (>100 g/day) appears a common acquired cofactor in βTT patients with IO, while NGS (up to now performed in 13/17 patients) did not reveal potentially pathogenic variants out of the known H63D in HFE. Nine patients started treatment with "mini-phlebotomies" and four of them have reached the iron depletion. None of the patients experienced a worsening of anemia during the treatment.
Summary and Conclusions: In our region, the occurrence of clinically significant iron overload is not rare in βTT subjects, and is likely multifactorial in most cases, with high alcohol intake and the H63D variant appearing as the most frequent cofactors contributing to further hepcidin suppression. Preliminary results suggest mini-phlebotomies as a valuable approach for this peculiar category of patients.

E1488

DIAGNOSIS OF FUNCTIONAL IRON DEFICIENCY: HEPCIDIN AS NOVEL BIOMARKER *VERSUS* CONVENTIONAL CLINICAL BIOMARKERS

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Background: Hepcidin is a peptide hormone, which is produced in the liver and excreted in the kidneys. It is considered as a key regulator of human iron homeostasis, lowering the serum iron in the blood circulation by binding and down regulating the iron exporter protein ferroportin in the basolateral site of duodenal enterocytes and reticuloendothelial macrophages. High hepcidin serum-levels decrease the iron transport out of the enterocytes as well as the ability of macrophages to export recycled iron from senescent enterocytes.
Aims: The aim of the present study was to investigate, whether hepcidin is a useful additional clinical biomarker to reflect the actual human iron status, especially functional iron deficiency (ID), as a state of inadequate iron supply to erythropoietic precursor cells in the bone marrow despite the presence of storage iron in the hepatocytes/macrophages.
Methods: This study was approved by the Ethical Committee of Upper Austria (Linz, Austria), and is in accordance with the current version of the Helsinki Declaration. All in all, 233 consecutive hospitalized adult patients with suspected ID, were included. In total, 33.91% (n=79) were male and 60.09% (n=154) were female. The median age was 69.0 (range: 20.0-93.0) years. Patients below 18 years of age were excluded from the study. All subjects were investigated for hepcidin, reticulocyte hemoglobin content (CHr), soluble transferrin receptor (sTfR)/log ferritin ratio (*i.e.* Thomas plot), sTfR, ferritin, transferrin saturation (TSAT), C-reactive protein (CRP) and for complete blood cell count. The hepcidin measurements were performed with the recently launched European Community (CE)-marked enzyme-linked immunoabsorbent assay (ELISA) (Hepcidin-25 bioactive ELISA; DRG Instruments GmbH, Marburg, Germany). Functional ID was defined as a CHr <28 pg. Separate logistic regression models were calculated with all potential biomarkers to evaluate and compare the predictive performance with respect to functional ID in patients without (CRP ≤0.5 mg/dL) and with (CRP >0.5 mg/dL) acute-phase reaction, respectively.
Results: The hepcidin measurements correlated with parameters of iron metabolism. There was a positive correlation with serum ferritin (p <0.0001, Pearson correlation coefficient 0.2608) and TSAT (p=0.0349, Pearson correlation coefficient 0.1383), and a negative correlation with transferrin (p <0.0001, Pearson correlation coefficient -0.6021), sTfR (p <0.0001, Pearson correlation coefficient -0.3736), and iron (p=0.1617, Pearson correlation coefficient -0.0920). There was also a positive correlation between CRP and hepcidin (p <0.0001, Pearson correlation coefficient 0.2581). One hundred seventeen patients with CRP >0.5 mg/dL showed a distinctly higher hepcidin median value (35.60 [range: 4.27-80.03] ng/mL) as compared to 116 patients with CRP ≤0.5 mg/dL (18.55 [range: 3.77-73.01] ng/mL). With respect to functional ID, sTfR/log ferritin ratio and sTfR were of better positive predictive value (PPV) (sTfR/log ferritin ratio: 58.33 and 70.83%; sTfR: 60.00 and 60.00%) than when compared to hepcidin (PPV: 37.74 and 42.86%) and ferritin (PPV: 27.54 and 46.15%) in both subgroups.
Summary and Conclusions: In the logistic regression modelling and when compared with the sTfR/log ferritin ratio or the sTfR measurements, hepcidin as well as ferritin, both biomarkers are known as acute-phase reactants, were only of low predictive value to reflect functional ID.

E1489

CORRELATION OF GENOTYPE WITH PHENOTYPE IN BETA THALASSAEMIA INTERMEDIA IN SRI LANKA

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Background: Previous studies on thalassaemia in Sri Lanka revealed that a

third of patients attending thalassaemia clinics in Sri Lanka have intermedia phenotype and has shown to be presented with a wide phenotypic diversity. The majority of them have Hb E beta thalassaemia, but a significant minority has been shown to have co-inheritance of beta thalassaemia minor with extra alpha globin genes.
Aims: In our previous study we have studied a group of beta thalassaemia intermedia patients without E beta thalassaemia for the molecular basis of intermedia phenotype. In this study we investigated the possibility of correlating the preliminary genetic analysis results in our thalassaemia intermedia study cohort with the phenotype with aiming to unravel phenotypic diversity with genotypic heterogeneity.
Methods: Fifty (50) unrelated thalassaemia intermedia patients were identified from the five main thalassaemia centers in the Island with mostly presented to the adult thalassaemia clinic at Ragama. Clinical severities of the intermedia patients were assessed and they were categorized into "mild", moderate and severe phenotypic groups according to the clinical severity. DNA analysis of the beta globin gene, alpha globin gene and SNPs of Hb F regulators were performed by Southern blot analysis, GAP PCR, MLPA and DNA sequence analysis of PCR products.
Results: Seventeen (34%), of the total thalassaemia intermedia population were homozygous or compound heterozygous for beta mutations. Five of the homozygotes carried two mild beta alleles including a rare promoter mutation-90 C>T, Hb variant alleles of Hb G-Szuhu and Hb G-Coushatta. Nine inherited with two sever beta alleles with alpha gene deletions either one or two. Of the other three individuals who carried two beta alleles with normal alpha gene, one had single nucleotide polymorphismxmn I +/+. Individuals who had inherited with two mild beta alleles and a sever mutation with Hb G-Szuhu and Hb G-Coushatta almost invariably had a mild phenotype. Those individuals who had inherited two severe beta alleles with alpha gene deletions invariably had a severe phenotype. It was unable to explain the phenotype in two individuals in this group with the existing genetic data. Thirty three (66%) of the total thalassaemia intermedia population were heterozygous for beta mutations IVSI-5 G>C (n=12), IVSI-1 G>A (n=11) being the commonest. Twenty eight of the heterozygotes carried excess alpha genes and had a mild to moderate phenotype. In this group of individuals who had inherited with single beta allele with normal alpha genes, the genetic study could not explain the phenotype in five individuals.
Summary and Conclusions: Thalassaemia intermedia patients in Sri Lanka showed a considerable heterogeneity both phenotypically and genotypically. The clinical outcome of our thalassaemia intermedia patients were mostly explained by the genotypes linked to the alpha and beta gene cluster. About three fourth of the cases found with beta thalassaemia intermedia phenotypic had mutations on the alpha and beta globin gene clusters. Co-inheritance of additional alpha globin genes and beta thalassaemia minor was accounted to be the most common finding for their clinical presentation. However in a minority, the existence of other causative genetic determinants remains to be molecular defined.

E1490

EVALUATION OF EMERGENCY CARE FOR ADULT SICKLE CELL PATIENTS: A MONOCENTRIC STUDY

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Background: Pain relief is the key point for patients admitted for sickle cell crisis in emergency care units.We present a prospective monocentric trial aiming to improve sickle cell patient support in our hospital
Aims: The primary objective was the evaluation of the pain relief in adult patients admitted for sickle cell crisis in our emergency department. Upon admission, a standard protocol based on international and local guidelines with hyper-hydration and high doses of intravenous morphine was used. Morphine was administered according to patient's EVA (Analogic visual Evaluation) pain scale. We in parallel conducted a hetero-evaluation of the patient's pain by the nursing team. The secondary objectives were the evaluation of morphine dose required for pain relief, patient's satisfaction and the length of hospitalization. This study also comprised of an ancillary part aiming to determine a predictive score of crisis severity based on standard laboratory parameters.
Methods: This observational prospective mono centric study was approved by the local ethics committee. Written informed consent was obtained from patients during routine hematology follow up visits or more rarely in the emergency care setting. Patients were enrolled from February 2013 until February 2015. Pain was evaluated through EVA upon admission, after 1 hour, 3h, 6h and then every 6 hour until pain relief. Patient satisfaction was analyzed with a questionnaire developed by our psychologist and graded from 1 (very bad) to 5 (very good).
Results: 104 observations were obtained for 51 patients as some patients had been hospitalized more than once. Mean waiting time before the start of treatment was 18.6 minutes. More than 65% of patients waited less than 10 minutes. Morphine doses used were less than those mentioned in literature and in our local guidelines. Pain was systematically under scored by the nurse team (hetero EVA). Unexpectedly, women received significantly (p<0.001) more morphine than men. Mean patient's satisfaction score was good (3.8).There was a significant correlation between morphine dosage, waiting time until start of treatment and patient satisfaction. We observed an improvement in patient satisfaction as the study moved along. Analysis of laboratory data and length of hospitalization are still ongoing.
Summary and Conclusions: The main aim of our study was to reinforce collaboration between the different teams taking care of patients in an emergency care setting as well as during hospitalization. This multidisciplinary approach allows a dramatic improvement in patient satisfaction during the study. A significant inverse correlation was found between morphine dosage, waiting time until treatment and patient satisfaction. We observed that continuous education of medical and paramedical staff is mandatory to improve quality of care in this case.

E1491

IMPROVING MACROCYTOSIS DIFFERENTIAL DIAGNOSIS BY RDW-SD

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Background: Macrocytosis is defined as a mean corpuscular volume (MCV) >100fL. Blood cells morphology and biochemical parameters are essential to differentiate megaloblastic anemia (MA) from other causes of macrocytosis.The red cell distribution width (RDW) evaluates the degree of anisocytosis. It can be expressed as a coefficient variation of the MCV (RDW-CV).
Aims: The objectives of this study were to compare the sensitivity of RDW-CV and RDW-SD to detect anisocytosis, in the presence of macrocytosis, and their impact in the differential diagnostic of MA.
Methods: We analyzed 152 samples with macrocytosis (78 with anemia; 74 without; 26 suggestive of MA). They were divided in 8 groups: on chemotherapy, hydroxyurea, alcoholic/hepatic disorders, hemolytic anemia, aplastic anemia, myelodisplastic syndrome, hypothyroidism, and other disorders. The samples were processed in HORIBA, Abbott and Sysmex instruments, in order to monitor the reproducibility and the ability to screen out of range values. The reference values were calculated for each equipment (150 blood donors). Data were analyzed with descriptive statistics, t-student, ANOVA, ROC (GraphPad Prism5).
Results: The objectives of this study were to compare the sensitivity of RDW-CV and RDW-SD to detect anisocytosis, in the presence of macrocytosis, and their impact in the differential diagnostic of MA. Reference values span from the minimum to maximum obtained values: RDW-CV 12.03-14.49%; RDW-SD 36.5-46.68fL. Hemoglobin(Hb) values were reproducible (p=0.8727), but MCV, RDW-CV and RDW-SD differ significantly (p<0.0001). Patients with anemia: Hb median 10.2g/dL[5.4;11.7]; MCV105.8±7fL, RDW-CV14.9±2.4% and RDW-SD56.9±9.8fL; patients without anemia: Hb median 13.5g/dL[12;16.8], MCV103±5.4fL, RDW-CV13.3±1.2% and RDW-SD50.2±5.1fL. It was found more cases of MA in "other disorders" group (n=10/63), followed by "hydroxyurea" (n=7/20), and "chemotherapy" (n=4/28). MA and non-MA parameters means showed no significant differences (p>0.05). However, RDW-CV were borderline (MA 14.9±2; non-MA 15.8±3.1) and RDW-SD were elevated (57.3±11.1; 60±11.8, respectively). Regarding to ROC analysis, it was found a better anisocytosis discrimination for RDW-SD, since it presented a higher area under the curve (AUC) than RDW-CV (0.97 and 0.79, respectively). The best cut off for RDW-CV was 14.45% (sensitivity-84.8%; specificity-82.69%) and for RDW-SD was 44.35fL, with improved results for sensitivity and specificity (93.75% and 94.23%, respectively).
Summary and Conclusions: In macrocytosis, RDW-SD is more sensitive than RDW-CV, improving anisocytosis discrimination. For this reason, RDW-SD may become helpful when MA is considered, as it triggers, earlier than RDW-CV, an alert that requires further actions, like blood smear review and vitamin B12/folic acid quantification.

E1492

PROSPECTIVE STUDY ON THE EFFECT OF FOK-I GENE POLYMORPHISM ON BONE HEALTH IN YOUNG PATIENTS WITH B-THALASSAEMIA MAJOR

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Background: Low bone mass, a major cause of morbidity in patients with b-thalassaemia major (b-TM) has a multifactorial pathogenesis with genetic factors greatly contributing to it. Fok-I vitamin D receptor (VDR) polymorphism has been significantly related to low bone mineral density and can serve as a useful genetic marker in predicting bone disease.
Aims: We sought to estimate the degree of genetic contribution of Fok-I gene polymorphism of VDR to the evolution of bone mass in patients with b-TM assessed with imaging techniques and biochemical parameters of bone metabolism over a period of two years.
Methods: Sixty-four children and young adults (33 males and 31 females) with mean decimal age of 23.20±5.41 (range: 9.25- 32.41 years) were recruited in this study. All patients were genotyping for Fok-I gene polymorphism and were

assessed with both Dual energyx-ray Absorptiometry (DXA) and Quantitative Ultrasound Sonography (QUS) at baseline and two years after. Z-scores were calculated based on normal age and sex matched Caucasian population. Metabolites of vitamin D, intact PTH, total calcium, inorganic phosphorous and alkaline phosphatase were measured at the serum pre transfusion.
Results: No deviation of the genotype distribution from the Hardy-Weinberg equilibrium was noticed: FF genotype accounted for 44.29%, ff genotype for 12.86% and heterozygosity (Ff) for 42.85%. A moderate proportion of patients had decreased DXA Z-scores (Z-score ≤-2) predominately in total hip (31%) and secondary in lumbar spine (15.6%). On the contrary, just a few patients (7.8%) had decreased QUS values measured at tibia. Patients being homozygous for the f allele had apparently higher BMD z-scores compared with those carrying the F allele in homo- or heterozygosity, however, with a difference that did not reached significance. Interestingly enough, a significant deterioration in BMD Z-scores measured at femur (FF: p=0.004 Ff: p<0.001, ff: p=0.024) and total hip (FF: p=0.022, Ff: p=0.005) was recorded for all type of genotypes, except for ff genotype and with regards to the total hip DXA values. In contrast, QUS values measured at tibia were significantly improved in patients carrying the FF genotype (p=0.035). An increased prevalence of serum $25(OH)D_3$ deficiency (59.4%) and $25(OH)D_3$ borderline (12.5%) was recorded. When stratified according to $25(OH)D_3$ levels, no statistically significant difference in the evolution of DXA or QUS parameters was observed. Regarding DXA Z-scores measured at hip, a significant deterioration was recorded in all groups, independently of the status of $25(OH)D_3$.
Summary and Conclusions: Fok-I gene polymorphism of VDR seems to have an impact on bone health of patients with beta-thalassaemia major in contrast to vitamin D levels. No level of agreement was observed between the two imaging techniques in identifying thalassaemic patients with impaired bone status.

E1493

GROWTH DIFFERENTIATION FACTOR-15 AND CARDIOVASCULAR DISEASE IN THALASSEMIA INTERMEDIA
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Background: Heart disease is the leading cause of mortality and one of the main causes of morbidity in β-thalassemia. Growth differentiation factor-15 (GDF-15), a member of the transforming growth factor-β superfamily, is a marker of ineffective erythropoiesis in several anemias.
Aims: To determine the level of GDF-15 in children and adolescents with TI in relation to clinical characteristics, iron overload, hemolysis and therapy and to assess its value a potential marker for cardiovascular complications and subclinical atherosclerosis.
Methods: Thirty-five TI patients without symptoms for heart disease were studied. Patients were subjected to detailed medical history and thorough clinical examination with special emphasis on disease duration, evidence of renal, hepatic or cardiac disease, splenectomy status. For transfusion status, patients were classified as: transfusion-dependent (patients on regular-interval transfusion protocols [once every 1-3 months for a pre-transfusion hemoglobin of ≥8 g/dL] initiated mainly for failure to thrive in childhood, bone deformities, progressive splenic enlargement, persistent worsening anemia; and non transfusion dependent (patients who never received transfusion or required incidental transfusions for transient severe anemia secondary to infections or surgery). GDF-15 was measured and correlated to echocardiographic parameters and carotid intima media thickness (CIMT).
Results: A total of 11 patients (31.4%) were splenectomized. By echocardiography, 16 patients (45.7%) had pulmonary hypertension risk and 12 patients (34.3%) had systolic left ventricular dysfunction. TI patients had significantly higher tricuspid regurgitation velocity (TRV), p<0.001, and lower left ventricular ejection fraction and fractional shortening than controls (p<0.001). CIMT was significantly increased among patients compared to controls (p<0.001). The median (IQR) of GDF-15 level in TI patients was 1500 (1000-2850) pg/ml compared to 110 (80-200) pg/ml in controls (p<0.001). Transfusion dependent TI patients had higher GDF-15 than non-transfusion dependent patients (p<0.001). TI patients with splenectomy, pulmonary hypertension risk, and heart disease had higher GDF-15 levels than those without. Hydroxyurea-treated patients had lower GDF-15 levels than untreated patients. ROC curve analysis revealed that the cutoff value of GDF-15 at 1500 pg/mL could differentiate patients with and without heart disease with a sensitivity of 87.5% and specificity of 100%, AUC 0.945; p<0.001. GDF-15 levels were positively correlated with age (r=0.463, p=0.005), disease duration (r=0.567, p<0.001), transfusion index (r=0.767, p=0.005), indirect bilirubin (r=0.345, p=0.043), LDH (r=0.612, p=0.002) and serum ferritin (r=0.949, p<0.001), while negatively correlated to hemoglobin (r=-0.408, p=0.015). Moreover, positive correlations were observed between GDF-5 and TRV (r=0.620, p<0.001) as well as CIMT (r=0.499, p=0.024).
Summary and Conclusions: Ineffective erythropoiesis as reflected by high GDF-15 levels is the hallmark of disease process in TI. GDF-15 may be considered a risk marker for pulmonary and cardiovascular complications as well as subclinical atherosclerosis in children and adolescents with β-TI. Levels are closely related to markers of hemolysis and iron overload. Elevated levels of GDF-15 may have clinical implications on treatment besides defining TI patients at risk of cardiac complications. A marker of ineffective erythropoiesis as GDF-15 may be a potentially valuable tool for monitoring the response to therapy, thus, promote earlier intervention and improve clinical outcomes in TI patients.

E1494

SOLUBLE FMS LIKE TYROSINE KINASE-1 (SFLT-1) : AN EARLY MARKER FOR GLOMERULAR DYSFUNCTION IN SICKLE CELL NEPHROPATHY
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Background: Given the potential role of angiogenesis in the pathophysiology of sickle cell nephropathy (SN), novel biomarkers are needed for early diagnosis of SN before irreversible kidney damage.
Aims: Our aim was to determine the relationship between serum level of the anti-angiogenic factor soluble FMS-like tyrosine kinase-1 (sFLT-1) and other biomarkers of renal damage as microalbuminuria (MA), for early diagnosis of SN.
Methods: 47 Sickle cell disease (SCD) patients and 49 healthy controls were enrolled. Microalbumine was determined in patients' urine sample. Blood samples were tested for sFLT-1, serum creatinine and various hemolysis and inflammation markers. Also, peripheral blood monocyte expression of sFLT-1 was measured using real time PCR.
Results: Serum level of sFLT-1 (pg/ml) in SCD patients was higher than the control level (Median/range/IQR=142/ 60-1300/61 pg/ml *vs* 125/ 110-187/52 pg/ml respectively), (*p*=0.006). The median (range) of sFLT-1 level was higher in SCD patients with MA compared to SCD patients with normoalbuminuria (NA), 185(140-1300) *vs* 125(60-189) mg/g respectively), (p=0.004). There was significant positive correlation between serum level of sFLT-1 and MA, lactate dehydrogenase (LDH) and indirect bilirubin (r=0.59, 0.39, 0.30 & *p*=<0.001, 0.007, 0.041, respectively). The sensitivity of sFLT-1 in early detection of renal affection in SCD was 93.6%, while the specificity was 68.6%. Finally, we identified the monocytes as a possible source of increased sFLT-1 in SCD patients.
Summary and Conclusions: sFLT-1 may constitute a novel renal biomarker that can predict SN, enabling early diagnosis to prevent progression of renal damage.

E1495

ORAL IRON-BASED PHOSPHATE BINDER, FERRIC CITRATE, INCREASES IRON MEASURES IN DIALYSIS-DEPENDENT AND NON-DIALYSIS DEPENDENT PATIENTS WITH CHRONIC KIDNEY DISEASE
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Background: Ferric citrate is an iron-based phosphate binder with demonstrated significant improvement in serum phosphate control and in iron markers including serum iron, transferrin saturation and serum ferritin in both dialysis and non-dialysis (ND)-dependent CKD patients.
Aims: We evaluated the temporal change in serum iron measures relative to the amount of iron administered as ferric citrate in both ND-dependent CKD and end-stage renal disease (ESRD) subjects.
Methods: In a previous 52-wk Phase 3 clinical trial evaluating efficacy and safety of ferric citrate, a subset of dialysis subjects on ferric citrate did not receive IV iron (n=48). Serum iron parameters were measured and% change from baseline was calculated. Serum iron parameters were also evaluated in subjects participating in a Phase 2 clinical trial of ND-dependent CKD patients administered ferric citrate for 12 wk (n=61) to determine the safety and efficacy of ferric citrate to control serum phosphorus and transferrin saturation (TSAT). The subjects were not receiving IV iron or erythropoeisis stimulating agents for the duration of the trial. In both trials, the percent change of serum iron parameters was calculated per gram of iron administered (based on cumulative dose for the duration of the trial). Each tablet of ferric citrate contains 210 mg elemental iron and the dose of ferric citrate was titrated to control serum phosphorus to treatment target.
Results: In both clinical trials, ferric citrate significantly improved serum iron and TSAT levels with significantly increased hemoglobin in the ND-dependent CKD subjects and maintained hemoglobin in the ESRD subjects. TSAT, hemoglobin and iron administered in the ND-dependent CKD subjects are depicted in the Figure 1 as mean±SEM. The percent change in serum iron in the ND-dependent CKD subjects was 0.47±0.09% (mean±SEM) per gram of iron administered as ferric citrate and% change in TSAT was 0.58±0.09% per g iron administered. In the ESRD subjects, the% change in serum iron and TSAT were lower than that seen in the ND-dependent CKD subjects at 0.05±0.02% per g iron (mean±SEM) and 0.05±0.02% per g iron administered respectively. The average dose of iron administered was 1.2 g/day for 12 weeks (~5-6 tablets/day of ferric citrate) in the ND-CKD subjects and 1.4 g/day (~7 tablets/day of ferric citrate) for 52 weeks in ESRD subjects.

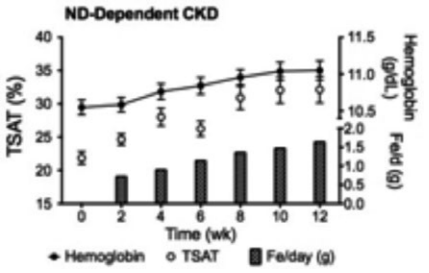

Figure 1.

Summary and Conclusions: Treatment with oral ferric citrate yielded significant and sustained improvement in iron measures in both dialysis-dependent and ND-dependent CKD subjects. The changes in both serum iron and TSAT are small relative to the iron administered, attesting to low fractional absorption of iron. In addition, the percent increase in iron parameters decreased over time indicative of physiologic negative feedback mechanisms regulating the amount of intestinal iron absorption.

E1496

THE SPECTRUM OF A-THALASSEMIA AND B-THALASSEMIA MUTATIONS IN SOUTH WEST IRAN

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Background: Carrier screening, genetic counseling, prenatal diagnosis and aborted affected fetus are successful programs in reducing new delivery of β-thalassemia major and severe form of α- thalassemia in Iran**.** As both alpha and beta thalassemia are highly prevalent in south west Iran. The increasing knowledge about mutation types might be an important guide for genetic counseling sectors.
Aims: The purpose of this study is to detect the accurate frequency and type of Alpha and Beta mutations in pre marital candidate couples.
Methods: A total of 600 couples (1200 individuals) were recruited in this study. Five milliliter of EDETA and clot blood were drawn and obtained from each subject. Cell blood count, Hemoglobin electrophoresis was done for everybody. The common deletional alpha thalassemia was determined by polymerase chain reaction based direct amplification assay.DNA sequencing was done to detect beta and non deletional alpha mutations. DNA study for beta mutation was performed on each individual with MCV <80, MCH <27 and Hb A2>3.5.However, alpha mutation was conducted with MCV <85, MCH <27.5 and Hb A2<3.5.
Results: The frequency of carrier state for beta thalassemia and alpha thalassemia (silent, minor and Hb H) were 7 and 15% respectively. The mutation at codon 36-37 (-T) was found the first mutation with a frequency of 21.2%. The other mutations, at decreasing frequencies ranging from 18.3 to 1.1 were: IVS I-110:18.3%,IVSII-I :14.8%,CD8 (-AA):5.6%,IVSI-I: 4.2%,CD 44(-C): 4%,CD5:4%,CD39(C-T):3.4%,IVS1-5:3.2%,CD8-9:2.6%,CD82=83:2.6%,-28:2.6%,IVSI-6:2.4%,-88:1.9%,25bp del: 1.6%,IVSII-848:1.1%,IVSI-(-24):1.1%,IVSI(-17):1.1. The prevalence of the other mutations was very rare. The alpha mutations with a decreasing frequency was as follow:-α3.7 heterozygote: 38.2%,-α3.7 homozygote: 11.6%,-Med: 3.8%, CD 19:3.5%, Poly A4:2.5%,-α3.7/-Med:2.5%,Poly A6:2.3%,-α4.2 heterozygote: 1.6%, IVSI -Donor site: 1.5%, 5NT-del: 1.4%,-20.5:1.2%. The frequency of the other mutations was below 1%.
Summary and Conclusions: This study shows the high prevalence of alpha and beta thalassemia in south west Iran. The most alpha and beta thalassemia mutations were-α3.7 and CD 36-37 respectively. This is in concordance with Amin Doosti-Irani *et al.* study. The most alpha thalassemia mutation is in the line of most Iranian and Arabian countries studies. But the predominant beta mutation in our study is not in the top list of Najmabadi H *et al.* study. Both alpha and beta thalassemia are highly prevalent in south west Iran. The increasing knowledge of health sectors about alpha and beta thalassemia frequency and mutation types will enable them to manage better genetic counseling.
Key Words: South West Iran, Alpha mutation, Beta mutation.

E1497

DO WE HAVE TO CHELATE PATIENTS WITH THALASEMIA MAJOR HAVING FERRITIN LEVELS <500 MG/L?

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Background: In developing countries ıron load can be determined by measuring ferritin levels. By following ferritin levels, alterations of iron status can be predicted. The general recommendation is to decrease the dose of DFX or stop chelation when the level of ferritin is <500 µg/L There is no study regarding the use of deferasirox or deferipron at low ferritin levels. But case reports related to iron toxicity with low ferritin levels had been published.
Aims: So, we aimed to evaluate the toxicity due to chelation therapy at ferritin levels of <500µg/L.
Methods: Children followed up at our pediatric hematology outpatient clinic with the diagnosis of Thalasemia Major are involved to the study. The charts of patients having regular blood transfussions and deferasirox therapy were reviewed retrospectively and they were evaluated for hepatotoxicity and nephrotoxicity when their ferrin levels were <500µg/L.
Results: Of 21 patients, 22 times ferritin levels became <500 µg/L (106 µg/L - 495 µg/L). In 16 of these low levels of ferritin, chelation dose was decreased, but in 6 of them the dose was not decreased due to the fact that te results were brought late by the patients. The ages of patients under chelation therapy while their ferritin levels were low was between 58 month and 21 years (median 9 years). Deferasirox was used in 20 patients, 1 patient was having deferipiron. The duration that patients have ferritin <500µg/L was between 22weeks and 91 weeks. None of the patients had any clinical and laboratory (BUN, Kreatinin, AST and ALT) derrangement.
Summary and Conclusions: Ferritin levels may change as a response to inflammation, liver function abnormalities and ascorbate deficiency. There are studies showing no significant correlation between cardiac iron load and ferrtin levels. Even with moderately elevated ferritin levels iron toxcity may ensue. So, it is a must to predict the toxicity of chelation therapy and continue chelation. There was no clinical and laboratory influence of deferasirox therapy for patients having ferritin <500µg/L. But larger controlled series are needed for saying deferasirox therapy is safe at low ferritin levels.

E1498

KLF10 GENE EXPRESSION AS A SECONDARY MODIFIER IN BETA THALASSEMIA AND SICKLE CELL DISEASE (SCD) PATIENTS AND A PHARMACOGENOMIC BIOMARKER FOR HYDROXYCARBAMIDE TREATMENT RESPONSE

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Background: Many genes have been uncovered which could modify γ- globin chain production and hence level of fetal hemoglobin (HbF) ameliorating the phenotype of beta hemoglobinopathies and SCD as well as the response to hydroxycarbamide (HU). KLF10 indirectly increase gamma globin chain synthesis.
Aims: the aim of the study was to evaluate the frequency of different genotypes for KLF10 gene in B-TM, B-TI,SCD patients compared to healthy controls via DNA extraction and PCR and to assess its relation to disease phenotypes, (HU), clinical and laboratory response.
Methods: This case control study included 80 patients; 48 beta thalassemia major (B-TM), 16 sickle cell disease (SCD) and 16 thalassemia intermedia (B-TI) patients (in a ratio 3:1:1), compared with 50 matched controls. All included SCD and B-TI patients were on stable dose of HU therapy. Assessment of KLF10 gene relation to phenotype was done using baseline HbF, transfusion index, while evaluation of response to HU therapy was done through comparing the frequency of vaso-occlusive crises and transfusion 2 years before and after start of therapy as well as an increase more than 20% in baseline HbF level.
Results: The frequency of mutant KLF10 genotype and the mutant allele (T) was significantly higher among B-TM (30%) compared to B-TI, SCD patients (0%,7.1%)and control(6%) (p=0.04). There was no significant difference between different KLF10 genotypes among B-TM patients as regards age at onset of disease, initial HbF%, transfusion index and mean pretransfusion Hb in last 2 years prior to the study(p=0.12, p=0.08, p=0.73, p=0.59, p=0.42 respectively), nor between homozygous SCD patients for normal KLF10 and mutant KLF10 gene as regard age of onset of disease, the percentage of patients who suffered from avascular necrosis, stroke, acute chest syndrome (p=0.69,p=0.27,p=0.18,p=0.15respectively). Yet Homozygous SCD patients for normal KLF10 gene had significantly lower TI/OR decrease transfusion frequency longer interval between transfusions (p=0.002). The percentage of clinical and laboratory responders and non responders to HU between different KLF10 genotypes among B-TI, SCD patients was comparable (p=0.679), (p=0.931). The frequency of mutant KLF10 genotype and the mutant allele (T) was significantly higher among B-TM (30%) compared to B-TI, SCD patients (0%,7.1%) and control (6%) (p=0.04). There was no significant difference between different KLF10 genotypes among B-TM patients as regards age at onset of disease, initial HbF%, transfusion index and mean pretransfusion Hb in last 2 years prior to the study(p=0.12, p=0.08, p=0.73, p=0.59, p=0.42 respectively), nor between homozygous SCD patients for normal KLF10 and mutant KLF10 gene as regard age of onset of disease, the percentage of patients who suffered from avascular necrosis, stroke, acute chest syndrome (p=0.69, p=0.27, p=0.18, p=0.15 respectively). Yet Homozygous SCD patients for normal KLF10 gene had significantly lower TI/OR decrease transfusion frequency longer interval between transfusions (p=0.002). The percentage of clinical and laboratory responders and non responders to HU between different KLF10 genotypes among B-TI, SCD patients was comparable (p=0.679), (p=0.931).
Summary and Conclusions: Although KLF10 gene has not a standalone role

as an HbF modifier, yet our data supports its importance in ameloriating phenotype among beta hemoglobinopathies. Further evaluation of KLF10 gene expression among B-TI, SCD patients as a potential pharmocogenomic marker to differentiate between responder and non responder to HU is warranted.

E1499

THINKING OF HEREDITARYxEROCYTOSIS: THE FIRST STEP TO THE DIAGNOSIS

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Background: Hereditaryxerocytosis (HX) or Dehydrated Hereditary Stomatocytosis is a rare autosomal dominant hemolytic anemia (1 in 50000 live births), resulting from a red blood cell (RBC) membrane permeability disorder. HX has a wide range of clinical phenotypes: while some patients are asymptomatic, others have compensated hemolysis, with slight macrocytosis and reticulocytosis, mild splenomegaly and/or gallbladder lithiasis. In spite of not requiring frequent transfusion, most of the HX patients have iron overload. In the peripheral blood smear (PBS) a variable number of cup-cells can be observed, but are frequently overlooked.
Aims: To review the clinical features and hematological parameters on patients with HX in order to understand the most important steps to the diagnosis of this rare disease, and also to show the high phenotypic variability of HX.
Methods: Collection of all the clinical and laboratory data of all the patients with HX diagnosis (confirmed by the presence of *PIEZO1* mutations) followed in our department.
Results: Our population consists in 7 patients, with male predominance (n=5) and median age at diagnosis of 30 years (0-59). This group of patients showed a heterogeneous disease phenotype: mean hemoglobin of 13,2 g/dL (10,4-14,8), mean globular volume of 96,5fl (87,7-105), mean corpuscular hemoglobin concentration of 34,6 g/dL (33,1-35,3), mean reticulocyte count of 232,8 G/L (94,9-600), mean total bilirrubin of 27,3 µmol/L (9-50), mean LDH between normal values (330 U/L (99-493)) and ferritin of 574,7ng/ml (168-1489). On clinical presentation only one patient showed the classical signs and symptoms associated with HX, as can be observed in Table 1. One patient presented with *hidrops fetalis* at birth but with Hb of 19g/dl and in a few weeks develops hemolytic anemia with macrocytosis, reticulocytosis, hyperferritinemia and liver and spleen enlargment while, in the opposite side of the clinical spectrum, 2 patients were asymptomatic, showing only a slight macrocytosis or reticulocytosis and hyperferritinemia. The only common feature to all patients was the presence of cup-cells in the PBS and 6/7 had hyperferritinemia. Five patients had family history of anemia and/or hyperferritinemia.

Table 1.

Patients	1	2	3	4	5	6	7
Anemia	**Yes**	**Yes**	No	No	No	No	No
Macrocitosis	**Yes**	**Yes**	No	No	**Yes**	No	No
Reticulocitosis	**Yes**	**Yes**	**Yes**	**Yes**	No	**Yes**	No
Cup-cells	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
Hyperferritinemia	**Yes**	**Yes**	**Yes**	No	**Yes**	**Yes**	**Yes**
Splenomegaly	**Yes**	No	**Yes**	**Yes**	No	No	No
Hepatomegaly	**Yes**	No	No	No	No	No	**Yes**
Previous cholecystectomy	No	**Yes**	**Yes**	**Yes**	No	No	No

Summary and Conclusions: Reviewing the data on these 7 patients with HX due to *PIEZO1* mutations we could observe a high phenotypic variability and that the main steps to the diagnosis were the clinical suspicion and the identification of cup-cells in the PBS. The high association between HX and hyperferritinemia misleads its diagnosis, which is overlooked on the etiological workup of hyperferritinemia. In the era of molecular biology, we want to emphasize the importance of the RBC morphology to the diagnosis of HX and the current tendency to ignore the PBS observation is probably the main cause for the disease underdiagnosis. The diagnosis of HX is essential because, unlike the most congenital hemolytic anemia, the splenectomy is contraindicated, since it is associated with severe thromboembolic disease.

E1500

EFFECTIVENES OF MAGNETIC RESONANCE IMAGING FOR MEASURING OF PITUITARY IRON OVERLOAD IN PATIENTS WITH THALASSEMIA

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Background: In patients with b-thalassemia major iron deposition resulting from complications related with morbidity and mortality. Iron accumulation starts in the reticuloendothelial system and subsequently accumulates in all parenchymal organs. One of the affected parenchymal organs is pituitary gland. Although thalassaemic patients are chelated adequately, pituitary siderosis may lead to hypopituitarism.
Aims: The aim of this study was to evaluate the magnetic resonance imagine (MRI) signal intensity of pituitary gland if it was suitable to measure pituitary siderosis.
Methods: The ratio of the average anterior pituitary gland signal intensity to that of the pterygoid muscles and that of nasopharyngeal fat was assessed in 60 patients with b-thalassemia major by using signal intensity ratio of T1-weighted MRI. All patients were taking deferasirox. In all patients, an endocrine evaluation was performed, including measurements of spontaneous gonadotropins, thyroid hormones, growth hormone and insülin like growth factor. Also liver iron content (LIC) was measured with R2 MRI.
Results: A total of 60(M/F=34/26) pediatric patients with mean age of 11.5±4.5(range 1.8-19.0) years were enrolled the study. The mean serum ferritin level and LIC values were 1127.1±615.7 ng/ml and 2.1±1.2 mg iron/g dry tissue weight (dw) respectively. Any patients had high LIC level. The measurements of anterior pituitary gland wasn't correlated median age of the patients and median of other hormone levels. Only TSH level and pituitary-to-fat ratio was correlated (r=0.32, p=0.033). Nine patients (15%) had pubertal retardation and six patients (10%) subclinical hypotiroidsm. There wasn't difference between the patients who had and hadn't hypotyroidism in terms of anterior pituitary MRI. Also there wasn't difference between the patients who had and hadn't pubertal retardation in terms anterior pituitary MRI.
Summary and Conclusions: Our data suggest that iron accumulation of anterior pituitary gland measurement by using MRI wasn't succesful. This may be related that there were no patient who had high level of LIC. So the patients' anterior pituitary glands may not be affected by iron accumulation due to priority in iron accumulation of the reticuloendothelial system.

LB2089

ASSESSMENT OF THE EFFECTS OF ANEMIA ON CARDIOVASCULAR FINDINGS IN OBESE ADOLESCENTS

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Background: Obesity that develops during childhood is a major risk factor in terms of cardiovascular diseases in further life. In obese adolescents, anemia may develop due to inflammation of obesity and iron deficiency anemia and it may be a risk factor that affects the cardiological progress negatively in these patients.
Aims: The purpose of this study is to assess the effects of anemia on cardiovascular findings in obese adolescents by using echocardiography.
Methods: Twenty nine obese adolescent patients with anemia and 33 obese adolescents without anemia who were 12-18 years old were echocardiographically investigated in the Pediatric Cardiology Outpatient Clinic. Thirty three adolescents who had similar age and gender and whose body mass indexes were between 3-85 percentiles were included as the control group. Patients data were obtained from the relevant departments and the hospital patient files. All patients were examined according to their cardiac risk factors, blood pressures, pulse rates, and physical examination findings. Blood counts, liver and renal function tests were noted. Serum iron parameters, thyroid function tests, fasting glucose, lipid profiles, insülin, and CRP values of the obese adolescents were recorded. The patients were examined according to their diet, presence of intolerance to the effort, sleep apnea, thrombosis, and fatigue symptoms. Echocardiographic parameters of all patients were examined.
Results: There were not any difference between groups in terms of age and sex. Anemia was mild (mean Hb: 11.5±0.79 g/dL) in the anemic obese group. Hypertension, presence of intolerance to the effort, and fatique were statistically significantly high in both obese groups compared to the control group. There was statistically significant difference between obese groups in terms of diet and cardiac pulse rate. Anemic obese adolescents were dieting more in order to loose weight and tachycardia was found at a higher rate. In laboratory examinations of anemic obese and obese groups, a significant difference was detected in terms of average values of ferritin, CRP, and fibrinogen. Ferritin was found significantly low, CRP and fibrinogen levels were found significantly high in the anemic obese group. According to M-mode echocardiographic examination results, a significant difference was detected in terms of averages of interventicular diastolic diameter, left ventricular posterior wall diastolic diameter, left ventricle mass, and left ventricle mass index in obese patients compared to the control group; there was no significant difference in these parameters between the anemic and nonanemic obese patients. Ratio of mitral peak early diastolic flow velocity to peak early diastolic myocardial velocity (Mitral E/E') by tissue Doppler echocardiography was slightly higher in both of the obese groups.
Summary and Conclusions: Early symptoms of subclinical left ventricular diastolic dysfunction were detected in obese adolescents. Mild anemia did not show any difference except higher cardiac pulse rate. Since severe anemia may change the results and be one of the cardiovascular risk factors in obese adolescents, it is necessary to avoid low iron diet programs in these children.
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Stem cell transplantation - Clinical

E1501

ACTIVATION OF MONOCYTES AND DENDRITIC CELLS IN PATIENTS WITH ACUTE GRAFT-*VERSUS*-HOST DISEASE AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Allogeneic hematopoietic stem cell transplantation (HSCT) is the treatment of choice for a variety of hematologic malignancies. Graft-*versus*-host disease (GVHD) is a key contributor to treatment related morbidity and mortality and consequently limits the efficacy of HSCT. GVHD may involve liver, skin, gastrointestinal tract or other organs leading to severe and life-threatening organ damage as well as to a high risk of opportunistic infections. Early diagnosis of GVHD and the consequent initiation of the adequate treatment are important factors that contribute to a favourable outcome.
Aims: Several studies indicate that innate immune responses play an important role in development of acute GVHD. Thus, we are interested in activation state of monocytes and dendritic cells in HSCT patients asking whether there are differences in activation of these cell types as early indicators of GVHD and maybe useful for the early identification of patients at risk of developing GVHD.
Methods: In this retrospective study, we analysed cryoconserved peripheral mononuclear cells (MNCs) from 17 patients with acute GVHD and 16 patients without GVHD. All patients had received HSCT after conditioning treatment with fludarabine, melphalan and alemtuzumab and gave informed consent for blood analyses. The following surface markers were analysed on monocytes and dendritic cells (DCs) by multi-color flow cytometry using fluorochrome conjugated monoclonal antibodies: TREM-1, CD86, HLA-DR, CD16, CD14, CD83, CD40, CD11c, CD123. In addition serum samples from a subset of 13 acute GVHD patients and 8 controls were analysed by enzyme-linked immunosorbent assay (ELISA) to detect soluble TREM-1 (sTREM-1). For statistical analyses, two groups were compared by Pearson's test for correlations and unpaired Student's t-test considering $p<0.05$ as statistically significant.
Results: In flow-cytometry analysis we found an increased expression of CD83 on plasmacytoid DCs (125.1 +/- 26.97 *vs* 38.06 +/- 20.65) as well as on myeloid DCs (122.5 +/- 27.33 *vs* 10.13 +/- 12.86) in patients with acute GVHD. We observed no differences in the expression of the surface markers (CD86, CD40, TREM-1) on monocyte subpopulations. Nevertheless, sTREM-1 ELISA revealed a significant difference between patients with acute GVHD and the controls (244.7 +/- 35.89 *vs* 101.4 +/- 18.83). Laboratory parameters as C-reactive protein (CRP) correlated positively with sTREM-1 levels in patients with acute GVHD (r=0.692). Other laboratory parameters *e.g.* eosinophils or platelets showed no significant differences between the groups.
Summary and Conclusions: We found an increased expression of CD83 on DCs in patients with acute GVHD. Furthermore, sTREM-1 levels in this group were significantly higher as well as CRP and s-TREM-1 levels showed a positive correlation. Other parameters as eosinophils, platelets or monocyte phenotype were not informative as markers for GVHD. However, the small number of patients and the retrospective character of our study are currently limiting the possibility to draw clearcut conclusions. Nevertheless, we conclude that ELISA of sTREM-1 could be a new marker to identify patients and initiate early treatment for GVHD, and warrant further prospective studies to evaluate the clinical value of this new marker.

E1502

COMPARISON OF CONDITIONING REGIMENS FOR RELAPSED/REFRECTORY LYMPHOMA PATIENTS UNDERGOING AUTOLOGOUS STEM CELL TRANSPLANTATION: BEAM *VS* HIGH DOSE ICE

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Background: High-dose chemotherapy in conjunction with auto-SCT is widely recognized as the preferred modality of treatment for patients with relapsed or refractory Hodgkin disease or non-Hodgkin lymphoma at the time of chemosensitive relapse. Despite different drug combinations and conditioning regimens before auto-SCT, the optimal choice is undetermined. Several studies have been made to identify a regimen with superior antitumor activity and acceptable toxicity.
Aims: In this retrospectical analysis, we compared the efficacy and the toxicity of the BEAM and high dose ICE conditioning regimens in relapsed NHL and relaps/refractory Hodgkin Lymphoma patients.
Methods: A total of 83 patients with HL or NHL treated with Auto-SCT at our center between 2004-2013 are analyzed retrospectively. Patients were considered eligible for transplantation, if they met the following inclusion criteria: age between 16 and 65 years, ECOG performance status 0-2 (WHO (World Health Organization)), left ventricular ejection fraction greater than 55%, creatinine clearance greater than 60 ml/min and adequate lung and liver function. The Bearman Regimen Toxicity Scale is specifically used to rate the complications caused by chemotherapy during hospitalization for SCT.
Results: 52 patients (%62,7) received BEAM, 31 patients (%37,3) received hd-ICE. Between two groups there is no difference by age, diagnosis, transfused CD 34 yield, disease status at transplant, refractory case distribution, ECOG score at transplant. The descriptive values are in Table 1. The Renal toxicity in any grade BEAM group is%4 (1 grade I and 1 grade III) by the way the renal toxicity in ICE group is%32 (6 with grade I, 1 with grade II, 3 with grade III) (p=0,01). The GIS toxicity with any grade in BEAM group is%25 (10 with grade 1, 3 with grade 2) while in ICE GIS toxicity is%22,5 (4 with grade 1, 3 with grade 2) (p=0,415). The mucositis in BEAM group in any grade is% 26,3 while in ICE is%25 (p=0,857). Hepatotoxicity in BEAM group is%10, there is no hepatoxicity in ICE group. (p=0,041). Transplant related mortality rate is%2,4 (2 patients, 1 BEAM group, 1 ICE group). The main cause of death in ICE group is renal failure, while in BEAM group it is sepsis. In the BEAM group the mean neutrophil engraftment occurred on 11,2 days (9-21 day) while in ICE group 9,77 days (8-14 day) (p=0,047). The mean platelet engraftment in BEAM group is 14,2 days (8-41 days) in ICE group the mean is 11,4 days (8-24 days) (p=0,024)When the total patients analyzed, the CR rate in 100 days after transplant in the BEAM group is%63,5, in the ICE group%45,2 (p=0,062).In refractory Hodgkin group CR rate in 100.day, in BEAM group is%6,1, in ICE group% 7,1 (p=0,844). The partial remission rate for refractory Hodgkin group BEAM is%14, ICE is%33,3 (p=0,047).1 year overall survival rate in BEAM group%87,2, in ICE group% 90,4. 1 year PFS in BEAM group%72,7 in ICE group%58.(p=0,039). If the refractory Hodgkin group is excluded 1 year PFS in BEAM group%81,7, in ICE group%74,2. (p=0,345).In ICE group after 6,3 years follow-up, OS is%54, PFS is%45. In BEAM group after 6,3 year follow up OS is%65,3 PFS%51,9. (p=0,041).

Table 1. Descriptive factors of BEAM and hdICE group.

	Total (n=83)	BEAM (n=52)	ICE (n=31)
Age	36,7 (16-63)	39,3	32,5
Male	51 (% 61)	35	16
Female	32 (%39)	17	15
NHL	34	24	10
HL	49	28	21
Relapsed HL	30	16	14
Refractory HL	19	12	7
Mean CD 34	5,35x10^6/kg	4,55 x10^6/kg	6,64 x10^6/kg
ECOG score	0,86 (0-2)	0,91	0,83
Disease status at transplant			
CR	19	13	6
PR	45	27	18
Refractory	19	12	7

Summary and Conclusions: Despite the common use of BEAM and hdICE regimens as conditioning regimens for auto-SCT in patients with lymphoma, there have been few reports comparing these two regimens.The high dose ICE conditioning regimen provides us a shorter neutrophil and platelet engraftment time, a lower hepatotoxicity and a higher response rate for refractory Hodgkin patients whereas a marked increase in renal toxicity and a lower PFS and OS in contrast to BEAM. According to our study analysis, Hd ICE as a conditioning regimen for auto-SCT should be considered for refractory Hodgkin Lymphoma patients. Prospective and randomized studies require defining the most efficient conditioning regimens for relapsed/refractory lymphoma patients.

E1503

ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR NON-MALIGNANT HEMATOLOGICAL DISORDERS

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Background: Allogeneic hematopoietic stem cell transplantation (allo-HSCT) from a geno-identical matched sibling (MSD) is one of the most successful therapies in patients with non-malignant hematological disorders.
Aims: The aim of this study was to illustrate and report the out-come of allo-HSCT in different non-malignant hematologicconditions treated at our Institute.
Methods: This study included 273 patients with severe aplastic anemia (SAA), 152 patients with B-Thalassemia major (BTM), 31 patients with Fanconi's anemia (FA), 20 patients with congenital immunodeficiency diseases (ID), and 13 patients with inherited metabolic disor-ders (IMD) allografted from a MSD. This study included 273 patients with severe aplastic anemia (SAA), 152 patients with B-Thalassemia major (BTM), 31 patients with Fanconi's anemia (FA), 20 patients with congenital immunodeficiency diseases (ID), and 13 patients with inherited metabolic disorders (IMD) allografted from a MSD. All donors were siblings and at least 6/6 HLA matched. PBSC Donors were injected subcuta-

neously with (G-CSF, 10ug/kg daily for 5 days) and mobilized PBSC was collected the day of last injection.
Results: In SAA, the 8-year overall survival (OS) of the whole group patients was 74%. OS was significantly better in patients conditioned with fludarabine and cyclophosphamide (Flu/Cy) than in those who received cyclophosphamide and antithymo-cyte globulin (Cy/ATG) (p=0.021). Acute graft-*versus*-host disease (aGVHD) grade II-IV occurred in 15% while chronic GVHD (cGVHD) occurred in 28%. In BTM, the 12-year dis-ease-free survival (DFS) of the whole group of BTM patients was 72.4%. DFS was 74% for peripheral blood stem cell (PBSC) group compared to 64% in the BM stem cell group. The inci-dence of graft rejection was significantly lower in patients who received PBSC than in those who received BM (9% *vs* 25%) (p=0.036). AGVHD grade II-IV and cGVHD occurred in 15% and 12% of the whole group of BTM patients respectively. In FA, the 5-year OS was 64.5%. Graft rejection occurred in 10% of patients. Grade II-IV aGVHD occurred in 16% while cGVHD occurred in 4%. In ID, the 5-year OS was 62%. Graft rejection occurred in two (10%) patients. Three patients (15%) developed grade II-IV aGVHD, 2 of them progressed. In SAA, OS was significantly better in patients conditioned with fludarabine/ cyclophosphamide (Flu/Cy) than in those who received Cy/ATG (p=0.021). Acute graft-*versus*-host disease (aGVHD) grade II-IV occurred in 15% while cGVHD occurred in 28%. In BTM, DFS was 74% for peripheral blood stem cell (PBSC) group compared to 64% in the BM stem cell group. The incidence of graft rejection was significantly lower in patients who received PBSC than in those who received BM (9% *vs* 25%) (p=0.036). AGVHD grade II-IV and cGVHD occurred in 15% and 12% of the whole group, respectively. In FA, the 5-year OS was 64.5%.Graft rejection occurred in 10% of patients. Grade II-IV aGVHD occurred in 16% while cGVHD occurred in 4%. In ID, graft rejection occurred in 2 (10%) patients. Three patients (15%) developed grade II-IV aGVHD. In IMD, OS was 46% at 5 years. Graft rejection occurred in 8% of patients.
Summary and Conclusions: Allo-HSCT provides a higher DFS rate over conventional therapies for patients with non-malignant hematological disorders with prolonged survival.

E1504

FERTILITY IN OUR ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANT PATIENTS: RESULTS FOR 23 YEARS OF EXPERIENCE
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Background: Survival after allogeneic hematopoietic stem cell transplantation (Allo-HSCT) has increased due to effective supportive treatment. Infertility is a major late side effect after transplantation. However, healthy pregnancies and births were reported previously.
Aims: In this paper, we aim to present our patients' fertility after Allo-HSCT.
Methods: We retrospectively evaluated the fertility in 122 (14%) eligible patients from 849 patients who underwent Allo-HSCT between 1989-2012 at Ankara University Hematology Department and survived 2 years or more after transplantation and desired pregnancy. We used Pearsonchi-square test to compare groups. P<.05 was considered statistically significant.

Table 1. Distribution and comparison of fertile and infertile patients.

Parameters	Fertility Yes % (n=26)	Fertility No (n=96)	P
Diagnosis			
Acute leukemia (AML, ALL, sec leukemia) 0.014*	38.4% (10/26)	57.3% (55/96)	
Chronic MPD (CML, PMF)	42.3% (11/26)	28.2% (27/96)	
Bone marrow Failure (AAA, PNH, MDS)	19.3% (5/26)	5.2% (5/96)	
Others (Lymphoma, Myeloma)	0% (0/22)	9.3% (9/96)	
Relative *vs* non-relative donors	100% (26/26)	96% (92/96)	0.88
Chemotherapy prior to ASCT 0.032*	77% (20/26)	93% (89/96)	
Radiotherapy prior to ASCT	0% (0/26)	3% (3/96)	0.45
Myeloablative conditioning regimen	96% (25/26)	90% (86/96)	0.48
TBI in conditioning regimen	0.4% (1/26)	13% (12/96)	0.29
Acute GVHD	35% (9/26)	39% (37/96)	0.82
Chronic GVHD	54% (14/26)	65% (62/96)	0.37
Disease relapse	12% (3/26)	31% (30/96)	0.04*

Results: In 122 patients (56 F/66 M), the mean age during Allo-HSCT in female patients was 33.48±8.41, whereas 32.85±7.92 in male patients. 35 patients had (29%) a child whereas 16 patients/patient partners (13%) had history of pregnancy complications prior to transplantation. The mean follow-up period after transplantation was 119 months (range 18-300 months). From totally 31 pregnancies (23 partners of male patients *vs* 8 female patients), 6 of the female patients (11%) and 20 of the male patients (30%), had a child after Allo-HSCT. From 23 pregnancies of male patients partners, there were 4 abortus, 1 stillbirth, 1 gestational diabetes, 1 gestational hypertension; whereas from 8 pregnancies of female patients there were 2 abortus, 1 preeclampsia, 1 gestational diabetes were detected as pregnancy complications. The mean time from Allo-HSCT to pregnancy was 68 months (range, 4-144) in male while 63 months (range, 8-108) in female patients. All patients who had given birth after Allo-HSCT had received transplants from full-match HLA sibling donors or relatives. 9 of 56 female patients (16%) had regular menstrual cycles after transplantation. Several parameters compared between fertile *vs* unfertile patients were shown in Table 1.
Summary and Conclusions: Treatments prior to Allo-HSCT may have damaging effects on gonadal tissue and induce infertility as previously reported. The benign nature of the initial diagnosis, lack of chemotherapy regimen before transplantation, early age and no relapse of the primary disease contribute to fertility in our study. Unexpectedly, we found no relation between the myeloablative conditioning regimen, radiotherapy prior to Allo-HSCT, TBI usage and frequency, and development of acute and chronic GVHD with infertility. Fertility preservation of recipients should be considered before transplantation.

E1505

MESNA'S CONCENTRATION IN URINE CAN BE MAINTAINED BY CONTINUOUS INTRAVENOUS INFUSION OF MESNA: A BETTER METHOD TO PREVENT HEMORRHAGIC CYSTITIS IN HSCT
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Background: The clinic protective effect of continuous intravenous infusion of MESNA on cyclophosphamide-induced HC in HSCT has been demonstrated by our team. But what is the mechanism? This work was supported by the National Natural Science Foundation of China (No. 30901367, 31070866), Outstanding Young Teachers' foundation of SMU (C1031694).
Aims: To detect the MESNA's Concentration in urine and serum, hoping to illustrate the mechanism.
Methods: (1) In preliminary prospective study: 66 allogenic HSCTs were prospectively randomized into the MESNA continuous i.v. (continuous) group and the MESNA intermittent (0, 3, 6, 9h) i.v. (intermittent) group. (2) In retrospective study: 394 allogenic HSCTs were retrospectively analyzed between both groups. (3) The MESNA concentration in urine and serum of both groups were detected by HPLC. The three observation points of both group (n=5) were as follows: Before CTX and MESNA, immediately after CTX administration, 12h after the beginning of CTX.
Results: (1) Preliminary prospective study (32 *vs* 34 cases) shows that HC occurs 34.38% *vs* 0 within +30d (P=0.001), and 40.63% *vs* 5.88% within +60d (P=0.002) between intermittent and continuous group. (2) Retrospective study (160 *vs* 234 cases) shows that HC occurs 18.75% *vs* 7.26% within +30d (P=0.001) and 28.13% *vs* 12.82% within +60d (P=0.000) between intermittent and continuous group. The mean HC occurrence time are +19.77d *vs* +27.20d (P=0.037), respectively. (3) The MESNA concentration in urine is as low as zero in intermittent group 12h after CTX; while in continuous group, it varied between (439.47±34.34) μM and (268.21±24.51) μM in urine, as well as between (56.62±1.10) μM and (37.06±2.95) μM in serum.
Summary and Conclusions: Continuous i.v. of MESNA is efficient in the prevention of HC in HSCT. Keeping enough MESNA concentration in urine is the key factor in HC prevention.

E1506

PHARMACOKINETICS, PHARMACODYNAMICS, SAFETY, AND EFFICACY STUDY OF PLERIXAFOR IN CHINESE PATIENTS WITH NON-HODGKIN LYMPHOMA (NHL)
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Background: Plerixafor, a small molecule antagonist of the chemokine receptor 4, has been approved in US and Europe for use in combination with granulocyte-colony stimulating factor (G-CSF) to mobilize hematopoietic stem cells to the peripheral blood (PB) for collection and subsequent autologous transplantation in patients with NHL and multiple myeloma.
Aims: This study was designed to investigate for the first time the safety, pharmacokinetics (PK), pharmacodynamics (PD) and efficacy profiles of plerixafor with G-CSF in Chinese NHL patients.
Methods: Eligible NHL patients were given G-CSF (10 μg/kg/day subcuta-

neously) for 4 days in the morning and randomized to receive plerixafor 240 µg/kg or placebo subcutaneously on the evening of day 4. Apheresis was initiated around 1 hour after the next morning dose of G-CSF (approximately 9 to 10 hours post dosing of plerixafor/placebo). This regimen was repeated until 5x 10^6 CD34+ cells/kg were collected or for up to day 8. Venous blood samples were collected at specific time points post the first dosing on the evening of day 4 for assessing plasma plerixafor concentration and PB CD34+ cells count. Patients with ≥2 × 10^6 CD34+ cells/kg collected, underwent pre-transplantation chemotherapy followed by transplantation within 2 months.
Results: A total of 32 Chinese NHL patients [15 males, 17 females, median age 28 years, mean weight 68.5 kg, ECOG PS 0-1] were randomized and treated in this study. Overall, the demographics and baseline characteristics of these patients were similar in the 2 treatment arms. Plerixafor was rapidly absorbed after the first subcutaneous administration in these patients with peak plasma concentrations occurring at a median t_{max} of 0.47 hours and then declined with a mean terminal half-life of 3.61 hours. Mean plasma C_{max}, AUC_{0-10}, AUC, CL/F and Vz/F were 786 ng/mL, 2580 ng.h/mL, 3010 ng.h/mL, 5.73 L/h and 29.0 L, respectively. The PB CD34+ cell count generally did not change significantly over time following the placebo treatment. However, following the first plerixafor dosing on day 4, a mean 2.1 to 2.7-fold increase of the PB CD34+ cell count from baseline was observed from 2 to 10 hours with the maximum at 8 hours post dosing. The incidence of adverse events was similar between the 2 treatment arms and no unexpected adverse events were observed. 71.4% (10/14) patients in the plerixafor plus G-CSF arm, and 5.6% (1/18) patients in the placebo plus G-CSF arm achieved the target of ≥5x 10^6 CD34+ cells/kg collected.
Summary and Conclusions: The PK and PD profiles of plerixafor in Chinese NHL patients were comparable to those of NHL patients observed in previous studies. Plerixafor with G-CSF regimen was safe and effective in mobilizing CD34+ cells for transplantation in these Chinese NHL patients.

E1507

THE ROLE OF STEM CELL TRANSPLANTATION FOR PATIENTS WITH CHRONIC MYELOID LEUKEMIA (CML) RESISTANT TO TYROSINE KINASE INHIBITORS WITH BCR-ABL KINASE DOMAIN MUTATION T315I

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Background: Treatment of CML based on the use of tyrosine kinase inhibitors (TKIs). Resistance to TKIs therapy is associated with the development of kinase domain mutations. The threonine-to-isoleucine mutation at codon 315 of the breakpoint cluster region/v-abl Abelson murine leukemia viral oncogene protein fusion Bcr-Abl (T315I) is insensitive to currently available TKIs. In 2012, ponatinib (Iclusig®) was approved for treatment patients who have failed first-line therapy, have the T315I mutation, or have progressed, and became an alternative to SCT.
Aims: Evaluate the role of Stem Cell Transplantation (SCT) in the treatment of patients CML with T315I mutation.
Methods: 14 patients with TKI-resistant CML who had T315I mutations underwent 16 transplantations. At the time of SCT, 5 patients were in chronic phase, 4 patients were in accelerated phase, 1 patient was in blast crisis phase; and 4 patients were in second chronic phase. All patients had received TKI and had become resistant: 4 patients received imatinib, 6-nilotinib, 4-dasatinib. The median time from CML diagnosis to SCT was 39 months (range, 14-119 months), and the median time from fist detection of T315I to SCT was 10 months (range, 4-72 months). In 6 cases, donors were HLA-identical siblings, 8-unrelated donors. 12 patients were male, 2-female, age was 17-55 years (median 33.5 years).Conditioning regimen: in 4 cases was myeloablative, 12-reduced-intensity conditioning. Source graft in 5 cases was bone marrow (BM), 9-peripheral stem cells (PBSC), 2 BM+PBSC.
Results: Survived 5 patients of 14. The best responses after SCT were a complete molecular response (CMR) in 6 patients, and complete cytogenetic response (CCyR) in 1 patient, The best outcome was for patients who underwent transplantation in chronic phase, and all of those patients remained alive and in complete molecular remission: 3 patients in the 1 st and 1-in the 2nd reached after abolition of the basic immunosuppression and resumption of TKIs. In 1 patient reported relapse after SCT and achieving CMR-progress in blast crisis phase, currently under chemotherapy in combination with TKIs. Duration of observation for living patients to date 1- 66 months, median 7 months. Overall survival of patients who underwent SCT was 43.8%, with a median-up follow 7 months.
Summary and Conclusions: The current results indicated that SCT is an effective strategy for patients with CML who have the T315I mutation, particularly in earlier stages.

E1508

GRANZYME B EXPRESSION IN T-REGULATORY CELLS ON DAY +14 AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION (ALLO-HSCT) IS A STRONG PREDICTOR OF ACUTE GRAFT-*VERSUS*-HOST DISEASE (GVHD)

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Background: As we previously reported on ASH (ASH2014, №5871) : we found out that percent of CD4+CD25highGranzymeB+ cells among CD4+cells at day +30 after allo-HSCT in patients who never developed aGVHD was statistically higher (9,49%±2,79; p=0.003) than in patients at day of aGVHD onset (3,8%±1,78) and who developed aGVHD in future after day +30 (3,38%±1,47).
Aims: Find an easy way to assess of aGVHD development
Methods: Peripheral blood samples were collected in EDTA-tubes at day +14 after allo-HSCT. Because of WBC mediana-0.28*10^9/L (0.02-0.8) we use PBMC from 15 patients with hematological malignancies (AML=12, ALL n=1, LPD=1, CML=1, CMML=1) after allo-HSCT (n=9 with standard immunosuppression (MMF+CSA+ATG), and n=6 with cyclophosphamide (CY) on day+3,+4. All patients in CY group was in advanced or high risk disease at a time before allo-HSCT. The anti-CD4-APC-Cy7, anti-CD25-APC, anti-CD127-FITC and anti-Granzyme B-PE (Becton Dickinson, USA) antibodies were used to determine T-regulatory cells population as $CD4^+CD25^{high}$ (Gregg *et al.*, 2005) and $CD4^+CD25^{high}CD127^{low}$. We did not include anti-FoxP3 antibodies as FoxP3 is not so specific in humans and particularly after allo-HSCT due to technical difficulties, several isoforms and etc. $CD4^-$ lymphocytes ($CD8^+$ and NK-cells certainly containing granzyme B) were used as internal positive control. Geometric mean fluorescence intensity (gMFI) was used to assess Granzyme B expression. 50000 of $CD4^+$ cells were analyzed on a BD FACSCanto II (Becton Dickinson, USA).
Results: Four patients (26, 6%) (n=2 (22%) in standard IST group and n=2 (33%) in CY group) have developed aGVHD (II-IV) at a median time of +32 day (20-54) after HSCT. As we can see on chart 1: in a group with standard immunosuppression therapy (IST) the level of Granzyme B was higher in patients who never developed aGVHD in future (1653±273 gMFI units) in CD4+CD25highCD127low and (1646±287) in CD4+CD25high cell population. In a group without standard immunosuppression-only CY on day +3, +4 at dose 50 mg/kg. (Luznik *et al.*, 2008) the results was inverse. Patients who developed aGVHD in future have a higher level of Granzyme B expression (3716 gMFI units in CD4+CD25highCD127low and 4673 gMFI units in CD4+CD25high population). This data show us that we can use low granzyme B expression in T-regulatory cells on day +14 after allo-HSCT as aGVHD predictor only in a group with standard IST (Figure 1).

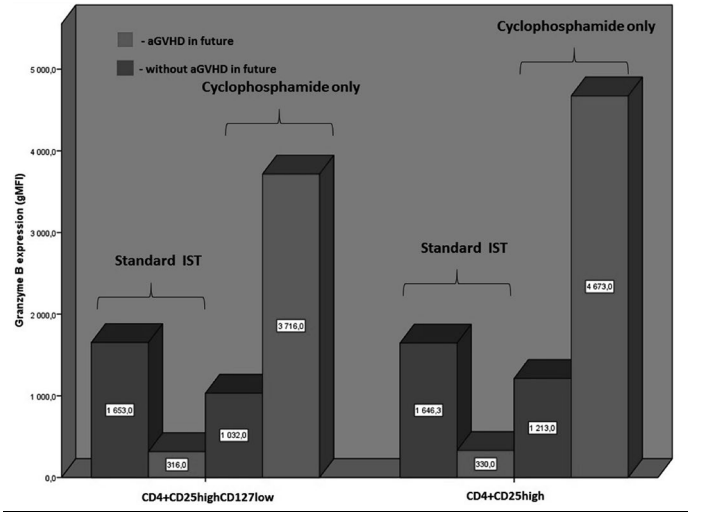

Figure 1.

Summary and Conclusions: Despite the fact that the analyzed group is small, we suggest that low expression of Granzyme B in Treg cells (despite assessment method) after allo-HSCT in patients with standard IST may predict aGVHD onset. This fact, of course, needs further investigation.

E1509

THE ROLE OF TREGS AND TIM3 IN HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Tregs in DC-CIK infusion play a critical role in immunosuppressive.
Aims: (1) To detect CD4+CD25+CD127-T cells (Tregs) from DC-CIK, which were infused to patients with high-risk or refractory hematologic malignancies after allogeneic hematopoietic stem cell transplantation(allo-HSCT) and to dis-

cuss the relationship between Tregs,Tim3 and aGVHD by detecting proportions of Tregs and expression of Tim3 in patients' peripheral blood.
Methods: (1) Detected the percentage of Tregs in DC-CIK by flow cytometry and compared the occurrence of aGVHD with patients who developed aGVHD but without DC-CIK infusion. (2) Sorted Tregs from DC-CIK by flow cytometry, using MTT assay and mixed lymphocyte reaction to research the inhibition function of Tregs from DC-CIK *in vitro*. (4) Detected the expression of Tim3 on CD4+ T cells, CD8+ T cells, CD3-CD56+ cells and CD4+CD25+ T cells in patients with or without aGVHD, respectively.
Results: (1) 32 patients with high-risk or refractory hematologic malignancies were treated with DC-CIK after allo-HSCT. 25 cases of DC-CIK were donor-derived and 7 cases were receptor-derived. (2) When the percentage of Tregs in DC-CIK was less than 5%, we defined as low proportion group, while high proportion group was defined when the percentage was more than 10%. 5%>10% was defined as middle proportion group. 10 cases were in low proportion group, 16 cases in middle proportion group and 6 cases in high proportion group. (3) 4/32(12.5%) patients with DC-CIK treatment developed I-II aGVHD and 3 of them were in low proportion group, 1 in middle proportion group. 10/27(37.03%) patients without DC-CIK treatment developed aGVHD. 7 of them developed I-II aGVHD and 3 developed III-IV. The incidence and degree of aGVHD in DC-CIK treatment group was significantly lower than none DC-CIK treatment group (P<0.05). (4) The percentage of Tregs in patients with aGVHD was lower than patients without aGVHD (P<0.05). (5) The Tim3 expression on CD4+ T cells, CD8+ T cells and CD4+CD25+ T cells were higher in patients with aGVHD than without aGVHD (P<0.05). While the expression was no significantly difference in CD3-CD56+ cells between the two groups(P<0.05).
Summary and Conclusions: (1)DC-CIK infusion after allo-HSCT can reduce the incidence of aGVHD and do not influence the effect of graft *versus* leukemia (GVL) at the same time, thus being a promising method to improve the cure rate of leukemia and providing a novel approach to cell biology treatment. (2) Tregs negatively regulate immune reaction post allo-HSCT on the occurrence and progress of aGVHD while Tim3 can promotes the occurrence and progress of aGVHD through positively regulating immune reaction. Further research will be needed to provide approach to reduce recurrence of aGVHD and gain more methods on the preventation and treatment of aGVHD.

E1510

SUCCESSFUL STEM CELL MOBILIZATION AND AUTOLOGOUS STEM CELL TRANSPLANTATION AFTER PRETREATMENT CONSISTING OF BENDAMUSTINE, PREDNISONE AND BORTEZOMIB IN PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA
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Background: Bendamustine is a bifunctional alkylating agent with low toxicity that produces both single- and double-strand breaks in DNA, and shows only partial cross resistance with other alkylating drugs. Treatment of patients with newly diagnosed multiple myeloma (MM) using bendamustine and prednisone in comparison to melphalan and prednisone results in superior complete response rate and prolonged time to treatment failure. So far, however, reliable information on stem cell toxicity and mobilization of stem cells for autologous stem cell transplantation (SCT) after induction treatment with a combination of bendamustine, prednisone and bortezomib (BPV) is missing.
Aims: Examining feasibility of induction therapy with Bendamustine, Prednisone and Bortezomib (BPV) for Stem Cell Mobilization and Autologous Stem Cell Transplantation in 35 Patients with newly diagnosed/untreated Multiple Myeloma.
Methods: A retrospective analysis of peripheral blood stem cell mobilization and autologous SCT was performed in 35 patients with MM who had received at least one cycle of a BPV induction therapy consisting of bendamustine 60mg/m^2 on days 1 and 2, bortezomib 1.3mg/m² on days 1, 4, 8 and 11, and prednisone 100mg on days 1, 2, 4, 8 and 11 between October 2008 and May 2014. The mobilization regimen consisted of cyclophosphamide 4 g/m^2 and G-CSF (2x5µg/kg). Apheresis was started as soon as peripheral CD34$^+$ counts exceeded 20x10^6/L with a harvest target of 8x10^6 CD34$^+$/kg. The minimal accepted target was 2x10^6 CD34$^+$/kg. The transplantation conditioning therapy consisted of melphalan 200mg/m^2.
Results: A median number of two (range 1-5) BPV cycles were given. The majority of patients (n=31, 89%) responded with 2 sCR, 5 nCR, 11 VGPR, and 13 PR. Three patients had MR, and 1 SD. Stem cell mobilization and harvest was successful in all patients. In 19 of 35 patients (54%) a single apharesis was sufficient to reach the target. The median number of aphareses was one (range 1-4) and the median CD34$^+$ cell-count/kg was 13.5 (range 3.2-33.1)x10^6. All patients received an autologous SCT. Engraftment was successful in 34 of 35 patients. The median time to a leukocyte count >lx10^9/L was 11 days and the time to untransfused platelet count of >50x10^9/L was 13 days. 34 patients (97%) responded after the autologous SCT with 11 sCR, 2 CR, 7 nCR, 7 VGPR, and 7 PR. The progression free survival at 18 months was 87% and overall survival was 92%.
Summary and Conclusions: Conclusion: Stem cell mobilization and autologous SCT is feasible in MM patients who have received BPV induction therapy.

E1511

MINIMAL RESIDUAL DISEASE MONITORING BY FLOW CYTOMETRY IN CLL POST ALLOGENEIC STEM CELL TRANSPLANT (ALLOHSCT)
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Background: Recent studies have demonstrated improved outcomes in patients with CLL who achieve MRD negativity within 12 months of alloHSCT.
Aims: We aimed to quantitate residual CLL after alloHSCT for the purposes of directing post-transplant chemo-immunotherapy to optimise outcomes.
Methods: We recently published a method for a single tube 10 colour flow cytometry assay to detect MRD in CLL and have used it for the quantitation of MRD post alloHSCT in a cohort of 11 patients transplanted for CLL between 2011 and 2014 at our institution.
Results: Sixty-eight MRD assessments were performed (median 5 per patient, range 1-13), the majority on PB (80%). Three patients died within the first 12 months post-transplant due to transplant related complications. Six patients attained MRD negative status very early post-transplant (range 1-6 months) and remain MRD negative and in remission at a median follow-up of 24 months (range 6-48 months). Two patients with persistent MRD at all times up to 12 months post-transplant were treated pre-emptively in response to rising MRD by tapering immunosuppression and administering escalating doses of DLIs, both without and on the final occasion with a preceding cycle of lympho-depleting chemotherapy. One patient has achieved MRD levels below 0.01% in PB within 22 months post alloHSCT. The other patient (17p- disease) has responded to treatment and has had stable residual disease up to 19 months post alloHSCT and now has progressive disease and has started treatment with ibrutinib.
Summary and Conclusions: Monitoring MRD at regular intervals gives a dynamic assessment of disease trends that is more meaningful than a single MRD assessment at 12 months post-transplant and may be a better indicator of disease trajectory. The application of MRD monitoring to guide pre-emptive immune interventions or targeted therapies shows promise in preventing clinical relapse post alloHSCT and warrants further investigation.

E1512

HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR PRIMARY IMMUNE DEFICIENY DISEASES: A SINGLE CENTER EXPERIENCE
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Background: Primary immunodeficiency diseases (PID) are rare heterogeneous genetic disorders that can result in serious complications such as life-threatening infections. Hematopoietic stem cell transplantation (HSCT) is the only curative treatment for many of PID.
Aims: The aim of this study is to present the outcome of HSCT for PID in a single center experience in Kayseri in Turkey.
Methods: Herein, we present a retrospective analysis of 20 patients who underwent HSCT for PID from 2010 to 2015. The outcomes of PID patients were analyzed retrospectively.
Results: In a 5-year period, 20 patients (Female=10, Male=10) with PID who underwent HSCT were screened retrospectively. In the presented study, the patients were included with combined immunodeficiency (CID) (n=6), chronic granulomatous disease (n=2), hemophagocytic lymphohistiocytosis (n=4), Griscelli syndrome type 2 (n=2), B-cell immunodeficiency plus bone marrow failure (n=2), severe congenital neutropenia (n=1),x-linked lymphoproliferative disease (n=1), T-cell deficiency plus relapsed Non-Hodgkin Lymphoma (n=1), and leukocyte adhesion deficiency type 1 (n=1). Eleven patients, six patients, two patients, and one patient received related HLA matched HSCT, haplo HSCT, unrelated matched HSCT, and miss matched HSCT, respectively. The median age of the diagnosis of PIDs was 10.5 months (3-33 months). The median age of HSCT was 21 months (6-80 months). The median follow-up was 3.5 months (range, 2-17 months). Eleven, six, and one patients received matched, haploidentical, and mismatched HSCT have survival rates 73% and 50%, and 100% with overall survival of 65%, respectively. In the CID patients, Immunoglobulin dependency has been observed only one patient with Artemis gene defect. The mean neutrophil engraftment was 14.25±3.08 days. The mean platelet engraftment was 24.7±11.4 days. The ratio of GVHD was 25%.
Summary and Conclusions: These results showed that haploidentical HSCT as well as HLA-matched related HSCT may provide good results for PID again. It may be concluded that HSCT can be performed with an encouraging outcome in developing countries such as Turkey in patients with PID.

E1513

DECITABINE BRIDGING ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN THE TREATMENT OF MDS RETROSPECTIVE EFFICACY ANALYSIS
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Background: A retrospective analysis of decitabine bridge therapy followed by allo-HSCT in 25 patients with MDS from July 2010 to December 2013, the First Affiliated Hospital of Soochow University, Department of Hematology.
Aims: To evaluate the efficacy of DAC (decitabine) bridge therapy followed by allo-HSCT (allogeneic hematopoietic stem cell transplantation) in patients with MDS.
Methods: We compared the 25 patients with the same period 33 patients who receive other treatments followed by allo-HSCT,comparing patients' mCR rate, OS and GVHD after DAC bridge therapy.
Results: With decitabine bridge therapy,64% patients achieved marrow complete remission,and the control group was 15.1% (64% *vs* 15.1%) (P<0.05). Decitabine bridging group of early transplant-related mortality than the control group (4% *vs* 18.2%), but the difference was not statistically significant (P=0.106). To follow-up deadline, decitabine group mortality was 12%, the control group was 30.3% (P<0.05). Decitabine group of 2-year OS was 83%, the control group was 59% (P<0.05). Patients with IPSS intermediate-1 receiving decitabine bridging the OS rate was higher, and transplant-related mortality rate less than the control group, the differences were statistically significant. GVHD occurred decitabine bridging group was 56% and the control group aGVHD incidence of 48.5% (P=0.713). aGVHD extent decitabine group is less than the control group (P<0.05). The incidence of occurrence of cGVHD was 41.7% in the control group, the control group was 31.6%.
Summary and Conclusions: decitabine bridging therapy followed by allo-HSCT in the treatment of myelodysplastic syndrome is safe and effective.

E1514

THE OUTCOMES OF HAPLOIDENTICAL HEMATOPOETIC STEM CELL TRANSPLANTATION PERFORMED WITH UNMANIPULATED DONOR STEM CELLS AND POST-TRANSPLANT CYCLOPHOSPHAMIDE IN HIGH RISK CHILDREN

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Background: The patients who have a rare frequency of HLA group with a low chance for a matched donor or need urgent HSCT, haploidentical transplantation from the relative donors promises as a good alternative. In recent years, some of haploidentical transplantations are performed with unmanipulated bone marrow or peripheral blood stem cells which the GVHD prophylaxis includes a more intense chemotherapy approach. This approach is now more popular by post-transplant high dose cyclophosphamide (PTCY). However, there is limited experience in children.
Aims: In this study, we present the children who transplanted haploidentically in our center, with unmanipulated bone marrow or peripheral blood stem cells and GVHD prophylaxis with post-transplant high dose cyclophosphamide.
Methods: We assessed 16 HSCT in 15 patients retrospectively, who underwent haploidentical related HSCT with unmanipulated bone marrow or peripheral blood stem cells and used post-transplant high dose cyclophosphamide for GVHD prophylaxis. Five of patients were male and 10 were female between 3.5-17 years old, median 9 years old. One patient was AML developed after Ewing Sarcoma and one was ALL progressed from CML. Another patient was transplanted for aplasia developed after a haploidentical transplantation from his mother which had performed with CD34 positive selection. One patient underwent second haploidentical transplantation because of relaps. Four patients were in the first complete remission, five were in the second and three were in the third in the transplantation date. Donors were their mother in ten transplantation, the father in five, and a sibling in one patient. For the conditioning regimen BU+FLU+Etoposid was used for seven patients and two patients with BU+FLU+ATG/, BU+CY+Mel,/BU+FLU+ATG and FLU+CY+Mel, and one patient with BCNU+Etoposid+ARA-C+Mel. Fifty mg/kg CY on the 3rd and 5th day and CsA or tacrolimus with MMF or MP were also used for GVHD profilaxis. The source of stem cells was bone marrow in six transplantation while bone marrow and peripheral blood stem cell were used in ten transplantation. The median count of TNC, MNC, CD34+ and CD3+ was $12{,}9x10^8$ (4,1-39), $8{,}8x10^8$ (4,8-14,3), $5{,}9x10^6$ (2,5-16) and $2{,}9x10^8$ (1,1-7,8), respectively.
Results: All patients engrafted with a median of 16 days (16-23) and 18 days (11-37) for neutrophil and thrombocyte recovery, respectively. Grade 1 acute GVHD developed in one patients, and grade 2 in 4 and 3 in 3 patients, while limited chronic GVHD developed in two patients. Three patients had hemorrhagic cystitis and four developed VOD. Two patients were lost in the first 100 days because of sepsis (TRM 12, 5%). After 100 days, a patient died of progression of the primary disease and one patient was lost because of pneumonia. One patient with diagnosis of Hurler showed graft rejection. The rest ten patients (66.6%) are disease-free (OS is 73.3%) and on the follow up of median 10 months (between 4-20 months).
Summary and Conclusions: The advantages of using unmanipulated hematopoetic stem cells are low laboratory costs, no worry for waiting for a suitable donor, and less risk for graft failure or T cell deficiency. Our results are consistent with these circumstances in our high risk patients and encouraging for haploidentical HSCTs. However our results should be accepted as a preliminary report with these few patients. We think that, it should be studied for more patients and also for comparing with other haploidentical HSCT modalities with longer follow up.

E1515

THE EBMT RISK SCORE CAN PREDICT THE OUTCOME AFTER ALLOGENEIC HEMATOPOIETC CELL TRANSPLANTATION FOR ACUTE MYELOID LEUKEMIA AMONG EGYPTIAN PATIENTS

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Background: Allogeneic hematopoietic stem cell transplantation (HSCT) is a potentially curative therapy for many patients diagnosed with acute myeloid leukemia (AML). However, the success of this procedure has been limited by high transplant-related mortality. Moreover, it is a high-cost procedure and can present a financial challenge for patients and health care systems in developing countries. Risk stratification and outcome prediction among AML patients receiving transplantation are required to guide decision making. The European Group for Blood and Marrow Transplantation (EBMT) risk score, previously devised for chronic myeloid leukemia, has been validated as a simple tool to predict the outcome of allogeneic HSCT for other acquired hematological disorders including AML.
Aims: The aim of this study was to evaluate the predictive validity of the EBMT risk score to the outcome of allogeneic HSCT in a cohort of Egyptian patients diagnosed with AML.
Methods: This is a retrospective single-center observational study. It was conducted using data of 291 consecutive patients diagnosed with AML and underwent first allogeneic HSCT between January 1997 and December 2008 at Nasser Institute Hospital. Patients diagnosed with AML-M3 were excluded. Median age of all patients at time of transplantation was 21 years (range, 1 to 57 years); with 114 patients (39%) aged 18 years or younger and 113 patients (39%) were female. HCT was performed in first complete remission for 209 patients (72%) and in second remission or beyond for 82 patients (28%).The majority of patients (89.6%) received myeloablative condition regimen. All donors were HLA-identical siblings. Peripheral blood stem cells were used as graft source in 96.2% of patients. Median follow-up duration among survivors was 12 months (range, 0-107 months). We calculated the EBMT risk score for all the patients and the overall survival (OS) and transplant-related mortality (TRM) were analyzed. Required parameters for the calculation of the score were extracted from patients' database and files.
Results: In univariate analysis the EBMT risk score was a significant variable predictive of OS. Survival decreased with increasing EBMT risk score. The projected 2- year OS rates were 71%, 50%, 37%, 25%, and 0% for scores 0, 1, 2, 3 and 4/5, respectively (p<0.0001). The EBMT risk score also was highly predictive of TRM. The 2-year cumulative incidence of TRM rates were 23%, 48%, 54%, 65% and 100% for scores 0, 1, 2, 3, and 4/5, respectively, (P<0.0001). In multivariate analysis the EBMT was confirmed as prognostic factor for both OS (p=0.03, HR=0.5, 95%CI=0.27-0.92) and TRM (p=0.0007, HR=0.39, 95%CI=0.23-0.67) (Figure 1).

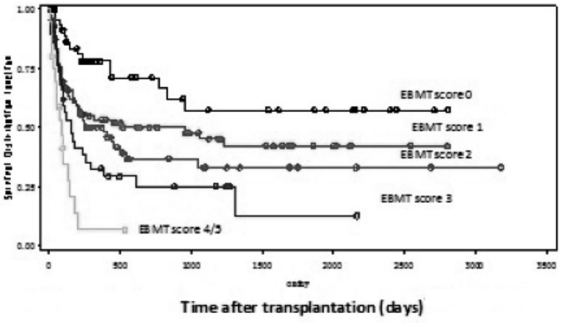

Figure 1. Probability of survival for patients who underwent allogeneic HSCT for AML at Nasser Institute, by the EBMT risk score.

Summary and Conclusions: The EBMT riak score is shown to be predictive of the outcome after allogeneic HSCT among Egyptian patients with AML and can be incorporated into risk-adapted decision-making before transplantation.

E1516

FACTORS AFFECTING SURVIVAL IN PATIENTS WITH ACUTE LEUKEMIA WHO RECEIVED DONOR LYMPHOCYTE INFUSION IN THE TREATMENT OF FIRST RELAPSE AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

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Background: Leukemia relapse after allogeneic stem cell transplantation (SCT) remains an important problem. Beside the best treatment option in patients with relapsed acute leukemia is not certain; cytoreductive chemotherapy followed by donor leukocyte infusion (DLI) is one of the treatment modality in relapse patients.
Aims: In this study we evaluated the factors affecting on overall survival (OS) in allogeneic SCT patients who received DLI after first relapse.
Methods: Acute leukemia patients who were in their first relapse after allo-SCT were evaluated retrospectively. The data of 54 patients [26 acute myeloid lekemia (AML) and 28 acute lymphoblastic leukemia (ALL)] between March 2004 and March 2013 was collected from patients' files and also confirmed with the data by computed file system. In this retrospective study 54 relapsed patients in their first relapse after allo-SCT who received fludarabine based chemotherapy followed by DLI were evaluated.
Results: The probability of OS after diagnosis for AML at 1, 3, 6, 12 and 24 months were 96%, 92%, 76%, 56% and 27%, respectively and for ALL were 100%, 92%, 81%, 62% and 25%, respectively. The median OS was 17 (1-66) months for AML and 10 (3-39) months for ALL; there was not a statistically significant difference between these patients (p>0,05) at the end of follow up. A total of 10 patients developed acute GVHD after DLI (18.5%). GVHD was seen more frequently in the patients who received more than one DLIs than the patients who received only one DLI (p=0.7), there was not a significant increase at probability of OS in patients who had acute GVHD after DLIs (p>0.05). Increased number of DLIs (p=0,001), and increased time period from transplantation to DLI (p=0,005) were predictive for survival in cox multiple regression analysis.
Summary and Conclusions: The chemotherapy regimens especially including lymphocyte depleting agents, such as fludarabin, before DLI may be effective in the treatment of patients with relapsed acute leukemia after allo-SCT. This study showed that increased number of DLI improves the OS, and efforts to enhance the number DLIs should be considered in leukemia patients that relapsed after allo-SCT.

E1517

CD4+ CELL COUNT ON DAY+30 PREDICT OVERALL SURVIVAL AND TRANSPLANT RELATED MORTALITY IN ACUTE LEUKAEMIA PATIENTS AFTER ALLOPBSCT

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Background: Allogeneic peripheral stem cell transplantation (alloPBSCT) from a related or unrelated donor is a well established strategy for patients with acute leukaemia. However, this procedure is associated with increased morbidity and mortality because of graft *versus* host disease (GvHD), immune reconstitution impairment and a high risk of infections.
Aims: In our study we evaluated the lymphocyte subset recovery after alloPBSCT and its impact on transplant related mortality (TRM) and overall survival (OS) in acute leukaemia patients.
Methods: We evaluated the immune reconstitution of CD3+/CD4+, CD3+/CD8+ and NK cells performed at 30, 100, 180 and 360 days after alloPBSCT in 122 patients with acute leukaemia. Patients were transplanted with unmanipulated PBSC from an HLA matched related donor (MRD) (n=85) or an HLA (8/8) matched unrelated donor(MUD) (n=37). Median age was 38 years (range 18-61); diagnoses were acute myeloid leukaemia (n=96) and acute lymphoblastic leukaemia(n=26); 80% of patients underwent myeloablative conditioning (busulphan, cyclophosphamide in MRD and busulphan, cyclophosphamide and ATG in MUD) and 20% underwent reduced intensityconditioning (busulphan, fludarabine, ATG).
Results: The median counts of CD3+/CD4+ were 98, 160, 200, 262 µl at 30, 100,180 and 360 days, respectively. The median counts of CD3+/CD8+ were 180, 350, 500 and 670 µl at 30, 100, 180 and 360 days, respectively. The median counts of NK cells were 110, 260, 270 and 260 µLat 30, 100, 180 and 360 days, respectively. Considering the entire patient population the median cell count rose above 200 µl after day 180 for CD3+/CD4+ and after day 45 for CD8+cells; the median NK cells count rose above 100 µl after day 30. The median CD3+/CD4+ cell count on day +30 for the entire patient population was 98 µl (range 20-190) and TRM at 2 years was significantly higher in patients not achieving this CD4 cell count (38% *vs* 15%, p<0.001); patients with a low CD3+/CD4+ count (<98 µl) on day +30 had a higher risk of dying of infections (30% *vs* 11%, p>0.003). Median OS in patients not achieving CD4+ of 98 µl at 30 days was 40 months while median OS in patients with more than 98 CD4+ at 30 days was not reached. Univariate analysis at 100 days showed a significant association between CD4 cell recovery and transplant from a related donor (p=0.003), myeloablative conditioning (p=0.04) and absence of aGvHD (grade II-IV)(p=0.002). In multivariate analysis, transplant from a related donor (p=0.05) and absence of aGvHD(grade II-IV) (p=0.002) were significantly associated with a better CD4 cell recovery. CD3+/CD8+ and NK cells recovery did not correlate with a different TRM risk or OS.
Summary and Conclusions: Our results support the relationship between immune reconstitution of CD4+, OS and TRM. The CD4+ cell count on day +30 is able to predict OS and TRM after myeloablative alloPBSCT in acute leukaemia patients.

E1518

HBV CLEARANCE IN HBSAG+ PATIENTS AFTER ALLOGENEIC STEM CELL TRANSPLANTATION IS HERALDED BY GVHD OF LIVER

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Background: Alloimmune reaction has been reported to be effective in clearing chronic hepatitis B (HBV) infection. However, the successful rates of HBV surface antigen (HBsAg) clearance after allogeneic hematopoietic stem cell transplantation are not clear.
Aims: We surveyed the seroconversion rate and chronologic change of HBsAg titers retrospectively from the hematologic malignancy patients with HBsAg+ underwent allogeneic stem cell transplantation at our transplant unit.
Methods: Between 2001 ad 2013, we have twelve patients of HBsAg+ out of 210 patients undergoing allogeneic stem cell transplantation. All of them were under anti-HBV prophylaxis with either lamivudine or entecavir and included myeloablative or reduced intensity conditioning regimens for AML, ALL, NHL, HL, and multiple myeloma patients.
Results: In these twelve HBsAg+ patients, 3 from HBsAg+ donors and one of them got seroconversion with HBsAg cleared. The other 9 patients received stem cells from HBsAg- and anti-HBsAb+ donors with 5 of them got successful clearance of HBsAg and HBV DNA undetectable (55.5%) with the earliest seroconversion seen on sixth month after transplantation. Three of them got malignant disease recurrence in the first year. None of our patients got symptomatic flare of hepatitis B or even fulminant hepatitis. In four patients, we can see the clearance of HBsAg is heralded by graft *versus* host disease with low or undetected HBV DNA (Figure 1).

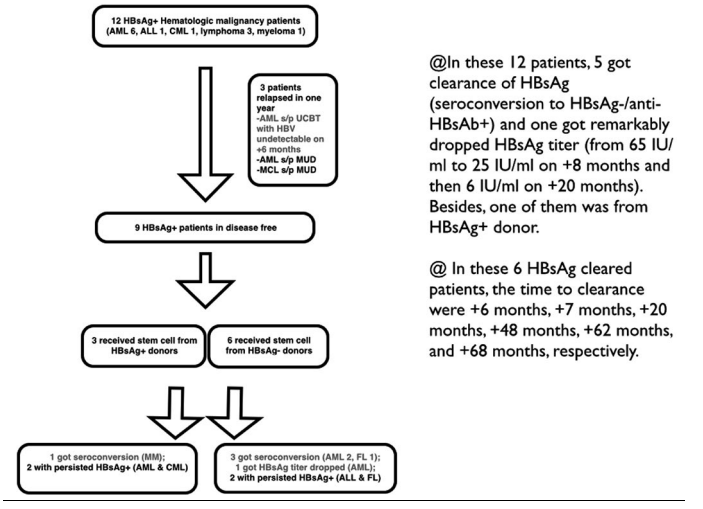

Figure 1. Disposition of HBsAg+ patients underwent allogeneic stem cell transplant in cohort 1.

Summary and Conclusions: HBsAg+ hematologic malignancy patients can attain high successful rate of seroconversion when underwent allogeneic stem cell transplantation from HBsAg-/anti-HBsAb+ donors via graft *versus* host effect. The risk of fulminant hepatitis flare is minimal in these patients whom anti-HBV prophylaxis performed.

E1519

OUTCOMES OF CORD BLOOD TRANSPLANTATION WITH NON-TOTAL BODY IRRADIATION CONDITIONING REGIMEN CONSISTING OF FLUDARABINE AND BUSULFAN FOR PATIENTS WITH ADVANCED HEMATOLOGYIC MALIGNANCIES

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Background: Cord blood transplantation (CBT) has increasingly become an alternative therapy in patients who lack suitable related or unrelated donors with advanced hematologic malignancies. Non-total body irradiation (TBI) conditioning regimen has a high degree of usability for medical institutions where TBI cannot be performed promptly. However, in contrast to bone marrow or peripheral blood stem cell transplantation, the optimal non-TBI conditioning regimen is not established in CBT yet.
Aims: We investigated the outcomes of CBT with non-TBI conditioning regimen consisting of fludarabine and busulfan in patients with advanced hematologic malignancies.
Methods: Patients who underwent their first CBT using single unit umbilical cord blood between April 2004 and December 2014 in Teikyo University Chiba Medical Center and Tsukuba Memorial Hospital were included for the present study. They were treated with non-TBI conditioning regimen consisting of fludarabine 30 mg/m^2 for 6 days and busulfan 0.8 mg/kgx 4 for 2 or 4 days. Graft-*versus*-host disease (GVHD) prophylaxis was provided by tacrolimus only.
Results: A total of 53 patients received CBT. The median age was 62 years old (range, 26-74 years). Acute myeloid leukemia was the predominant diagnosis (53%, n=28), followed by Non-Hodgkin lymphoma (n=8). The remaining diagnosis included multiple myeloma (n=7), myelodysplastic syndrome (n=6), primary myelofibrosis (n=2) and chronic myeloid leukemia (n=2). Only 16 patients (30%) were in remission at transplantation. Median numbers of infused total nucleated and CD34-positive cells were 2.9x 10^7 /kg (range, 1.9-5.5) and 0.58x 10^5 /kg (range, 0.2-2.1), respectively. The cumulative incidence of neutrophil engraftment was 62% (95% confidence interval [CI], 48%-74%), with a median time to recovery of 23 days (range, 14-47 days). The cumulative incidence of grade II-IV and grade III/IV acute GVHD was 22.9% (95% C.I., 12.5-35.0%) and 5.7% (95% C.I., 1.5%-14.3%), respectively. Twelve patients developed chronic GVHD and the cumulative incidence of chronic GVHD was 20.8% (95% C.I., 10.8-32.9%). At one year, the cumulative incidence of treatment related mortality was 42%. The leading cause of non-relapse mortality was infection (n=9) followed by graft failure (n=3), acute GVHD (n=3), chronic GVHD (n=2) and hemorrhage (n=2). At one year, overall survival was 25.0% (95% C.I., 13.9-37.6%). Univariate and multivariate analysis revealed that poorer overall survival was significantly associated with age ≥70 (p<0.01). Overall survival at 1 year among patients younger than 70 years old was 28% (95% C.I., 15.8-42.0%) (Figure 1 and Figure 2).

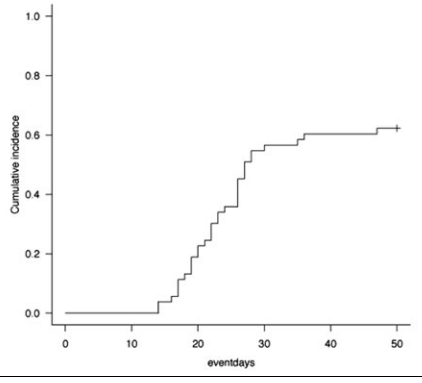

Figure 1. The cumulative incidence of neutrophil engraftment.

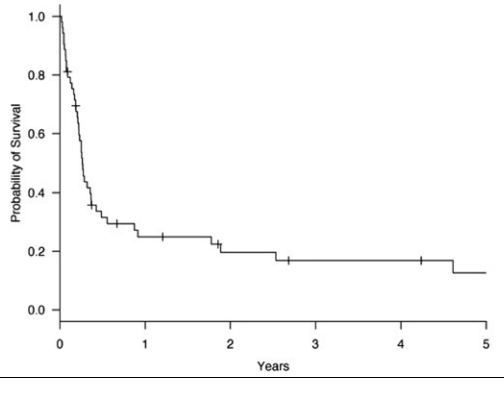

Figure 2. Kaplan-Meier estimates of overall survival.

Summary and Conclusions: Our results suggest that conditioning regimen with fludarabine and busulfan in CBT is a feasible option for patients younger than 70 years in medical institutions where TBI cannot be performed.

E1520

A PORTABLE MICROSCOPIC CELL COUNTER (ADAM II) FOR ENUMERATING CD34+ CELLS

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Background: Accurate determination of CD34+ cell numbers is of considerable clinical importance and essential in the field of stem cell transplantation. Several flow cytometry assays for enumerating CD34+ cell have been proposed, but differences in gating methods between users lead to variability in the results. Also flow cytometry assays require expensive instrumentation, have high reagent costs, and, in most cases, are technically difficult. Therefore, a new instrument that produces CD34+ cell counts more simply and reproducibly is required.
Aims: We previously developed a new CD34+ cell counting device (Adam II) that uses a microchip and microscopic cell counter. Our aim here was to evaluate the Adam II as a CD34+ cell counter by comparing it with a conventional flow cytometer.
Methods: We used peripheral blood stem cell (PBSC) samples from 17 patients with hematological malignancies and 11 cryopreserved umbilical cord blood (UCB) samples. Each individual donor provided written informed consent prior to us obtaining the samples, and the study was approved by the Institutional Review Board of Hanyang University Hospital. To assess the precision and linearity of the Adam II, CD34+ cell counts obtained with it were compared with those from flow cytometry. For measurements, samples were introduced into the Adam II CD34 tube containing lyophilized fluorescence mixtures. The reacted samples were then loaded into the chips and read. For flow cytometry, we used a Stem Cell Enumeration kit (BD Biosciences, Franklin Lakes, NJ) to stain the samples, and the relative proportions of CD34+ cells were obtained with a FACS Calibur (BD Biosciences). To assess linearity, samples were serially diluted with mononuclear cells separated from the peripheral blood of a healthy adult, and to assess reproducibility, some samples were counted 10 times, and coefficients of variations (CVs) were calculated.
Results: Linear regression analysis revealed a close correlation between the data for CD34+ cell fractions (%) obtained with the Adam II and those obtained with the FACS Calibur for both PBSCs (r^2=0.98) and UCBs (r^2=0.93). The two methods also gave very similar results for the viability of the PBSCs (r^2=0.94). A dilution test of the Adam II method gave a linearity coefficient of r^2=0.99, and the method proved to be reliable in the samples serially diluted over the expected range: thus, four PBSC dilutions at CD34+ cell concentrations of 0.44, 0.18, 0.08 and 0.03% had C*V*s of 7.86, 13.66, 20.83 and 38.46%, respectively.
Summary and Conclusions: We have established a close correlation between CD34+ cell counts and viability assays with the FACS Calibur and the Adam II. This suggests that the Adam II CD34+ cell counting device could be useful for stem cell assays given its advantages of reproducibility, accuracy, convenience, and low expensive.

E1521

LONG-TERM OUTCOMES FOLLOWING REDUCED-INTENSITY STEM CELL TRANSPLANTATION: THE CRITICAL IMPORTANCE OF THE DEVELOPMENT OF GRAFT-*VERSUS*-HOST DISEASE

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Background: Allogeneic stem cell transplantation (AlloSCT) is the most effective treatment for a wide variety of hematologic diseases. The development of reduced-intensity conditioning transplantation (RICT) has allowed alloSCT to be used in patients (pts) of advanced age or with comorbidities.
Aims: The study objective was to determine the incidence of acute/chronic graft-*versus*-host disease (GVHD), treatment-related-mortality (TRM) and relapse rate and to calculate Kaplan-Meier estimates of overall survival (KM OAS) following RICT.
Methods: An IRB-approved retrospective chart and data base review was performed on all pts that underwent RICT at Vancouver General Hospital between 05/01 and 12/13. Final analysis date was August 1, 2014.
Results: During the study period, 220 pts underwent RICT with all patients signing IRB-approved consent forms; there were 150 males and 70 females with median age of 58 years (range 18-68). Diagnoses were CLL (73 pts), lymphoma (42 pts), AML (40 pts), myeloma (25 pts), MDS (10 pts), myelofibrosis (8 pts), CML (4 pts) or other (18 pts). A total of 232 RICT were performed-209 pts had one, 11 pts had two [10 for graft failure (GF)] and 1 pt had 3 (for GFx 2) RICT. Conditioning was Fludarabine (Flu)/Busulfan (Bu) (71 pts), Flu/Cyclophosphamide (Cy) (64 pts), Flu/Bu/Campath (42 pts), Flu/Bu/ATG (37 pts) or other (18 pts); GVHD prophylaxis was Cyclosporine

and either Methotrexate (228 pts) or Mycophenolate (4 pts). Donor source was matched related (MRD, 140 pts) or unrelated (MUD, 59 pts) and mismatched related (7 pts) or unrelated (MMUD, 26 pts). Following RICT, 59%, 53% and 42% of patients did not require red cell, platelet or any transfusion support, respectively. Neutrophils >0.5 occurred a median of 18 days (range 0-138) post-RICT (19 pts never went <0.5). Acute GVHD occurred in 32% of MRD, 38% of MUD and 58% of MMUD pts and chronic GVHD occurred in 72%, 55% and 54% of these cohorts, respectively. GF was identified 13 times in 11 pts; 6 of these pts are alive and well. KM OAS after FluCy MRD RICT is 41% at 10 years, 51% at 5 years after BuFlu MRD RICT and 53% at 5 years after MUD/MMUD RICT. There are 86 pts alive in continuous complete remission (CCR), 85 pts relapsed (13 of whom are still alive in remission) and 48 pts (21%) died of treatment-related complications (TRM). MUD/MMUD pts receiving Campath had a lower incidence of acute (32%) and chronic (44%) GVHD compared to those receiving ATG (62% and 76%, respectively) but a higher incidence of relapse (50% *versus* 22%, respectively) and a lower 5-year OAS (48% *versus* 68%, p=0.14). In the entire cohort, 11% developed acute GVHD only, 39% chronic GVHD only, 26% both and 24% neither. Outcome was most favourable, and similar, for the "chronic only" and "both" groups (combined N=151) with 78 pts (52%) in CCR; relapse and TRM occurred in 45 (30%) and 27 (18%) pts, respectively (1 pt had GF). In the "acute only" group, 7 pts (27%) remain in CCR, 12 pts relapsed (46%) and 6 pts (23%) experienced TRM (1 pt had GF). In the "neither" group, 30 pts (55%) relapsed, 13 pts (24%) died of TRM, 11 pts (20%) had GF and 1 pt (2%) remains in CCR.

Summary and Conclusions: RICT results in long-term survival for many pts with hematologic malignancies with similar outcomes seen with MRD and MUD/MMUD. In the latter group, *in vivo* T-cell depetion with ATG is associated with better outcomes than with Campath due to a lower risk of relapse with ATG. Despite the morbidity associated with GVHD, its development (particularly the chronic form) is critical to a successful outcome with RICT.

E1522

AUTOLOGOUS STEM CELL TRANSPLANT IN AL AMYLOIDOSIS: AN EFFECTIVE TREATMENT FOR SELECTED PATIENTS

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Background: High-dose melphalan (HDM) followed by autologous stem cell transplant (ASCT) has been routinely used as a treatment option for systemic light chain AL amyloidosis since the first report in mid-1990s. The high treatment-related mortality (death before post-transplant day 100) reported in literature (11-40% in different papers) has decreased over the last years with the improvement in patient selection. Particularly, in the largest Mayo Clinic series of 422 subjects, patients with cardiac troponin-T <0.06 ng/L and NT-proBNP <5000 ng/L had TRM <1%; besides, patients achieving hematologic complete response/very good partial remission (CR/VGPR) had superior overall survival than those achieving hematologic partial response (PR) and no response.

Aims: To evaluate the outcome of the autologous stem cell transplant in patients with AL Amyloidosis

Methods: Twenty-one ASCT were performed between 2001 and 2014 in 16 patients with AL amyloidosis (10 male and 6 female), in our center. The median age at the time of ASCT was 58 years (range 38-68). Ten patients had 2 or more involved organs. All 16 patients had: normal troponin-I value, NT-proBNP <5000 ng/L, PS 0-1, cardiac ejection fraction over 50%, CO diffusion capacity over 50%; three had creatinine clearance <50 ml/min. Five patients collected stem cells after cyclophosphamide (1,5-4 g/m^2), the others after G-CSF alone. Seven patients proceeded directly to ASCT without "induction therapy". Five patients received CyBorD (5/5 obtained hematologic PR, 3/5 organ response) and 4 patients other induction regimen (without any response). Eleven patients were treated with ASCT as 1st line therapy, 5 in 2nd or 3rd line. Patients received melphalan 200 mg/m^2 (12 ASCT) or dose adjusted HDM (100-140 mg/m^2, 9 ASCT), depending on clinical decision. Five patients received double ASCT.

Results: Median follow up is 39 months (range 3-168); only 1 patient died (+48 months), for progression. All the others were alive at the last visit. Overall hematologic and organ response rate after ASCT were 81% and 56%, without significant differences between patients treated with "induction therapy" before HDM and patients that proceeded directly to ASCT. Two of 5 patients with double ASCT improved response after second course (after 82 and 144 months follow up they maintain a hematologic CR without organ involvement). Median hospitalization for ASCT was 24 days (range 17-45), without any threatening-life side effect. Eight of 16 patients needed new therapy for progressive disease after a median follow up of 22 months (range 6-52): 5 of them had achieved hematologic PR after ASCT, 1 patient achieved hematologic VGPR but no organ response and 2 had no hematologic response. Eight patients are in follow up without other therapy after ASCT: 1 had optained no response, 2 hematologic PR, 1 VGPR and 4 CR. These 4 patients in persistent hematologic CR performed ASCT as 1st line therapy and 3 of them have long follow up (+82, +144, +168 months).

Summary and Conclusions: HDM followed by ASCT is an effective treatment option in AL-amyloidosis, which may be effective even in patients non responding to previous treatment with non myeloablative drugs. According to the data of the literature, our experience suggests that a careful selection of patients is critical for good outcomes and that particular cardiac biomarkers (cardiac troponin-T <0.06 ng/L and NT-proBNP <5000 ng/L) are useful to guide the choice of therapy. Also in our patients, hematologic CR after HDM seems to be an important factor for long survival without other therapy. Therefore, consolidation therapy (IMiDs and proteasome inhibitors) should be considered for patients who do not obtain at least a VGPR/CR after HDM. Finally, in our experience the best results are in patients treated with ASCT as 1st line therapy.

E1523

TROUGH LEVEL MONITORING OF INTRAVENOUS BUSULFAN TO ESTIMATE THE AREA UNDER THE PLASMA DRUG CONCENTRATION-TIME CURVE IN PEDIATRIC HEMATOPOIETIC STEM CELL TRANSPLANT RECIPIENTS

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Background: Busulfan is an alkylating agent that plays an important role in myeloablative preconditioning regimens in the setting of hematopoietic stem cell transplantation (HSCT). A relationship between the busulfan dose intensity, expressed as the area under the plasma busulfan concentration *versus* time curve (AUC), and certain HSCT outcomes has been described. Although intravenous busulfan is considered to have a much more predictable pharmacokinetic profile than oral busulfan, there remains strong variation, particularly in children. Therapeutic drug monitoring for appropriate dose adjustment is therefore recommended in all patients treated with regimens containing high-dose busulfan.

Aims: Traditionally, the busulfan AUC is calculated using six plasma concentrations obtained within the six hours following busulfan infusion and before the second dosing of busulfan ($AUC_{0-\infty}$). The application of fewer samples to calculate the busulfan AUC would result in cost savings (workload, assay costs) and reduce patient inconvenience related to obtaining blood samples. In this study, we investigated the correlation between the busulfan concentration and $AUC_{0-\infty}$ to determine the best clinical method for monitoring the drug level.

Methods: Intravenous busulfan was infused via a central venous catheter for two-hour periods every six hours for a total of 16 doses over four days. Following the administration of a single dose of busulfan as a test dosing and the first dosing of busulfan for the conditioning regimen, blood samples were collected from each patient at six time-points (1, 2, 2.25, 2.5, 3 and 6 hours from the start of the infusion). The busulfan concentrations in the plasma were measured using high-performance liquid chromatography. The busulfan AUC from 0 to infinity ($AUC_{0-\infty}$) was calculated according to the trapezoidal method using MOMENT program, employing all six of the available data points. The relationships between the $AUC_{0-\infty}$ and plasma busulfan concentrations obtained at individual time points after intravenous administration were assessed using Pearson's correlation coefficient. Limited sampling strategies (LSSs) using one, two, or three plasma busulfan concentrations were developed by multiple linear regression. The associations between the actual $AUC_{0-\infty}$ and the predicted $AUC_{0-\infty}$ based on LSS were described using the adjusted coefficient of determination (r^2); values of >0.9 were considered to be acceptable. The degree of bias and precision of the LSS was measured using the mean prediction error (bias) and root-mean-squared prediction error (precision) methods. Bias and precision values of <15% were considered to be acceptable.

Results: Pharmacokinetics samples (total: 46 AUCs) were collected from all 29 patients. The mean (±standard deviation) $AUC_{0-\infty}$ was 1,124 (±321) μM min. The relationship between the AUC calculated using available all time-points (actual $AUC_{0-\infty}$) and the AUC predicted using each one time-point at 1 hour (C_1), 2 hours (C_2), 2.25 hours ($C_{2.25}$), 2.5 hours ($C_{2.5}$), 3 hours (C_3) or 6 hours (C_6) after the start of the two-hour infusion (predicted $AUC_{0-\infty}$). The $AUC_{0-\infty}$ predicted based on C_6 (trough level) was significantly correlated with, and not statistically different from, $AUC_{0-\infty}$ (r^2=0.929, P <0.0001, mean bias 0.282% (95% confidence interval: -2.01 to 2.57), precision: 7.91%). These results met the criteria for acceptability (adjusted r^2 >0.9, mean bias <15% and precision <15%). In contrast, the limited AUCs derived from the other sampling points (C_1, C_2, $C_{2.25}$, $C_{2.5}$ and C_3) did not meet the criteria. LSSs using one- (C_6), two- (C_2 and C_6), or three-sample (C_2, C_3 and C_6) were developed by multiple linear regression that showed excellent agreement with $AUC_{0-\infty}$.

Summary and Conclusions: We created the LSSs using one, two, or three time-point to estimate the $AUC_{0-\infty}$ in children receiving intravenous busulfan prior to HSCT. These $AUC_{0-\infty}$ well predicted the actual $AUC_{0-\infty}$ with good precision and little bias. In single-point sampling strategies, the $AUC_{0-\infty}$ predicted by the LSS (C_6) and $AUC_{0-\infty}$ using the six data points demonstrated excellent agreement.

E1524

A BENDAMUSTINE CONTAINING FLUDARABIN-BASED REDUCED INTENSITY CONDITIONING FOR ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN REFRACTORY/RELAPSED CLASSICAL HODGKIN LYMPHOMA

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Background: Autologous hematopoietic stem cell transplantation (autoHSCT) is the standard treatment for pts with relapsed and refractory cHL (rr-cHL) but 20-30% responders to second line chemotherapy eventually have relapse. Allogeneic hematopoietic stem cell transplantation (alloHSCT) is a method with high curative potential in rr-cHL, but the role of alloHSCT is still a subject of controversy because of high treatment-related mortality (TRM) of myeloablative conditioning regimens (CR). Reduced intensity conditioning (RIC) regimens have emerged as a potential option with acceptable TRM in chemosensitive pts. High toxicity of melphalan containing RIC in highly pretreated rr-cHL induces to look for a new ways for CR. Bendamustin has a good safety profile as a CR in non-Hodgkin lymphomas[1] and showed high efficacy as a salvage monotherapy in rr-cHL[2].

Aims: Efficacy and toxicity of a bendamustine containing RIC for alloHSCT in pts with rr-cHL

Methods: For 3-year period (2012-2014) 13 pts with rr-cHL alloHSCT with a bendamustine containing RIC have been performed. This RIC was modification of BFR regimen[1] and consist of intravenous (i.v.) fludarabine (Flu) at a total dosage of 90 mg/m2 over three days and bendamustine (Be) i.v. at 130 mg/m2/day during the same three days. Primary end-points were engraftment/rejection rate, organ toxicity, incidence of GvHD and infection complication. Secondary end-points were a progression-free survival (PFS), overall survival (OS) and treatment-related mortality (TRM) at 100 days and 2 years after alloHSCT. Pts characteristics are outlined in Table 1.

Results: The engraftment was achieved in 100% of pts with median day +20 (14-44). Rejection was not registered. Cumulative incidence of mucositis grade 3-4 was 7,7%, severe renal toxicity-7,7%. There was no severe hepatic toxicity, but VOD was observed in one patient. Infection complication: severe bacterial infection/sepsis were registered in 15% of pts, CMV reactivation in two pts and one of them had CMV reactivation before alloHSCT. Probable invasive aspergillosis (IA) according EORTC/MSG criteria had 3 pts before alloHSCT. The only one patient had relapse of IA at day +20. Two patient developed IA after alloHSCT at days +100 and +235 which were associated with GvHD and corticosteroid treatment. Cumulative incidence of aGvHD in study group grade 1-2 was 53%, 3-4-7,6%. Cumulative incidence of chGvHD was 38,5%, severe-15%. There was no TRM at 2 years follow up. Progression-free survival at 100 days after alloHSCT was 76,9%, PFS at 2 years was 69,2%. Complete or partial remission of HL at the moment of HSCT was associated with the best PFS at 2 years *vs* stable or progression disease (89% *vs* 25%, p=0,02). OS at 100 days and at 2 years after alloHSCT was 92,3%. One patient died from the progression of the underlying disease in early period after alloHSCT.

Table 1. Patient characteristics, n=13.

Median age,	years	30 (20-49)
Time from diagnosis to alloHSCT,	months	43 (27-119)
Lines of CT / autoHSCT	numbers	6 (4-7) / 10
Brentuximab	before alloHSCT	7
	after alloHSCT	2
Status of cHD at the moment of alloHSCT	CR	4
	PR	5
	SD	2
	PD	2
Donor type	MRD	5
	MUD	8
GvHD prophylaxis	Standard tacro/CsA + Mtx/MMF + ATG in MUD	4
	Cyclophosphamide-base Cy alone or Cy + tacro/CsA + MMF	8
Source of graft	BM	8
	PB	5
Median CD34+ count	millions	4,2 (1,4-7,2)
Median follow up	months	6 (3-33)

Summary and Conclusions: The FluBe RIC for alloHSCT had 100% engraftment rate in our study group with acceptable toxicity and absence of TRM and rejection. The administration of a bendamustine containing RIC was associated with good PFS at day 100 (76,9%), as well with promising long-term OS (92,3%) and PFS (69,2%), especially in chemosensitive pts (89% 2y-PFS).

Reference

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E1525

EFFICACY OF ORAL GLUTAMINE IN THE PREVENTION OF MUCOSITIS IN PATIENTS UNDERGOING HEMATOPOIETIC STEM CELL TRANSPLANTATION. EXPERIENCE OF A SINGLE CENTER

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Background: Mucositis is an inflammatory reaction produced by chemo and/or radiotherapy, which affects the whole gastrointestinal tract. The main symptoms are oral dryness, odynophagia, diarrhea and painful ulcerations. Glutamine is a non-essential amino acid that helps to maintain the integrity of the digestive tract. In high metabolic stress your intake exceeding the body's synthesis capacity and deficiency may occur. Some studies suggest that this amino acid can prevent the onset of mucositis.

Aims: Analyze whether the use of oral glutamine supplementation decreases the incidence of mucositis, severity and duration in patients undergoing hematopoietic stem cell transplantation (HSCT).

Methods: A retrospective observational study was performed reviewing 274 hospital histories of patients undergoing HSCT inxeral Cíes Hospital, from 2002 to 2010 and from May to December 2014. Mucositis degree was evaluated in patients who have received glutamine (YG) or not (NG) regarding the HSCT type and conditioning regimen. The data was processing using R statistical software using correlation matrices according the p Holms method.

Results: 147 males and 127 females were included, with a median age of 49 years (1-70). In the 191 autologous HSCT predominant diagnosis were myeloma and lymphoma (87'4%), most of them receiving high doses of melphalan -BEAM, MEL200- (79'6%). 83 were allogeneic HSCT and predominant diagnosis was acute leukemia, receiving busulphan more than two days 42'2%, total body irradiation (TBI) 21'7%, reduced intensity conditioning (RIC) 22'9% and others 13'2%. Mucositis was seen in 95'5% of the autologous HSCT, III-IV degree 43'1% and 35'3%, NG and YG groups respectively. 81'9% of allogeneic HSCT developed mucositis, III-IV degree 42'9% *vs* 39'6%, NG *vs* YG. Mucositis degree, duration and the use of opioids and parenteral nutrition (PTN) in both groups, as well as mucositis depending on conditioning regimen are shown in Table 1. The TBI and RIC groups were not evaluated by of the small number of patients in each group. As shown in the Table 1 there is a significantly relation between the use of glutamine and reducing severe mucositis in autologous HSCT. Correlation is also observed with duration of mucositis and opidoids use. Not so in the allogeneic group in which no significant differences probably related to the sample size and the existence of other factors that may influence their appearance are observed (methotrexate use for GVHD prophylaxis, oral GVHD,…).

Table 1. Mucositis degree, duration, use of opioids and parenteral nutrition in autologous and allogeneic HSCT, and grade of mucositis based on conditioning regimens.

	Autologous HSCT			Allogeneic HSCT		
	NG (n=123)	YG (n=68)	*P* value	NG (n=35)	YG (n=48)	*P* value
Grade of mucositis - I-II - III-IV	56 (45.5%) 53 (43.1%)	37 (54.4%) 25 (36.8%)	0.005	12 (34.3%) 16 (45.7%)	19 (39.6%) 21 (43.8%)	0.16
Duration (days)	9.3±2.9	8.1±3	0.007	10.9±4.7	12.2±4.5	0.14
Use of Opioids	87 (79.8%)	41 (67.2%)		25 (89.3%)	36 (90%)	
Duration (days)	8.9±2.8	8.1±2.9	0.04	10.3±3.8	10.6±4.1	0.11
Use of PTN	69 (63.3%)	28 (45.2%)		19 (67.9%)	23 (57.5%)	
Duration (days)	8.4±2.8	8.5±3.3	0.09	12.1±4	11.7±5.6	0.01
Conditioning regime	High doses of melphalan			Busulphan (>2 days)		
	NG (n=94)	YG (n=58)		NG (n=13)	YG (n=22)	
Grade of mucositis - I-II - III-IV	44 (46.8%) 41 (43.6%)	33 (56.9%) 19 (32.8%)	0.06	4 (30.8%) 7 (53.8%)	9 (40.9%) 12 (54.5%)	0.13

Summary and Conclusions: In the present study we have found a lower incidence of severe mucositis in patients who have received glutamine undergoing autologous SCT, so we would recommend its use. We cannot draw any conclusions from their use on allogeneic setting.

E1526

THIOTEPA FOR AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION (AHSCT) IN SOLID TUMORS

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Background: Thiotepa (*NN'N"*-triethylenethiophosphoramide) is a multifunctional alkylating agent known to produce a significant anticancer effect in a variety of hematological malignancies and solid tumors. Since its molecular weight is as low as 189 g/mol, a good penetration of thiotepa to central nervous system (CNS) has been reported. Because of the high concentration in cerebrospinal fluid, thiotepa is recognized as one of the key agents for CNS tumors, primary CNS lymphoma, and AHSCT. However, thiotepa is currently unavailable in Japan due to the problem in drug supply.
Aims: The aim of this study is to clarify the significance of thiotepa use for AHSCT in solid tumors in Japan.
Methods: Data were retrospectively analyzed using Japan nation-wide database by the Japan Society for Hematopoietic Cell Transplantation.
Results: Thiotepa was used for 1,083 of 3,850 patients who received AHSCT for solid tumors. The median age was 9 years (renge: 0-71), and 679 patients (63%) were younger than 16 years. The main indication was breast cancer (314 of 444), primary CNS tumors (241 of 486), rhabdomyosarcoma (153 of 416), neuroblastoma (147 of 1,017), germ cell tumor (61 of 540), Ewing sarcoma (54 of 298) and hepatoblastoma (46 of 139). Various preconditioning regimens were used, but the median dose of thiotepa was 600 mg/m3 of 12 mg/kg. More than 80% of patients were treated in combination of alkylators. Thiotepa+melphalan was the most frequently used regimen, with a total of 534 patients, particularly for CNS tumors, rhabdomyosarcoma, neuroblastoma, germ cell tumor, and hepatoblastoma. Thiotepa+cyclophospamide with or without other agents was used for 330 patients mostly for breast cancer. Thiotepa+etoposide with or without other agents was used for 97 patients preferentially for CNS tumors and Ewing sarcoma. The 5-year overall survival of patients transplanted with thiotepa was 52%, and was significantly better than that without thiotepa (48%, P=0.03). The survival benefit was evident in primary CNS tumor (P=0.001), Ewing sarcoma (P=0.02) and rhabdomyosarcoma (P=0.05), but not for other tumors including breast cancer, neuroblastoma, germ cell tumor and hepatoblastoma (Figure 1).

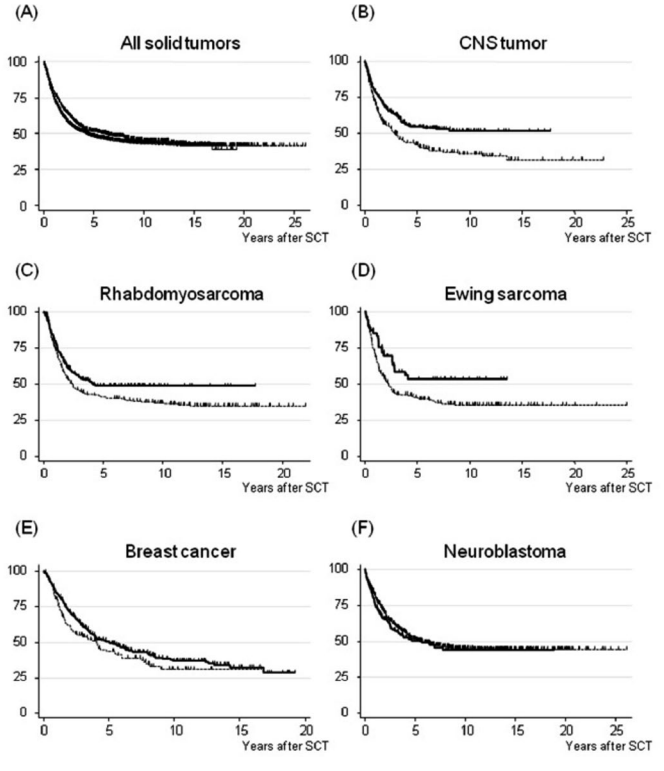

Figure 1.

Summary and Conclusions: Thiotepa is potentially useful for certain solid tumors as preconditioning for AHSCT. These findings should be confirmed in prospective studies.

E1527

DELAYED RECONSTITUTION AND IMMATURE PHENOTYPE OF NATURAL KILLER(NK) CELLS FOLLOWING POSTRANSPLANT CYCLOPHOSPAMIDE IN 30 UNMANIPULATED HAPLOIDENTICAL TRANSPLANTATION PATIENTS. A PROSPECTIVE ANALYSIS

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Background: NK cells exert an important role in postransplant immune reconstitution, relapse prevention, infection control and graft *versus* host disease(GVHD).They are the first lymphoid subpopulation to recover after a conventional bone marrow transplantation.Their reconstitution and maturation process resembles normal ontogeny, and is crucial for the development of their functional capacities. Unmanipulated Haploidentical transplantation (Haploid SCT) with postransplant cyclophosphamide is an emerging alternative for patients with no identical sibling donor.
Aims: We prospectively analyse NK reconstitution post Haploid SCT in 30 patients comparing with simultaneous 8 patients with HLA identical sibling donor (HLAid SCT), and its relationship with CMV reactivation and GVHD.
Methods: Between November 2012 and July 2014,30 patients received Haploid SCT and 8 HLAid SCT in Gregorio Marañon Hospital.Peripheral blood was used in all cases.GVHD prophylaxis included high dose Cyclophosphamide on +3,+4/cyclosporineA/mycophenolatemofetil for Haploid and cyclosporine A/Methotrexate for HLAid. Table1 shows patients characteristics,with no relevant differences between the two groups, except for longer engraftment times and lower acute GVHD incidence in Haploid group.For analysis of NK reconstitution flow cytometry on FC500/Navios Beckman Coulter® was used. Total NK cells, CD56 intensity and expression of NKG2A, NKp30, Nkp46 and NKG2D was studied at +15, +30, +60, and +90.For comparison Mann-Whitney U-test was used.
Results: Median NK cells/mm3 on +15 and +30 were significantly lower on Haploid than HLAid group (0 (0-2) and 34,5 (16-177) *vs* 60 (52-125) and 230 (122,5-387,7); p<0.01),being similar on+60 and +90 (112 (52-225) and 147 (84,2-211,7) for Haploid *vs* 95 (42-119) and 101 (63,2-177,7) for HLAid; p ns).NK cells reached normal levels on +30 in HLAid group,while in +60 in Haploid patients.High percentages of immature CD56bright(CD56b) NK cells and high expression of immature receptor NKG2A appeared on Haploid on +30 with further decrease (CD56b 77% (68.5-88.5), NKG2A 84% (77-93) on +30, 38% (25-48) and 72% (61-85,5) on +60, 35% (23-43) and 62%(53,7-77) on +90). Compared with HLAid patients,CD56b cells and NKG2A expression were significantly higher (p<0.05) on Haploid group on +30, +60 and +90. Expression of activating receptors NKP46/ NKp30,and inhibitor NKG2D,linked to NK maturation,were only decreased in Haploid group on +15 (p<0.05),with appropriate expression and no differences on +30, +60 and +90.Expression of NKP46 tended to be higher in Haploid on +30 (81% (54,5-92,5) *vs* 59,5% (42,5-69,5)), (p 0.05).In Haploid group, patients with acute GVHD II-IV had lower NK cells/mm3 at +30 (Median 28,5 *vs* 132) and +60 (Median 67,5 *vs* 103) than those with GVHD 0-I; p ns.No differences in receptor expression was detected between patients with and without GVHD or CMV reactivation.

Table 1.

		Haploid	HLAid	p
Sex	M/F	21/9	4/4	0.4
Age	Median	39,5 (29.75-48.75)	56.5(33.25-61.65)	0.12
Diagnose				0.19
	AML	9	3	
	ALL	3		
	CML	4		
	NHL	5	3	
	HL	7		
	MDS	1	1	
	Aplasia	1		
	MM		1	
Disease status				0.4
	CR	18	3	
	PR	3	2	
	Active disease	9	3	
Conditioning				0.7
	Submyeloablative	16	5	
	Myeloablative	14	3	
CD34+ x10e6/Kg		5,3 (4,3-6)	5.7 (4.8-8.1)	0.25
Days Neutrophils>500/mm3		17 (16-19)	15(14.2-16.7)	0.05
Days Platelet>20000/mm3		30 (28-40)	12 (10-13)	0.00
Acute GVHD				0.01
	0	9	0	
	I	7	4	
	II	12	1	
	III-IV	2	3	
Chronic GVHD		1	1	0.3

Summary and Conclusions: After Haploid SCT with postransplant Cyclophosphamide NK cell reconstitution is delayed comparing with HLAid SCT,reaching normal levels at +60. NK maturation in Haploid is also different. NK cells remains in an immature phenotype with high proportion of CD56b NKG2A+ cells longer than in HLAid patients. Interestingly cytotoxic capacity seems preserved, with activating and inhibitor no KIR receptor expression, normally linked to maturation, similar to HLAid patients from +30 onwards. Patients with clinically significant GVHD had worse NK reconstitution in terms of lower total counts in the first 60 days. These observations bring interest about the possible benefit of early NK infusions on Haploidentical setting to overcome this impaired NK reconstitution.
Acknowledgments: Foundation Mutua Madrileña.

E1528

UTILITY OF CHIMERISM ANALYSIS IN DETECTION OF POST-TRANSPLANT DISEASE RELAPSE IN PATIENTS WITH MYELOID NEOPLASMS UNDERGOING REDUCED INTENSITY CONDITIONING ALLOGENIC HEMATOPOIETIC CELL TRANSPLANTATION

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Background: Reduced intensity conditioning (RIC) has expanded allogeneic hematopoietic stem cell transplantation (allo-HCT) to elderly patients and to those with additional comorbidities. However, relapse of myeloid neoplasms after RIC allo-HCT remains a major problem. Although chimerism analyses are frequently used for post-transplant surveillance, their utility in the detection of relapse is controversial.
Aims: We sought to determine the utility of peripheral blood (PB) and bone marrow (BM) chimerism analyses for the detection of post-transplant disease relapse after RIC allo-HCT.
Methods: After IRB approval, consecutive patients with myeloid neoplasms (MDS and AML) who underwent their first RIC allo HCT between 2005 and 2014 were reviewed. Clinical and laboratory data were retrospectively abstracted. BM biopsies (including chimerism studies) that were done for post-transplant surveillance, as well as concurrent CBC, and PB (sorted and unsorted) chimerism results that were done within 5 days of the BM biopsies were analyzed.
Results: A total of 137 patients, 59% male, with a median age of 59 years (range 18-69), were identified. Ninety-two (67%) had AML and 45 (33%) had MDS. After a median follow up of 19 months, 29 (21%) patients (21 with AML and 8 with MDS) had disease relapse. Of these, 14 (48%) relapsed within the first 100 days, 8 (28%) between day 101 and 200, and 7 (24%) after day 200. A total of 316 bone marrow biopsies were reviewed. Of these, 53 (17%) were consistent with disease relapse (41 with AML and 12 with MDS). As shown in the Table 1, full donor chimerism was still present concurrent with BM relapse within a respectable number of cases (32% of unsorted PB, 43% of lymphoid-sorted PB, 79% of myeloid-sorted PB, and 15% of unsorted BM chimerisms). Furthermore, mixed donor chimerism without the morphological or cytogenetic evidence of relapse was also seen in 37% of lymphoid-sorted PB and 7% of unsorted BM chimerisms. Unsorted BM and PB chimerisms had the best sensitivity for identifying disease relapse (85% and 78%, respectively), whereas unsorted PB and myeloid-sorted PB chimerisms had 100% specificity. CBC abnormalities preceded the relapse in 25 (86%) out of the 29 patients, with circulating blasts in 13 (45%), isolated thrombocytopenia in 10 (34%), and pancytopenia in 5 (17%), being early predictive markers. Negative change in serial chimerisms prompted an evaluation for possible relapse in only 1 (3%) patient. In 4 (14%) patients, relapse was not suspected, but was diagnosed on a routine BM biopsy that showed only cytogenetic relapse (3 patients) and at an extramedullary site (laryngeal chloroma) without BM involvement (1 patient). The overwhelming majority of patients (15 out of 21 AML and 8 out of 8 MDS relapses) had circulating blasts at the time of relapse confirmation on BM biopsy.

Table 1. Results of 316 concurrent* BM biopsy and chimerism studies performed in 137 AML/MDS patients after RIC allo-HCT.

		PB (unsorted) chimerism results available (N = 88)		Lymphoid-sorted PB chimerism results available (N = 81)		Myeloid-sorted PB chimerism results available (N = 82)		BM (unsorted) chimerism results available (N = 260)	
		No relapse on BM Bx (N = 79)	Relapse on BM Bx (N = 9)	No relapse on BM Bx (N = 67)	Relapse on BM Bx (N = 14)	No relapse on BM Bx (N = 68)	Relapse on BM Bx (N = 14)	No relapse on BM Bx (N = 226)	Relapse on BM Bx (N = 34)
Chimerism result	Full donor	N = 79	N = 2	N = 42	N = 6	N = 68	N = 11	N = 210	N = 5
	Mixed donor	N = 0	N = 7	N = 25	N = 8	N = 0	N = 3	N = 16	N = 29
	90-99%	n = 0	n = 1	n = 7	n = 1	n = 0	n = 2	n = 15	n = 4
	80-89%	n = 0	n = 0	n = 6	n = 2	n = 0	n = 0	n = 1	n = 2
	70-79%	n = 0	n = 1	n = 5	n = 4	n = 0	n = 1	n = 0	n = 1
	< 70%	n = 0	n = 5	n = 7	n = 1	n = 0	n = 0	n = 0	n = 22
		PB (unsorted)		Lymphoid-sorted PB		Myeloid-sorted PB		BM (unsorted)	
Test Sensitivity**		78%		57%		21%		85%	
Test Specificity**		100%		62%		100%		92%	

* Performed within 5 days of each other
** Sensitivity and specificity for diagnosis of relapse are calculated based on chimerism results of full vs. mixed donor

Summary and Conclusions: Chimerism studies, and in particular myeloid-sorted PB chimerism, have a limited role in the detection of post-transplant relapse in patients with myeloid neoplasms. Close monitoring of CBC results, including early detection of thrombocytopenia, circulating blasts, and pancytopenia still remains a very effective strategy.

E1529

BUSULFAN THERAPEUTIC DRUG MONITORING IS NEEDED IN POPULATIONS WITH GENETIC HETEROGENEITY UNDERGOING ALLOGENEIC STEM CELL TRANSPLANTATION

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Background: Busulfan (Bu) based preparative regimens in allogeneic stem cell transplantation are commonly used. Previous studies have shown that Bu at a fixed dose of 3.2 mg/Kg/day (FBD) given intravenously decreases variability in drug pharmacokinetics and this decreases the dependency on therapeutic Bu drug monitoring (TDM). We hypothesized that in a population with genetic heterogeneity, like Oman, the FBD may not be adequate and TDM is still needed.
Aims: Therefore, we planned to compare the Bu dose given using TDM with the FBD of 3.2 mg/Kg/day.
Methods: We retrospectively reviewed consecutive patients who received allogeneic stem cell transplantation at Sultan Qaboos University Hospital during the period of January 2003 to December 2014. Included patients received intravenous Bu based preparative regimen using TDM. Patients with acute leukemia, myelodysplasia, thalassemia major and sickle cell disease were included in this study. We used Bland-Altman plot to compare the daily dose between the TDM and FBD using 3.2 mg/day. We then normalized the dose using patients' weights (mg/Kg/day) and assessed the impact of age, gender, weight, height and serum bilirubin on the difference between the normalized TDM and FBD.
Results: Sixty-one patients (Males 28, Females 33) with a median age of 16.5 y (Range: 2.7-55.9) were included. The median and the interquartile range (IQR) for weight, height and serum bilirubin were 49 Kg (IQR: 23.2-62.5), 152 cm (IQR: 130-164) and 10 micromol/l (IQR: 4-17) respectively. Indication for transplantation was acute leukemia/myelodysplasia in 30, sickle cell disease in 23 and thalassemia major in 8 patients. The mean difference in daily dose between the TDM based and FBD was 40 mg with clinically significant differences (Bland-Altman agreement limits: -39 to 119; see Figure 1). The median normalized difference between the TDM based and FBD methods was 4.3 mg/kg/d (Range: 2.4-8.1). In the multivariable linear regression model, weight (p=0.0278) and gender (p=0.0288) were statistically significant predictors of the normalized difference with a coefficient of determination of 47% for the model.

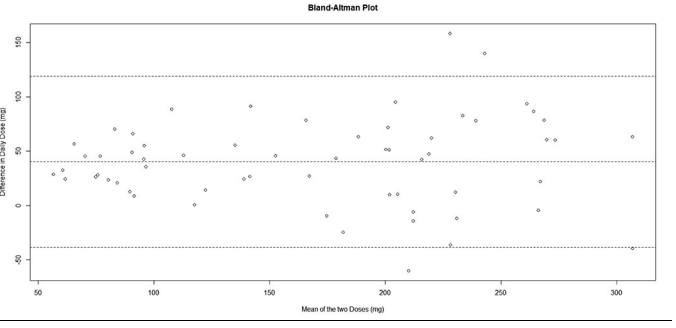

Figure 1.

Summary and Conclusions: TDM remains important for appropriate dosing of Bu in preparative regimens of allogeneic stem cell transplantation especially in populations with genetic heterogeneity. Although, the gender and weight can help predict the difference, the unexplained variance remains high. We recommend Bu TDM to be used as a standard of care.

E1530

HEMATOLOGICAL RECOVERY AFTER INTRA-BONE MARROW CORD-BLOOD TRANSPLANT WAS ENHANCED THROUGH LOCAL ENGRAFTMENT OF INJECTED SITE. A SINGLE CENTER PROSPECTIVE PHASE I/II TRIAL IN JAPAN

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Background: Cord-blood transplantation (CBT) is an effective option for hematological diseases. However, high rate of graft failure and delayed engraftment were the major limitations of CBT and as a consequence, CBT is associated

with a high risk of morbidity and mortality. Intrabone-marrow CBT (iBM-CBT) has been proposed as a measure which can solve the problems.
Aims: We conducted phase I/II prospective single-center trial of iBM-CBT with 15 cases to evaluate its safety and effectiveness. And another aim of this study was to identify the site of early hematopoiesis after iBM-CBT.
Methods: 15 adult patients (median age 59 years, range 32-64) with hematological malignancies (AML, 8; ALL, 4; NHL,3) were enrolled in University of Tsukuba Hospital, Tsukuba, Japan. Of them, three patients were not in remission. HLA matching was 5/6, and 4/6, for 5, and 10, respectively. Unwashed units were used to avoid loss of the cord-blood cells. The cord blood was divided in four syringes, and was infused in the superior-posterior iliac crest bilaterally under general anesthesia for one case, or local anesthesia for fourteen cases. Median transplanted cell dose was 2.8×10^7/kg (range 2.0-5.0). The primary endpoint was injection-related complication and the probability of hematological recovery after iBM-CBT. Secondary endpoints included the incidence of transplant-related mortality (TRM), acute GVHD, overall survival (OS), and disease-free survival (DFS). We compared donor chimerism in the iliac bone (the injected site) with that in the sternal bone (the remote site from the injection) 14 days after intra-bone marrow injection. This trial was registered on the UMIN website, number UMIN000006175.
Results: Between October 2011 and July 2014, 15 consecutive patients underwent iBM-CBT. Transient hypotension was observed after intrabone injection in one case, which was thought to be due to vasovagal reflex and the sedatives. The neutrophil recovery (≥0.5×10^9/L) and platelet recovery (≥5.0×10^9/L) were seen in 14 cases (93%), and 13 cases (87%), respectively. The median time to neutrophil and platelet recovery were 17 days (range 12-27) and 44 days (range 32-99), respectively. Acute GVHD developed in 34% (grade II-IV), and 0% (grade III-IV). TRM within one year was not observed. Estimated 1-year OS and DFS were 79% and 67%, respectively. The early donor chimerism was significantly higher in the iliac bone (median 95.2%, range 67.3-99.7), where cord blood had been injected, compared to the sternal bone (median 94.4%, range 22.3-98.8) (P=0.006; Wilcoxon signed-rank test) (Figure 1).

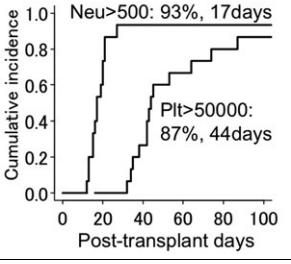

Figure 1.

Summary and Conclusions: Our data suggest that iBM-CBT was well tolerated and can promote prompt hematological recovery. Local anesthesia is sufficient to control pain associated with the procedure and the cord blood does not have to be washed. The dominance of donor chimerism in the iliac bone before engraftment suggests that local engraftment just after iBM-CBT contributes to early hematopoiesis of the recipients.

E1531

EXCELLENT LONG-TERM OUTCOME OF AUTOLOGOUS TRANSPLANTATION WITH INTERMEDIATE-DOSE INTENSIVE MELPHALAN (100 MG/M2, MEL100) FOR MULTIPLE MYELOMA: A SINGLE CENTRE STUDY
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Background: It is generally accepted that high-dose melphalan therapy, followed by autologous hematopoietic cell transplantation, should be the treatment of choice in younger transplant-eligible patients with multiple myeloma (MM). The optimal therapeutic approach in elderly patients with MM remains unclear. Newer agents, including bortezomib and lenalidomide, have become available, and their use should be considered in this population. We carefully evaluated transplant-eligibility in both young and older patients (50s to 70s), and considered the treatment procedure: reduced intensity melphalan conditioning that reported several study. Here we report on the excellent long-term outcome of autologous tandem transplantation with intermediate-dose intensive melphalan in patients with MM.
Aims: We aimed to determine the long-term efficacy, and associated adverse events, of autologous tandem transplantation with intermediate-dose intensive melphalan for MM.
Methods: We retrospectively analysed data from 37 patients, treated at our institution with pre-planned 2 or 3 courses of intermediate-dose intensive melphalan (100 mg/m^2, MEL100) followed by peripheral blood stem cell (PBSC) transplantation, between 2000 and 2009. Eligibility criteria for inclusion were as follows: (1) histologically and clinically confirmed symptomatic MM; (2) aged 40-79 years; (3) transplant-eligible; (4) adequate organ function with Eastern Cooperative Oncology Group performance status <3; and (5) provision of written informed consent. The median age of patients was 60 years (range, 43-77 years). Pre-transplant disease status, according to the Durie and Salmon system, was as follows: stage I (n=5), stage II (n=6), stage III (n=28). The rate of response better than very good partial response (VGPR) was 29% at pre-transplant with dexamethasone alone or VAD (Vincristine-Adriamycin-Dexamethasone) regimen. All cases of PBSC mobilization were performed with cyclophosphamide plus granulocyte-colony stimulating factor. Five patients underwent allogeneic hematopoietic cell transplantation after tandem autotransplantation, because of disease progression.
Results: The 5-year overall survival (OS) was 50%, and cumulative incidences of relapse and non-relapse mortality were 26% and 24%, respectively. All patients who were followed up for more than 5 years post-transplantation are alive. Complete remission was achieved in 16 (37%) patients. The median day of neutrophil engraftment was day 11; there were no cases of engraftment failure. Only 2 patients died within 100 days post-transplantation (1 case of sepsis and 1 case of disease progression). Surprisingly, survival during the follow-up period was higher in patients carrying a 13 chromosome deletion (13q-) (10/11, 91%), compared with patients with a normal karyotype (9/26, 34%).
Summary and Conclusions: These data demonstrate the effectiveness of 2 or 3 courses of intermediate-dose intensive melphalan followed by stem cell transplantation in older patients with MM. Thus, the MEL100 regimen is not only feasible in older patients, but also prolonged overall survival. Patients with a 13q deletion showed a particularly good response to this treatment, particularly considering the poor prognosis often associated with such deletions. Although recent data shows the potential of novel treatments such as proteasome inhibitors and immunomodulating agents, the treatment protocol reported here should be considered to achieve prolonged progression-free survival.

E1532

IMPACT OF DONOR AGE ON PERIPHERAL BLOOD AND BONE MARROW HEMATOPOIETIC STEM CELL HARVEST
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Background: Hematopoietic stem cell transplantation (HSCT) is still one of the most important treatment modalities for the patients with hematologic malignancies. For allogeneic HSCT, increasing difficulty in proper donor selection leads to broaden the range of donor age than before. However, the exact clinical impact of donor age on peripheral blood (PB) and bone marrow (BM) hematopoietic stem cell (HSC) harvest is unclear.
Aims: This study was conducted to evaluate the impact of donor age on PB and BM HSC harvest.
Methods: We retrospectively reviewed the yields of allogeneic PB or BM HSC harvest from 384 healthy sibling or unrelated donors who donated their HSC to adult patients receiving allogeneic HSCT at Severance hospital between Jan. 2000 and Feb. 2015. For PB-HSCT, granulocyte colony-stimulating factor (G-CSF), either filgrastim or lenograstim, was injected subcutaneously with doses of 10 μg/kg/day either as a single or split from 4 days before harvest to the end of the procedure.
Results: Among 384 donors, 290 (75.5%) donated PB HSC and 85 (22.1%) BM HSC. Nine (2.3%) sibling donors provided both BM and PB stem cells due to insufficient number of BM stem cell in initial harvest. Median age was 37 (range 14-73) years, and the number of donors with age <50 years was 244(84.1%), 50-59 years 34 (11.7%), and ≥60 years 12 (4.1%). Donors with older age needed more sessions of harvest (Table 1. p=0.032) to complete the harvest. The median CD34+ cell counts at the first day of harvest was also significantly different according the age of donor; 4.03 (range 0.34-24.55)x 10^8 in patients with age <50 years, 2.85 (range 0.16-10.21)x 10^8 in 50-59 year, and 1.89 (0.64-3.37)x 10^8 in ≥60 years (p <0.001). The median total CD34+ cells collected were also reversely correlated with the age of donor (p <0.001). Two of 12 (16.7%) donors over 60 year-old did not collect more than 1.4x 10^8 CD34+ cells finally which are probably needed to give >2.0x 10^6/kg CD34+ cells in 70kg recipients. The age of donors providing BM HSC was younger (median 33, range 13-56 years) than those providing PB stem cells. The number of donors with age<50 years and ≥50 years was 88 (93.6%) and 6 (6.4%), respectively. The median CD34+ cell counts at the first day of harvest were 1.56 (range, 0.06-8.11)x 10^8 and 1.08 (range, 0.31-1.37)x 10^8 in donors with age <50 years and ≥50 years, respectively (p=0.015). The median counts of total collected CD34+ cells after completion of harvest were 1.81 (range, 0.06-8.11)x 10^8 and 1.08 (range, 0.31-1.37)x 10^8, respectively (p=0.03). Median CD34+ cells/kg body weight of recipients were significantly higher in donors with age<50 year than in those with age ≥50 years (2.59 *vs* 1.81x10^6, p=0.025).
Summary and Conclusions: This study showed that the majority of elderly donor could provide sufficient PB or BM HSC for allogenetic HSCT, but number of collected HSC was reversly correlated with the age of donor. Especially because the risk of insufficient harvest was increased in PB HSC donor with

age >60 and in BM HSC donor with age >50, more consideration should be given in case of these elderly donors.

Table 1.

PBSCH / Age group	<50 (244)	50 -59 (34)	≥60 (12)	*p*-value
Median age, years (range)	34.0 (14-49)	53.0 (50-59)	63.5 (61-73)	
Gender, male, % (n)	57.1 (140)	51.4 (18)	33.3 (4)	0.241‡
Apheresis session, 1/2/3. %	59.8/39.8/0.4	50.0/47.1/2.9	33.3/58.3/8.3	0.032‡
1st day CD34+ cells collected, median (x 10^6, range)	4.03 (0.34-24.55)	2.85 (0.16-10.21)	1.89 (0.64-3.37)	<0.001†
Total CD34+ cells collected, median (x 10^6, range)	4.57 (1.34-24.55)	4.04 (0.26-11.16)	2.94 (1.17-3.77)	<0.001†
Total collected CD34+ cells <1.4 x 10^6, % (n)	1.2 (3)	0 (0)	16.7 (2)	0.017‡

BMH / Age group	<50 (88)	≥50 (6)	*p*-value
Median age, years (range)	33 (13-49)	53 (51-56)	
Gender, male, % (n)	63.6 (56)	66.7 (4)	>0.999‡
Harvest session, 1/2, % (n)	85.2/14.8 (75/13)	100.0/0.0 (6/0)	0.589‡
1st day CD34+ cells collected, median (x 10^6, range)	1.56 (0.06-8.11)	1.08 (0.31-1.37)	0.015¶
Total CD34+ cells collected, median (x 10^6, range)	1.81 (0.06-8.11)	1.08 (0.31-1.37)	0.003¶
1st day CD34+ cells/kg recipient, median (x 10^6, range)	34.5 (20)	44.4 (12)	0.007¶
1st day TMNC/kg recipient, median (x 10^8, range)	3.22 (0.06-36.8)	3.05 (1.36-9.21)	0.882¶

Table 1. CD34+ cell yields in donors receiving stem cell harvest from peripheral blood or bone marrow according to age groups.

Abbreviations: PBSCH, peripheral blood stem cell harvest; BMH, bone marrow harvest; TMNC, total mononuclear cells.
† Kruskal-Wallis test
‡ Fisher's exact test
¶ Mann-Whitney's U-test

E1533

BASELINE PERIPHERAL BLOOD CD34+ CELL COUNT DOES NOT INFLUENCE PLERIXAFOR SUCCESS RATE

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Background: Plerixafor, a CXCR4 chemokine antagonist, has been shown to significantly improve blood stem cell collection mediated by granulocyte-colony-stimulating factor (G-CSF). Peripheral blood CD34+ cell counts (PB CD34+) after different mobilization schemes have been shown to correlate with apheresis yields and are widely used as the main variable to start apheresis. However, doubts remain if there are predictive factors that can be used before starting plerixafor administration in order to ration the use of this expensive drug.
Aims: To investigate if lower baseline PB CD34+ correlate with inferior collection success rates.
Methods: We retrospectively analyzed 62 patients that received plerixafor between 13-10-2008 and 31-12-2014. Five patients were excluded due to insufficient data. Plerixafor was given as part of a preemptive strategy or salvage therapy after failure to mobilize or collect sufficient CD34+ cells following chemomobilization. It is the standard operating procedure at our centre to target a collection of 2 × 10^6 CD34+ cells/kg for lymphoma and of 4 × 10^6 CD34+ cells/kg-enough for two autologous stem cell transplants (ASCT)-for multiple myeloma (MM) patients. However, for the purpose of this analysis, the 2 × 10^6 CD34+ cells/kg target was considered as a collection success for all patients. Plerixafor was given at a dose of 0.24 mg/kg subcutaneously 11 hours prior to apheresis in conjunction with G-CSF (10 µg/kg/day). Baseline PB CD34+ were determined after 4 G-CSF administrations and before the first plerixafor was given.
Results: Of the 57 patients, 33 (57.9%) were males. Median age was 57 years [18-67]. In 12 patients (21.11%) the diagnosis was Hodgkin Lymphoma, in 14 (24.56%) Multiple Myeloma, in 30 (52.63%) Non-Hodgkin Lymphoma and in 1 (1.75%) Waldenström Macroglobulinemia. In 20 patients (35.09%), Plerixafor was used preemptively. The PB CD34+ after the first plerixafor correlated with the initial PB CD34 (r=0.60, p<0.001). The number of CD34+ cells collected after the first apheresis also correlated with PB CD34+ at baseline (0.60, p<0.001) and, to a higher degree, to the PB CD34+ after the first plerixafor (r=0.78, p<0.001).The number of CD34+ cells collected after the first apheresis was higher in patients with PB CD34+ ≥5 /mL at baseline (2.9±0.46 *vs* 1.8±0.29x10^6 cells /kg, p=0.02). The number of plerixafor administrations needed to achieve a total collection of ≥2.0x10^6 cells/kg was higher in patients with a baseline PB CD34+ under 5 /mL (2.1±0.13 *vs* 1.6±0.15, p=0.0094). The percentage of patients achieving this goal after a single administration of plerixafor was more than two-fold higher when the baseline count was ≥5 cells /mL (47.8% *vs* 20.6%, p=0.030; 50.0 *vs* 25.0% for Myelomas and 47.1 *vs* 19.2% for Lymphomas). However, the ratio of patients achieving a collection of ≥2.0x10^6 cells/kg both after the first apheresis (47.1% *vs* 56.5%, p=NS) and at the end of all the aphereses with plerixafor (64,7% *vs* 78,3%, P=NS) was unaffected by the initial PB CD34+. The optimal cut-off point to predict for better collection results as defined above was 4.795 cells /mL at baseline, with a sensitivity of 66.7% and a specificity of 61.1%.
Summary and Conclusions: In our series, higher baseline PB CD34+ correlated with faster and higher PB CD34+ increase and therefore the need for a lower number of apheresis. Nevertheless, it does not significantly influence the probability of reaching our goal of sufficient CD34+ cells for one ASCT.

E1534

AUTOLOGOUS PERIPHERAL BLOOD CD34+ CRYOPRESERVED WITH 5% DIMETHYL SULFOXIDE COMPARED TO 10%: BETTER VIABILITY OF CD34+ AND NO NEUROLOGICAL SIDE EFFECTS

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Background: Dimethyl sulfoxide (DMSO) is extensively used as a cryoprotectant in stem cell preservation, but is associated with toxicity and adverse reactions in the transplant recipient. Cryopreservation using 5% DMSO instead of 10% DMSO is a strategy to reduce the adverse effects.
Aims: We compare the use of 5% DMSO *versus* 10% DMSO in terms of median and viability of CD34+ cells infused, haematological engraftment, number of febrile neutropenia and treatment related mortality (TRM).
Methods: 118 consecutive patients with haematological malignancies submitted to autologous transplant were included in the study. 81 (69%) were stored in 10% DMSO (from January 2012 to September 2013) and 37 (31%) in DMSO 5% (from October 2013 to January 2015).

Table 1. Results of cryopreserved HPC and haematological recovery.

	Group 5% DMSO	Group 10% DMSO	p
Median CD34+ infused cells (x10^6/Kg) (range) >4 x 10^6/Kg (N,%)	4,47 (1,91-8,35) 20 (54%)	3,85 (1,17-12) 33 (47%)	0,317
Median viability CD34+ (range) > 70% (N,%)	99% (94-100) 37 (100%)	81,5 (31-99,6) 62 (82%)	0,004
Median days to >0,5 ANC x10^9/L (range) > 12 days (N,%) Median days to >20 platelets x 1^09/L (range) > 14 days (N,%)	10 (8-16) 2 (6%) 12 (9-26) 10 (30%)	10 (6-27) 6 (12%) 13 (5-42) 15 (29%)	0,34 0,8
Median days of hospitalization from day 0 > 24 days (N,%)	21 (14-36) 8 (22%)	21 (12-69) 10 (20%)	0,89
Febrile neutropenia	25 (68%)	52 (66%)	0,853
Neurological toxicity	0	3 (4%)	0,23
Mucositis	28 (76%)	57 (76%)	0,97
Diarrhea	22 (59%)	47 (63%)	0,74
Renal toxicity	2 (5%)	2 (5%)	0,23
Hepatic toxicity	2 (5%)	2 (5%)	0,44

Results: Patients were similar in terms of median age, diagnosis, number of previous treatments, disease status at transplant. Median time to transplant from diagnosis was similar in the two groups but higher number of patients in DMSO 10% were transplanted after one year from diagnosis (85% *vs* 43%, p<0,001). Median of CD34+ cells infused was similar in the two groups, Table 1. Reduction in DMSO concentration to 5% was associated with higher CD34+ cells viability (99% *vs* 86%; p<0,008). No significant difference in median time to neutrophil (ANC) and platelets (PLT) engraftment was demonstrated in the two groups of patients, Table 1. DMSO concentration did not influence median day of hospitalization and febrile neutropenia. No differences in terms of mucositis, diarrhea, renal or hepatic toxicity were observed. No neurological side effects were observed in the group of patients with DMSO 5%, instead 3 events (4%) were registered in the DMSO 10% group, (p=0,23). After 14 months of median follow-up (range, 1-37), the estimated 1y TRM was 6% in the DMSO 5% *vs* 13% in DMSO 10%, p=0,45 (Figure 1).

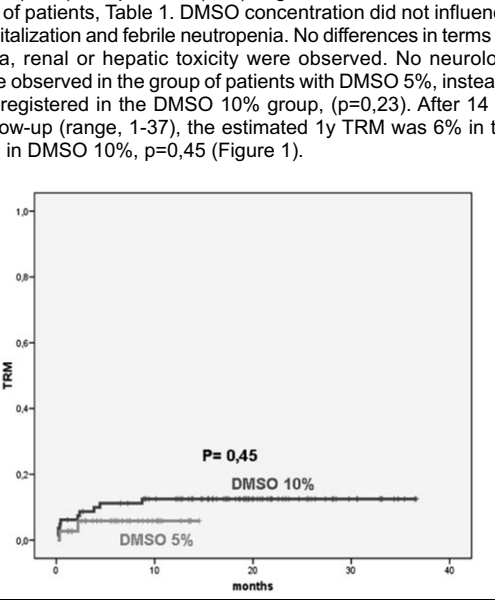

Figure 1.

Summary and Conclusions: Our results suggest that reduction in DMSO concentration to 5% was associated to higher viability of CD34+ cells infused without affecting median CD34+ cells infused and haematological reconstitution. At the same time, an equivalent 1y EFS was experienced between the two groups of patients studied. Interesting, neurological side effects were not observed in DMSO 5%. Further studies and longer follow-up is needed to evaluate fully efficacy and long term safety of cryopreservation of cells with 5% DMSO.

E1535

SINGLE *VERSUS* TANDEM AUTOLOGOUS STEM CELL TRANSPLANTATION IN MULTIPLE MYELOMA-RETROSPECTIVE STUDY

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Background: High-dose chemotherapy followed by autologous stem cell transplantation (ASCT) is the main treatment of Multiple Myeloma (MM). However, an alternative approach with tandem ASCT has been studied but response rates, progression free survival (PFS) and overall survival (OS) are not well established and conflicting results had been published.
Aims: Compare single *versus* tandem ASCT after high dose chemotherapy in terms of responses, PFS and OS.
Methods: Retrospective study of all cases (n=176) of MM treated with high-dose chemotherapy followed by ASCT (single or tandem) in our center during six years (January/2008 to December/2014). The initial intention to treatment was tandem ASCT. However, the majority of patients only had collected peripheral blood progenitor cells (PBPC) for one ASCT or refuse a second ASCT. We performed a demographic and clinical survey of the population and evaluated response to induction chemotherapy (before the ASCT) and to single or tandem ASCT (2 and 6 months after ASCT) according to the criteria of the International Myeloma Working Group (IMWG). We performed the analysis of OS and PFS by the Kaplan-Meier method/log-rank test, Chi-square test.

Table 1. Main characteristics of study population.

	All patients	Tandem ASCT	Single ASCT	p-value
Number of Patients	176	50	126	
Age at Diagnosis				
Median years (range)	57 (30-69)	54 (38-64)	59 (30-69)	0.01
Gender (N)				
Male	90	22	68	0.25
Female	86	28	58	
Hemoglobin (g/dl)				
Median (range)	10.9 (5.5-15.5)	10.8 (7.3-15.1)	11.0 (5.5-15.5)	0.45
Missing data	75	20	55	
Platelets (x10^9/L)				
Median (range)	203 (35-402)	227 (147-402)	197 (35-367)	0.28
Missing data	81	21	60	
Bone Marrow Plasm Cells (%)				
Median (range)	30 (0- ≥90)	31 (0-88)	30 (4-≥90)	0.42
Missing data	64	17	47	
Serum β_2 – Microglobulin (mg/dl)				
Median (range)	2.9 (1.15-33.1)	2.4 (1.2-9.1)	2.9 (1.2-33.1)	0.27
Missing data	86	26	60	
Serum Creatinine (mg/dl)				
Median (range)	0.9 (0.5-11.3)	0.9 (0.5-11.3)	0.9 (0.5-7.7)	0.05
Missing data	77	21	56	
Citogenetics (N)				
High risk*	14	5	9	
Standard-risk	67	24	43	1.00
Missing data	95	21	74	
Durie-Salmon Stage				
IA	30	5	25	
IB	3	2	1	
IIA	36	16	20	0.93
IIB	1	0	1	
IIIA	81	25	56	
IIIB	17	2	15	
Missing data	8	0	8	
ISS				
I	67	25	42	
II	45	10	35	0.07
III	32	7	25	
Missing data	32	8	24	
M component (N)				
IgG	87	27	60	
IgA	42	11	31	
IgM	1	0	1	0.94
IgD	4	1	3	
Light Chain	39	10	29	
Non- secretory	3	1	2	
Presence of Plasmocytomas (N)	41	8	33	0.17
Number of Induction Regimens Prior to ASCT (%)				
1	132	38	94	0.18
2	36	8	28	
3	8	4	4	
Radiotherapy	34	8	26	0.40
Induction Response				
RC	50	14	36	
VGPR	26	5	21	0.61
PR	98	31	67	
SD	1	0	1	
Missing data	1	0	1	

NOTES: *t(4;14) and del 17p; RC - complete response; VGPR – very good partial response; PR – partial response; SD – stable disease

Results: Fifty patients underwent tandem ASCT and 126 single ASCT with a median follow-up of 42 months (9-132). Median age was 57 years (30-69) with a slight male prevalence (51.1%). Tandem ASCT group had a lower median age (54 *vs* 59, p=0.01) but no other demographic or clinical differences were found between the two groups (Table 1). Type of response to induction chemotherapy were also similar. After transplant, rates of response were also comparable between the two groups: the tandem ASCT group had 56.2% complete response (CR), 20.8% very good partial response (VGPR) and 22.9% partial response (PR), while the single ASCT group had 62.3% CR, 11.3% VGPR and 25.5 PR and 0.9% without a response (p=0.92). The PFS at 3 years and 5 years was higher in patients undergoing tandem ASCT (75.8% *vs* 59.0% and 68.5% *vs* 33.3%, p=0.04), but showed no OS difference between the two groups: tandem ASCT *vs* single ASCT at 3 and 5 years (100.0% *vs* 96.0% and 79.0% *vs* 74.2%, p=0.27). These results were similar even after excluding CR to induction chemotherapy: PFS at 3 and 5 years was 73.9% *vs* 49.1% and 67.2% *vs* 32.0%; p=0.021; OS was, respectively, 100.0% *vs* 89.9% and 69.2% *vs* 70.0%, p=0.75. Transplantation related mortality was absent in the two groups.
Summary and Conclusions: In our study, tandem ASCT improved PFS when compared with a single ASCT, even in those who not achieved a CR after the induction chemotherapy. However, it failed to significantly prolong overall survival.

E1536

IS ALLOGENEIC STEM-CELL TRANSPLANTATION AN OPTION FOR PATIENTS WITH ACUTE LEUKEMIA AND MYELODISPLASTIC SYNDROME TRANSPLANTED WITH ACTIVE DISEASE? A CENTER EXPERIENCE

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Background: The prognosis of patients with refractory/relapsed acute leukemia (rAL) or myelodisplastic syndrome (MDS) is poor. Although patients with refractory disease are not good candidates for allogeneic hematologic stem cell transplantation (HSCT), this could be the only curative option. Due to the absence of reports on long-term follow up in this population, transplant indication in those patients is a permanent focus of debate.
Aims: To evaluate the outcome and efficacy of salvage HSCT for rAL and MSD.
Methods: We examined retrospectively all the patients transplanted at our Unit since1999 to 2003 who recived a HSCT in this situation.Diagnosis was in 74 patients (myeloid/lymphoblastic n=36/10) and MDS (n=28).Criteria of refractoriness was defined as patients had never achieved a complete remission (CR). Feasibility and efficacy of HCT was analyzed in terms of related mortality (TRM), event free survival (EFS) and overall survival (OS).

Table 1.

	n (%)
Patients sex	
Male	37 (50%)
Female	37 (50%)
Nº of prior regimes	
≤2	49 (66%)
>2	17 (24%)
Untreated	8 (10%)
HCST characteristics	
Conditioning regimen	
Myeloablative	22 (30%)
Reduced intensity	52 (30%)
Stem cell source	
Peripheral blood	69 (%)
Bone marrow	3 (%)
Cord blood	2 (%)
Donor	
Related	45 (60%)
Unrelated	22 (32%)
Haploidentical	6 (8%)
GVHD prophylaxis	
calcineurin inhibitors-regimens (+MMF or +methotrexate	38 (52%)
tacrolimus and rapamicine	25 (34%)
other inmunosupresion	10 (14%)

Results: The main characteristics of the patients at transplant were: median age 55 years (14-69); disease status at transplant: refractory 37 (50%), relapse 17 (23%), untreated 8 (10%) and aplastic 12 (30%); median of blasts at transplant was 10% (5-72). 70 (94%) of the patients had evaluable pretrasplantation cytogenetics: 12 favorable (16%), 29 intermediate (40%) and 29 unfavorable (39%) (according MRC and IPSS-R). Median time from diagnosis to HCT was 7 months (1-160). All the patients but one engrafted. Median time to reach >500 granulocytes and >20000 platelets were 17 (10 to 41) and 12 (6 to 70) respectively. The incidence of acute-GVHD (aGVHD) was 61% (14% grade III-IV) with median onset of 26 days (8-130); in evaluable patients, incidence of chronic-GVHD (cGVHD) was 45% (24% severe) with a median onset of 163 days (84-451). (other characteristics in Table 1). Response to transplant was evaluated at days +21 or 28, +56 and +100. At day +100: 43 patients (58%) were in complete remission (CR), 13 (17%) have progressed and 18 (24%) have died,mostly due to disease (n=12). With a median follow up of 30 months among patients alive, the estimated OS and EFS at 6 monthsat 1 year and 3 years was 63%, 50%,37% and 47%, 38% and 30% respectively. The overall TRM was 17% (10% at day +100). In the univariate analysis, to maintain CR at +100 and to develop cGVHD were the only factors associated with a better OS and EFS (p<0.05); these variables mantained their significance in the mul-

tivariate analysis. Although patients with favourable cytogenetics findings had a trend to a better relapse free survival as compared with intermediate and unfavourable (74% *vs* 41%; p=0.1), we couldn't demonstrate its impact in OS and EFS in the univariate and multivariate analysis. Status disease at transplant and blast percentaje has not significance in both OS and EFS also. The main cause of death was progression disease (n=29, 40%). Median time to disease progression was 183 days (6 to 4773). Considering those patients who were on CR at +100, 76% of them (33/43) maintained the CR and 53% of them (23/43) remained alive and disease free at last follow up to (156 months). Analysing the influence of cGVHD in this selected group of patients on CR at +100, the estimated probability of relapse at 1 year was 43% in those without cGVHD (n=14) *vs* 11% in those who developed cGVHD (n=29) (p=0.02).
Summary and Conclusions: Our results indicate that patients with rAL or MDS could benefit from HSCT even with high leukemia burden and should be considered as transplant candidates. We have shown that HSCT can induce long-term disease remission and possibly cure in those who developed cGVHD especially who are on CR at +100.

E1537

RETROSPECTIVE AUDIT OF A SINGLE CENTRE USE OF FRESH FROZEN PLASMA WITH OR WITHOUT PLASMA EXCHANGE TO REDUCE ISOAGGLUTININ TITRES PRIOR TO MAJOR ABO MISMATCHED HAEMATOPOIETIC STEM CELL TRANSPLANTS

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Background: ABO major mismatched transplants are known to be associated with various immune mediated complications such as immediate hemolysis, delayed red cell engraftment and pure red cell aplasia. Reduction of incompatible (recipient) isoagglutinins pretransplantation has been thought to reduce the incidence of these complications, though the optimal strategy for this remains unclear. Use of donor group fresh frozen plasma (FFP) infusions with or without plasma exchange (PLEX) has been one of the strategies used, but the literature on the safety and efficacy of this method has been sparse.
Aims: The aim of our retrospective audit was to assess the efficacy of donor-type FFP infusion on anti-A and/or B isoagglutinin reduction and clinical outcomes associated with this strategy of isoagglutinin reduction.
Methods: All patients undergoing major or bidirectional ABO-mismatched bone marrow (BM), peripheral blood (PBSC), and cord blood (UCBT) hematopoietic stem cell transplantation (HSCT) between 2010 -2014 were identified from our institutional database. All patients received daily donor-type FFP infusion till day of stem cell infusion if their antidonor isoagglutinin titres (Anti-A or Anti-B titres) were ≥1:16, and PLEX was considered on the day of stem cell infusion if the antibody titres remained ≥1:16. In addition, for all major ABO incompatible HSCTs, the stem cell product was red cell-depleted, while for bidirectional ABO incompatible HSCTs, the stem cell product was red cell- and plasma-depleted.
Results: We identified 45 patients, 33 who received a major ABO mismatched (73%) and 12 (27%) who had a bidirectional ABO mismatched HSCT during this period (Table 1). Amongst these patients, 43 (96%) had an underlying malignant disease whilst only 2 had a benign condition (Figure 1).

Table 1. ABO donor recipient pairs in the study.

ABO Incompatibility	Total Number of Patients tested	Blood Group		No. of patients
		Recipient	Donor	
Major	33	O	A	18
		O	B	9
		O	AB	3
		A	AB	0
		B	AB	3
Bidirectional	12	A	B	4
		B	A	8

Of the 45 patients identified, 29 patients (65%) required FFP with a median of 3871 ml (Range: 750ml-10 900 ml) received per patient. Median antibody titres were 1:64 (Range: 32-1024) before intervention and 1:32 (Range: 2-256) post intervention. Eight of the 29 patients had reduction of antibody titres to ≤16 and did not need PLEX. Amongst the other 21 patients, 11 went on to receive PLEX, while 10 did not due to either patient or physician choice. No significant major side effects from FFP infusion was noted in all patients though 5 patients (17%) suffered minor infusion reactions such as urticaria. No episodes of immediate hemolysis or disseminated intravascular thrombosis were seen in any of the patients at the time of the graft infusions. Incidence of pure red cell aplasia was 4% (n=2). There were no significant major side effects from FFP infusion, though 5 patients (11%) suffered minor reaction like urticaria. There were no episodes of immediate hemolysis or disseminated intravascular thrombosis seen in any patient at the time of ABO-incompatible graft infusions. Incidence of pure red cell aplasia was 4% (n=2). There were 11 early transplant deaths (24%) and 2 suffered graft failure (4%).

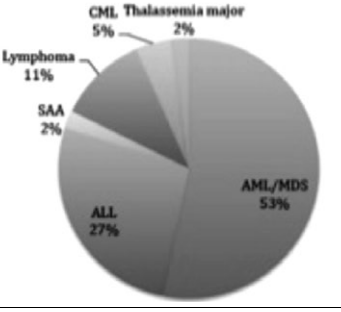

Figure 1. Indications for tranplant.

Summary and Conclusions: Our findings suggest that that the use of donor-type FFP was safe, and was able to reduce anti-donor issoagglutinins by one fold dilution (from median 1:64 to 1:32). Interestingly, despite only 50% of patients who were recommended to receive PLEX actually receiving it, there did not appear to be an increase in severe infusional reactions, hemolysis or PRCA incidence in the patients in our study. Our findings confirm the safety and efficacy of donor-type FFP in reducing isoagglutinin titres, but also question whether the threshold at which PLEX is recommended may be raised especially with the current concomitant use of red cell depletion in the stem cell products.

E1538

BONE MARROW EOSINOPHILIA CORRELATES WITH TREATMENT OUTCOMES BOTH AFTER AUTOLOGOUS AND ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Many studies have shown the beneficial influence of eosinophils (Eo) in diverse neoplasms independent of other standard prognostic factors. Besides, eosinophilia in the blood and tissue is a common feature of graft *versus* host disease (GvHD) and contributes to the allogeneic response, as revealed in investigations in animal models and clinical observations. Data from a number of cohorts indicate that elevated Eo blood counts could be associated with better outcomes following allogeneic hematopoietic stem cell transplant (HSCT). However, whether the degree of bone marrow eosinophilia influences transplant outcomes has not been established yet, neither the significance of eosinophilia after autologous transplantation.
Aims: The aim of the study was to evaluate how the existence and degree of peripheral blood (PB) and bone marrow (BM) eosinophilia after auto-HSCT and allo-HSCT influenced transplant outcomes.
Methods: A total of 310 consecutive pts who received HSCT at the National Hematology Hospital, Sofia were included in this retrospective study. Auto-HSCT was performed in 246 pts, 148 male and 98 female, at a mean age of 48.5 years (ranging 19-67), while allogeneic-in 64 patients, 45 male and 19 female, including 35 from related and 29 from unrelated donors, at a mean age of 37.4 years (20-59). PB and BM Eo were evaluated at day 100 and at a next time point depending on the follow-up schedule. Eosinophilia was defined as an Eo count >0.5x10(9)/L in PB and >3.5% in the BM. Information about the following events was recorded: peripheral cell engraftment, acute (a)GvHD and chronic (c)GvHD, viral reactivation, and deaths. Overall survival (OS) was defined as the time from transplantation until death from any cause.
Results: A biphasic pattern of eosinophilia was noted after HSCT. The first peak occurred prior to day 100 then the second one, beyond day 100. At day 100 PB eosinophilia was detected in 4.9% of all pts, while increased BM Eo were seen in 27.7%, without any correlation between PB and BM counts, with no significant difference between auto- and allo-HSCT and regardless of the donor type. No differences in engraftment, viral reactivation, aGVHD or cGVHD depending on Eo counts were established. At this time point PB eosinophilia did not correlate with OS in neither groups, however increased BM Eo were associated with better outcomes in the overall cohort of allo-transplanted pts (100% *vs* 51% OS in pts with low Eo, and median not reached *vs* 24 months, respectively; log rank p=0.001). Beyond day 100 PB eosinophilia was rarely seen in only 2.3% of all pts, while BM Eo were increased in 33.8% regardless

of the type of HSCT. Similarly, PB eosinophilia did not correlate with OS in neither groups of pts, however increased BM Eo were associated with longer OS both in auto-transplanted (100% *vs* 85.7% OS in pts with low Eo, mean 72 *vs* 63 months, respectively; log rank p=0.03), and in allo-transplanted pts (94% *vs* 61.5% OS in pts with low Eo, mean 41 *vs* 27 months, respectively; log rank p=0.007) (Figure 1).

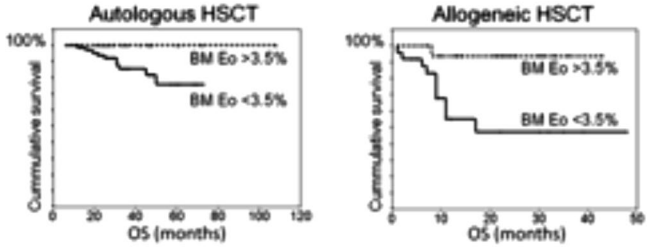

Figure 1. Overall survival curves for patients with and without BM eosinophilia beyond day 100.

Summary and Conclusions: Better outcomes could be expected in patients with BM eosinophilia regardless of the PB Eo counts and the type of transplantation. Interestingly, based on the results common mechanisms might be speculated in allogeneic and autollogous HSCT, however the pathophysiology behind eosinophilia remains to be investigated. Further studies are also warranted to elucidate the causative mechanisms behind the clinical benefits of increased eosinophils.

E1539

AUTOLOGOUS PERIPHERAL BLOOD HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT) FOR MULTIPLE MYELOMA WITHOUT CRYOPRESERVATION OF STEM CELL,SINGLE CENTER EXPERIENCE

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Background: Hematologic malignancy currently represents the main indication for HSCT. Clearly, autologous and allogeneic HSCT are established therapies in many of hematologic malignancies. High dose therapy (HDT) supported by autologous HSCT are the preferred choice for lymphoproliferative disorders and multiple myeloma. Several clinical trials have shown the superiority of high-dose therapy (HDT) and autologous stem cell transplantation over conventional dose therapy for patients with multiple myeloma.
Aims: There is limited experiences with noncryopereserved autologous transplantation.In this report we will describe our experiences with this method.
Methods: During 10 years we treated 152 patients with multiple myeloma (mean age=53 range: 31-70) with a conditioning regimen of Melphalan 140-200 mg/m^2. Patients were treated by intensive chemotherapy followed by reinfusion of non- cryopreserved autologous stem cells. The source of stem cell in all patients was peripheral blood. All apheresis products were kept in a conventional blood bank refrigerator at 4°C for 2 -3days before infusion.
Results: The median time of hospitalization was 19days (range: 16-37).The median time to platelet count >20×10^9/L was 14 days (range: 10-35). Also, the median time to absolute neutrophil count >0.5 × 10^9/L was 11 days (range: 9-22). All the 152 patients were engrafted and there was not graft failure in this study group. Responses (complete and partial response) were seen in all the 152 patients. Transplant-related mortality in this study group was 1.3%.
Summary and Conclusions: All patients in our study showed hematopoietic engraftment after receiving the HDT and non-cryo preserved stem cell. According to our experiences and results we concluded HDT and autologous stem cell transplantation without cryopreservation is effective and safe method which simplifies the procedure and is feasible and cost saving in our patients with diagnosis of multiple myeloma.

LB2095

ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR CHILDREN WITH HEMOPHAGOCYTIC SYNDROME: SINGLE CENTER EXPERIENCE

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Background: Hematopoietic stem cell transplantation (HSCT) is the only curative treatment for patients with primary hemophagocytic lymphohistiocytosis (HLH) and for patients with secondary HLH who fail to respond to therapy.
Aims: To observe the conditioning regimen, efficacy, side effects of HSCT for HLH.
Methods: We retrospectively reviewed children with HLH who received HSCT. From April 2011 to February 2015, a total of 15 allo-HSCT in 14 cases (9 were females) were evaluated including 13 cases with familial HLH and a case with secondary HLH related otoimmun disease who fail to respond therapy. The median age was 19 months (3 months-9 yr). Eight patients received HLA-matched related HSCT, five received HLH-match unrelated HSCT and two received unrelated cord blood transplantation with conditioning regimen of etoposide, busulphan, cycphosphamid and ATG (ATG in 13 pts). Cyclosporine (in 9 pts) or cyclosporin and methorexate (in 6 pts) were used for prevention of graft *versus* host disease (GVHD).
Results: The mean overall survival time was 11 months (1-46 months). Eleven patients were successfully engrafted. Acute GVHD occurred in 5 cases, including 3 cases with skin, a case with skin+liver, and a case with gastrointestinal GvHD. Chronic GVHD (cGVHD) occurred in 3 cases. Hepatic veno-occlusive disease were developed in 6 (40%) patients. Five cases died after allo-HSCT. Three patients died (reactivation of disease=1, infection=1, other=1) before day +100, and another 2 patients died (reactivation of disease=1, infection=1) after day 100. In four HSCT, failed to engraft (a case developed recurrent HLH and died from infection after a second HSCT).
Summary and Conclusions: The allo-HSCT is successful in treating primary and secondary HLH who refractory to therapy.

LB2096

POST TRANSPLANT ERYTHROCYTOSIS IN ELEVEN CASES

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Background: Post transplant erythrocytosis (PTE) is not an expected complication after allogeneic hematopoietic stem cell transplantation (allo-HSCT). Only Mahmood *et al.* reported PTE in 3 aplastic anemia cases in literature.
Aims: We aim to present 11 PTE diagnosed as; 4 acute myeloblastic leukemia (AML), 2 paroxysmal nocturnal hemoglobinuria (PNH), 1 myelodysplastic syndrome (MDS), 2 chronic myeloid leukemia (CML), 1 primary myelofibrosis (PMF), 1 fanconi aplastic anemia patient.
Methods: We retrospectively evaluated 11 PTE patients from 945 allo-HSCTs in our center.
Results: Patients' characteristics are shown in Table. All patients are male. They have transplanted from full matched related donors except one patient has 1 mismatch and received stem cells from unrelated donor. PMF and fanconi aplastic anemia patients received stem cells from bone marrow, others transplanted by peripheral blood stem cells. 1 CML patient relapsed after allo-HSCT but followed in remission with tyrosine kinase inhibitors. None of them had either leukocytosis or thrombocytosis. All of the patients had hyperviscosity symptoms with no organomegaly. Erythropoietin levels in the upper limit of normal in all patients. No JAK2V617F mutation positivity was detected. 2/11 patients had bronchiolitis obliterans (BO) and 5/11 diagnosed as chronic liver Graft *vs* Host Disease (GVHD) during follow-up. None of them are smokers. All patients received cyclosporine (CsA) for GVHD prophylaxis.

Table 1.

Disease	Age	Pre transplant status	HLA Match/donor	GVHD prophylaxis	Conditioning Regimen	GVHD (Graft vs Host Disease) site	Post transplant status	Hb (g/dl) /Hct (%) and time after transplant
AML	38	CR	Full/related	CsA+MTX	MA	Skin Liver	Remission	17.9/52, 5 years
PNH	29	Aplastic	Full/related	CsA+MTX	RIC	Skin	Remission	17.6/51, 3 months
AML	49	2.CR	Full/related	CsA+MTX	MA	Skin, GI,Lung eye	Remission	17.5/54.5 , 4 years
MDS	17	Transfusion dependent	1 Mis match/unrelated	CsA+MP	MA	Skin, liver,GI	Remission	17.7/51.7 , 4 years
PNH	26	Aplastic	Full/related	CsA+MMF	RIC	-	Remission	17.5/55, 5 years
AML	33	2.CR	Full/related	CsA+MTX	MA	Liver	Remission	17.6/51, 6 years
Fanconi Aplastic Anemia	21	Transfusion dependent	Full/related	CsA/MMF+MTX	RIC	GI	Remission	17.5/51.6 , 1 year
CML	43	Active disease	Full/related	CsA+MTX	MA	GI,Liver,Eye	Remission	17.3/51.2 , 12 years
AML	32	2.CR	Full/related	CsA/MMF+MTX	MA	Eye, Liver,Lung	Remission	16.7/50.7 , 6 years
PMF	57	Transfusion dependent	Full/related	CsA+MTX	MA	-	Remission	17.7/50.4 , 2 years
CML	45	Active disease	Full/related	CsA+MTX	MA	-	Relapsed/remission with TKI	16.8/50.2 , 11 years

Table 1. Patients' characteristics (CR: Complete Remission, GI: Gastrointestinal, RIC: Reduced intensity Conditioning, MA: Myeloablative, MTX: Methothrexate, TKI: Tyrosine Kinase Inhibitor)

Summary and Conclusions: Liver GVHD, BO and cyclosporine could lead to secondary erythrocytosis however there is no evidence for causality. All of our patients were male which is a risk factor for erythrocytosis similar to hypertension and smoking. Remarkably, all of the patient except one had erythrocytosis a year after allo-HSCT. Further surveillance of the cases will improve our knowledge over erythrocytosis.

Stem cell transplantation - Experimental

E1540

IMMUNOPHENOTYPIC CHARACTERIZATION OF HUMAN MOBILIZED HEMATOPOIETIC STEM AND PROGENITOR CELLS: BIOLOGICAL AND CLINICAL IMPLICATIONS

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Background: In the last years, peripheral hematopoietic stem and progenitor cells (HSPC) have been widely used for transplantation procedures, instead of bone marrow HSPC. The advantages consist in a quicker hematological and immunological reconstitution and in a reduced morbidity and mortality. Unfortunately, very few is known about the proportions of HSPC in the peripheral CD34+ cells.
Aims: In the present study, we intend to investigate the composition of circulating HSPC subpopulations and analyze whether the differences in the number of re-infused precursors could influence the hematopoietic engraftment after an hematopoietic stem cell transplantation.
Methods: Multicolor flowcytometry was run to examine 9 base-line peripheral blood (PB) samples and 5 bone marrow (BM) samples from healthy donors and 33 mobilized peripheral blood (mPB) samples from hematological patients prior CD34+ cells harvesting.
Results: Common myeloid progenitors (CMP) showed a higher ratio in PB compared to BM (47.8%±9.5 *versus* 27.6%±9.5) while granulocyte-macrophage progenitors (GMP) were lower in PB compared to BM (10.3%±6.9 *versus* 23.8%±7.2). Progenitors fractions were equally distributed in BM (27.6%±9.5 for CMP, 23.8%±7.2 for GMP and 27.6%±16.2 for megakaryocyte-erythroid progenitors -MEP). No significant differences were noticed between PB and BM hematopoietic stem cells (HSC) (1.54±1.22 in PB *versus* 2.11±0.69 in BM) (Figure1a). Comparing base-line PB and mPB, no significant differences were shown between the subpopulations (Figure1b). Among mPB samples, cyclophosphamide chemotherapy mobilized a higher ratio of CMP (61.1%±12.05) and a lower percentage of GMP (11.09%±4.74) unlike patients who had received other drugs (CMP 49.06%±14.12 and GMP 27.59%±10.92). No differences were found in HSC proportions (Figure1c). In the two patients mobilized with the anti-CXCR4 Plerixafor instead of C-GSF only, more elevated proportions of GMP were released: 37.8% in patient#1 and 33.8% in patient#2 compared to the average 16.31% of the "G-CSF only" mobilized samples (Figure1d). In the analysis of CXCR4 expression among the subpopulations, we observed a significant higher mean fluorescence intensity of this marker on GMP (Figure1e). Furthermore, a strong correlation between the number of total peripheral CD34+ cells and mobilized CMP, GMP and MEP was shown (Figure1f); no correlation was displayed with mobilized HSC. White blood cells (WBC) count exhibited significant correlation with the number of mobilized HSC (Figure1g), but not with CMP/GMP/MEP. The next step was to detect possible relationships between the number of re-infused progenitors or HSC and the hematological recovery in patients receiving an auto-transplantation conditioned by high dose chemotherapy. A tendency to inverse correlation was shown between the number of re-infused progenitors and the days of aplasia (Figure1h-1m), as well as between the number of re-infused CMP and MEP and platelet levels during aplasia time (Figure1o-1q).

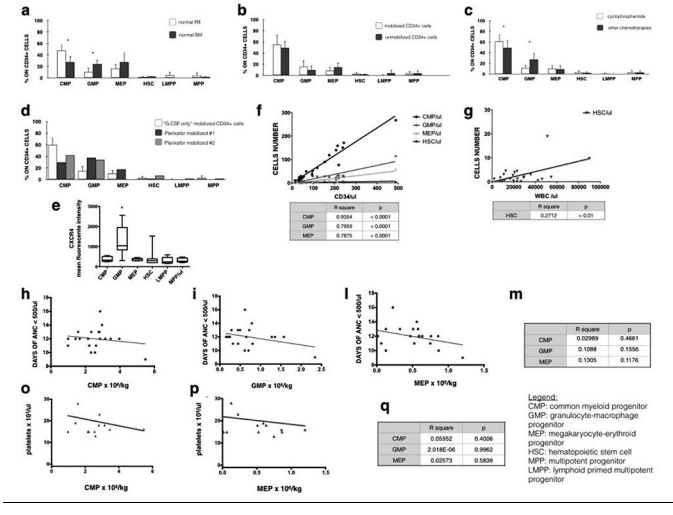

Figure 1.

Summary and Conclusions: With these data, we confirmed the heterogeneity of HSPC composition between PB and BM; chemo and mobilization regimens can strongly influence the proportions of mobilized HSPC. Furthermore, the number and type of re-infused progenitor could effect the engraftment kinetic and the hematological recovery. Further studies on mobilized HSPC and the correlation with clinical data will provide new sensitive tools for improving the outcomes of hematopoietic stem cell transplantation.

E1541

EXPRESSION OF TH17/TREGS AXIS ASSOCIATED REGULATORY FACTORS IN PATIENTS WITH ACUTE GRAFT-*VERSUS*-HOST DISEASE

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Background: Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is one of the most valid methods for the treatment of malignant hematological diseases. Graft-*versus*-host disease (GVHD)□which remains the main complication after allo-HSCT□leads to the high transplant-related mortality. GVHD occurs as a result of T cell activation. Acute GVHD (aGVHD) is suggested to be predominantly related to T helper 1 (Th1) responses. Recent investigations have discovered the possible role of Th17 cells and regulatory T cells (Tregs) in the development of aGVHD. Th17 cells are found to have a direct role in the development of aGVHD, and adoptive transfer of *in vitro*-differentiated Th17 cells is capable of inducing lethal aGVHD. Clinical and experimental studies have suggested that Tregs could prevent and treat aGVHD, while preserve graft-*versus*-tumor activity. However, the controls of Th17/Tregs axis on the occurrence and development of aGVHD are not fully understood.
Aims: To investigate the expression levels and clinical significance of Th17/Tregs axis associated regulatory factors in patients with aGVHD.
Methods: The expression levels of Th17/Tregs axis associated regulatory factors (IL-17A, IL-23R, RORc, STAT-1, STAT-3, Foxp3, CD25, CTLA-4, GITR and TLR8) were analyzed in peripheral blood from 20 patients with aGVHD at the following two time points (at the onset of aGVHD and two weeks after the treatment), using real-time reverse transcription polymerase chain reaction with SYBR Green I staining. Fifteen patients responded effectively to the treatment within two weeks, and 5 did not respond within two weeks. Twenty healthy donors were selected for the control. The β_2-microglobulin gene was used as an endogenous reference, and the relative mRNA expression level of each gene was evaluated by the $2^{-\Delta Ct}$×100% method.
Results: The expression levels of RORc, STAT-1 and STAT-3 genes in the untreated patients with aGVHD were significantly higher than that in the healthy donors (P<0.001, P<0.001 and P<0.001), while the expression levels of IL-17A and IL-23R genes were similar between the untreated patients with aGVHD and the healthy donors (P=0.147, P=0.190). The expression levels of GITR and TLR8 genes in the untreated patients with aGVHD were also significantly higher than that in the healthy donors (P=0.003, P<0.001). The expression levels of Foxp3, CD25 and CTLA-4 genes were similar between the untreated aGVHD patients and the healthy donors (P=0.298, P=0.934 and P=908). The expression level of TLR8 gene in the patients with aGVHD was significantly decreased after effective treatment (P=0.035). The expression levels of IL-17A, IL-23R, RORc, STAT-1, STAT-3, Foxp3, CD25, CTLA-4 and GITR genes in untreated patients with aGVHD had no significant change after effective treatment (P=0.778, P=0.875, P=0.730, P=0.177, P=0.925, P=0.397, P=0.778, P=0.470 and P=0.638). The expression level of Foxp3 gene was significantly decreased after ineffective treatment (P=0.043). The expression levels of IL-17A, IL-23R, RORc, STAT-1, STAT-3, CD25, CTLA-4, GITR and TLR8 genes were similar in patients at aGVHD onset and after ineffective treatment (P=0.686, P=0.500, P=0.686, P=0.345, P=0.225, P=0.345, P=0.345, P=0.893 and P=0.345).
Summary and Conclusions: Th17/Tregs axis associated regulatory factors might influence in the occurrence and development of aGVHD□

E1542

MECHANISMS OF FATAL CARDIOTOXICITY FOLLOWING HIGH-DOSE CYCLOPHOSPHAMIDE THERAPY AND A METHOD FOR ITS PREVENTION

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Background: High-dose cyclophosphamide (CY) is a mainstay in most conditioning regimens for hematopoietic stem cell transplantation. Recently, administration of posttransplantation of CY in high doses has been attracting attention as novel strategy for preventing graft-*versus*-host disease. CY is activated by the hepatic cytochrome P-450 (CYP) enzyme system to form 4-hydroxycyclophosphamide (HCY), which is in equilibrium with aldocyclophosphamide (AldoCY). AldoCY decomposes to form cytotoxic phosphoramide mustard and the byproduct acrolein. Alternatively, aldoCY is oxidized to the inactive metabolite *o*-carboxyethylphosphoramide mustard (CEPM) by aldehyde dehydrogenase1 (ALDH1). The dose-limiting toxic effect of CY, observed only after administration of high doses, is cardiotoxicity.
Aims: Since the mechanism underlying this phenomenon has not yet been elucidated, and no definitive risk factors have yet been identified, we investi-

gated the cardiotoxic mechanisms of high-dose CY. To determine preliminary mechanisms that may prevent the occurrence of CY-induced cytotoxicity in H9c2 embryonic rat cardiomyocytes cell line, we also evaluated the protective effects of potential cardioprotectant agents.
Methods: A rat cardiac myocardial cell line, H9C2, was exposed to CY metabolized by S9 fraction of rat liver homogenate mixed with co-factors (CYS9). The degree of cytotoxicity was then evaluated by MTT assay, LDH release, production of reactive oxygen species (ROS), and incidence of apoptosis. We also investigated how the myocardial cellular effects of CYS9 were modified by antioxidant N-acetylcysteine (NAC), isorhamnetin (ISO), and β-ionone (BIO) a CYP inhibitor. Quantifying CY and CY metabolites, using LC/MS/MS, we assayed culture supernatants of CYS9 with or without potential cardioprotectant agents (PCA). In addition, we observed the cytotoxicity of CYS9 and the protective effects of PCA using a live-cell imaging system.
Results: Assay results for MTT showed that treatment with CY (125-500 μM) did not induce cytotoxicity. CYS9, however, exhibited myocardial cytotoxicity when CY concentration was 250 μM or more. After 250 μM of CY was metabolized in S9 mix for 2 h, the concentration of HCY was 18±4, CEPM 27±5 μM, and acrolein 31±1 μM. NAC, ISO, and BIO all inhibited CYS9-induced cytotoxicity. When treated with ISO or BIO, metabolism of CY was significantly inhibited. Pretreatment with NAC, however, did not inhibit the metabolism of CY. Compared to control samples, we observed no difference in HCY, a significant increase of CEPM, and a significant decrease of acrolein. Furthermore, NAC pre-treatment did not affect intracellular ROS levels produced by CYS9. Live-cell imaging also confirmed that CYS9 induced acute cytotoxicity, which was inhibited by NAC.
Summary and Conclusions: We ascertained that, when metabolized *in vitro* by S9, CY would produce similar levels of metabolic substance concentrations as *in vivo*. When H9C2 was pre-treated with NAC, ISO, and BIO, each inhibited cell damage by CYS9. When treated with ISO and BIO, we observed that CY metabolism itself was inhibited. This result suggests that cardiac cell damage may depend on individual differences in CY metabolic capacity. As preventative drugs, however, both BIO and ISO seem likely to interfere with antitumor effects and are thought to be unsuitable. Pre-treatment with NAC did not inhibit CY metabolism: compared to control samples, while HCY concentration was similar, CEPM concentration statistically significantly increased. Less acrolein, however, was present after exposure to NAC. The increase in CEPM suggests that scavenging of acrolein by NAC prevents inhibition of ALDH1. Consequently, through a mechanism related to its ability to inhibit acrolein, NAC may attenuate cardiotoxicity associated with high-dose CY.

E1543

SCA1+ MESENCHYMAL STROMAL CELLS INHIBIT GRAFT-*VERSUS*-HOST DISEASE IN MICE AFTER BONE MARROW TRANSPLANTATION

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Background: Mesenchymal stromal cells (MSCs) have therapeutic potential for the prevention and treatment of graft-*versus*-host disease (GVHD). Although the treatment of GVHD using MSCs has been studied in phase I/II and phase III clinical trials, the results of clinical trials seem contradictory. MSCs comprise several subpopulations, which have not been individually assessed for their role in GVHD suppression. However, previously conducted pre-clinical experiments or clinical trials for investigating the effects of MSCs on GVHD have predominantly used MSCs mixtures. We speculate that the heterogeneity of MSCs mixtures may be responsible for the contradictory outcomes of previous studies. Some MSCs subpopulations may be immunosuppressive, whereas others may be immune stimulatory. Therefore, it is necessary to explore the therapeutic effects of MSCs subpopulations which remain unknown. Sca1 is a marker expressed by hematopoietic progenitors and stem cells. Hu *et al.* previously identified bone-associated $CD45^{-}Ter119^{-}CD31^{-}Sca1^{+}$ cells as a common progenitor of niche mesenchymal cells in adults. Thus, Sca1 is also a cell marker that is expressed by a primitive subpopulation of adult MSCs.
Aims: In this study, we aimed to assess the immunosuppressive effect of bone-associated $Sca1^{+}$ MSCs on acute GVHD (aGVHD) and elucidate the related mechanisms in a MHC-mismatched mouse model of allogeneic hematopoietic stem cell transplantation (HCT).
Methods: The clinical manifestation of aGVHD was evaluated by the loss of body weight, diarrhea, clinical scores, survival period and pathological changes of target organs. The infiltration of T cells and the expression of CD80/86 in target organs were analyzed by flow cytometry. The expression of CTLA-4 in splenocytes was tested by Real-time PCR.
Results: Our results showed that: (1) Non-cultured $Sca1^{+}$ MSCs decreased the severity of aGVHD and prolonged the survival period of allogeneic HCT recipients; (2) The effect of $Sca1^{+}$ MSCs on aGVHD was slightly better than that of MSCs mixture, but not in a statistically significant way; (3) Infusion of $Sca1^{+}$ MSCs reduced donor T cell infiltration into GVHD target organs; (4) The expression of CD80 and CD86 on splenic dendritic cells was decreased in $Sca1^{+}$ MSCs injected recipients; (5) The expression of cytotoxic T-lymphocyte antigen-4 (CTLA-4), a negative regulator of T cells, was elevated in the recipient splenocytes.
Summary and Conclusions: Bone-associated $Sca1^{+}$ MSCs subpopulation suppressed GVHD and should be considered as a potential strategic treatment of aGVHD.

E1544

IN VITRO EXPANDED HUMAN CD4+CD25+FOXP3+ REGULATORY T CELLS IN NUTRIENT-DEPRIVED MEDIUM AS A POTENTIAL ARMAMENT IN THE PREVENTION OF GRAFT-*VERSUS*-HOST DISEASE

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Background: Allogeneic haematopoietic stem cell transplantation (HSCT) has been used to treat some of haematological malignancies and inherited or acquired non-malignant diseases. Unfortunately, graft-*versus*-host disease (GVDH) occurred approximately 15% in transplant recipients and decreases the success of allogeneic HSCT. At present, no effective treatment can completely prevent the GVHD from allogeneic HSCT patients. CD4+CD25+FoxP3+ regulatory T cells (Tregs) have been shown to be important in maintaining immune homeostasis and preventing autoimmunity. However, 5% to 10% Tregs could be measured in human CD4+ T cells and few Tregs would convert to conventional activated T cells because of losing FoxP3 expression. It had been reported to correlate with the occurrence and severity of GVHD in some study.
Aims: In order to study the potential use CD4+CD25+FoxP3+ Tregs for the prevention of GVHD, we attempt to evaluate the better efficient method to increase the number of induced Treg cells (iTregs) in the donor's PB and stabilize the FoxP3 in iTreg cells.
Methods: PBMC were prepared from blood of healthy donors by Ficoll-Hypaque density gradient centrifugation. T cells were isolated by negative selection. CD4+ cells were harvested, and then activated with anti-CD3/CD28 beads in the presence of IL-2, TGF-β and retinoic acid (RA) containing RPMI1640 medium. During the Tregs induction, the activated T cells were performed under low nutrient supplement (5% FBS) for three days then refreshed the cells into the full nutrient supplement (10% FBS) for another four days. The harvested cells were analyzed by flow cytometry method with fluorescence-conjugated CD-antibodies, including CD4, CD25, CD127 and FoxP3. The protocol was shown in Figure 1A.

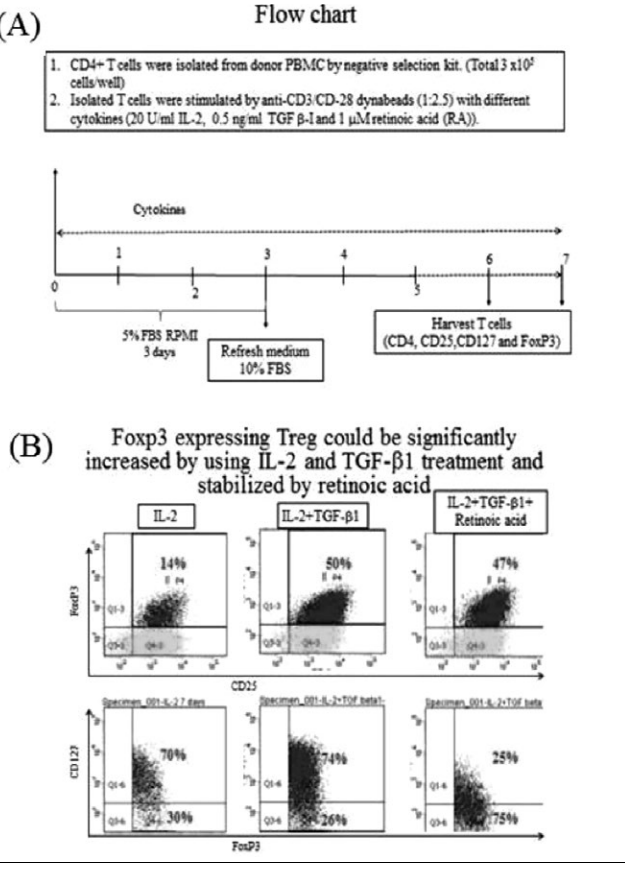

Figure 1.

Results: Our data showed that FoxP3+Tregs in PBMC were increased from 3.5% to 30% under IL-2 (20U/ml) and TGF-β (0.5ng/ml) containing medium, after T cell activation. Most importantly, the number of FoxP3+Treg cells was

increased from 30% to 50% with the 3-day-nutrient deprivation in advance, comparing with the full nutrient supplement for 7 days. The addition of retinoic acid (1mM) stabilized the FoxP3+ Tregs during this incubation period. Besides, FoxP3+ and CD127 - shown in Figure 1B, the combination of RA and TGF-β increased the functional iTreg cells to >70% (from 30% to 75% in this representative data). Finally, activated T cells differentiated to FoxP3+ Treg cells and stabilized by RA significantly, under this particular treatment.
Summary and Conclusions: Our study showed that the combination of IL-2, TGF-β and RA in 3-day-nutrient-deprived medium could induce naïve CD4+CD25+ cells to express and stabilize FoxP3 markedly. Further, we will develop the iTreg suppression assay to clarify the biological function of iTregs *in vitro*. GVHD mouse model will be established by using allogeneic HSCT to verify iTreg's function *in vivo*, too.

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E1545

PROGNOSTIC SIGNIFICANCE OF IMMUNOLOGIC RECOVERY AFTER AUTOLOGOUS PERIPHERAL BLOOD STEM CELL TRANSPLANTATION IN PATIENTS WITH LYMPHOMAS

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Background: Autologous peripheral blood stem cell transplantation (APBCST) is now established therapy for patients (pts) with refractory and relapsed lymphomas (Hodgkin lymphoma-HL and non-Hodgkin's lymphoma-NHL). A lot of factors influence on post transplant outcome: performance status, activity of disease at time of APBSCT and chemosensitivity to salvage therapy.
Aims: The aim of our study was the analysis of immunologic recovery after APBSCT as another important factor with prognostic significance.
Methods: A total 28 consecutive pts with lymphomas (16 with NHL and 12 with HL) at Department of Haematooncology and Bone Marrow Transplantation at Medical University of Lublin were recruited to this study. In NHL group there were 5 female and 11 male (median age 50.5) and in HL group there were 7 female and 5 male (median age 27.5). The day before myeloablative regimen blood samples for flow cytometry analysis were taken. In the transplantation procedure pts were received BEAM (27 pts) or CBV (1 pt) regimen. Next blood samples were taken at 30 and 100 day after transplantation procedure. At 100 day after APBSCT pts underwent clinical evaluation with CT or PET to establish activity of disease (Complete Remission-CR or not Complete Remission-nCR).
Results: Analysis of flow cytometry results compared with disease activity showed at 100 day after APBSCT: higher level of T helper cells (CD3+CD4+; CD3+CD4+CD45RO+; CD4+CD25high) in pts with CR compared with nCR (p=0.0069; p=0.0025, p=0.0095); higher level of dendritic cells (BDCA1+CD19-) in pts with CR compared with nCR (p=0.017), and higher level of T regulatory cells (Foxp3+CD4+CD25high in pts with CR compared with nCR (p=0.0095).
Summary and Conclusions: Our analysis of immunologic recovery after APBSCT showed that CR status in pts with lymphomas is associated with higher level of T helper cells, dendritic cells and T regulatory cells. Such ascertainments encourages to searching new methods of immune reconstitution acceleration and enrichment of transplant source with those cells.

E1546

COMPARISON BETWEEN HEALTHY DONOR-DERIVED BONE MARROW MESENCHYMAL STEM CELLS BM-MSCS AND HUMAN DERMAL FIBROBLASTS: IMPACT ON CLINICAL APPLICATIONS OF HMSCS

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Background: The results of clinical applications of human Mesenchymal Stem Cells (hMSCs) depend on clinical and biological factors. The quality of stem cell culture will affect success rate. Stem cell culture contamination by fibroblasts can be happened. The identification of the differences between these two cell types plays an important role to improve the quality of the therapeutic uses of hMSCs.
Aims: Our present work is the first work to compare the phenotype and functional properties of primary human Dermal fibroblasts HDF to hMSC from Bone Marrow BM to reflect the impact of the possible contamination of bone morrow derived hMSC (BM-hMSC) by fibroblasts.
Methods: In this study, hMSCs were obtained from the BM of (Healthy donor, n=5) and compared to primary HDF. The following parameters were used for this comparison: cell morphology, cell proliferation tests, cell cycle, immunophenotype, pluripotent and differentiation capacity into osteoblastic and adipogenic lineages. Genes expression profile was determined in BM-MSCs and HDF by Q-PCR.
Results: Our results show that both of BM-hMSCs and HDF cells have homogenous fusiform, fibroblast-like appearance. Human derived fibroblasts showed, significantly (p<0.001), high proliferative ability than hMSCs, which induces a shortening of doubling time (27.3-/+3h *vs* 44.5-/+ 8h for HDF and BM-MSCs, respectively; p<0,0001). The analysis of cell cycle show that HDF cultures contained, significantly p<0.001, two-fold higher cell number at synthesis stage (S) than in BM-hMSC cultures. Both cell types share the same immunophenotypic feature for these surfaces markers CD45(-), HLA-DR(-), CD73(+), CD90(+), CD105(+), and CD166(+) but CD146 and CD10 were differently expressed on BM MSCs *vs* HDF. Both of cell types have a potential to differentiate into osteoblastic and adipogenic lineages showed by specific stains.This result was confirmed by the expression levels of Runx2, PAL and Osterix (osteoblasts); and PPAR-γ2 (adipocytes). However, expression of these genes in osteoblasts and adipocytes derived from HDF remains, significantly p<0.05, two times lower than the expression in osteoblasts and adipocytes obtained in BM-MSCs differenced cells.
Summary and Conclusions: Our results confirm the similarities of morphology and phenotype of both HDF and BM-MSCs. The differentiation potential of BM-MSCs can not be used to distinguish between HDF and BM-MSCs as we showed that HDF could differentiate into other cell types as osteoblasts and adipocytes. The genes expression profile in osteoblasts and adipocytes derived from BM-MSCs and HDF can be used to distinguish between these tow cell types as we found the genes expression in osteoblasts and adipocytes derived from HDF were significantly lower than in BM-hMSCs. These results may explain the unexpected results of hMSCs in therapeutic applications. In this current study, new marker was identified to discriminate HDF from BM-hMSCs in order to improve the quality of the therapeutic uses of hMSCs.

E1547

COMPARISON OF RED BLOOD CELL AND PLASMA DEPLETION EFFICIENCIES OF TWO METHODS FROM CORD BLOOD: AUTOMATED SEPAX (BIOSAFE) AND MANUAL CELLEFFIC (KANEKA)

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Background: Volume reduction is a routine procedure in cord blood (CB) banking. Two major automated systems have been in use for depletion of excess plasma and red blood cells (RBC) from CB, the most commonly used SEPAX (Biosafe) and AutoXpress Platform (Thermogenesis) systems. Both automated systems were proven to be efficient post processing, yielding high Total Nucleated Cell (TNC) recovery rates as well as viabilities. Nonwoven matrices are being used clinically as filters and scaffolds owing to their ability to trap cells. A novel filtration system was described by KANEKA Corporation (Japan) for processing of CB. This filtration system uses a nonchemical- coated/nonwoven polyester fabric filter, which traps CD34+ cells through affinity without the need of centrifugation or potentially toxic chemicals.
Aims: To compare Red Blood Cell and Plasma Depletion Efficiencies of Two Methods from Cord Blood: Automated SEPAX (Biosafe) and Manual Celleffic (Kaneka).
Methods: Twenty one CB units collected in utero from consented maternal donors were included in the study. CBU plasma and red cell depletion was performed either with only Celleffic or with both Sepax/Celleffic systems. CBUs with below criteria were accepted: 1. CBUs with collection volumes of ≥40 mL and ≤100 mL (with CPD) were chosen for Celleffic only group and CBUs with collection volumes >100 mL were chosen for Sepax *vs* Celleffic comparison group and processed as follows: CBU was split equally in half and after assesment of pre TNC and pre CD34+ cell counts for each split bag, half of the initial CBU was processed with automated SEPAX (Biosafe) and the other half with Celleffic (Kaneka) 2. All CBUs and maternal donors were negative for infectious disease markers 3. CBUs were processed within 48 hrs after collection. Post process TNC, CD34+ cell, mononuclear cells (MNC), RBC and neutrophil recovery rates as well as viability were assessed for all groups and compared for the split CBUs. The comparative results of two processing methods were analysed statistically using Student's T-test.
Results: CBU with collection volumes ≤100 mL (n=12) were processed only with Celleffic whereas CBU with collection volumes >100 mL (n=9) were processed in both systems. There were no significant differences for any of the pre-processing values of the parameters analyzed between two groups (Celleffic only and Celleffic *vs* Sepax). Figure 1 indicates depletion and recovery results of variables after processing with only Celleffic. Figure 2 denotes the comparison between these two systems for the same five parameters. Celleffic was favorable in terms of RBC depletion rates (p=0.008) while Sepax was found to be superior for post TNC and MNC recovery rates (p=0.008 and p=0.015, respectively). There were no significant differences between two systems regarding CD34+ hematopoietic stem cell (HSC) recovery and NRBC cell depletion rates (p=0.674 and p=0.236, respectively). Post processing TNC viability after Celleffic was slightly higher than after Sepax (96% and 94%, respectively; p=0.017).

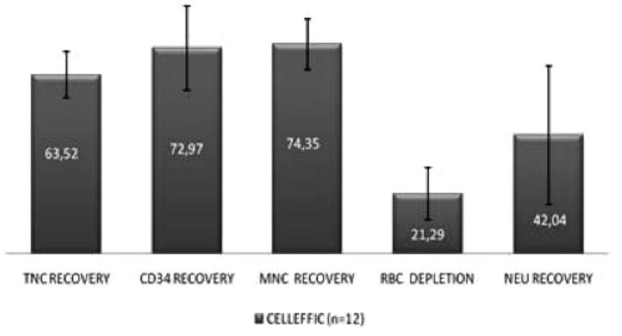

Figure 1. Mean and SD values in percentages of cell recovery (TNC, HSC, MNC, Neutrophil) or red blood cell depletion.

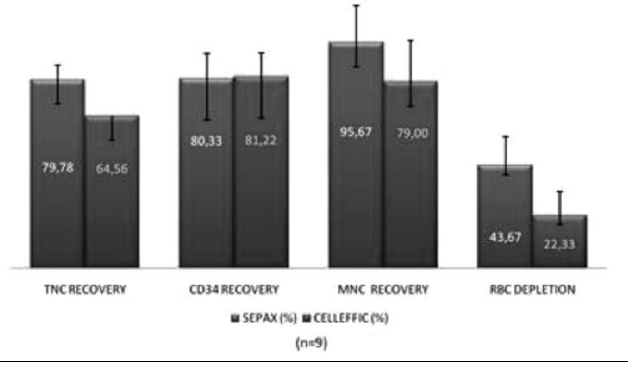

Figure 2. Comparison of two systems in terms of recovery or depletion.

Summary and Conclusions: The filtration method developed by Kaneka is claimed to be simple, cost-effective, and nontoxic without requiring costly equipment mostly suitable for developing laboratories with an average turn over rate. This small sized prospective parallel study shows that both methods are comparable in terms of HSC recovery. The Cellefic system has a higher red cell depletion capacity whereas Sepax was more effective for TNC/MNC recovery. Colony forming potential as well as post thaw viability/recovery rates of both systems are currently being analyzed.

E1548

THE EFFECTS OF -ADRENERGIC AGONIST OF ISOPROTERENOL ON MIR-886-3P AND MIR-23A EXPRESSION IN HUMAN MESENCHYMAL STEM CELLS

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Background: Mobilization of Hematopoietic Stem Cells (HSCs) for transplantation and the importance of -adrenergic signals in induction of this process, have been well investigated. However, little is known about the role of -adrenergic signals in mobilization of HSCs and factors influenced by these signals. The chemokine Stromal Derived Factor 1 (*SDF-1*) which is expressed by human bone marrow-derived mesenchymal stem cells (hMSCs), has a key role in mobilization of HSCs and *miR-886-3p* and *miR-23a* can regulate the expression of this chemokine.
Aims: In this study, To investigate the role of *miR-886-3p* and *miR-23a* in mobilization process, The expression of both miRNAs were evaluated in hMSCs treated by Isoproterenol (a -adrenergic agonist).
Methods: In this study, hMSCs were isolated and cultured from human bone marrow. After doing flowcytometric analysis, the cells were treated with 100 M Isoproterenol. Total RNA was extracted at 12 and 48 hours post treatment, and also from untreated hMSCs as a control. Then *miR-886-3p* and *miR-23a* expression levels were quantified by quantitative Reverse Transcriptase PCR.
Results: The expression level of *miR-886-3p* was increased significantly at 12 and 48 hours post treatment (P<0.05). In addition, the expression level of *miR-23a* was decreased at 12 hours post treatment and increased significantly at 48 hours post treatment (P<0.05).
Summary and Conclusions: Isoproterenol induces *miR-886-3p* in hMSCs. miR-23a is firstly decreased and then increased by treating with Isoproterenol. So both miRNAs can contribute in mobilization process.

Thrombosis and vascular biology

E1549

SOLUBLE FMS-LIKE TYROSINE KINASE-1 IN CHILDREN AND ADOLESCENTS WITH THALASSEMIA INTERMEDIA: RELATION TO PULMONARY VASCULOPATHY AND SUBCLINICAL ATHEROSCLEROSIS

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Background: The study of vascular biomarkers has greatly enhanced our understanding of the underlying pathophysiology of atherosclerosis including soluble biomarkers and physiological parameters such as measures of arterial stiffness and endothelial function. The hypercoagulability in TI has been attributed to several factors, including a procoagulant activity of hemolyzed circulating red blood cells, increased platelet activation, coagulation factor defects, depletion of antithrombotic factors, and endothelial inflammation. Iron-mediated endothelial dysfunction may be mediated either directly through the inactivation of endothelium-derived nitric oxide (NO) or indirectly through the promotion of reactive oxygen species formation. Soluble fms-like tyrosine kinase-1 (sFLT-1) is a member of the vascular endothelial growth factor receptor (VEGFR) family. By adhering to and inhibiting VEGF and placenta growth factor, it induces endothelial dysfunction.
Aims: We assessed the level of sFLT-1 in children and adolescents with TI, correlating it with markers of hemolysis and iron overload as well as cardiovascular complications.
Methods: Thirty-five patients were studied stressing on history of cardiac disease, splenectomy, transfusion history and chelation/hydroxyurea therapy and serum ferritin. sFLT-1 levels were measured by enzyme linked immunosorbent assay. Ehocardiography and measurement of carotid intima media thickness (CIMT) were done for all subjects.
Results: TI patients had significantly higher Tricuspid Regurgitation Velocity (TRV) with lower ejection fraction and fractional shortening than controls (p<0.001). CIMT was significantly increased among patients compared with control group (0.43±0.02 mm *versus* 0.36±0.02 mm; p<0.001). sFLT-1 was significantly higher in TI patients compared to control group (median [IQR], 110 [80-155] pg/mL *versus* 70 [60-90] pg/mL; p<0.001). Splenectomized patients as well as those who had pulmonary hypertension had higher sFLT-1 levels than those without (p<0.001). Hydroxyurea-treated patients had lower sFLT-1 levels than untreated patients. Significant positive relations were observed between sFLT-1 and indirect bilirubin (r=0.357, p=0.035) as well as serum ferritin (r=0.879, p<0.001) while levels were negatively correlated with hemoglobin (r=-0.398, p=0.018). TRV and CIMT were positively correlated with sFLT-1 levels (p<0.001). ROC curve analysis revealed that the cut-off value of of sFLT-1 at 110 pg/mL could differentiate patients with and without pulmonary hypertension with a sensitivity of 86.96% and specificity of 100%, AUC 0.944; p<0.001.
Summary and Conclusions: Measurement of sFLT-1 as a marker of vascular dysfunction in β-TI may provide utility for early identification of patients at increased risk of pulmonary and cardiovascular complications. The relation between sFLT-1 and CIMT provides a link between endothelial dysfunction and subclinical atherosclerosis.

E1550

ASSOCIATION OF THE DYNAMIC STATUS OF COAGULATION FACTORS WITH L-ASPARAGINASE ADMINISTRATION IN THE INDUCTION PHASE OF PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA AND LYMPHOBLASTIC LYMPHOMA

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Background: Although L-asparaginase (L-asp) is one of the most important medication used in pediatric acute lymphoblastic leukemia (ALL) and lymphoblastic lymphoma (LBL) treatment, it has been associated with several adverse effects, including thrombosis. Thrombosis is caused by multiple factors, including prothrombic properties of tumor cells, genetic factors, indwelling central venous catheters, corticosteroids, and L-asp. L-asp plays a major role in thrombosis because it reduces the synthesis of hemostatic factors in the liver. The timing of thrombosis is generally late in the induction phase; however, little is known regarding the dynamic status of hemostatic factors.
Aims: This study was conducted to analyze the dynamic status of hemostatic factor levels in the induction phase of ALL treatment, including L-asp and to determine the association between L-asp and thrombosis.
Methods: We retrospectively analyzed 69 pediatric patients (61 with ALL and eight with LBL) who received Berlin-Frankfurt-Munster chemotherapy at a single institution between October 2009 and November 2014. All patients received an intramuscular injection of *Escherichia coli*-derived L-asp (5000 U/m^2/injec-

tion) on days 12, 15, 18, 21, 24, 27, 30, and 33. One patient had cerebral venous thrombosis. Hemostatic factors analyzed in this study included fibrinogen (Fib), alpha-2-plasmin inhibitor (α_2-PI), antithrombin-III (AT-III), plasminogen (PLG), and platelets (Plt). Although platelets are not an L-asp dependent factor, their levels were analyzed because of their association with hemostasis. We analyzed the levels of each hemostatic factor at time point (TP)1 (at diagnosis), TP2 (day 12, before the initial L-asp administration), TP3 (day 22, after the fourth L-asp administration), and TP4 (after protocol Ia). Additionally, we analyzed the periods required by each hemostatic factor level to decrease below the normal range and to recover to the normal range following the initial L-asp administration; this is defined the period analysis. All data were analyzed by one way analysis of variance.
Results: In TP1, Plt decreased below the normal range because of the myelosuppression of tumor burden. Fib increased above the normal range because of the proinflammatory effects of tumors, while other factors were in the normal range. In TP2, Plt levels remained suppressed, Fib decreased below the normal range, and AT-III increased above the normal range, while α_2-PI and PLG were in the normal range. In TP3, the levels of all factors decreased. In TP4, Plt and Fib levels recovered to the normal range, while AT-III, PLG, and α_2-PI were below the normal range. Our period analysis showed the following results: median periods for each hemostatic factor that decreased below the normal range were as follows: Plt, 0 days; Fib, 0 days; α_2-PI, 3 days; AT-III, 5 days; and PLG, 4 days. During the early induction phase, Plt and Fib levels decreased significantly faster than AT-III and α_2-PI levels ($p < 0.05$). Additionally, the median periods required by each hemostatic factor to recover to their normal ranges were as follows: Plt, 21.5 days; Fib, 29 days; α_2-PI, 29 days; AT-III, 30.5 days; and PLG, 31 days. During the late induction phase, Plt levels recovered significantly faster than AT-III and PLG levels; Fib levels recovered significantly faster than PLG levels ($p < 0.05$).
Summary and Conclusions: During the early induction phase, bleeding risk, rather than thrombosis, may increase because Plt and Fib levels decreased below their normal ranges, which occurs faster than the decrease in the levels of antithrombic factors, including AT-III and PLG. During the late induction phase, the thrombotic risk may increase because of the faster recovery of Plt and Fib levels as compared with that of PLG and AT-III levels. One patient developed cerebral venous thrombosis on day 35 in protocol Ia, which confirmed our hypothesis. Therefore, clinicians need to be more cautious regarding the possibility of thrombosis in the late induction phase.

E1551

UPPER AND LOWER EXTREMITY DEEP VEIN THROMBOSIS IN CHILDREN:A SINGLE CENTER EXPERIENCE

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Background: Deep Vein Thrombosis (DVT) in children can be caused by a combination of several risk factors such as poor blood circulation, damage to the veins and thrombophilia.
Aims: Our aim is to evaluate the causes, risk factors and clinical outcome of upper (UEDVT) and lower extremity (LEDVT) DVT in our center.
Methods: We retrospectively evaluate their clinical and laboratory results by the way of searching their paper based & electronic files.
Results: Our database revealed 352 children having various types of thrombosis between January 2009 to December 2014 and 70 out of 352 were diagnosed as DVT. There were 44 male and 26 female with a median age of 60 months (minimum 1 month- maximum 216 months)in this group. The location of UEDVT involved internal jugulary vein (n=16) and superior vena cava(SVC) (n=5) whereas LEDVT include femoral and iliac veins(n=39), popliteal veins(n=10). More than half of these DVTs were associated with a cathether related thrombosis on which 4/5 SVC, 15/16 jugulary vein thrombosis and 21/49 LEDVTs. All except 5 patient having an underlying chronic diseases such as malignancy, nephrotic syndrome, immune deficiency, congenital cardiac disorders, metabolic diseases. One out of these 5 patient was diagnosed as a hereditary protein C deficiency after having a recurrent LEDVT and pulmonary thrombosis. Recurrent thrombotic episodes(n=13) was observed in 10 patients and 9 of these patient having LEDVT had 12 recurrent episodes including (6 pulmonary thrombosis, 4 LEDVT, 1 renal venous thrombosis and 1 sinevenous thrombosis). One patient who initially had UEDVT experienced recurrent episode of SVC thrombosis. Factor V Leiden mutation was found to be heterozygous in 15/50 and normal in 35/50. Homozygous prothrombin 20210A mutation were detected in 2 out of 50 and 8/50 were heterozygous. Homozygous MTHFR C677T and MTHFR A1298C mutation was found in 3/50 and 8/23 respectively. PAI 4G/5G heterozygous mutation was found in 15/22 patient. Most of them(%65) initially treated with LMWH and TPA were used as a thrombolytic agent in 6 of them without any complication. Vena cava inferior filter was used in 3 patients having LEDVT. During the follow up period 2 had postphylepitic syndrome and 1 had an amputation and 9 patient deceased because of the primary disesase.
Summary and Conclusions: Our single center experience showed that children having spontenous DVT is very rare and most of these DVT episodes were due to underlying disorder and associated risk factors (aquired and hereditary).

E1552

RECURRENCES AFTER SPLANCHNIC VENOUS THROMBOSIS: RISK FACTORS AND EFFECT OF DIFFERENT TREATMENTS IN A RETROSPECTIVE MONOCENTER COHORT OF 154 PATIENTS

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Background: Non-cirrhotic non-malignant splanchnic vein thrombosis (SVT) is a rare clinical entity associated with heterogeneous underlying disorders; chronic myeloproliferative neoplasms (MPN) and thrombophilia are the leading systemic causes of SVT. Long-term treatment is a clinical challenge and the optimal duration of anticoagulation is uncertain.
Aims: To assess in a retrospective cohort of non-cirrhotic non-malignant SVT patients the risk factors for recurrent thrombosis and the efficacy of antithrombotic long-term secondary prophylaxis.
Methods: We analysed a retrospective cohort of 154 patients with a first SVT referred to our Thrombosis Center between 1995 and 2014 (M/F 68/86, median age at SVT 45 years, range 1-83); 12% of patients had hepatic vein thrombosis and 88% thrombosis of one or more sites of the spleno-mesenteric-portal venous axis. SVT was unprovoked in 84 patients (54%); inherited or acquired thrombophilia was diagnosed in 56 patients (36%). Forty-seven patients (30%) had diagnosis of overt MPN; JAK2 V617F mutation was present in 50 of 131 tested patients (38%). Long-term antithrombotic treatment was given to 112 patients: 80 (53%) were receiving anti-vitamin K treatment (AVK), 16 aspirin (ASA) (10%), and 8 AVK+ASA (5%); 50 patients (32%) discontinued any prophylaxis after 6-12 months of AVK treatment. The probability of recurrent thrombosis after SVT and the impact of different risk factors or treatments were estimated by the Kaplan-Meier method and a multivariate proportional hazards regression model.
Results: During a total observation time of 748 years, 25 patients (16%) had a recurrent thrombotic event: 15 a recurrent SVT, 7 a venous recurrence in other sites, and 3 an arterial recurrence. The overall incidence of recurrences was 3.3% pts-years. The cumulative probability of recurrence was 6.1% at 1 year, 16.2% at 5 years, and 25.7% at 10 years. Recurrence occurred in 10 cases during AVK treatment (2.1% pts-years) and in 15 cases without AVK (5.3% pts-years). The univariate hazard ratio (HR) for recurrent thrombosis in patients without AVK was double than in patients on treatment (HR 2.02, 95%CI 0.97-5.28). A multivariate proportional hazards regression model having as dependent variable the recurrence of thrombosis included as covariates gender, age >45 yrs at SVT, family history of venous thrombosis, absence of provoking causes of SVT, presence of thrombophilia, diagnosis of overt MPN, cytoreductive treatment, AVK or ASA treatment. Male gender and age >45 years were associated with a higher risk of recurrence (HR 2.6, 95%CI 1.04-6.53, and HR 1.18, 95%CI 1.18-8.97, respectively); AVK treatment was significantly effective in preventing recurrences (HR 0.35, 95%CI 0.13-0.91). A modified model with the variable JAK2 V617F and without the redundant variable MPN was applied on the 131 patients checked for the mutation, retaining only the presence of JAK2 V617F as positive predictor of recurrence (HR 6.7, 95%CI 1.76-25.44) and confirming the efficacy of AVK (HR 0.15, 95%CI 0.04-0.57). A sub-analysis of the patients with overt MPN or carriers of JAK2 V617F confirmed the efficacy of AVK, whereas the use of ASA had no significant impact in preventing recurrences.
Summary and Conclusions: In this monocenter cohort thrombophilia and MPN/JAK2 V617F mutation were confirmed to be the systemic risk factors for SVT more represented. Male gender, age >45 years, and the presence of JAK2 V617 mutation resulted independent risk factors for recurrence. Long-term treatment with AVK decreased the rate of recurrences at least by 65% in the overall cohort as well as in the MPN subgroup.

E1553

THROMBIN INDUCES VASCULAR LEAKAGE THROUGH MACROPHAGE MIGRATION INHIBITORY FACTOR AND AUTOPHAGY DURING SEPSIS

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Background: Sepsis is a disease, of which the systemic inflammation-induced vascular leakage and disseminated intravascular coagulation leads to multiple organ failure. Thrombin, a serine protease mediating critical pathological roles during sepsis, not only activates coagulation cascades but also acts as a cytokine to induce vascular hyper-permeability and inflammation. In previous study, it has been demonstrated that thrombin could induce endothelial cells to secret macrophage migration inhibitor factor (MIF). MIF is another critical pro-inflammatory cytokine secreted in sepsis, and it has also been indicated to induce the formation of autophagy. However, the interplay between MIF and autophagy on thrombin-induced vascular hyper-permeability during sepsis has not yet been revealed.
Aims: We aim to investigate whether thrombin-induced vascular hyper-permeability is through MIF-mediated autophagy in sepsis.
Methods: Human microvascular endothelial cell line (HMEC-1) was co-treated with thrombin with or without MIF/autophagy inhibitors and the permeability

was monitored by real-time cell analyzer (RTCA) and trans-well permeability assay *in vitro*. We also used lipopolysaccharide (LPS)-intraperitoneally injected mice as endotoxemia mice model to test whether MIF and autophagy inhibitors could rescue LPS-induced vascular leakage and septic shock. To measure vascular leakage level of endotoxemia mice model, Evans blue dye was intraperitoneally injected into mice and the vascular leakage of the mice was determined by the concentration of Evans blue dye in peritoneal lavage.
Results: We found that thrombin activity in plasma was significantly increased in endotoxemia mice. In addition, treating HMEC-1 cells with thrombin could induce MIF secretion and autophagy formation within 30 minutes and accompanied with VE-cadherin translocation. Next, we used MIF and autophagy inhibitors to rescue endothelial barrier dysfunction. Blocking MIF by ISO-1, p425 and anti-MIF antibodies could rescue thrombin-induced endothelial hyperpermeability both in RTCA and trans-well assay. Blocking the autophagolysosome formation by bafilomycin A1 (BafA1) and chloroquine (CQ) partially block thrombin-induced endothelial hyper-permeability in RTCA. Co-treatment of PI3K inhibitor (3-methyladenine, 3-MA) or ROS scavenger (N-acetyl-L-cysteine, NAC) with thrombin effectively attenuated thrombin-increased endothelial permeability in trans-well permeability assay.In addition, inhibition of MIF could block the thrombin-induced autophagy, revealing that thrombin-induced autophagy is mediated by MIF. Furthermore, in endotoxemia mice model, peritoneal injection of LPS increased the MIF secretion, while bovine serum albumin (BSA) did not show similar effect. Injection of ISO-1 and BafA1 with LPS eliminated LPS-induced vascular leakage, indicating that this effect was mediated by MIF and autophagy.
Summary and Conclusions: Our results suggest that thrombin-induced vascular hyper-permeability is mediated by MIF and autophagy and this study may provide potential therapeutic target for treating sepsis in the future.

E1554

BLOOD RHEOLOGY DISTURBANCES INCREASES VTE-RISK IN CHILDREN WITH ACUTE LYMPHPBLASTIC LEUKEMIA
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Background: Central hemodynamics and blood rheology disorders are well-known factors leading to thrombosis. High risk of venous thromboembolism (VTE) have accompanied acute lymphoblastic leukemia (ALL) like another malignancies. Besides the chemotherapy has negative effect on cardiac function which can lead to blood velocity decrease. And hemorheological changes could be caused due to proinflammatory cytokines negative influence on red blood cells aggregability.
Aims: To investigate blood rheology features as VTE risk factor in children with acute lymphoblastic leukemia.
Methods: The study's population consist 48 children (age <17.6 y.o.). In all patients we investigated BNP level. Whole blood viscosimetry (shear rates 5-300 s^{-1}), plasma viscosimetry (shear rates 250 s^{-1}), erythrocytes aggregability and deformability were investigated. All patients had not any symptomatic organs failures.
Results: From 48 patients 1) in 8 cases BNP was elevated more 82 ng/L (up to 208 ng/L), and 2) in 6 patients had thrombosis and three from them were with increased BNP. All cases thrombi had revealed at the area of central venous line. All patients had normal plasma viscosity (1,1-1,5 mPa*s) and unimpaired erythrocyte deformability. Despite it whole blood viscosity was increased by shear rates 5-300 s^{-} and mainly by shear rates 5-75 s^{-1}(eta by 5s^{-1}: 4,2-7,9 mPa*s; eta by 75s^{-1}: 2,3-4,1 mPa*s). The last assumes erythrocytes hyperaggregability in all patients.
Summary and Conclusions: As a whole, hemorheological profile is impaired moderately in children with ALL. Totally increased blood viscosity and erythrocytes hyperaggregability and hidden ventricle overload (presumably, this is a temporary) signed by elevated BNP allows together to aggravate circulatory disorders. We assume that the revealed hemorheologic features could be one trigger to start of VTE despite standard antithrombotic prevention in children with acute lymphoblastic leukemia. The study is continued now.

E1555

ADAMTS-13 A NOVAL MARKER LINKING BETWEEN MICRO AND MACRO- VASCULAR DISEASE IN YOUNG TYPE -1 DIABETES PATIENTS
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Background: Several observations that vascular damage and endothelial dysfunction occur early in the course of diabetic microangiopathy have been reported.The net effect of all these changes is to convert the endothelium from a thromboresistant to a thrombogenic surface and, consequently, impairment of coagulation and of anticoagulant pathways. Recently it was demonstrated elevated level of VWF in type1 diabetics which is degraded by a metallo protease, ADAMTS-13. It's hypothesized that elevated VWF level was due to reduced level of ADAMTS-13**.** Deficiency of Von Willebrand factor (VWF)-cleaving protease (ADAMTS13) causes platelet thrombosis in the microcirculation.
Aims: to determine whether diabetic micro-angiopathy was associated with abnormally modulated haemostasis and to demonstrate whether a correlation existed between the thrombotic tendency, as measured by ADAMTS-13 levels and macrovascular complications measured as dyslipdemia and CIMT.
Methods: Seventy children and adolescents with type 1 diabetes attending the Pediatric Diabetes Clinic, Pediatric Hospital, Ain Shams University were compared with 40 age- and sex-matched healthy controls. The mean age of patients was 12.6±4.9 years and with a M/F ratio of 1.2/1.Detailed medical history with special emphasis on disease duration and insulin therapy, thorough clinical examination,blood pressure (BP) measurement, as well as screening for diabetic complications, was performed. Laboratory assessment of high-sensitivity C-reactive protein, Lipid profile, albumin/creatinine ratio, renal functions and glycosylated hemoglobin (Hb A_1c) were assayed. Carotid intima-media thickness was measured using ultrasound as well as ADAMTS 13 level using ELISA technique.
Results: The mean cIMT was higher in diabetics than controls (0.6 mm±0.1 *vs* 0.4 mm±0.1, p=0.000). Moreover, it was higher in diabetics with positive microalbuminuria compared to normo-albuminuric patients (mean 0.7 mm±0.1 *vs* 0.6 mm±0.1, p=0.018). cIMT was found to positively correlate with: age in diabetics (r=0.76, p=0.000,), body mass index (r=0.82, p=0.000). In diabetics, mean aggregate cIMT positively correlated with duration of diabetes (r=0.66, p=0.000), mean systolic blood pressure SDS(r=0.82, p=0.000) as well as Hb A_1c (r=0.40, p=0.004) and correlated negatively with high density lipoprotein -cholesterol (HDL-C) (r=−0.88, p=0.000).The mean ADAMTS-13 serum levels were significantly lower in diabetics compared to controls with the lowest values in complicated patients. Mean ADAMTS-13 levels were negatively correlated with ACR(r=2.1,p<0.05), mean CIMT(r=1.9,p<0.01), HbA1c (r=2.4,p<0.05)and mean random blood glucose(r=2.7,p<0.001). Moreover mean ADAMTS 13 levels were negatively correlated with TG,TC,high sensitivity CRP and LDL(p<0.01). Multiregression linear analysis showed that, UACR, and hs-CRP were independently related to ADAMTS 13 levels in type 1 diabetics (p<0.05).The mean ADAMTS 13 levels were related to severity of MVCs as patients with combined or more than one MVCs had significantly lower levels compared to patients with single MVCs.
Summary and Conclusions: ADMTS13 levels were eleveted and young type1 diabetics and related to metabolic control, dyslipidemia, CIMT as well as severity of MVCs.

E1556

PROGNOSTIC SIGNIFICANCE OF THE ABSOLUTE MONOCYTE COUNTS IN LUNG CANCER PATIENTS WITH VENOUS THROMBOEMBOLISM
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Background: Lung cancer is one of the most prevalent types of cancer in which VTE occurs among malignancies presenting with VTE. Advanced disease stage, elderly, adenocarcinoma, smoking history, comorbidities, and chemotherapy have been reported to be risk factors for VTE in patients with lung cancer. VTE in lung cancer is associated with an increased risk of mortality
Aims: We investigated the clinical significance of the absolute monocyte count (AMC) as a predictor of the response to anticoagulation and survival in lung cancer patients with venous thromboembolism (VTE).
Methods: We retrospectively reviewed 1,707 patients with pathologically proven lung cancer who visited the hospital between July 2008 and May 2014. Among them, the clinical data of patients newly diagnosed with VTE and treated with anticoagulation were compared between the low and high AMC groups according to the median value of AMC (640/μL) at the time of VTE diagnosis.
Results: The incidence of VTE was 7.9% during the study period. Most of the patients had non-small-cell lung cancer (82.1%), stage IV (64.2%), and pulmonary thromboembolism (76.1%) and were incidentally diagnosed with VTE (76.9%). The patients' characteristics and laboratory values were not significantly different between the low and high AMC groups. Among patients available for evaluation of the response to anticoagulation, the high AMC group was significantly more refractory to anticoagulation than the low AMC group (no response to anticoagulation: 21.7% *vs* 6.8%, respectively; p=0.044). Additionally, the high AMC group showed worse overall survival (OS) than the low AMC group (median: 9.6 months *vs* 5.9 months; p=0.038). On multivariate analysis, high AMC, low albumin, and advanced stage were independent poor prognostic factors for OS.
Summary and Conclusions: High AMC is associated with refractoriness to anticoagulation and poor prognosis in lung cancer patients with VTE.

E1557

PREDICTIVE POTENTIAL OF MARKERS OF COAGULATION, FIBRINOLYSIS AND ANGIOGENESIS IN CANCER PATIENTS
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Background: In cancer setting hemostatic system exhibits recognized pleiotropic effects in the processes of tumor initiation, growth, metastasis and angiogenesis. However, the predictive potential of various hemostatic markers regarding treatment efficacy and disease progression remains disputable.
Aims: To determine dynamics of the procoagulant activity of tissue factor bearing microparticles (MP-TF) throughout chemotherapy, its correlation to levels of soluble urokinase plasminogen activator receptor (suPAR) and angiopoietin-2 (Ang-2).
Methods: Single institution prospective observational study including 128 cancer patients who underwent chemotherapy in 2013/2014 for breast (44), lung (28), colon (37), ovarian (19) cancers. Patients were subsequently followed up: before chemotherapy (ChTh) initiation, after three cycles, after six cycles and three months post therapy cessation. A control group of 30 healthy volunteers was studied as well. Written informed consent was obtained from both study groups. Serum levels of suPAR and Ang-2 were determined by ELISA and MP-TF procoagulant activity was measured by a combined immune-chromogenic method (Zymuphen MP-TF).
Results: MP-TF procoagulant activity was twice higher in cancer patients compared to controls (0.9421vs.0.4300 pg/ml, p<0.0001). Sequential testing of MP-TF in patients with lung, colorectal and ovarian cancers revealed decrease in their procoagulant activity during treatment followed by a significant rise in activity after therapy cessation (0.9421 *vs* 1.4103 pg/ml, p<0.0001). No significant dynamics in MP-TF was observed in patients with breast cancer (0.9814 *vs* 0.8733 pg/ml, p=0.785). SuPAR and Ang-2 showed marked two- to threefold decrease during ChTh that is sustained after its cessation. MP-TF activity correlates negatively with suPAR and Ang-2 (ρ_{ang2} -0.259, ρ_{suPAR} -0.296, p=0.0001). All three markers had AUC >0,70 and therefore could be identified as reliable diagnostic tool for the presence of malignant disease. Amongst them MP-TF had greatest potential as independent predictor (Beta 0,408, p<0.0001).
Summary and Conclusions: MP-TF procoagulant activity, suPAR and Ang-2 could serve as surrogate markers for biologic activity and therapeutic efficacy.

E1558

DURABLE LONG TERM REMISSIONS WITH EARLY USE OF RITUXIMAB IN PATIENTS WITH THROMBOTIC THROMBOCYTOPENIC PURPURA (TTP)

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Background: Adult onset acquired thrombotic thrombocytopenic purpura (TTP) is a life-threatening disease mediated by autoantibodies directed against ADAMTS13. Therapeutic plasma exchange (TPE) and immunosuppressive therapies have largely remained the mainstay of treatment. Several studies have reported promising results with early use of rituximab.
Rituximab is a safe and effective treatment for newly diagnosed TTP, and has shown in a prospective, multicentre trial to decrease the number of plasma exchanges (PEX) required to achieve remission, to decrease the length of inpatient stay and to reduce the risk of relapse by over 80% when compared to historical controls.
Aims: We report our experience with early use of rituximab in patients with initial severe manifestations (cardiac or neurological involvement) or those who were failing to show early response to TPE from a tertiary care centre in Saudi Arabia.
Methods: This is retrospective data of ten patients with TTP who received rituximab either for refractory disease or sever manifestations of TTP since 2006 to 2014 at our teritary care hospital, saudi Arabia.
Results: Total of ten patients with TTP received rituximab either for refractory disease or sever manifestations of TTP since 2006 to 2014.There were six females (60%) and four males (40%). The median age was 33±12.08. Nine of the ten patients (90%) had neurological involvement ranging from headache, dizziness, confusion, seizures and focal neurological signs. Four patients (40%) had renal impairment. Two patients (20%) had associated systemic lupus erythematosis(SLE). Five patients (50%) were refractory to plasma exchange while the rest received early rituximab due to cardiac or neurological involvement at treating physician's discretion. Four patients received Rituximab within 5 days of diagnosis. Seven patients received weekly four doses of Rituximab at 375mg/m2 while two patients received eight and five doses weekly. One patient with SLE received two doses at 1000mg each as per Rheumatology protocol for the benefit of treatment of concurrent SLE. Nine out of ten (90%) achieved complete response; no subsequent relapses occurred with median follow-up of 16 months (range, 6-62 months). There was one death during the early course of disease due to delayed presentation. Number of mean plasma exchanges in early rituximab group who received Rituximab within five days was 13.5 while it was 17.4 in those who received later than day 5. Number of mean total plasma volumes exchanged in early Rituximab group were 11.1 (range 7.8-15.5) compared to 16.15 in later group (range 11.2-25.2). There were no serious immediate complications noted. On longer term clinical follow up, we have not found any increased tendency of infections or any case of leukoencephlopathy.
Summary and Conclusions: The results suggest that early use of Rituximab is associated with decrease number of plasma exchanges, increased duration of remission and decreased risk of relapse with excellent safety profile as suggested by earlier studies. We also noticed maximum benefit is seen if Rituximab given at the earliest. We need further randomized controlled trials testing efficacy of upfront Rituximab. Based on these observations, our group is planned for prospective study for the early use of rituximab.

E1559

ASSESSMENT OF LOW MOLECULAR WEIGHT HEPARIN ANTICOAGULANT EFFECT IN CHILDREN USING THROMBODYNAMICS GLOBAL HEMOSTASIS ASSAY

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Background: *In vitro* studies show that thrombodynamic (TD) method is sensitive for anticoagulant effect of low molecular weight heparins (LMWH), but there is still no information of its utility in anticoagulation monitoring in children with deep venous thrombosis (DVT) during active cancer treatment.
Aims: to assess anticoagulant effect of LMWH in children with DVT using Thrombodynamic assay.
Methods: Twenty-one children aged 5 months to 13 years with objectively confirmed DVT during active cancer treatment were enrolled in this prospective observational study. All children were prescribed for LMWH treatment starting with 100 IU/kg/bid on the day of thrombosis detection. Anti-Xa activity and novel global hemostasis test Thrombodynamics were used to assess coagulation status in all patients before and on the 4th day of anticoagulation. Hypercoagulability in TD was defined when clot growth velocity (V) was above reference range, hypocoagulability -when V was below reference range. For all calculations, OriginPro 8.0 (Microcal Software, Northampton, MA, USA) have been used.
Results: The day of thrombosis detection 13 patients had hypercoagulability in TD: V=30.3-45.5 um/min (median 33,9 um/min, normal range is 22-29 um/min in children), while 8 had normal results. After 4 days of LMWH therapy TD revealed significant shift (*P<<0.05, pair-sample t-test) to hypocoagulability range: V=6.3-33.9 um/min (median 9.7 um/min); anti-Xa activity=0.14-0.79 IU/ml (median 0.43 IU/ml). There was a strong correlation between anti-Xa activity and value of 1/V during LMWH treatment: Spearman correlation coeff.=0.77 (Sign. 0.0001).
Summary and Conclusions: Thrombodynamics reveal significant shift from hypercoagulability range on thrombosis to hypocoagulability in consequence of anticoagulant treatment with LMWH that is in good agreement with anti-Xa activity.

E1560

CRP, D-DIMER, LEUKOCYTE, BLAST AND PLATELET COUNTS AT THE TIME OF ALL DIAGNOSIS AS POSSIBLE BIOMARKERS FOR EARLY CEREBRAL VEIN THROMBOSIS

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Background: Thrombotic complications in cancer patients are mostly venous, cerebral vein occlusion (CVT) representing often the most drastic consequences in patients' lives. A majority of thromboses occurs during the first months following ALL diagnosis and introduction of treatment (Vu *et al.* 2015). They possibly have an adverse impact on the overall survival (Ku *et al.*, 2009). Older age, presence of a catheter, comorbidities as well as introduction of asparaginase (ASP) have been linked to deep venous thrombosis in leukemia patients. However, very limited data exists on biomarkers already available at diagnosis of ALL predicting the risk of thrombosis during the early treatment course of ALL.
Aims: We performed a multicenter retrospective study aiming at characterizing the incidence and patterns as well as possible predictive factors for thrombosis during the multiagent treatment of ALL before allogenic stem cell transplantation.
Methods: We reviewed the charts of 161 consecutive patients diagnosed with ALL and recruited to a national treatment study (ALL2000) in four university hospitals and one central hospital in Finland during years 2000-2012.
Results: In all, 31 patients (19%) suffered from thrombosis during the three months follow-up time. 13 (42%) of the thromboses occurred within two

months after ALL diagnosis. 32% (n=10) of the thrombotic events were localized in patients' cerebral veins detected by MRI and seven of them took place remarkably early in the treatment course and before the introduction of ASP (cycle 3, day 8). None of the patients with CVT had been diagnosed with leukemia in central nervous system. Plasma CRP, fibrinogen degradation product (DD), and blood hemoglobin, leukocyte, blast and platelet counts recorded at ALL diagnosis were also investigated. In patients acquiring thrombosis a trend, nearly reaching statistical significance, towards a lower platelet count and higher CRP, DD, leukocyte and blood blast count at leukemia diagnosis was detected. This was even more pronounced in patients who suffered from CVT.

Summary and Conclusions: A large proportion of thromboses possibly affecting regular ALL treatment and patients' survival occurs very early after the leukemia diagnosis despite low platelet counts at diagnosis and during the multiagent cytostatic treatment. At diagnosis, high CRP, high DD, high leukocyte and high blood blast count as well as low platelet count possibly reflect the role of ALL disease itself in provoking thrombosis, especially CVTs. In order to confirm these findings prospective studies are still called for.

E1561

CHILDREN WITH CEREBRAL INFARCT DUE TO CRANIOCERVICAL ARTERIAL DISSECTION IN A SINGLE CENTER

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Background: Children with craniocervical arterial dissection is being recognized increasingly in the recent years and it is reported to be associated with a high morbidity rate in the childhood period. Treatment is challenging however anticoagulation and antiplatelet treatment are the treatment options. CAD is an important cause of childhood arterial ischemic strokes which occured between 7.5%>20%.

Aims: Our aim is to evaluate our patients who had had arterial ischemic stroke secondary to craniocervical arterial dissection in our center.

Methods: We retrospectively reviewed our patients who had had arterial ischemic stroke secondary to craniocervical arterial dissection. All of these patients' diagnoses were based on cranial and neck magnetic resonance (MR) imaging, MR angiography and computed tomography (CT) angiography in presence of appropriate clinic setting and a past history of trauma.

Results: Of the 305 children with thrombosis diagnosed in our center, 64 were acute arterial cerebral thrombosis and of those 4(6.2%) patients were diagnosed as arterial dissection. In all of these patients past history revealed a trauma and all patients had evidence of cerebral ischemia at the time of diagnosis. Hereditary prothrombotic risk factors were homozygout MTHFR in 2 patient and heterozygout FV Leiden mutation in one patient. Recurrent stroke was not observed in our group.

Summary and Conclusions: Long term mortality rates of childhood onset arterial ischemic stroke were reported as 2-11% and overall rate of intracranial hemorrhage was shown to be about 5% in this group of patients. In our ischemic stroke group, cervicocranial dissection rate (6.2%) seems to be lower than the rate reported in the literature which may be up to 20% of strokes in childhood and adolescence. This may be due to underdiagnosis of these patients, in that diagnosis should be based on primarily clinical suspicion and vascular imaging of the neck and brain. Children who developed neurological abnormalities after a blunt trauma to the head and/or neck should alert the physicians to have the possibility of carotid artery injury. It is important to remember that ischemic infarcts are usually not visible on CT during the first 12 hours of the acute stage. Cranial MR imaging especially with introduction of the diffusion-weighted imaging have been much more sensitive in detection of the acute ischemic lesions.Furthermore detection of extracranial dissections usually require demonstration of an intramural thrombus which is hyperintense on subacute stage especially on fat-saturated T1-weighted imaging or an intimal flep as a linear structure traversing the lumen.

E1562

A BESPOKE 'APP' FOR THE INVESTIGATION AND MANAGEMENT OF DEEP VEIN THROMBOSIS

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Background: Deep vein thrombosis (DVT) is a common condition that may lead to potentially fatal complications, such as pulmonary embolism. The development of an application, the 'app', guides clinicians to the appropriate diagnostic management and treatment for patients with suspected DVT in acute setting. The 'app' offers guidance on the two-level DVT Wells score, D-dimer measurement and ultrasound imaging requests. It uses formal clinical decision rules in conjunction with blood tests to enable a more structured clinical diagnosis of DVT. It offers guidance on the management and treatment of DVT covering contraindications/cautions for anticoagulation therapy, prescription dosing and patient counselling information. Information from the 'app' is pre-populated in the discharge summary facilitating discharge with accurate summary of investigations and management. The 'app' has been developed in accordance with hospital and national guidelines with an aim to standardise the pathway ensuring patient quality and safety.

Aims: To improve the pathway for the investigation and management of patients with suspected DVT with electronic technology to ensure patient safety, quality and experience.

Methods: Stakeholders (clinical, radiology and nursing staff; information and 'app' development teams) agreed on the vision and requirements such as objectives; data metrics for diagnosis, management, patient and staff satisfaction, workflow and time; clinical aspects (ward area, patient exclusions, pathway); ethics approval and technical system (user device, software, server, network, configuration criteria, access rights) to deliver an electronic decision-support safety system, in an 'app' format, to guide clinicians to the appropriate diagnostic investigations and treatment for patients with suspected/confirmed DVT. Timelines for implementation phrases and go-live date were set. Stages for development, testing, installation, further validation, training and monitoring were performed. Supporting materials and user guides were created. The 'app' will be trialled for 3 months to assess benefits and performance compared to the current paper system using a DVT proforma. This timeframe will provide feedback whether our vision is delivered and if our strategies are working for better patient care, accurate and relevant investigation and appropriate management.

Results: *Audit results for investigation and management of DVT (WS: Wells Score; USS: Ultrasound):* 92% (47/51) of patients had a history and examination recorded; 18% (9/51) of patients had a WS; 100% (6/6) of patients with a DVT likely WS had USS within 4 hours of request or therapeutic enoxaparin and USS within 24 hours; No (0/2) patients with DVT likely WS had a repeat USS 6-8 days with positive D-Dimer and negative USS; 100% (3/3) of patients with DVT unlikely WS had a D-Dimer test; 50% (1/2) of patients with DVT unlikely WS and positive D-dimer had USS within 4 hours or therapeutic enoxaparin and USS within 24 hours; 100% (4/4) of patients with confirmed DVT had appropriate anticoagulation therapy.The audit showed the lack of documentation of the two-level DVT Wells score, thus difficult to evaluate whether patients were management appropriately due to limited data available and confirming non-compliance to hospital guidance. Results will be compared to an audit post implementation of the 'app'.

Summary and Conclusions: Clinical teams are aware the current paper system is not effective and have developed and introduced a novel decision-support pathway, in an 'app' format, to guide the clinician step-by-step for appropriate investigation and management of suspected and confirmed DVT, with a mission to 'see, feel and change'.

References

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E1563

EFFICACY OF THROMBOPROPHYLAXIS IN THE PREVENTION OF THROMBOSIS IN PREGNANCY. EFFECTS ON THE INCIDENCE OF MISCARRIAGES

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Background: The etiology of gestational vascular complications (GVC) is not accurately known, however it has been proposed that activation of haemostasis may cause poor placental development and thrombosis. Antithrombotic prophylaxis with LMWH and/or aspirin could prevent the development of CVG by controlling of hemostasis during pregnancy.

Aims: To analyse the type and dose of thromboprophylaxis in a group of pregnant women. To study the relationship between antithrombotic prophylaxis and GCV development.

Methods: We studied 162 pregnant women referred to the Hematology consultation in HURJC and HUFJD. All of them were pregnant at the time of consultation. Thrombotic and CVG risk factors were estimated and it was decided to have expectant management or to start prophylaxis with LMWH +/- aspirin, according to our protocol. The following data were collected: prophylactic medication and dose, obstetric history, history of miscarriages, history of thrombosis, prior pregnancy complications and thrombophilia.

Results: The mean age was 35.7 years (range=22-43); mean gestational age at first visit was 12.6 weeks. 51.2% (n=83) women had no living children; and 69.9% (n=114) had have abortions. 63.6% (n=103) were referred with a history of abortion, 72.7% (n=83) in the 1st trimester, 18.5% (n=10) in the 2nd and 8.8% (n=21) in the 3rd. In addition, 70 women had other pregnancy complication history: preeclampsia (8.5% n=6), implantation failure (18.6% n=13), and fetal death (22.8% n=16). In this group 19.8% (n=32) had at least

one thrombotic event before pregnancy. 58% (n=94) had thrombophilia: 13.8% C677T homozygous, 26.5% FV Leiden, 16% PT20210A and 8.5% AFS. 47 patients (29%) had complications during pregnancy or childbirth. 49% of newborns experienced some complications, being the most common: prematurity (n=27) and low weight (n=15). There was no perinatal death. Risk factors (previous thrombosis, maternal age, recurrent miscariages, presence of thrombophilia) were studied and treatment was prescribed as indicated. In 53.3% of the patients prophylaxis was started in the first trimester). LMWH prophylaxis was effective in preventing thrombosis during pregnancy, (statistically significant p <0.0001). 108 (88.5%) of women treated with LMWH had a live birth. Although didn´t found no statistically significant relationship between antithrombotic phrofilaxys and prevention of abortions or fetal loss, 87.9% (n=73) women with no living children who received LMWH prophylaxis during pregnancy were able to have a live birth (p=0.394).
Summary and Conclusions: In our study group LMWH prophylaxis was effective in preventing thrombosis. Although there is no statistically significant relationship between antithrombotic prophylaxis and prevention of abortions or fetal loss, 87.9% of women with no living children who were prescribed with LMWH were able to have a live birth.

E1564

THROMBIN GENERATION (TG) POTENTIAL AND ACTIVATED PROTEIN C (APC) RESISTANCE IN PATIENTS ON CHRONIC ORAL ANTICOAGULANT THERAPY WITH VITAMIN K ANTAGONIST (VKA)

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Background: Atrial fibrillation (AF) and cardiac valvular prosthesis (CVP) expose patients to high risk of stroke that significantly contribute to morbidity and mortality. In these diseases despite the clinical benefit of oral anticoagulation with VKA (*i.e.* Warfarin) for thrombosis prevention, major bleeding may be frequent and devastating**.** The dose of VKA is determined for each patient by monitoring the Prothrombin-International Normalized Ratio (PT-INR). Some recent studies showed that, though with in therapeutic range PT-INR some patients can suffer serious bleeding complications or recurrent thromboses, suggesting that PT-INR do not always reflect the real bleeding or thrombotic risk and therapeutic effect of VKA.
Aims: This prospective study aim to characterize the TG potential in a group of AF or CVP patients on VKA, in order to investigate whether a correlation exists between TG and PT-INR and whether TG might be useful to identify subjects at higher bleeding risk.
Methods: Thirteen patients with AF (6M/7F, median age 77 years, (59-86)) and 7 patients (7M, median age 65 years (58-76)) who underwent CVP intervention were enrolled at the Hemostasis and Thrombosis Center of Papa GiovannixXIII Hospital, Bergamo, Italy, after gave informed written consent. All patients were on VKA treatment. To be included into the study the patients should have at least 65% in-range PT-INR in the previous year and 100% in-range PT-INR in the previous 3 months. Enrolled patients were prospectively followed up for one year. TG was performed by the Calibrated automated thrombogram (CAT) in platelet-poor plasma collected at the same day of PT-INR control (13 samples/each patient). TF 5pM was used as a trigger of TG assay. TG curves were described in terms of lag-time, endogenous thrombin potential (ETP), peak height and time to peak (ttP). ETP-based APC resistance was evaluated by CAT assay in the presence of 2 nM APC and results expressed as normalized APC sensitivity ratio (nAPCsr). Thirty healthy subjects (14M/16F) with no antiplatelet, anticoagulant or, for women, no oral contraceptives or hormone replacement therapies, acted as a control group.
Results: TG assay showed that lag-time and ttP were significantly prolonged while ETP and peak height were significantly lower in patients compared to controls (p<0.01), particularly in CVP patients. PT-INR correlate positively with lag-time (R=0.58, p<0.000) and ttP (R=0.61; p<0.000) and negatively with ETP (R=0.78; p<0.000) and peak height (R=0.78; p<0.000). The correlation between PT-INR and ETP and peak remain statistically significant also after correction for age. Interestingly, patients with similar PT-INR showed wide differences in TG value. nAPCsr, fixed at 1 in controls, was significantly (p<0.01) reduced in patients (0.6±0.14) suggesting that patients on VKA were more sensitive to the anticoagulant activity of APC. During follow-up, a total of four bleeding events were registered: two minor bleedings in AF patients, and two bleedings (one minor and one major) in CVP patients: all these patients presented with in-range PT-INR but very low TG potential.
Summary and Conclusions: The variability of TG values in patients with similar PT-INR and the occurrence of bleeding complications in patients with in-range PT-INR but very low thrombin generation values, suggest that TG assay can be more sensitive in detecting a hemorrhagic phenotype. This strategy can be useful in monitoring those patients initiating and/or receiving multiple antithrombotic drugs.

E1565

POLYPHARMACY IN PATIENTS TREATED WITH NON-VITAMIN K ANTAGONIST ORAL ANTICOAGULANTS (NOACS) AND THEIR IMPLICATION IN THE PERIOPERATIVE SETTING. RESULTS FROM THE "REAL LIFE" COHORT

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Background: The use of NOACs has increased especially in elderly patients polymedicated. The drug-drug interactions and polypharmacy are a significant problem mainly affecting the geriatric population. Some studies have considered this issue, but most of them focused only on relevant interactions. It is important to evaluate the impact of polymedication on NOACs patients in special situations such as the perioperative setting and to determine whether some of the commonly used drugs may modulate the anticoagulant effect of NOACs.
Aims: To determine the degree of polypharmacy and the incidence of possible drug interactions between daily used drugs and NOACs in perioperative setting and whether they affect NOACs plasma concentrations or not.
Methods: From June 2014 to December 2014 we consecutively reviewed 33 patients diagnosed of atrial fibrillation treated with NOACs that needed to undergo a surgical procedure. Perioperative management was performed following the PM guidelines from the Spanish Forum of anticoagulants and Anaesthesia. NOACs plasma concentrations were measured the day of the procedure using the Direct Thrombin Inhibitor Assay from IL (Bedford-MA-USA) for Dabigatran and the Technoclone anti-Xa assay from Technoclone (Vienna-Austria) for Rivaroxaban and Apixaban. Every drug each patient had daily taken was recorded and assessed considering the drug transport (P-glycoprotein) and metabolism either if they are cytochrome substrates, inhibitors or inducers.
Results: A total of 33 patients were included. Median age was 74 years (range 61-94), 17 (51.5%) were female. From them, 8 patients received dabigatran, 20 rivaroxaban and 5 apixaban.The number of medications consumed ranged from 2 to 16 and averaged 6.9 (SD+/-3.13) drugs per patient. The most frequent were proton pump inhibitors, hypogycemics, antihypertensive, statins and beta-blockers. A considerable number of routine taken drugs share the cytochrome for its metabolization with NOACs, none of them are strong inhibitors or inducers of cytochrome or transport P-glycoprotein as if to provoke a change on NOACs plasma concentrations. We just found five drugs that modulated the NOACs effect (Amiodarone, Atorvastatin, Carvedilol, Diltizem and Omeprazole). Some of the considered moderate inducers or inhibitors of either cytochrome or P-glycoprotein did not show any modulation of NOACs plasma concentrations. No association between the number of taken drugs and bleeding events was found.
Summary and Conclusions: In a real clinical setting we found that the commonly used drugs would have little impact on NOACs plasma concentrations. So we might conclude that NOACs are safe in polymedicated patients, even in special situations such as the perioperative setting.

E1566

IMMOBILIZATION AS A RISK FACTOR IN VENOUS THROMBOEMOLISM. DIFFERENCES BY SEX AND AGE

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Background: Many studies recognize the importance of immobilization as a risk factor for venous thromboembolism (VTE), the OR ranging from 5.5 to 11.1 depending on the series. Retrospective studies have shown that 59% of cases of VTE occur in hospitalized patients (35% for nonsurgical causes). It has also been reported that 42% of pulmonary embolism (PE) initiated during hospitalization. Blood flow in the venous valves is slow, especially in the lower limbs; an effect is accentuated with immobilization. This is the reason why a prolonged bed rest may be a risk factor.
Aims: Study immobilization as a risk factor for venous thromboembolism, and analyze differences according to the characteristics of the patient and the thrombotic event
Methods: We studied 438 patients with VTE. In this group immobilization was defined as non-surgical patients bed-ridden at least four days, two months prior to the diagnosis of thrombosis.
Results: 53% (n=232) of patients included in the study were male and 47% (n=206) female, with a mean age of 55.2 (±17.9) years. The distribution of patients according to thrombosis localization was: DVT-LL 49.4% (n=217), 26.9% PE (n=118), DVT+PE 8.8% (=39), DVT-UL 2.3% (n=10), SVT 3.9% (n=17) and VT-UL 8.7% (n=38). Immobilization was determined as a trigger and risk factor for thrombotic event in 32 patients (7.4%), and was associated with the development of venous thrombosis with an OR of 2.84 (CI 95%: 1.35 to 6.71, p=0.0048). The relationship with VTE according to thrombosis localization is shown in Table 1. Immobilization was a risk factor for SVT and DVT-UL. Immobilization was a risk factor for thrombosis in men but not in women

(Table 2). In addition, it was a risk factor for patients younger than 55 years but not in older patients (Table 2).

Table 1. Association between immobilization thrombosis localization.

GROUP	OR	CI (95%)	p
TVP-MMII	3,46	(1,54-8,53)	0,002
TEP	1,60	(0,48-4,90)	0,427
TVP-MMII+TEP	6,94	(2,16-21,3)	0,02
TV-LI	1,99	(0,29-8,43)	0,425

Table 2. Association between immobilization and VTE. Analysis by sex and age.

GROUP	OR	CI (95%)	p
Women	2,40	(0,84-2,58)	0,1054
Men	3,33	(1,21-11,7)	0,0181
<55 years	6,90	(1,95-43,8)	0,0013
≥55 years	1,53	(0,60-4,42)	0,3822

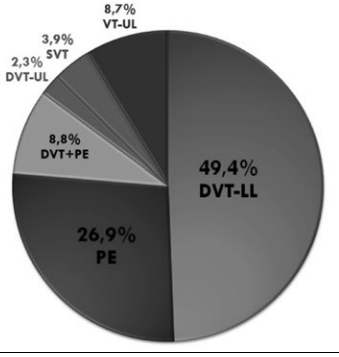

Figure 1.

Summary and Conclusions: Immobilization is an important risk factor for the development of thrombotic events with greater relevance in male and younger patients.

E1567

PROPHYLAXIS FOR VENOUS THROMBOEMBOLIC DISEASE IN PREGNANCY AND POSTPARTUM PERIOD

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Background: Venous thromboembolism (VTE) remains one of the leading direct causes of maternal death. Risk stratification is difficult and recommendations for prophylaxis have low grade of evidence. Risk factors for VTE and prophylaxis guidelines have been highlighted by the Royal College of Obstetricians and Gynaecologists (RCOG). Thromboprophylaxis is a cost-effectiveness way to reduce VTE high morbi-mortality.
Aims: To determine the risk factors of VTE during pregnancy and postpartum period present in our population, and to assess compliance with a written alert used in our clinical practice.
Methods: We conducted a prospective, descriptive and analytical study at Hospital de Clinicas, Montevideo, Uruguay between 1/2014 to 2/2015. We enrolled hospitalized pregnant and puerperal patients and stratified them, through a written alert in the medical record, in high, intermediate or low risk for VTE. We used a local modification of the RCOG guidelines (2009) to identify those patients at risk and to guide prophylaxis with low-molecular-weight heparin (LMWH).
Results: We included 348 patients in whom the risk stratification for VTE through written warning and according thromboprophylaxis were performed. 38,2% (n=133) were pregnant and 61,7% (n=215) were puerperal. Median age: 23 years (range 15-43). 3,4% (n=12) were at high risk and 36,4% (n=127) at intermediate risk. All high risk patients received adequate pharmacological prophylaxis with LMWH. Of the 127 intermediate-risk patients, 66,9% (n=85) received adequate pharmacological prophylaxis. VTE was developed in only 1 patient of the intermediate group who received prophylaxis with LMWH. Non bleeding complications were observed.
Summary and Conclusions: Awareness of the thrombotic risk, as conferred by an easy and suitable risk assessment has the potential to improve VTE prophylaxis in pregnancy and puerperal patients. In our service, 39,8% of admitted patients had criteria for pharmacological prophylaxis for VTE.

E1568

THE CALIBRATED AUTOMATED THROMBOGRAM AS A USEFUL TOOL IN DETERMINATION OF PROTHROMBOTIC PHENOTYPE

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Background: The assessment of prothrombotic phenotype is important to determine optimal primary and secondary prophylaxis in patients with thrombophilia. Application of integral global assays promises to be useful in this aspect. The Calibrated Automated Thrombography (CAT) is known to determine haemostatic misbalance and it reflects the action of both procoagulant and anticoagulant factors.
Aims: Aim of our study was to determine intermediate phenotype in asymptomatic carriers of known prothrombotic mutations and in thrombophilic patients with diagnosed thrombotic complications.
Methods: Genotyping was done by PCR and restriction fragment length polymorphism analysis. Factor I 455 G/A, factor II (FII) 20210 G/A, factor V 1691 G/A (FVLeiden), factorxII 46 C/T, PAI-I 675 4G/5G, TPA 311 I/D, GpIa 807 C/T, GpIbα 434 C/T, GpIIIa 1565 T/C, platelet receptor P2RY12 H1/H2, MTHFR 677 C/T, endothelial nitric oxide synthase 786 T/C, ApoE E2/E3/E4, AGT 704 T/C, ACE 287 I/D and angiotensin receptor 1 1166 A/C polymorphisms were detected. Homocysteine (Hcy) was measured by high-performance liquid chromatography with fluorescence detection. Hyperhomocysteinemia (HHcy) was defined as Hcy levels more than 95% percentile measured in age and sex matched control group. Lupus anticoagulants (LA) were diagnosed according to the SSC-ISTH criteria: a sensitive APTT, the DRVVT, and confirmatory tests. Activities of FVIII, protein C and antithrombin were measured. The study involved 100 persons: 85 patients with VTE and/or recurrent pregnancy loss and 15 asymptomatic carriers of known prothrombotic mutations-7 FVLeiden heterozygous, 6 FII20210G/A heterozygous and 2 double heterozygous. CAT was done according to Hemker *et al.* at 5 pM TF and 4 μM phospholipids in platelet poor plasma (PPP) with PPP plasma+/-TM reagent.
Results: From the parameters of the thrombin generation curve (Lag time, endogenous thrombin potential (ETP), peak thrombin (PT), time to peak), as well as ETP and PT inhibition, no one showed significant correlation with FVIII activity, studied polymorphisms and/or HHcy. None of the patients demonstrated antithrombin or protein C deficiency. Among 85 patients with VTE and/or recurrent pregnancy loss 18 patients had FV Leiden (FVL) mutation, 5-FII20210G/A mutation, 1 was heterozygous for both mutations, 5 had LA, 10-increased FVIII activity, 19-moderate HHcy. Significant correlation with LA ($p<0,05$) was found for lag-time ($R=0,45$), ETP ($R=-0,45$) and PT ($R=-0,44$) both in presence and absence of TM. ETP and PT reduced in the presence of TM. Values below 21% for ↓ETP and/or 14% for ↓PT *i.e.* activated protein C resistance (APCR) were found in 100% of FVL and LA patients, 90% of asymptomatic carriers of FVL and 50% of patients with increased FVIII. Abnormal ↓PT was more sensitive to detect APCR. FII 20210G/A carriers demonstrated increased ETP (*i.e.* >2114 nMmin in the absence of TM and >1433 nMmin in the presence of TM-values above 95% persentile of controls) in 80% of cases. APCR was found by CAT in 20% of FII 20210G/A carriers.
Summary and Conclusions: We consider CAT to be useful in evaluating haemostatic abnormalities in thrombophilia patients. In cost-effectiveness aspect in a number of cases it could be preferable to a standard approach that includes a wide panel of genetic and functional tests. Clinical utility of abnormal CAT results in asymptomatic carriers of factor V Leiden and prothrombin G20210A mutations needs further elucidation, but we suggest that an integrated approach defining an individual's intermediate phenotype could help in identifying individuals with high risk for thrombotic event that would benefit from active primary thromboprophylaxis.

E1569

LMWH PROPHYLAXIS THROMBOTIC EVENTS IN HODGKIN LYMPHOMA PATIENTS

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Background: Patients with Hodgkin Lymphoma(HL) have a high risk of venous thrombotic complication. LMWH is used for thrombosis prophylaxis throughout the therapy. However this prophylaxis do not prevent all thrombotic complications thus routine laboratory monitoring is not recommended for low-dose

LMWH. The problem of adequate laboratory diagnostic to identify high-risk group and to prevent thrombotic complications is still remains.
Aims: The aim of this was to examine the state of hemostatic system in primary patients with HL and to identify the number of thrombotic risk factors (RF) in these patients. The evaluation of the effectiveness of heparin therapy remains important.
Methods: Twenty four primary patients (median 31 yr, 18-60 yrs, 12 men and 12 women), staging by Ann Arbor II-8, III-7, IV-9 patients, were enrolled in this study. The number of thrombotic RF by NCCN scale is from 3 to 7. The patients were treated by BEACOPP-14. LMWH was administered in doses 70-113 IU/kg 2 times a day. aPTT, PI, TT, fibrinogen and D-dimer levels, and the global hemostasis assays Thromboelastography (TEG) and Thrombodynamics (TD) were performed to evaluate the hemostatic state of patients on diagnosis (before oral contraceptives for women), on 1 and 8 day of all chemotherapy cycles.
Results: Hypercoagulation was detected in 75% of primary patients with at least one of the tests before treatment (45% by TD, 55% by TEG, 16% by aPPT), 40% by TEG and TD, 20% by aPPT and TD, 13% by aPPT and TEG. D-dimers were higher in 33% of patients. Throughout the chemotherapy cycles in patients with ineffective LMWH prophylaxis was revealed the significant($p<0.05$) increase in global assays parameters (TD V(velocity)>25 um/min, TEG alpha>40 deg) and in D-dimer level compared to patients with effective prophylaxis (medians 387ng/ml (min 62-max 5109) and 195ng/ml (min 53-max 3571) respectively). Blood samples were collected after 4 hours of LMWH injection. Despite low-dose LMWH prophylaxis the D-dimer level was significantly($p<0.05$) increased from 1 cycle (median 213 ng/ml, min 71-max 5109) to 8 cycle (median 349ng/ml, min 79-max 4376) of chemotherapy.
Summary and Conclusions: Primary patients with HL revealed hypercoagulation by global hemostasis assays, TD and TEG, before chemotherapy. Despite LMWH prophylaxis nearly 40% of patients remained hypercoagulation state by global hemostatic assays and D-dimer level. The combination of global assays parameters, D-dimer level and thrombotic RF is prospective in indicating high-risk group and monitoring and correction of LMWH prophylaxis in patients with HL.

E1570

CATALASE C−262T GENE POLYMORPHISM AS A POSSIBLE RISK FACTOR FOR VENOUS THROMBOEMBOLISM IN THE POPULATION OF NORTH-WESTERN RUSSIA

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Background: The role of genetic mechanisms in most cases of venous thromboembolism (VT) is still unclear. We have recently shown, that in addition to known molecular determinants of inherited thrombophilia, genetic variations associated with endothelial dysfunction (ED) could be involved in pathogenesis of VT. Oxidative stress is an important mechanism of ED development which occurs due to imbalance between reactive oxygen species production and antioxidant system activity. Catalase is an important enzyme of antioxidant system which activity depends on the variation(s) in the promoter of the respective gene.
Aims: To evaluate the role of catalase C−262T gene polymorphism as a possible risk factor for VT in the population of North-Western Russia.
Methods: Retrospective study involved 300 patients with VT (147 men and 153 women, mean age 42.3±12.6 years) and 203 sex- and age-matched healthy controls (HC). All individuals originated from the North-Western region of Russia and gave informed consent for participation in the study. Catalase C−262T gene polymorphism was discriminated by PCR-RFLP technique. The differences in genotype distributions between the groups were estimated by Fisher`s exact test. Odds ratios (OR), their 95% confidence intervals (CI) and p-values were calculated using the GraphPad Prism software.
Results: Catalase −262T allele was more frequently seen in VT patients than in HC (44.3% *vs* 34.9%, OR=1.5; 95% CI: 1.0-2.1; p=0.042). Heterozygosity for this polymorphism was present in 39.0% of VT cases compared to 30.0% in controls (OR=1.5; 95% CI: 1.0-2.2; p=0.046). Catalase −262T allele occurred more frequently in the group of 200 patients with early-onset disease (at age 45 or less) than in those who suffered from the first VT episode after 45 years old (46.0% *vs* 40.0%, respectively). The frequency of homozygous −262TT genotype was 2-fold higher in young patients than in those with late-onset VT (6.5% *vs* 3.0%, respectively). When compared to HC group, the presence of the catalase −262T allele has been proved to be a significant risk factor for VT development in young patients originated from the North-Western region of Russia (OR=1.6; 95% CI: 1.1-2.4; p=0.026). At the same time, the difference between the group of patients with late-onset VT and HC for the proportion of individuals positive for −262T variant was not statistically significant (40.0% *vs* 34.9%, respectively, OR=1.2; 95% CI: 0.8-2.0; p=0.45).
Summary and Conclusions: We suggest that catalase C−262T gene polymorphism is a possible risk factor for VT development in young patients from the North-Western region of Russia.

Transfusion medicine

E1571

EVALUATION OF ABO BLOOD GROUP DISCREPANCIES IN A TERTIARY CARE CENTRE IN SPAIN

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Background: ABO incompatible blood transfusion is associated with major morbidity and mortality. Solving ABO discrepancies is an essential step towards a safe transfusion practice.
Aims: To evaluate the frequency, main causes and clinical impact of ABO blood group discrepancies detected in our hospital in a two-year period of time.
Methods: We retrospectively analyzed all ABO discrepancies recorded in Ramón y Cajal Hospital Blood Bank from 1st January 2013 to 31st December 2014. The results included both in-patients and out-patients but not blood donors. ABO blood group determination in our centre includes forward and reverse group using column agglutination technology (AutoVue Innova, 0.8% Affirmagen A1, B Grouping Red Blood Cell, and ABO/Rh Reverse cassette). When ABO discrepancies are detected, sample and reagent suitability is checked and the cited automatized test is then repeated. Once confirmed, manual red cell (Anti-A, Anti-B Diagast) and serum (A1, B cells Affirmagen 3.5%, Ortho Clinical Diagnostics) tube testing at different temperatures (immediate centrifugation, room temperature and at 4ºC) is performed. If unexpected positive reactions are seen in the serum test, a 3-cell antibody screen (Surgiscreen, Ortho Clinical Diagnosis) is also carried out. When relevant, antibody identification panels (11- cell microcolumn Liss-Coombs and papain-treated red cells panels, DianaGel-Grifols), direct antiglobulin test (polyspecific and monospecific anti-IgG and C3d, Menarini reagents) and adsorption and elution tests (acid glycine elution or 56ºC elution) were also undertaken. When necessary, samples were sent to the Blood Regional Centre for molecular analysis. The causes for the discrepancy were assessed as well as the clinical impact of the subsequent resolution of the ABO blood group problem.
Results: A total of 155 patients presented ABO discrepancies. The main causes for the discrepancy were: ABO major incompatibility allogeneic stem cell transplantation (ASCT) (9.7%), ABO minor incompatibility ASCT (12.3%), both major and minor incompatibility ASCT (1.3%); "A" subgroups (6.5%), out-of-group transfusion (2.6%), acquired immunodeficiencies (18.7%), alloantibodies (3.2%), autoantibodies (2.6%), cryoagglutinins (4.5%), technical errors (11%), age-related (6.5%) and others (19.4%). In the "A" variants group, 6 A_2B were detected (5 with anti-A_1 active at 37ºC) and 4 A_2 were detected (2 with anti-A_1 active at 37ºC). The alloantibodies identified were anti-M (n=4) and anti-H (n=1). The "others" group consisted of samples with diminished serum testing results in column agglutination technology but normal reverse grouping in test tube at lower temperatures (4ºC). Solving ABO discrepancies lead to specific blood unit selection and preparation in 51 patients through antigen phenotype match (n=5), compatible blood cross-match (n=49), not allowing O transfusion (anti-H: 1 case) and pre-warming of blood and minimizing low temperatures during cardiac surgery (1 cryoagglutinin with cardiac valve surgery). No hemolysis was observed in the patients who received blood transfusions.
Summary and Conclusions: Currently, ABO incompatible ASCT is the main cause of ABO discrepancy in our hospital followed by acquired immunodeficiency and by technical issues. Automatized ABO discrepancies should be confirmed in test tube method and at low temperatures in the serum test. ABO discrepancy resolution lead to specific transfusion practice decisions in about a third of these patients.

E1572

USE OF A PEGYLATED CARBOXYHEMOGLOBIN BOVINE IN SEVERE LIFE-THREATENING ANEMIA

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Background: Patients who cannot receive blood transfusions in the presence of severe anemia can sustain significant morbidity and mortality. Religious beliefs and hemolytic reactions are the most common reasons for not transfusing a patient. Pegylated bovine carboxyhemoglobin (Sanguinate; SG), a therapeutic drug, has been used in 5 such patients including a patient who developed a severe hemolytic reaction following stem cell transplantation. SG is a therapeutic drug designed to carry carbon monoxide (CO) and oxygen in the mammalian vasculature for delivery to tissues to synergistically treat inflammation and vasoconstriction and other comorbidities characterized by tissue hypoxia.
Aims: Perform post-hoc analysis of clinical findings; their relationship to individual patient diagnosis/associated comorbidities and SG intervention.
Methods: Five patients with hemoglobin (Hb) levels under 3.5 g/dL were treated with repeated doses of SG. Diagnoses included AML, hemolytic reaction, acute

chest syndrome and sickle cell crisis. Patients ranged in age from 19 to 61. A unit of SG consists of 500 mL (40 mg/mL). Total doses ranged from 1 unit/day for 2 to 4 days up to 8 units given over 9 days.
Results: No adverse events associated with SG were reported. Each investigator reported clinical improvements in their patient following administration of SG, despite continuing low Hb levels. The number and size of allowed blood draws were restricted; therefore analysis of clinical chemistries was limited. Patients with reported extreme fatigue or neurological deficit each demonstrated increases in responsiveness or self-reported comfort within close temporal association with SG treatment. One patient had a significant increase in cerebral oximetry and another showed improvements in cerebral blood flow measured by TCD. A patient with hyperhemolysis reported feeling well with no pain and no shortness of breath despite severe anemia (Hb 2.5) and had improved mobility following a total of 8 units of SG. A patient who had undergone a stem cell transplant developed immune hemolytic anemia, with resulting extreme fatigue and tachycardia. He received 4 once-daily units of SG, during which time his heart rate normalized and was reported to be alert and oriented.
Summary and Conclusions: Evidence indicates that SG has potential therapeutic benefit in patients who have life-threatening anemia and cannot receive blood transfusions for personal or medical conditions. As vasoconstriction, inflammation, and oxygen deprivation are significant factors in severe hemolysis and ischemia, SG through the therapeutic actions of CO and oxygen may interrupt the ischemic cascade and improve clinical symptoms of hypoxia due to severely low hemoglobin levels. The improved clinical status in these patients suggests that SG has potential utility in patients with severe anemia who are unable to receive blood transfusions. Since submission to ASCO2015, preclinical and *in vitro* studies with SG have been shown to demonstrate anti-inflammatory activity as well as active CO and oxygen transfer. SG is currently in clinical development. A protocol is under development to treat patients for whom blood transfusion is not an option.

E1573

CLINICAL COURSE AND OUTCOME OF NEWLY DIAGNOSED ACUTE LEUKEMIA PATIENTS PRESENTED WITH HYPERLEUKOCYTOSIS IN THAILAND: A 10-YEAR SINGLE-CENTER RETROSPECTIVE STUDY

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Background: Hyperleukocytosis is defined as peripheral WBC count ≥100x 10^9/L or blast count ≥50x 10^9/L. It is one of the most emergent life-threatening conditions in acute leukemia (AL) and confers early and high mortality, low complete remission (CR) rate and high relapse rate. Patients with hyperleukocytosis may suffer from vascular infiltration of CNS and lungs, and fatal bleeding in vital organs. Most such patients belongs to monoblastic subtype of acute myeloid leukemia (AML). Current treatment would include prior intensive leukapheresis followed by intensive chemotherpy.
Aims: To study an incidence, clinical and laboratory presentation, treatment modalities and outcome of newly diagnosed AL patients presented with hyperleukocytosis.
Methods: This was a retrospective, descriptive chart review study. All newly diagnosed AL patients presented with hyperleukocytosis in Siriraj Hospital, Mahidol University, Thailand during 2000-2009 were included. Data collection comprised demographic data, presenting clinical manifestation, initial WBC and blast count, chemistry panel, types of AL (AML, acute lymphoblastic leukemia (ALL) and subtypes), treatment modalities with complications, and outcome including overall survival (OS) and disease-free survival (DFS).
Results: Of all 1,232 AL patients, 136 patients (66 male) were collected. Median age was 46.52 years (range 13-87), and AML:ALL was 118:18 of which M4, M1 and M5 were the majority subtypes (34, 26 and 20, respectively). Normal karyotype was found in 48 of 74 patients. Presentation (%) with fever, alveolar hemorrhage, hypoxemia, acute respiratory distress syndrome, ischemic and hemorrhagic stroke, headache, dizziness/ vertigo, blurred vision, and abnormal bleeding were 29.4, 2.9, 12.5, 3.7, 2.9, 2.2, 12.5, 4.4, 6.6 and 42.6, respectively. Median WBC and blast count (x 10^9/L) were 201.42 (58.59-978.00) and 159.90 (0.54-806.40), respectively. Only 129 patients received treatments with either leukapheresis (2), chemotherapy (120) or combination of both (7). CR rate was 34.85% after front-line treatment, and median OS and DFS were 2.86 and 7.87 months, respectively. Major causes of death (%) were infection, bleeding, stroke and respiratory failure for 47.8, 16.2, 5.1 and 5.1, respectively.
Summary and Conclusions: Hyperleukocytosis as initial presentation in acute leukemia is associated with poor prognosis and overall survival. More rapid, emergent and appropriate treatment is required to improve outcome.

E1574

DETECTION OF UNEXPECTED RED CELL ALLOANTIBODIES BY THE ENZYME/NACL GEL TEST ONLY

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Background: In the patient's pretransfusion testing, the antibody screening helps in detecting the presence of significant red blood cell (RBC) alloantibodies. Performing a low ionic strength solution (LISS) indirect antiglobulin test (IAT) at a minimum couple of donor's reagent RBC expressing all the clinically significant antigens, is considered as the only obligatory step. The additional step of hemagglutination testing by using enzyme treated RBC could further contribute in determining the presence of unexpected alloantibodies.
Aims: The determination of the frequencies/ specificities of "enzyme only" detected RBC- alloantibodies in the routine RBC antibody screening.
Methods: In a general hospital of 300 beds (>80% oncology patients), 10523 blood samples, were submitted for routine pretransfusion testing, during a 41-months-period (11/2010- 5/2014). According to National Blood Bank Center's guidelines for blood group serology, all samples were tested for ABO blood group, RhD antigen and RBC unexpected alloantibodies. The RBC antibody screening was carried out with a 3-cell panel RBCs LISS IAT on Liss/Coombs cards, and also with an enzyme test (ET) with a 3-papaine-treated-cell panel on NaCl cards, according to the manufacturer's instructions. In the case of a positive screening test result, an antibody identification test was carried out, using an 11-cell panel LISS IAT and an 11 papaine-treated cell panel test (ET). Results were considered "inconclusive" either when there was no specific alloantibody identified or when the test reaction was too weak to interpret accurately. A positive screening test followed by a negative panel investigation was considered "false positive" result. The inconclusive results, the false positive ones, as well as the cases of autoantibodies were categorized as "unwanted positive reactions".
Results: During the study period, 10523 samples were tested. Positive RBC antibody screening (positive IAT/ positive ET, or negative IAT/ positive ET) was recorded in 168 patients. Negative IAT/ positive ET screening was found in 54.76% of them (n=92, 47.8% men, 52.2% women). A history of previous transfusion, was recorded in 50 patients (54.34%), while 34 (36.95%) of them had never been transfused before and 8.69% (n=8) had an unknown history of transfusion. A history of labor or miscarriage was recorded in 44 women (91,66%). The frequencies/specificities of RBC antibodies detected only by the ET technique are shown in Table 1.

Table 1.Frequencies and specificities of RBC antibodies detected with the ET technique only.

ANTIBODY DETECTION	FREQUENCY	PERCENTAGE
Specific antibodies		
• ANTI E	26	28.26
• ANTI Cw	3	3.3
• ANTI D	2	2.2
• ANTI C	2	2.2
• COMBINED ANTI E & ANTI c	1	1.1
• ANTI Jkb	2	2.2
• ANTI Lea	6 (natural occuring 5/6)	6.52
• ANTI Leb	1	1.1
• ANTI P1	1	1.1
Unwanted positive reactions		
• AUTO ANTIBODIES	6	6.52
• NON SPECIFIC	17	18.47
• INCONCLUSIVE	19	20.65
• FALSE POSITIVE	6	6.5
TOTAL	92	100

Summary and Conclusions: 1. The ET technique helps in detecting clinically significant RBC alloantibodies (*e.g.* anti Jkb) which could be missed if we used the IAT technique only (early phase of alloimunization? low- titter antibodies?). 2. Larger studies could lead to more data about the frequency/ specificity of detected RBC alloantibodies and help in defining the necessity or not of performing the ET technique in general patient population or among specific subpopulations.

E1575

SUCCESSFUL COMPATIBLE PHENOTYPE TRANSFUSION PROTOCOL TO PREVENT ALLOIMMUNIZATION IN SICKLE CELL DISEASE

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Background: Transfusion of red blood cells (RBCs) is used to treat and prevent complications of sickle cell disease (SCD). Alloimmunization to non-ABO antigens is commonly encountered in part due to antigen disparity between blood donors and patients for ethnic reasons and in part due to the frequency of pro-inflammatory factors present at time of transfusion.

Aims: We report our experience in one single reference center.
Methods: We retrospectively analyzed pediatric patients with SCD and at least one RBC transfusion in Gregorio Marañón Hospital (pediatric reference center for SCD patients in Madrid) through a query of blood bank database. Patients were excluded if they were alloimmunized prior to be transfused in our center. Transfusion indications included acute complications, therapy for prevention cerebral vascular events and pre-stem cell transplantation. Since 2009 a pediatric RBC matching protocol is applied at our blood bank for every new SCD patient who is going to have a hypertransfusion regimen. It entails matching for ABO, D, C, E, c, e and Kell. Before first transfusion, fully phenotype is performed (ABO, D, C, E, c, e, Kell, Jk^a, Fy^a and S) and registered in the database. If further alloimmunization occurs extended matching is employed.
Results: A total of 32 patients (19 males, 13 females) with HbSS and history of one or more RBCs transfusion between 1998 to 2015 were included; 23 were African, 8 African-American and 1 Asian. The median age of the first transfusion was 4 years (5 months-16 years). Of the 32 patients, 13 (40.63%) were on chronic transfusion therapy for prevention cerebral vascular events (6) or pre-stem cell transplantation (7); 19 (59.37%) were transfused because of acute complications such as acute chest syndrome (6), splenic sequestration (6), vaso-occlusive crisis (4) and acute febrile illness (3); 11 received bone marrow transplantation and 19 were on chronic Hydrea treatment; of the 32 patients, 24 (75%) were transfused under the pediatric RBC matching protocol, and 8 (25%) were transfused matching only ABO and D. Median transfusion per patient was 11.5 RBCs (1-57) with total transfusions of 482 RBCs resulting in the formation of 5 new RBC alloantibodies in four patients (AntiKpa, AntiM+S, AntiK, AntiFy^a). The global alloinmunization rate was 1.037% per 100 units transfused and the alloinmunization under pediatric RBC matching protocol was 12.5% per patient; no case was associated with hemolytic transfusion reaction. The characteristics of alloimmunized patients are shown in Table 1. The median number of RBCs transfused pre new antibody was 3 (2-17) and there was no clear association between the number of units transfused and alloinmunization. Of the 4 alloimmunized patients, 2 were on chronic transfusion and 2 were transfused due to acute complications, 3 of them were transfused with Rh (C,E,c,e) and Kell matched RBCs, and the other one was transfused before the matching protocol was use.

Table 1. Characteristics of alloimmunized patients with SCD (N=4).

CHARACTERISTICS	PATIENT 1	PATIENT 2	PATIENT 3	PATIENT4
SEX	Male	Female	Male	Male
AGE ALLOINMUNIZATED	4 years	10 years	2 years	5 months
DATE FIRST TRANSFUSSION	31.06.07	7.06.14	12.06.98	7.09.10
UNITS RBC TRASFUSSION PRE ALLOIMMUNIZATION	17 RBCs*	1RBC pre anti-M 8 RBCs pre anti-S	3 RBCs	2 RBCs
TIME BETWEEN FIRST TRANSFUSION AND ALLOIMMUNIZATION	32 months	1 month 6 months	1 month	4 months
SPECIFICITY ALLOANTIBODY	Anti-Kpa.	1ª. Anti-M. 2ª. Anti-S.	Anti-K	Anti- Fy^a
CAUSE OF TRANSFUSSION	Acute Chest Syndrome.	Prevention Cerebral Vascular Event.	Prevention Cerebral Vascular Event and Pre Stem Cell Transplantation.	Vaso-occlusive crisis.
APPLIED RBC MATCHING PROTOCOL BEFORE ALLOIMMUNIZATION	YES	YES	NO	YES

*RBCs: red blood cells.

Summary and Conclusions: As previously described, we confirm that limited RH (C,E,c,e) and K phenotype matched transfusion could be enough to successfully reduce the incidence of alloantibody production in SCD. With this protocol we manage a very low alloinmunization rate (1.037% per 100 units transfused), with only 5 alloimmunizations in 4 patients which did not jeopardize subsequent transfusions. This scenario allows routine transfusion protocols to prevent SCD complications.

E1576

THE MIRNA PROFILE OF PLATELETS STORED IN A BLOOD BANK AND ITS RELATION TO CELLULAR DAMAGE FROM STORAGE

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Background: Millions of blood products are transfused each year, and many lives are directly affected by transfusion. Platelet concentrate (PC) is one of the main products derived from blood. Even under good storage conditions, PC is likely to suffer cell damage. The shape of platelets changes after 5 to 7 days of storage at 22°C. Taking into consideration that some platelet proteins undergo changes in their shape and functionality during PC storage.
Aims: Characterize the most highly expressed miRNAs in PC using high coverage sequencing, examine quantitative changes in miRNA levels during and after storage in a blood bank and validate by real-time quantitative polymerase chain reaction (RQ-PCR) most expressed miRNAs in 40 PC bags tubes to propose candidate miRNAs as biomarkers of PC storage damage.
Methods: Sixteen PC bags were collected and each PC bag tube was cut into six equal pieces to perform experiments with platelets from six different days of storage. Thus, on the first day of storage, 1/6 of the tube was used for miRNA extraction, and the remaining 5/6 was stored under the same conditions until extraction of miRNAs on each the following five days. Samples were sequenced on an Illumina Platform to demonstrate the most highly expressed miRNAs. Two miRNAs, mir127 and mir320a, were selected from miRnome and two were selected based on literature to validated by real-time quantitative PCR (RQ-PCR).
Results: Our sequencing results suggests the use of the miRNAs mir127 and mir320a as biomarkers to assess the "validity period" of PC bags stored in blood banks for long periods. Preliminary validation results suggest statistical difference the four selected miRNAs from the fourth day storage ($p<0.05$). Thus, bags can be tested on the 5th day of storage for the relative expression levels of this miRNAs (Figure 1).

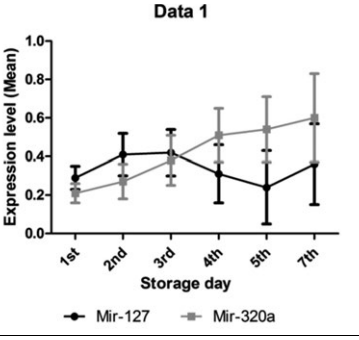

Figure 1.

Summary and Conclusions: We suggest using candidate miRNAs as molecular biomarkers of storage damage that can be used as tools to evaluate the quality of stored PC. The use of miRNAs as biomarkers storages damage is unprecedented and will contribute to improved quality of blood products for transfusions.

E1577

MONITORING HEMOSTATIC POTENTIAL OF PLATELETS DURING STORAGE OF PLATELET CONCENTRATES FOR TRANSFUSION

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Background: Platelet concentrates (PCs) are extensively used either prophylactically to prevent bleeding in onco-hematology patients with thrombocytopenia, or therapeutically to control active bleeding. For a successful transfusion, platelets in PCs should have a good hemostatic potential to have an effective and immediate action.
Aims: In this study we evaluated variations in platelet activation and aggregation properties during standard storage of PCs and the influence of blood group on these changes.
Methods: Seventy pooled buffy-coat-derived PCs (O group=43, A group=27) were analyzed on the day of preparation (D0) and after 3 day-storage (D3) at 22°C on lateral agitation. Platelet aggregation was assessed by Born method (300,000 plts/ul) in response to collagen (5mg/ml), TRAP-6 (40mM) or arachidonic acid (1mM). Levels of degranulation markers (Platelet factor 4 (PF4), β-Thromboglobulin (β-TG), Thrombospondin-1 (TSP-1) and Vascular Endothelial Growth Factor (VEGF)), together with soluble form of membrane surface proteins, *i.e.* P-selectin and glycoprotein V (sGPV), were evaluated in PC supernatants by ELISA. Data were analyzed according to blood group and statistical analysis performed using SPSS statistic data editor.
Results: Platelet aggregation potential after three days of storage was significantly ($p<0.01$) decreased in response to TRAP-6 (-35%) and collagen (-58.5%). Moreover, platelets from A group showed lower aggregation capacity to all agonists used, both in D0 and D3, compared to those from O groups (p=ns). Analysis of PC supernatants showed that levels of the soluble form of the two platelet membrane surface molecules, P-selectin and GPV, significantly ($P<0.01$) increased from D0 to D3, respectively of 49% and 123%, probably due to platelet degranulation and to the presence of protease activity in PC bags. Similarly, level of α-granule proteins significantly ($p<0.01$) raised during storage. We observed marked differences in percentage increment according to the type of α-granule proteins and blood groups analyzed. Particularly, PF4 and β-TG, which levels significantly ($p<0.001$) correlates ($r=0.932$), raised respectively about 140% and 110%, and the increase was higher in A group; TSP-1 level increased only about 33% and mainly in O group; finally, VEGF level raised about 180% in both blood groups. All these data suggest the occurrence of a differential and regulated release of platelet α-granules subsequent

to activation. A significant (p<0.05) inverse correlation was found between the decrease of aggregation potential induced by TRAP-6 and the increase in PF4 (r=-0.335), β-TG (r=-0.415) and sGPV (r=-0.420) levels.
Summary and Conclusions: During storage, platelets become activated and change their hemostatic phenotype; this reflects in an increased release of α-granules content and a lower ability to aggregate in the presence of agonists. Platelets of A group seem to be more sensitive to these phenomena. The significant correlation found between degranulation and platelet aggregation suggests that measurement of these proteins can be a valuable surrogate of platelet aggregation for evaluation of platelet hemostatic potential. Further studies are required to define the strength of these markers to identify PCs that could not display a full functionality and reactivity in the recipient.

E1578

INDIVIDUALIZED GCSF/PLERIXAFOR PBSC MOBILIZATION SCHEDULE ALLOWS SUCCESSFUL HARVEST ALSO IN POOR MOBILIZERS LYMPHOMA PATIENTS

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Background: Autologous peripheral blood stem cell (PBSC) transplantation has a fundamental role in first line treatment of multiple myeloma (MM) and has been shown to improve complete remission and overall survival rates in relapsed Non Hodgkin Lymphomas (LNH). GCSF with or without chemotherapy represents the conventional PBSC mobilization regimen; according to standard criteria apheresis start at level of 20x10^6/L CD34+ cells. Nevertheless, 10% to 20% of patients fail the harvest of an adequate amount of PBSCT.
Aims: The combined administration of Plerixafor and GCSF at steady state or during haematological recovery after chemotherapy allows a sufficient PBSC harvest even in this group of poor mobilizers. In fact, many authors demonstrated that, in poor mobilizers, CD34+ PBSC reach a peak 9 hours after plerixafor administration and in this time-frame the CD34+ cell count may become sufficient to start apheresis. The aim of our study was to assess the feasibility and safety of a regimen combining GCSF (10 µg/kg, on days 1-4) and plerixafor (0,24 mg/kg on day +4), with leukaphereses started at a lower level of circulating CD34+ cells (10x10^6/ L).
Methods: Patients and methods: Between July 2014 and February 2015 we enrolled 10 pts affected with LNH. Seven were male e 3 female, median age was 48 (range 36-62). Seven pts were proven and 3 were predicted very poor mobilizer. The CD34 mobilization profile was evaluated by testing CD34+ levels every 3 on day +4 of CGSF alone (6 patients) or starting on day +10 during recovery post DHAP chemotherapy (3 patients). In case an increase of CD34+ cells of at least 10 /microl was not reached, we administered plerixafor on day +5. Thereafter we tested CD34+ count every 3 hours and we began leukapheresis as soon as CD34 count reached 10/mL.
Results: Results: All patients successfully performed leukaphereses and reached the target value of CD34 (2.x 10^6/kg) with one (in 5 pts) or two (5 pts) procedures. Median value of cd34+ cells/ul at +3, +6, +9, +12 hours at day +5 were 15.5, 15.8, 19.11, 16.8, respectively; median value of CD34 harvested at day +5 was 1.7x 10^6/kg (range 0,86-7,63). Five pts needed a second plerixafor dose and apheresis and the median value of CD34/ul at +3, +6, +9, +12 hours were 13, 8.7, 8.27, 7.5, respectively; median value of CD34 harvested at day +6 was 1.2x 10^6/kg (range 0.61-3.18). In the overall series the median value of CD34 harvest was 2.66x 10^6 CD34/kg (range 1.97-7.67). 5 pts underwent to PBSC transplantation and the median of neutrophils and platelets engraftment were 12 and 18 days respectively.
Summary and Conclusions: Conclusion: An early (hour +3) evaluation of CD34+ levels after plerixafor allows detecting early CD34 increase that would be missed with standard monitoring protocols (hour +12). Moreover the early evaluation of Cd34 mobilization profile starting on day +4 allows identifying patients who are predicted to become poor mobilizers so that a further dose of plerixafor may be added to standard schedule. This strategy may be successful in rescuing patients that, according to standard criteria, would never reach the minimum level of CD34+ cells to start leukaphereses.

E1579

GENETIC RISK FACTORS IN HUMORAL IMMUNE RESPONSE TO PLATELET ANTIGENS HLA AND HPA SYSTEMS IN MULTITRANSFUSED HEMATOLOGICAL PATIENTS

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Background: Humoral immune response to alien antigens reduces number of compatible and, hence, safe blood components transfusions. Alloimmunization of recipients to HPA (Human Platelet Antigens) and HLA (Human Leukocyte Antigens) I class antigens is one of the common immunological complications in platelet transfusions. Molecules HLA II class play an important role in the initiation of humoral immune response as amino acid structure of peptide-binding sites of immune cells influences on presentation of alien peptide to T-lymphocyte's receptors. HLA-restricted mechanisms of alloimmunization are not investigated enough in transfusiology.
Aims: to investigate the association of the HLA and HPA genotypes with antibody formation to HLA and HPA systems in multitransfused Russian hematological patients.
Methods: serums of 228 multitransfused patients [100 with aplastic anemia (AA) and myeloproliferative syndrome (MDS), 76 with acute myeloblastic leukemia (AML), 52 with acute lymphoblastic leukemia (ALL)]; DNAs of 178 the same patients (57 with AA and MDS, 44-with ALL, 77-with AML). All investigated people were of Eastern European origin.HLA-DRB1,-DQB1 (low and high resolution) (Dynal, Protrans, BAG) and HPA-1,-2,-3,-4,-5,-6,-15 genotyping by PCR-SSP (BAG, Protrans). Patients were initially screened for HLA class I using standard microlymphocytotoxic test; and for HPA antibodies using ELISA with native and modified (without HLA) platelets. HPA-typed platelets were used for detection of anti-HPA antibodies specify. The statistical calculation was performed using Chi-square and Fisher exactly (in small groups) criteria, p value <0,05 were considered significant. HLA haplotype were estimated via maximum-likelihood analysis using the Arlequin software version 3.5.
Results: Alloimmunization to HLA and to HPA was detected in 70% patients with AA and MDS, 35,5% patients suffering AML, and 15% patients with ALL. Antibody formation depends on disease's forms which range on alloimmunization frequency as follow: AA-AML-ALL. Parallel investigations of HLA antibody formation and HLA genotyping were carried out in 124 patients (39 AA+MDS, 36 ALL, and 49 AML) from whom only 48 made HLA antibodies (20 AA, 22 AML, and 6 ALL). Risk factors for anti-HLA antibody formation were established as HLA-DQB1*02 and HLA-DQB1*03:01 with closely linked genes of superfamily HLA-DRB3*01 (HLA-DRB1*03,-*11,-12,-*13,-*14): 18/20 AA and 13/22 AML patients with anti-HLA and 6/19 AA and 7/27 AML without anti-HLA were HLA-DQB1*03:01 positive (p<0,001 and 0,04 accordingly). Absolute marker of alloimmunization to HLA was haplotype HLA-DRB1*13:03,-DQB1*03:01: 5/20 patients with AA (p=0,047), 4/22 patients with AML (p=0,035) and 3/6 patients with ALL (p=0,01) were positive for this one. 10/22 AML patients with antibodies and 4/27 without those were HLA-DQB1*02 positive (p=0,027). Genes HLA-DRB1*15 and HLA-DQB1*06 had protective effect for anti-HLA antibody formation in AML patients: 13/27 patients without HLA antibodies and 3/22 with antibodies were HLA-DRB1*15 positive (p=0,015); according HLA-DQB1*06 the relation was as 19/27 and 7/22 (p=0,016). Parallel investigations of HPA antibody formation and HLA typing were carried out in 114 patients (46-AA+MDS, 56-ALL, and 12-AML) from whom only 33 made HPA antibodies. We could detect the antibody specifies in 24 patients. Haplotypes of predisposition for specific HPA antibodies formation were determined as follow: to anti-HPA-1b in HPA-1a/1a patients-HLA-DRB1*07:01,-DQB1*02; to anti-HPA-5b in HPA-5a/5a patients-HLA-DRB1*13:01,-DQB1*06 and HLA-DRB1*13:03,-DQB1*03:01, to anti-HPA-2b in HPA-2a/2a patients-HLA-DRB1*15,-DQB1*06:02/*06:14.
Summary and Conclusions: HLA tissue type profiles together with HPA typing will be informative in predicting the alloimmunization in multitransfused patients. It's very important in patients needed long term platelet transfusion support.

E1580

STUDY OF AN ALTERNATIVE PYROGEN TEST FOR BLOOD PRODUCTS USING MONOCYTE ACTIVATION TEST

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Background: Blood product such as plasma derivatives is a drug manufactured by separating plasma protein components. For example, human serum albumin, immunoglobulin, coagulation factor etc. are plasma-derived preparations. Currently the rabbit pyrogen test is practiced for national lot release of final blood derived products. However, the replacement of rabbit test needs to be considered due to the high consumption of animals and ethical issues. While it costs many lives of rabbits, it does not line with the international trend which argues for minimization of animal testing, also represented as 3R. There also has been a need of replacement due to its impossibility in quantification of pyrogen, and also to its sensitivity to condition of animals and technique of examinant.. In this study, we established an assay method of alternative pyrogen test for final blood derived products using monocyte activation test as a quality control.
Aims: The aim of our study was to develop an alternative *in vitro* pyrogen test for final blood derived products.
Methods: Three organizations including MFDS collaborated for this joint research about monocyte activation test. We established the assay protocol of monocyte activation test according to the EP 2.6.30. In this research, human cryopreserved blood was used as a cell source of human monocyte and fever inducing cytokines were measured by ELISA method to determine the pyrogenisity of the samples. Three organizations shared the established protocol

and conducted the monocyte activation test for WHO-endotoxin international standard and representative non-endotoxin pyrogenes from gram positive bacteria, fungi. And then we also applied the assay method to final plasma derived preparations. We confirmed the possibility to replace of the rabbit pyrogen test by conducting the preliminary test.
Results: As a result, we established conditions and test method of monocyte activation test for endotoxin and non-endotoxin pyrogen standards. And we confirmed that not only endotoxin from gram negative bacteria but also non-endotoxin pyrogenes from other origins were detected by monocyte activation test using human cryopreserved blood and cytokine IL-1beta, IL-6 and TNF-alpha ELISA kit. In addition, we conducted monocyte activation test (MAT) based on human fever reaction to study on the possibility of its application to final blood products. Furthermore, data showing that MAT is capable of covering the total pyrogens and applicable to blood derived products.
Summary and Conclusions: By this study of alternative pyrogen test, especially monocyte activation test for national lot release of complete blood products, we predicted substitutability of the rabbit pyrogen test. The results of the research will be used as baseline data for introduction of *in vitro* pyrogen test which is mainly inspected for safety of blood product, and will also contribute to blood product quality improvement. However, further study is needed to confirm that the developed method can be used as an official quality control test.

E1581

INFILTRATION OF PLATELET RICH PLASMA OBTAINED IN OPEN SYSTEM IN PATIENTS WITH EPICONDILITIS

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Background: There is evidence that the infiltration of PRP improves function and pain in patients with chronic epicondylitis and is an alternative before considering surgical treatment.
Aims: To assess the efficacy and safety of treatment with platelet-rich plasma obtained in an open system in a group of patients with epicondylitis.
Methods: We included patients with a diagnosis of chronic epicondylitis (>6 months) with persistent pain despite proper rehabilitation treatment. All patients had rehabilitation; patients with persistent symptoms after four months were referred to the Interventional Rehabilitation Unit. A shoulder ultrasound was ordered to all patients, to exclude major elbow tendon ruptures and determine the degree of damage. They were evaluated according to the DASH scale before and after infiltration to estimate the results objectively. The infiltration was performed with ultrasound control. PRP was obtained from 12x3ml tubes of PB with 3.2% sodium citrate. Additionally, we obtained blood samples from each patient to carry out serology (HBsAg, anti-HIV type 1 and 2, HCV S, techniques HCV genomic nucleic acid amplification, syphilis, ABO group, regular antibodies). The samples were processed using a two sequential centrifugation, keeping them at room temperature. All manipulations were performed under sterile conditions in laminar flow cabin within a C/D room. The samples were processed immediately after extraction in the Blood Bank.
Results: To date, 12 patients have been treated with PRP. They received a minimum of 1 and a maximum of 3 infiltrations. All patients showed tendon thickening, decreased echogenicity and neovascularization in the ultrasound. In 6 cases intrasubstance rupture was found and one patient had microcalcifications. 9 patients had symptoms improvement (considered as a reduction of at least 25% in the DASH scale), that is 75% of the treated patients. In these 9 patients ultrasound improvement was documented 4 months after the end of the PRP treatment.Only one patient had a minor complication: a presyncopal episode after infiltration without hemodynamic instability. There are no other adverse reactions or complications associated with PRP treatment in the 12 month follow-up.

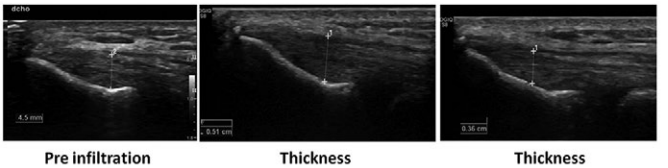

Figure 1.

Summary and Conclusions: Infiltration with PRP in patients with epicondylitis refractory to conservative treatment is safe and effective and can be considered as an option prior to surgery. In our patients signs of clinical and ultrasound improvement (tendon regeneration mediated by the PRP) was found one month after the first infiltration. Obtaining PRP in an open system has proved safe in the treatment of these patients.

LB2084

PREVALENCE AND TRENDS OF HBV, HCV, AND HIV SEROLOGICAL AND NAT MARKERS AND PROFILES IN SAUDI BLOOD DONORS

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Background: Epidemiologic studies on the prevalence of transfusion-transmitted infections (TTIs) in Saudi Arabia (SA) regions are limited.
Aims: This study investigated prevalence of HBV, HCV, and HIV using both serological and nucleic acid testing (NAT) methods to determine temporal and geographic trends among blood donors in Makkah. Our secondary objective was to realize the most suitable NAT format, without compromising sensitivity of NAT results by using individualized or mini-pool testing.
Methods: Serologic and NAT screening records of 22,963 blood donors from January 2011 to December 2014 were evaluated for HBsAg, Anti-HBc, Anti-HCV, Anti-HIV, HBV-DNA, HCV-RNA, and HIV-RNA. Prevalence rates were calculated for TTIs per hundred donations and additional analysis was conducted to examine donor profiles associated with positive serologic and NAT results. Known viral loads (<20 IU/ml for each HBV and HCV and <50 copies/ml HIV) diluted in negative plasma at 1:2, 1:4, 1:6, and 1:8 were evaluated by NAT screening.
Results: The overall serological prevalence of HBs-Ag, anti-HBc, anti-HCV, and anti-HIV were 0.7, 6.7, 0.44, and 0.07%, while molecular HBV-DNA, HCV-RNA, and HIV-RNA overall prevalence were 0.72, 0.05, and 0.03% respectively. There was a gradual decline in percentage of infected donor blood based on combined serological and/or NAT screening from 8.3% in 2011 to 6.8% in 2014 with an overall 7.4% (n=1,689) infected by one or more TTI during the period under study. The prevalence of HBV, HCV and HIV was unevenly distributed among different regions in SA. Analysis of donor serologic and molecular profiles revealed solitary anti-HBc- positive was the highest (6%) donor profile followed by anti-HBc-positive/HbsAg-positive/HBV-DNA positive donor profile at 0.6%, and solitary anti-HCV at 0.4%. Simulation of mini-pool NAT format by dilution of known viral loads at 1:6, resulted in 70% reduction in HBV detection, 50% for HCV, and 40% for HIV.
Summary and Conclusions: This is the first study to provide current data collectively comparing prevalence and trends of HBV, HCV, and HIV serologic and nucleic acid markers amongst Saudi blood donors. Makkah boasts one of the lowest TTIs prevalence in SA and to surrounding countries. The majority of seropositive and NAT-reactive blood donors are in a state of acute, chronic or resolved HBV infection. Individual donor NAT is the ideal methodology that should be applied in SA where diluted samples could compromise clinical sensitivity and blood safety.

LB2093

PLASMA EXCHANGE FOR ACUTE HUMORAL REJECTION OF RENAL ALLOGRAFT AFTER ABO-MATCH KIDNEY TRANSPLANTATION: 5-YEAR EXPERIENCE IN A UNIVERSITY HOSPITAL

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Background: Acute humoral rejection (AHR) of renal allograft after ABO-match Kidney Transplantation (KT) is one of the main causes of acute graft dysfunction and had a high risk of allograft loss. This entity often pose challenge to nephrologists and hematologists during practice.
Aims: To analyze the safety and effectiveness of plasma exchange (PE) in patients with AHR in our department.
Methods: Retrospective review of PE procedures carried out over 5 years (2008-2012) in patients with AHR. We collected clinical and analytical data and defined *response* as a reduction greater than 25% from nadir initial values of serum creatinine (SC), *Stable graft function* as decrease of less than 25% or increase over 25% of nadir initial values of SC and *no response* as a rise greater than 25% of values. *Early response*: response with 3 or less procedures of PE. *Graft failure*: restarting of hemodialysis.
Results: 15 patients (8♀y 7♂), median age: 44,64 (29-76) *y-o*. Median time between KT and AHR: 10,8 (0,57-148,7) months. Five patients had also histological findings of Acute Cellular Rejection (n=3), Chronic Humoral Rejection (*n=2*) and Tubular Acute Necrosis (*n=1*). Treatments before PE: monoclonal antibody anti-cd20 in 3 cases (in one case with MTPD bolus). Simultaneous treatments: none (*n=1*), IVIG (*n=1*); IVIG with MTPD bolus (*n=2*); IVIG with anti-cd20 (*n=6*), IVIG, MTPD and anti-cd20 (*n=4*); IVIG, anti-cd20 and Bortezomib (*n=1*). Median procedures done on each patient: 6 (5-12). During this period time 109 procedures were performed, we observed 7 sessions (6,4%) with technique-related adverse events, none of them was severe. One patient achieved *Response* and eleven *Stable graft function*. These 12 patients had the highest values of serum creatinine before PE (3,5 mg/dL *vs* 2,2 mg/dL, *p=0,041*) and a late development of AHR (38,3 *vs* 4,7 months after KT,

p=0,035). Variables related with better responses were, initial hypertension (*p=0,046*) and immunosuppressive *therapy* before PE (*p=0,024*). At the end of follow up, eight sustained graft function. Five patients reached *early response*. These patients were mainly women (57,1%), all of them had hypertension and more than 2 antibodies (see Table) in the immunohematological study before PE. At the end of follow up, three of them sustained graft function. After PE, all patients had immunosuppressive *therapy* (Tacrolimus, MF and Prednisone) and in one case, there was necessary to add a dose of anti-cd20. Only in two cases, antibodies turned negative after PE (1 for Class I and 1 for Class II). Random urine Protein/Creatinine ratio was normal in all patients before and after PE. There were no important differences between values of white blood cells, hemoglobin, platelet count, pH or albumin before and after PE. During 2,7 (0,21 - 6,24) years of follow up, three patients died (1 of unknown cause and 2 of infectious cause), one of them with functional graft. One out of 12 patients alive, had been transplanted for a second time (32 months after PE completion), and 33,3% had graft failure. Other abbreviations used (alphabetical order): Abs: Antibodies, DSA: Donor specific antibodies, IGIV: Intravenous Immunoglobulin, MF: Mycophenolate, MTPD: Methylprednisolone.

Table 1. Immunohematological study previous and after plasma exchange (PE).

Immunohematological study previous and after Plasma Exchange (PE)

	Previous PE			After PE		
	Abs Anti Class I	Abs Anti Class II	Abs Anti-MICA	Abs Anti Class I	Abs Anti Class II	Abs Anti-MICA
Global series						
Positive	10	11	4	9	11	4
DSA	6	4				
No DSA	4	7				
Negative	5	4	11	6	3	10
No data	0	0	0	0	1	1
Patients with response	7 (3 DSA)	9 (2 DSA)	2	6	9 No data: 1	2 No data: 1
Patients with early response	5 (2 DSA)	5 (1 DSA)	2	4	5	2

Abs: antibodies. DSA: Donor specific antibodies. MICA: Major-histocompatibility-complex class I-related chain A antigens.

Summary and Conclusions: Eighty percent of our series achieved response and 33% of them in an early way. These results are maintained long-term. Therefore, although AHR is a serious complication for transplant recipients, PE improve results regards renal graft survival, and is a safe therapeutic method. In addition, we observed that there is no correlation between response and the presence of antibodies in the immunohematological study.
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PUBLICATION ONLY

Acute lymphoblastic leukemia - Biology

PB1584

GENE THERAPY USING TRAIL-SECRETING HUMAN ADIPOSE TISSUE DERIVED MESENCHYMAL STEM CELLS (AD-MSCS) AGAINST LEUKEMIA

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Background: Mesenchymal Stem Cells (MSCs) are a group of adult stem cells naturally found in the body. These cells were reported from many sources. Bone marrow and adipose tissue are two main sources of human MSCs. MSCs have attracted considerable attention in the fields of cell and Gene Therapy due to their intrinsic characteristics and ability to differentiate into multiple lineages. Whereas BM has been the first recognized source of MSC, adipose tissue represents a valid reservoir of mesenchymal progenitors. Adipose tissue can be obtained in relevant amount and easily processed to release large numbers of adipose-derived MSC (AD-MSC). These cells may offer efficient tools for cell-based Gene Therapy approaches.
Aims: In this study, we evaluated whether AD-MSC could deliver TRAIL for leukemic cells treatment and induce apoptosis in leukemic cells.
Methods: Human AD-MSCs were isolated, characterized and transduced with a lentiviral vector encoding secretory tumor necrosis factor-related apoptosis-inducing ligand (TRAIL). TRAIL protein production verified by western blotting. TRAIL-ADMSCs were Co-cultured with HL-60 and AML-M3 patients MNCs. Apoptosis induction was studied by flow cytometry using annexin-FITC. We also studied MSCs migration potential by Trans-Well method.
Results: In coculture experiment of TRAIL secreting MSCs with leukemic cells we observed TRAIL-AD-MSC targeted HL-60 cells and AML-M3 mononuclear cells (MNCs). We observed significant apoptosis inducing potential by TRAIL-ADMSC in HL-60 cells and AML patients' mononuclear cells (MNCs) compared to control once (P<0.05). Maximum apoptosis induction in HL-60 cells was 25% which shows most of these cells have resistance to TRAIL induced cell death. Whatever in patients MNCs maximum induction of apoptosis was 45%. *in vitro* coculture experiments on Trans-well plate's migration assays showed that HL-60 cells culture but not FBS lacked growth media, supported the migration of hMSCs and enhanced their migration (P<0.05).
Summary and Conclusions: We found that stably TRAIL transduced ADMSCs could serve as constant source of TRAIL production. However this treatment modality didn't completely induced apoptosis in all HL-60 or MNCs nevertheless it indicate needs for finding cotreatments appropriate to type of resistance mechanism. We also observed that leukemic cells are able to production and releasing cytokines which cause migration of AD-MSCs toward leukemic cells and also AD-MSCs have relevant receptors on the cell surface to respond such cytokines. In conclusion these results suggest that human AD-MSCs have potential use as effective delivery vehicles for therapeutic genes in the treatment of hematologic malignancies.

PB1585

PEDIATRIC TEL-AML1-POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS SHOW INCREASED MN1 EXPRESSION

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Background: The *MN1* (Meningioma 1) gene is overexpressed in certain subtypes of acute myeloid leukemia (AML), including patients carrying translocations inv(16)(CBFB-MYH11) and t(8;21)(AML1-ETO) which target the core-binding factor (CBF) transcription factor complex. In consistence, ectopic *MN1* expression in mouse bone marrow (BM) cells cultured under myeloid conditions results in increased myeloid colony forming activity and myeloid leukemia. Despite this growing body of evidence describing *MN1's* involvement in AML development, it is unknown if *MN1* also plays a role in the pathogenesis of lymphoblastic leukemia.
Aims: We aimed to determine expression level of *MN1* in pediatric B-ALL as well as effect of ectopic *MN1* expression on primary mouse Pro B/pre B-cells *in vitro*.
Methods: Engogenous *MN1* expression in BM samples of 73 pediatric B-ALL patients and healthy controls (n=9), as well as in FACS isolated primary mouse pro B/pre B-cells, were analyzed using RT-qPCR. *MN1* was overexpressed in mouse pro B/pre B-cells using retrovirus and followed by methocult assays.
Results: Here we showed that compared with control BM, *MN1* expression was increased (2-fold or more) in 29 out of 73 (40%) pediatric B-ALL patient BM. Additional analysis of *MN1* expression in sub-groups within our cohort carrying different chromosome translocations showed that carriers of t(12;21)(TEL-AML1) (n=27), targeting CBF, expressed significantly more *MN1* than both healthy controls (P=0.02) and the group carrying the t(9;22)(BCR-ABL) (n=9) (P=0.001). In addition, there was a trend of increased *MN1* expression in TEL-AML1^{positive} patients (18 out of 27) as compared with patients that are negative for this translocation (n=30). Retroviral *MN1* overexpression increased the colony forming activity of mouse Pro B/Pre B cells *in vitro*.
Summary and Conclusions: Our results suggest that deregulated *MN1* expression contributes to the pathogenesis of pediatric B-ALL. Further investigation into the clinical and biological significance of elevated *MN1* expression in TEL-AML1^{positive} leukemia might provide insight into additional molecular mechanisms contributing to B-ALL and may lead to improved treatment options for patients.

PB1586

SCREENING FOR VARIATIONS IN EXONS 9, 10 AND 12 OF CD22 GENE IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: CD22, a B cell specific transmembrane protein, have commonly seen in hematological malignancies and plays a role in B cell survival, proliferation, and differentiation via B-cell receptor (BCR). BCR is an inhibitory co-receptor of B cells and B cell precursors that acts as a negative regulator of multiple signal transduction pathways critical for B cells. CD22 signaling is mediated through interactions with kinases and phosphatases which connect the cytoplasmic domain via phosphorylated tyrosine residues that locate within TAM and TIM motifs. CD22 protein SHP-1 (Src homology 2 domain-containing tyrosine phosphatase) function deficient mice showed disruption of the SHP-1 signaling network that can result in defective maturation in development of B-cell lymphoproliferative state and apoptosis. Studies of human CD22 demonstrated that its variations could cause CD22 molecule with inadequate functional and disturbance of mRNA expression. CD22 is expressed in 90% of chronic lymphoblastic leukemia patients, in 60-70% of B cell lymphoma patients, in 100% of hairy cell leukemia patients, in 96% of childhood acute lymphoblastic leukemia patients. Precursor B Cell Acute Lymphoblastic Leukemia (precursor B-ALL) the largest subset of acute lymphoblastic leukemia (ALL), is the most common form of childhood cancer. B cell expression of CD22 makes this a great target for the treatment of leukemia. CD22 that is located on chromosome 19q24 contains 14 exons. Studies by using DNA sequencing method were detected several homozygous mutations (transversions/transitions, deletions, and insertions) between exon 12 and intron 13 in CD22 gene and this region was called "mutational hotspot".

Tabella 1. Charcaterization of the variants in CD22 gene.

rs Number	Nucleotide Change	Localization	Amino acid change
Novel	T 2199 G	Intron 10	-
rs4805119	A2538G	Intron 12	-
rs4805120	A2646G	Intron 12	-
rs10413526	C2642G	Intron 12	-
rs10406539	G2557A	Intron 12	-
rs10413500	C2612G	Intron 12	-
rs10406069	G2318A	Exon 12	Gly-Asp

Aims: In this study we aimed to screen exon 9, 10 and 12 of CD22 gene that is mutational hotspot region in children with precursor B-ALL and to find possible genetic variations for molecular hematological analysis in childhood ALL.
Methods: In this study we aimed to screen exon 9, 10 and 12 of CD22 gene that is mutational hotspot region in children with precursor B-ALL and to find possible genetic variations for molecular hematological analysis in childhood ALL. Study population consisted of 115 patients aged between 1 and 15 years who were admitted to Lösante Hospital with the diagnosis of precursor B-ALL. Blood samples were collected with EDTA-containing tubes and DNA was extracted from peripheral blood and bone marrow leukocytes with MagNA Pure automatic DNA isolation instrument (Roche Diagnostics, Manheim, Germany). Amplification of gene was performed by PCR and all samples were screened

for the variants by SSCP. Samples showing band shifts were directly sequenced on an automated sequencer (Beckman Coulter, USA).
Results: We detected 7 variants in CD22 gene in our study population. There were no variants at exon 9. We report a novel mutation at exon 10 of CD22 (T2199G). The most of CD22 variants were described in exon 12; intron A2538G (rs4805119), intron A2646G (rs4805120), intron C2642G (rs10413526); intron G2557A (rs10406539); intron C2612G (rs10413500); exon G2318A (rs10406069). Table 1 shows summary of CD22 variant profiles.
Summary and Conclusions: The detected variants in this study seem to be the first screening results of studied gene in childhood precursor B-ALL patients in our country. Identified variants in the CD22 exons encoding the cytoplasmic domain could disturb mRNA stability and splicing mechanism of gene. Thus, a truncated CD22 protein or reduced expression levels of an intact protein might lead to development of leukemia. These results need to be confirmed by further exons on a larger number of patients.

PB1587

COMPARISON OF THE IMMUNOPHENOTYPE OF CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA AT DIAGNOSIS AND RELAPSE

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Background: Lineage switch was reported in relapsed acute leukemia.
Aims: Leukemia-associated immunophenotypes in patients with acute lymphoblastic leukemia (ALL) at diagnosis and relapse were investigated.
Methods: The immunophenotype of leukemia cells from 28 relapsed patients at diagnosis and relapse was detected by four-color flow cytometry (FCM). Isolated testicular relapse was excluded. Antibodies used in the panel were CD2, CD3, cyCD3, CD5, CD7, CD13, CD14, CD15, CD33, CD10, CD19, CD20, CD22, cyCD79a, CD34, CD117, MPO, TdT, glicophorin A, CD41 and HLA-DR. The classification was according to the European group for the immunologic group. Cut-off for positive antigens was ≥20%.
Results: Mean age at the diagnosis was 7.57±4.32 years and 71.43% (n=20) of the patients were male. Median time from diagnosis to relapse was 25 months (6 -138 months). According to BFM criteria 11, 9 and 8 patients had very early, early and late relapse, respectively. Sites of relapse were isolated bone marrow in 19, bone marrow and testis in 5, bone marrow and central nervous system (CNS) in 3 and isolated CNS in 1 patient. At presentation precursor B-ALL, T-ALL, and mix lineage leukemia was detected in 21, 3 and 4 patients respectively. Immunophenotypic switch occurred in 28.57% (n=8) of the relapsed patients (two patients from precursor B-ALL to T-ALL, three patients from precursor B-ALL to mix lineage leukemia, one patient from precursor B-ALL to AML-M5; and of the two patients with mix lineage leukemia one switched to precursor B-ALL and the other to T-ALL). There was no relation between immunophenotypic switch and site and time of relapse. HLA-DR expression decreased at relapse (50.99±32.01% at diagnosis, 38.67±27.4% at relapse, P=0.029). However, CD33 and TdT expression increased at relapse (CD33 from 34.02±32.26% to 58.05±31.28% and TdT from19.83±24.84% to 44.0934.86±%; p values were 0.006 and 0.031, respectively.
Summary and Conclusions: There are many hypotheses in lineage switch as physiological plasticity of the original clone or emerging of a new clone or expansion of secondary clone after eradication of the dominant clone by chemotherapy. In the present study, immunophenotypic switch was detected about one fourth of the patients at relapse. In addition, there were significant differences in the expression levels of HLA-DR, CD33 and TdT. Their clinical implications require further study.

PB1588

FREQUENCY OF COPY NUMBER ABNORMALITIES IN COMMON GENES ASSOCIATED WITH BCP-ALL CYTOGENETIC SUBTYPES IN BRAZILIAN CHILDREN

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Background: Copy number alterations (CNAs) in genes committed to B-cell precursors have been associated with poor survival in subgroups of B-cell precursor acute lymphoblastic leukemia (BCP-ALL).
Aims: Therefore, the aim of this study was to determine the frequency of gene deletions and the significance of copy number alterations in *IKZF1*, *CDKN2A/B*, *PAX5*, *EBF1*, *ETV6*, *BTG1*, *RB1*, *CSF2RA*, *IL3RA* and *CRLF2* within a cohort of Brazilian pediatric BCP-ALL patients.
Methods: Bone marrow and peripheral blood aspirates samples from 274 BCP-ALL patients were analyzed prior to any oncological treatment (period 2004-2011). The inclusion criteria were the quality of the frozen diagnostic material, having at least 30% blast cells, patients with ≤18 years old at the time of BCP-ALL diagnosis. The exclusion criteria were acute leukemia in children with Down syndrome, samples collected in heparin, and DNA of insufficient quality. DNA was analyzed by the SALSA multiplex ligation-dependent probe amplification (MLPA) kit (P335-A4). *IKZF1* deletion results were confirmed using a P202 *IKZF1* SALSA MLPA kit. The age at diagnosis and white blood cell (WBC) count were the criteria for assigning prognostic risk of ALL, according to the National Cancer Institute (NCI). The disease risk associated with CNA occurrence across overall and subgroups of patients was determined by calculating odds ratios (ORs) with a 95% confidence interval (CI). Overall survival (OS) was defined as the time from diagnosis to last event (death or alive). The OS analysis were obtained by the Kaplan-Meier method and log-rank test.
Results: Deletions/amplifications in at least one gene were identified in 83% of the total series. In children older than two years, there was a predominance of CNAs involving deletions in *IKZF1*, *CDKN2A* and *CDKN2B*, whereas *IL3RA* and *CSF2RA* had deletions that were found more frequently in infants ($P<0.05$). Multiple gene deletions occurred in the same patients. Sixty-three patients had both *CDKN2A* and *CDKN2B* deletions ($P<0.01$). Deletions of *CDKN2A* and *CDKN2B* also coincided significantly with deletions in *PAX5* ($P<0.01$) and *IKZF1* ($P<0.05$). *IKZF1* deletions were also found concomitantly with *BTG1* and *EBF1* deletions ($P<0.05$ and $P<0.01$, respectively). *ETV6* deletions overlapped with *PAX5*, *BTG1*, *EBF1* ($P<0.01$) and *CRLF2* deletions ($P<0.05$). Based on the cytogenetic subgroups, favorable cytogenetic subgroups (*i.e. ETV6-RUNX1* and hyperdiploiy) showed more deletions than other subgroups, specifically *ETV6* deletions ($P<0.05$). *TCF3-PBX1* was frequently deleted in *RB1*, and an absence of deletions was observed in *IKZF1* and genes localized to the *PAR1* region. *IKZF1* deletions ($P=0.01$, 30.2 months, 95% CI 8.5-52.0) and *RB1* deletions ($P<0.01$, 16.8 months, 95% CI 0.0-46.1) confered poorer OS to standard risk patients.
Summary and Conclusions: The results corroborate previous genome-wide data and are an important validation of the impact of *IKZF1* deletions in the prognosis of BCP-ALL. The obtained results emphasize the need for including screening of submicroscopic alterations as additional markers for risk stratification, especially in standard risk patients.

PB158

PROGNOSTIC VALUE OF GLUCOCORTICOID RECEPTOR GENE POLYMORPHISMS AND MRNA EXPRESSION IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA.

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Background: Therapeutic protocols used in adult acute lymphoblastic leukemia (ALL) are widely variable. However, glucocorticoids (GC) are essential components in its treatment. The antineoplastic actions of glucocorticoids can be partially attributed to glucocorticoid-induced apoptosis.
Aims: The aim of this study was to evaluate the distribution of the 3 prominent glucocorticoid receptor gene polymorphic variants (*Bcl*I, *N363S*, and ER22/23EK) among controls and Philadelphia-negative adult ALL patients. In addition, we aimed to investigate the association between glucocotiocid receptor mRNA isoforms expressions and the response to induction chemotherapy in the studied patients.
Methods: Fifty-two adults with newly diagnosed Philadelphia-negative ALL, aged 18 to 75 years and 30 healthy control subjects were enrolled in this study. Genotyping of *Bcl*I, *N363S*, and *ER22/23EK* polymorphisms was carried out by polymerase chain reaction restriction fragment–length polymorphism. Glucocotiocid receptor mRNA isoforms expressions were assayed by quantitative real time PCR.
Results: Fifty-two newly diagnosed Philadelphia-negative adult ALL patients were enrolled with a median age of 34 years (range 18-75). They were 35 (67.3%) males and 17 (32.7%) females. The incidences of *BclI, N363S*, and *ER22/23EK* polymorphic variants were 44.2%, 5.8% and 1.9% among patients and were 33.3%, 6.7% and 6.7% among controls, respectively with no statistically significant difference. The allelic frequencies of the mutant allele of *Bcl*I, *N363S*, and *ER22/23EK* polymorphisms in patients were 0.15, 0.02, and 0.004, respectively. The different genotypes of the polymorphisms were not related to glucocorticoid resistance. No association was found between the studied parameters and the expression of beta glucocorticoid receptor (GR) isoform. The alpha GR expression was associated with complete remission (P=0.03). Compared to GC sensitive patients, the expression of gamma GR mRNA was significantly higher in GC resistant patients (P=0.032). Moreover, the expression of gamma GR mRNA was significantly higher in non-responders *versus* responder patients(P=0.019).
Summary and Conclusions: The *BclI* polymorphic variants of the GR gene are the most frequent compared to *N363S* and *ER22/23EK* polymorphisms in Philadelphia-negative adult ALL patients. Polymorphisms of the GR gene were not associated with the sensitivity to glucocorticoids. Higher alpha GR and

lower gamma GR expressions were associated with achievement of complete remission while higher expression of gamma isoform was associated with glucocorticoid resistance. Our data suggest that pretreatment glucocorticoid receptor isoforms expression levels can be useful in predicting response to glucocorticoids and in anticipating achievement of complete remission in Philadelphia-negative adult ALL patients.

PB1590

EFFECTS OF SIRT6 INHIBITORS ON ACUTE LYMPHOBLASTIC T CELL LEUKEMIA

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Background: Sirtuins are NAD(+)-dependent deacetylases with documented roles in gene expression, metabolic pathways, apoptosis, cell survival, DNA repair, development, inflammation, and healthy aging. SIRT6 is a sirtuin family member which has been implicated in the control of glucose uptake and metabolism, cell metabolism, genomic stability and DNA repair, and in inflammation. Aside for the observation that SIRT6 chromosomal locus is a region prone to chromosomal breaks in human acute myeloid leukemia, no data are available concerning the possible involvement of SIRT6 in leukemogenesis.
Aims: Herein, we have selected a series of novel and specific quinazolinedione small molecules SIRT6 inhibitors named S602, S603, and S610 and we have analyzed their *in vitro* effects on the T-ALL cell line Jurkat upon stimulation with anti-CD3/CD28 antibodies coated immunomagnetic beads to mimic tumor microenvironment signaling of the T-ALL cells.
Methods: After 24, 48 and 72 hours of stimulation in presence or absence of SIRT6 inhibitors we performed: a) a viability test with propidium iodide to determine whether these inhibitors affect cell survival; b) an assay for evaluating the content of ATP to determine whether the inhibitors alter the mitochondrial metabolic activity and therefore proliferation; c) an ELISA for TNF-α released on harvested supernatants
Results: We found that none of these inhibitors induced cell death or inhibited cell proliferation at concentrations raging between 10 and 100 μM. On the contrary, a strong and concentration dependent inhibition of TNFα production triggered by CD3 and CD28 co-engagement was observed. Notably, a marked reduction in intracellular ATP levels in Jurkat cells treated with the SIRT6 inhibitors could also be detected after a 72 h incubation.
Finally, SIRT6 inhibition resulted in a decrease in TNFα production in Jurkat cells line triggered through CD3 and CD28 co-engagement
Summary and Conclusions: Altogether, these findings suggest that inhibitors of SIRT6 can selectively affect the synthesis of ATP and thus the metabolic state of T-ALL cells. These inhibitors could be an useful tool when used together with pro-apoptotic anti-blastic drugs because of their complementation activity, affecting different molecular targets that are relevant for T-ALL cell growth.
In addition, TNFα production inhibition is expected to favorably affect the tumor microenvironment and to reduce invalidating systemic manifestations of disease, such as fever and cachexia, which typically worsen the clinical outcome of T-ALL patients.

PB1591

ROLE OF FLOW-CYTOMETRIC IMMUNOPHENOTYPING IN PREDICTION OF BCR/ABL GENE REARRANGEMENT IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Among B-cell precursor acute lymphoblastic leukemia (B-ALL), t(9;22) (q34;q11) responsible for BCR/ABL fusion transcripts is the most common cytogenetic abnormality that occurs in around 20-30% of adult patients (pts). BCR/ABL-positive (BCR/ABL+) B-ALL has been typically associated with high expression of myeloid antigen such as CD13, CD33, CD66c. We analyzed 44 adult patients with B-ALL diagnosed in our center from December 2004 to December 2013, with a median age of 46 ys (range 13-78), 23 males and 21 females.
Aims: The purpose of this study was to establish by immunophenotyping which combination of antigens was most predictive for BCR/ABL gene rearrangement.
Methods: Flow-cytometric data were acquired by immunophenotypic B-ALL definition panels performed using FACSCanto cytometer and BDFACSCanto software. Arbitrary cutoff of 20% analyzed events that were brighter than the control stain, was required for an antigen to be considered positive. Levels of BCR-ABL fusion transcript were quantified in RT-PCR assay. Comparison between two groups were performed using the Mann–Whitney *U* (MWW) and Chi-square tests (CSQ) or Fisher exact test (FET) for continuous and dichotomic variables, respectively. Measure of association was expressed by Odds Ratio (OR). Cut-off value for the marker was selected using receiver operating curves (ROC). Multivariate analysis was performed using Logistic Regression (LR). P values lower than 0.05 were considered statistically significant (SPSS 15.0 version).
Results: BCR/ABL transcript was identified in 21 pts (47.7%). CD10 and CD34 was positive in the totality of BCR/ABL+ cases. BCR/ABL+ pts exhibited a greater median percentage of CD10 (96.8% vs 88.6%, MWW, P=0.030) and CD34 (98.3% vs 92.3%, MWW, P=0.013) expressions, but a lower median percentage of CD38 expression (92.6% vs 99.0%, MWW, P=0.001) than pts with BCR/ABL-. Considering Median Fluorescence Intensity (MFI), BCR/ABL+ cases presented a higher CD10 MFI (median of MFI, 11295 vs 1972, MWW, P=0.001) and a lower CD38 MFI (median of MFI, 919 vs 6747, MWW, P=0.006) than their counterpart. No significant difference were observed in CD34 MFI values comparison. By calculating ROC, the best cut-off for CD10 MFI was 5159 (AUC=0.787, 95% CI: from 0.65 to 0.92) and for CD38 MFI was 6284 (AUC=0.750, 95% CI: from 0.60 to 0.90). These cut-off values provided a sensitivity of 80% and a specificity of 74% for CD10 and a sensitivity of 83% and a specificity of 54% for CD38. Categorized CD10 MFI and CD38 MFI presented a strong association with BCR/ABL rearrangement [CSQ, P<0.001, OR 12.75 (95% CI: from 3.06 to 53.19); P=0.010, OR 0.14 (95% CI: from 0.03 to 0.59), respectively]. CD66c positivity was more frequent in BCR/ABL+ pts than in the others (81% vs 45%, FET, P=0.016). The co-expression of CD66c with CD13 or CD33 was more frequently present in BCR/ABL+ pts (44% and 39%, respectively) than in BCR/ABL- (13% and 6%, respectively)(FET, P=0.04). The sensitivity and the specificity of CD66c as sole marker were 81% and 55%, respectively. In opposite, the co-expression of CD66c/CD13 or CD66c/CD33 showed a lower sensitivity (44% and 39%, respectively) and a greater specificity (88% and 94%, respectively). Multivariate analysis in which categorized CD10 MFI was combined with categorized CD38 MFI and CD66c expression showed that CD10 was an independent predictor for the presence of BCR/ABL rearrangement (LR, P=0.003).
Summary and Conclusions: We suggest a screening panel predictive for BCR/ABL rearrangement in B-ALL by using the combination of CD38, CD10, CD34, CD66c, CD13 and CD33 expressions.

PB1592

EVALUATION OF MULTIPLEX LIGATION DEPENDENT PROBE AMPLIFICATION FOR IDENTIFICATION OF ACUTE LYMPHOBLASTIC LEUKEMIA WITH AN INTRACHROMOSOMAL AMPLIFICATION OF CHROMOSOME 21 IN A BRAZILIAN POPULATION

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Background: An intrachromosomal amplification of chromosome 21 (iAMP21) defines a unique subgroup of B-cell precursor acute lymphoblastic leukemia (BCP-ALL). The finding of three or more extra copies of the *RUNX1* gene by fluorescence *in situ* hybridization (FISH) is internationally used to define of an iAMP21. Recently, genomic profiling of chromosome 21 has been suggested for assisting the identification of an iAMP21.
Aims: In this work, we aimed to test the multiplex ligation-dependent probe amplification (MLPA) SALSA P327-A1 probe set for evaluating copy number alterations (CNA) on chromosome 21.
Methods: A series of consecutive diagnostic samples from Brazilian childhood BCP-ALL patients collected between the years 2002 to 2012 were selected according to availability of material of good quality providing that bone marrow aspirates harbored at least 30% of blast cells. The MLPA SALSA P327-A1 probe set was employed to detect an iAMP21 (MRC Holland, Amsterdam, The Netherlands). Test fragments of the P327-A1 probe set were designed to detect CNA of 24 genes distributed from centromeric to telomeric regions (q11.2-q22.3, 14.668-46.356) of chromosome 21. Relative copy numbers were obtained after normalization of peaks against cord blood derived controls. Relative peak heights between 0.75 and 1.3 were considered normal, while those below 0.75 and above 1.3 indicated losses or gains of genomic material, respectively. FISH was performed using the "LPH012 *TEL/AML1* Translocation, Dual Fusion Probe", designed to detect the *ETV6/RUNX1* fusion gene. Cases were considered positive for an iAMP21 when ≥5 *RUNX1* FISH signals were observed in at least 7% of metaphase nuclei (standard deviation +/- 2%).
Results: In 74 out of 368 BCP-ALL patients, gain of genetic material on chromosome 21 was detected. MLPA peak profiles segregated patients into three groups by hierarchical clustering. Next, patient material was subjected to FISH. Cells with ≥5 *RUNX1* FISH signals (n=9) were considered as "true iAMP21". By contrast those with 3-4 *RUNX1* signals (n=35) or a normal *RUNX1* FISH pattern (n=6) probably acquired additional copies of an intact chromosome 21, even though not detectable by FISH in all cases. Peak ratios of *RUNX1* and *DYRK1A* genes, located within the common region of amplification (CRA), were in "true iAMP21" patients higher, an opposite pattern was observed for telomeric *SNF1LK* and *TFF1* genes. Since we observed variations in MLPA peak heights between different patients, the correlation between *RUNX1, DYRK1A, ETS2* and *ERG* gene load and expression was tested. For illustration we stepwise

arranged cases from the lowest to the highest MLPA peak ratios and depicted corresponding delta CT (threshold cycle) values. Positive correlation between the MLPA peak ratios and CT values were observed, since those patients with high MLPA peak ratios were the ones with low gene expression. This observation particularly held true for the genes *RUNX1, DYRK1A,* and *ERG*.
Summary and Conclusions: Altogether, we confirmed that aberrations on chromosome 21 represent a high level of complexity and individuality and provided evidence for the existence of gene regulatory mechanisms that might modulate the expression of amplified genes. In BCP ALL with an iAMP21 we consider MLPA tests highly desirable for data replication of array based comparative genomic hybridization analyzes. MLPA profiles of high peak ratios of CRA genes might be especially helpful in case identification whenever metaphase nuclei for FISH are not available. However, we want to stress that a precise separation of BCP-ALL with an iAMP21 from hyperdiploid ALL with additional copies of chromosome 21 is still under revision. Control fragments binding on chromosomes that are usually over represented should be included in next generation MLPA probe sets.

PB1593

CIRCADIAN RHYTHM OF MELATONIN IS PRESERVED AT DIAGNOSIS AND DURING TREATMENT IN PEDIATRIC PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Melatonin, the main hormone secreted by the pineal gland in the human brain, has a strong impact on the sleep-wake cycle and is considered to be the general moderator of circadian rhythm and through this of several biological functions. It has also been shown to neutralize a number of free radicals and to enhance the activity of several antioxidative enzymes. Patients with acute lymphocytic leukemia (ALL) show significantly deranged oxidation homeostasis due to both the leukemic burden and the therapy. Alterations of circadian rhythm of melatonin secretion on these patients either at diagnosis or during therapy could enhance oxidative stress or affect bioavailability and efficacy of therapeutic agents.
Aims: Aim of the present study was to elucidate whether melatonin maintains its diurnal variation among pediatric patients with ALL from diagnosis and throughout their treatment. Additionally, correlation of melatonin's level with the oxidative/antioxidant status of these patients was also evaluated.
Methods: Twelve patients (median age 6.5 years old) with intermediate risk B-ALL, treated according to BFM-ALL95 protocol, were included in this study. Morning (10 am) and night (10 pm) samples were collected from each patient at 7 different time points, with a median follow up time being 8 months. More precisely, blood samples were collected at the time of diagnosis, at the end of the first part of Induction, at the beginning and at the middle of Consolidation and Reinduction cycles and before starting Maintenance therapy. Melatonin levels were measured in serum by an Enzyme Linked Immunofluorescent Assay (ELISA). Total oxidative stress was estimated by colorimetric assay by measuring the total quantity of lipid peroxides present in serum. Total antioxidative serum capacity was estimated by photometric assay by adding a specific quantity of hydrogen peroxide in the sample and then measuring the remaining amount, not neutralized by the natural antioxidants in the serum.
Results: Melatonin's secretion retained its circadian rhythmicity with higher levels (9.72±7.39 pg/mL) at night and lower during the day (8.1±6.19 pg/mL) throughout the study (P<0.05). In this respect, intrapatient AM and PM levels, as well as their difference were stable. Total Oxidative Capacity showed an inverse circadian rhythm pattern with higher levels measured in the morning (612.79±329.56 μmol/L) and lower in the night (531.50 ±321.75 μmol/L) samples (P<0.001). Of note the highest levels of oxidative stress (913.30 ±277.26 μmol/L) were measured at the time of diagnosis (P<0.001). This was the time point with the lowest, though not statistically significant, melatonin concentration. Moreover, morning levels of melatonin correlated with the oxidative *versus* anti-oxidative ratio (r 0.23, P<0.05).
Summary and Conclusions: This study depicts, for the first time to our knowledge in the literature, the daily variations of melatonin and oxidative stress in pediatric patients with ALL. Our results showed that melatonin levels maintain their circadian rhythm in our pediatric patients with ALL, both at diagnosis and throughout treatment. Furthermore, we portrayed that oxidative capacity exhibited a similar inverse circadian rhythm, also retained throughout the study. However, oxidative status presented significant fluctuations between distinct measurements, probably related to leukemic burden and the type and intensity of therapy. The above data are of significant importance as many physiological processes, like hormonal secretion and pharmacodynamics, are affected by the body's circadian rhythms.

PB1594

IMPLEMENTATION OF 8 COLORS MULTIPARAMETRIC FLOW CYTOMETRY (8-MFC) IN THE DIAGNOSIS AND MONITORING OF PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) IN A SINGLE CENTER

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Background: ALL is the most common childhood malignancy and the leading cause of pediatric cancer-related mortality. Minimal residual disease (MRD) is a powerful predictor of outcome in childhood ALL and allows stratification of patients into prognostic risk subgroups and the application of risk-directed therapy. MRD assessment is routinely performed by flow cytometry and quantitative real-time polymerase chain reaction (qRT-PCR).
Aims: the aim of our study was to implement 8-MFC to diagnose ALL and monitor MRD using EuroFlow Consortium approach and evaluate its correlation with molecular techniques.
Methods: a descriptive study of childhood ALL diagnosed in a single center between September 2013 and February 2015 and treated according to Spanish Pediatric Hemato-Oncology Society SEHOP-PETHEMA-2013 protocol. The initial diagnosis was established with the monoclonal antibodies panels proposed by the EuroFlow Group. Detection of MRD was based on leukemia-associated immunophenotype (LAIP) observed at diagnosis. Fusion genes ETV6-RUNX1, TCF3-PBX1 and BCR-ABL1 were analyzed by qRT-PCR following the guidelines of the Europe Against Cancer program.
Results: twenty-six patients were included (17 males, median age 6.5 years, range 1.1-16.7 years). Twenty-one cases were B-cell precursor ALL (B-pre-ALL) in common stage (CD10+) and 5 patients had T-cell ALL in cortical (CD1a+) stage. The most frequent LAIPs identified in B-pre-ALL were cross-lineage antigen expression (studying CD13, CD15, CD123 and CD66) and asynchronous antigen expression. ETV6-RUNX1 rearrangement was detected in six patients and TCF3-PBX1 rearrangement was observed in three cases. All patients had a good response to prednisone at day +8 and the evaluation on day +15 by morphology showed persistent disease (≥5% blasts) in 4 B-pre-ALL cases. At the end of induction IA all patients (24 cases evaluated) achieved morphologic remission (<5% blasts). The MRD by 8-MCF on day +15 was negative (<0.01%) in 8/26 cases (5 B-pre-ALL and 3 T-ALL), on day +33 in 21/24 cases and on day +78 in all cases (18 cases evaluated). Two follow-up samples were not evaluable by qRT-PCR analysis. The MRD study by qRT-PCR was concordant with the 8-MFC in 17/19 samples. In a patient with TCF3-PBX1 rearrangement the MRD on day +33 was negative by 8-MCF but positive by qRT-PCR (ratio TCF3-PBX1/ABLx10[4] 3 copies) but the assessment on day +78 was negative in both. In addition, a patient with ETV6-RUNX1 rearrangement had a negative MRD on day +33 by 8-MCF but qRT-PCR still detected a very low number of copies (ratio ETV6-RUNX1/ABLx10[4] 4.9 copies). The evaluation on day +78 for this patient is still pending.
Summary and Conclusions: implementation of MFC-8 has optimized the diagnosis and monitoring of MRD using less amount of sample for the analysis, representing a significant improvement in pediatric care. Furthermore, a good correlation of the MFC-8 MRD levels with molecular results was observed (89.5% of cases), suggesting a good complementarity of both techniques. Thus, MFC-8 confirms as a simple, fast and very useful tool to diagnose and follow MRD with high sensitivity in pediatric ALL patients.

PB1595

ABERRANT BAFF-R EXPRESSION AFFECTS APOPTOSIS RATE OF B LYMPHOBLASTS AFTER CORTICOSTEROID TREATMENT

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Background: BAFF and APRIL play crucial role in the survival, maturation and homeostasis of peripheral B-cells through their interactions with BAFF-R, TACI and BCMA receptors. While the BAFF/APRIL cytokine axis lacks functional roles in early stages of normal B cell development, recent evidence indicates that malignant lymphoblasts aberrantly express receptors of the BAFF-system (predominantly BAFF-R), suggesting a new role for these molecules in acute lymphoblastic leukemia (ALL) biology.
Aims: The **aim** of this study was to uncover any possible effect of BAFFR aberrant expression on the *in vitro* survival capacity of malignant lymphoblasts after treatment with conventional chemotherapeutic drugs.
Methods: Two ALL cell lines were included in the study: the pre-B-ALL cell line 697, expressing BAFF-R and carrying the E2A-PBX1 chromosomal translocation and the T- ALL Jurkat cell line, characterized by the absence of expression of any BAFF/APRIL receptor (served also as a negative control). Cells have been treated in the presence of chemotherapeutic agents that are currently being used in ALL therapeutic protocols (aracytine and dexamethasone). The hypotoxic dose of these drugs (aracytine: 0.08-40.0 μg/mL, dexametha-

sone: 0.01-200.0 μg/mL) was determined by evaluating the apoptosis rate of cells with flow cytometry, using an Annexin V-FITC/7-AAD kit (Beckman-Coulter), according to the manusfacturer's instructions. In order to determine if the presence of BAFF could affect the survival of ALL cell lines, cells have been treated with exogenous BAFF (5 ng/mL-800 ng/mL), in combination or not with one of the above agents.
Results: Dexamethazone induced apoptosis of both cell lines in a dose-dependent manner. Interestingly enough, exogenous BAFF significantly increased the dexamethasone-induced apoptosis of 697 cells; this effect was also obvious in the lowest dose of BAFF (5ng/mL). As expected, exogenous BAFF did not affect the rate of dexamethasone-induced apoptosis in Jurkat cells, which did not express BAFF-R. Concerning aracytine, treatment of both cell lines with BAFF did not alter the apoptosis rate caused by the drug itself.
Summary and Conclusions: The aberrant expression of BAFFR might play a crucial role in ALL biology and treatment.

Acute lymphoblastic leukemia - Clinical

PB1596

THE EFFECT OF PROLIFERATION INHIBITION AND APOPTOSIS OF BRD4 INHIBITORS JQ1 ON PH POSITIVE ACUTE LYMPHOCYTIC LEUKEMIA CELL AND ITS MECHANISM

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Background: It has been widely demonstrated that BRD4 knockdown or inhibition by JQ1 is associated with c-MYC downregulation and antileukemic activity. leading to suppression of the transcriptional program linked to proliferation and survival.
Aims: We evaluated the effect of proliferation inhibition and apoptosis of brd4 inhibitor JQ1 on Ph positive acute lymphocytic leukemia(Ph+ ALL) cell and its mechanism.
Methods: different concentrations of JQ1 are used on SUP-B15 cell and proliferation inhibition level were detected by MTT assaythe cell apoptosis rate were determined by flow cytometry(FCM) the expressions of BCR – ABLmRNA brd4mRNA mycmRNA P53mRNA were detected by real-time fluorescent quantitative PCR(RT-PCR).
Results: different concentrations of JQ1 can all inhibit SUP-B15 cell proliferation, inducing cell apoptosis apoptosis rate was significantly increased compared to control group, and depend of time and dose. half inhibitory concentration of 72h is about 1.0 umol/L.At the same time□JQ1 can down regulation BCR-ABLmRNA,brd4 mRNA, myc mRNA transcription levels, and up regulation the transcription level of p53mRNA.
Summary and Conclusions: JQ1 as brd4 inhibitor can down regulation the expression of brd4, thus affecting its downstream gene myc and p53, at the same time it can changeover the express of BCR-ABL,further achieve inhibition of cell proliferation, inducing cell apoptosis.

PB1597

THE EFFECTS OF JQ1 ON SUP-B15 CELL NOTCH1 PATHWAY AND ITS MECHANISMS

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Background: Philadelphia chromosome-positive (Ph+) acute lymphoblastic leukemia (ALL) has a poor prognosis. Advances of treatment due to the tyrosine kinase inhibitor imatinib have improved the cure rates.
Aims: The study was aimed to investigate the inducing effect of JQ1 on the apoptosis of Ph(+) human ALL Cell(SUP-B15),and whether the regulation of Notch1 pathway involved in the effect of JQ1 on SUP-B15 cells.
Methods: The SUP-B15 cell were treated with different concentrations of JQ1 for different time.The cell proliferation was analyzed with cytotoxicity test(MTT method).Cell cycle was detected by fluorescence microscopy and flow cytometry. The expression of Notch1 pathway was detected by real-time quantitative PCR by MSI2□Notch1□Hes1mRNA.
Results: The results indicated that JQ1 could significantly inhibite the viability of SUP-B15 cells treated with 0-4μmol/L in does-and time-dependent manner.JQ1 could induce S cycle arrest in does-dependent manner which was statistical different from the control at the same time ($P<0.05$).MIS2 Notch1 Hes1mRNA expression was down-regulated by JQ1 which was statistical different from the control($P<0.05$)
Summary and Conclusions: It is concluded that JQ1 could potently inhibit the growth and proliferation of SUP-B15 cells and the Notch1 pathway might be one of the important apoptosis mechanisms in Ph(+) ALL cells induced by JQ1.

PB1598

IS BERLIN-FRANKFURT-MUNSTER (BFM) REGIMEN FEASIBLE FOR TREATMENT OF ADULT ACUTE LYMPHOBLASTIC LEUKEMIA: RETROSPECTIVE ANALYSIS OF OUTCOME OF ADULT ALL TREATED WITH BFM AND HPER-CVAD PROTOCOLS

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Background: Multiple induction regimens have been developed for adult patients with acute lymphoblastic leukemia (ALL). But none have been directly compared in a prospective randomized trial.
Aims: In this report, we want to evaluate outcome of our adult ALL patients treated with BFM-like and Hyper-CVAD protocols retrospectively and also evaluate the feasibility of BFM protocol in adult patients in case of efficacy and tolerability.

Methods: In this study, we want to evaluate outcome of our 50 adult ALL patients treated with Berlin-Frankfurt-Munster (BFM) (20 patients) and Hyper-CVAD (30 patients) protocols between march 2006 and october 2012.
Results: The median age was 25 years in BFM, 30.5 years in Hyper-CVAD group with M/F ratio 15/5 and 17/13 respectively. 45% of patients in BFM group and 30.3% of patients in Hyper-CVAD group were under age of 25. The majority of cases were B cell in origin (80% in BFM, 70% Hyper-CVAD group). Complete remission after induction therapy was achieved in 95% and 96% of the patients, respectively. Median follow-up time was 37 months. Five-year survival rate was higher in BFM group with respect to Hyper-CVAD group (59%x 34%). There were also no complication which can cause a delay during Hyper-CVAD regimen. Both chemotherapy were well tolerated. None of patients died due to drug related toxicity. Only mild liver enzyme elevations were seen as toxicity in BFM which didn't cause any delay in therapy.
Summary and Conclusions: BFM regimen seems to be feasible for adult patients with ALL in case of tolerability and efficacy especially in young adults.

PB1599

THE RESULTS OF BFM 95 PROTOCOL IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA: A SINGLE CENTER EXPERIENCE FROM TURKEY

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Background: In the past, acute lymphoblastic leukemia (ALL) treatment success was 20-30%. But in the last three decades this rate has been increased by over 80%. This success achieved by contemporary protocols, improvements in the infrastructures, supportive treatments, and social and psychological supports. Over the last decade, our country has reached the same success level as other developed countries. ALL BFM 95 protocol is commonly used for pediatric ALL in European countries and also in Turkey.

Table 1. Demographic characteristics and treatment results of the 147 children receiving ALL-BMF-95 protocol.

Age (years)	5.3 (1-14)
Gender	
M (%)	80 (54.4)
F (%)	67 (45.6)
Risk Groups	
SR (%)	29 (19.7)
IR (%)	75 (51)
HR (%)	43 (29.3)
Blast Type	
Pre-B cell (%)	123 (83.7)
T cell (%)	24 (16.3)
Genetic Abnormality (n=45)	
t(12;21)	8
t(9;22)	6
t(4;11)	2
t(1;19)	1
t(1;14)	1
Trisomy 8	3
Trisomy 4,10,17	1
Monosomy 1	2
Others	21
Steroid Response	
Positive (%)	123 (83.6)
Negative (%)	24 (17.4)
Day 33 BM	
Remission (%)	144 (97.1)
No Remission (%)	3 (2.8)
Survival	
5-year EFS (%)	
SR	100
IR	84
HR	48.8
5-year OS (%)	
SR	100
IR	89.3
HR	53.5

SR: standard risk; IR: intermediate risk; HR: high risk; EFS: event-free survival; OS: overall survival; BM: bone marrow

Aims: We would like to present our results of 147 pediatric ALL patients who were treated with this protocol in our hospital within a 10-year period.
Methods: One hundred and forty-seven children with acute lymphoblastic leukemia (ages 1-14) who were treated with ALL BFM 95 protocol in the Lösante Hospital between the years 2000 to 2009 were reviewed. The risk groups were defined by the age and leukocyte count at diagnosis, steroid response of 8th day, bone marrow aspiration results of 15th and 33th day and cytogenetics/genetic alterations.
Results: Demographic characteristics, distribution of risk groups and survival rates for children with ALL receiving ALL BFM 95 protocol are shown in Table 1. The standard risk group showed excellent results with a rate of 100% for both EFS and OS. The rates of EFS and OS for B-cell ALL are around 90% when the SR and IR groups were taken together whereas they were 55.88% and 58.82 for HR group. The event free survival (EFS) and overall survival rate (OS) for 123 patients with B-cell phenotype were 82.25% and 83.1%, respectively. The rates of OS of T-cell IR and HR groups were 86.66% and 50%, respectively. Twenty-four patients with T-cell phenotype achieved 73.91% five-year OS and 69.56% EFS rate. Overall, five-year survival rate of 147 patients who received treatment in our center was 81% and event-free survival rate was 77% that is comparable to published results so far. Regarding the white blood cell count (WBC), the rates EFS and OS are higher in patients with low WBC (<20 000/μL). During high dose corticosteroid treatment, 8 patients showed hypertension and six patients showed hyperglycemia. Later on, patients who showed hyperglycemia diagnosed with diabetes mellitus and insulin treatment was initiated. One patient developed aseptic necrosis on femur two years after chemotherapy. PRES Syndrome was seen in two patients. Regarding the distribution of the patients included in the risk groups, we found that number of our patients in SR group were lower (19% *vs* 35%), equal in IR group (51% *vs* 53%), and higher in HR group (30% *vs* 12%). This result may be due to referred patients diagnosed with ALL to our center from other medical centers. We proudly observed that the rates for EFS and OS in our ALL-BFM 95 SR group were 100%. This excellent outcome could be explained by successful planning of the current chemotherapy protocol by BFM group and implementation of appropriate supportive treatments. Additionally, our treatment results of other risk groups suggest that our success rates are comparable to the results of centers using ALL-BFM 95 protocol in European countries and our country.
Summary and Conclusions: We are pleased to know that ALL-BFM 95 protocol improved survival in childhood ALL. However, the development of new strategies is still necessary in order to prevent relapses and to improve survival.

PB1600

NO ADVANTAGE OF ALLOGENEIC STEM CELL TRANSPLANTATION OVER MAINTENANCE CHEMOTHERAPY AS POST-REMISSION THERAPY IN ADULT PATIENTS WITH B-ACUTE LYMPHOBLASTIC LEUKEMIA IN OUR CENTRE

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Background: The optimal post-remission therapy for adult acute lymphoblastic leukemia (ALL) patients is not well established. Although allogeneic stem cell transplantation (allo-SCT) has been used in adult ALL for more than 20 years, its role remains controversial as demonstrated by conflicting results in many studies.
Aims: Compare allo-SCT vs maintenance chemotherapy as post-remission therapy in adult B-ALL patients.
Methods: We retrospectively analyzed 64 patients with *de novo* B-ALL in a single centre from 2006 to 2014.
Results: The median age at diagnosis was 47 years [17-70], 25(39.1%) patients with ≤40 years. The median white blood count (WBC) was $9x10^9$/L [1.0-254] and 17(26.6%) patients had hyperleucocytosis (>$30x10^9$/L). Forty (62.5%) cases corresponded to common-B ALL, 20 (31.3%) to a pre-B ALL and 4 (6.2%) were pro-B ALL. Thirty-three (51.6%) patients had an unfavorable karyotype: t(9,22) in 31 (48.4%) patients, t(4,11) in 1 (1.6%) and hypodiploidy in 1 (1.6%) patient. Thirty-four (53.1%) patients received remission induction therapy with the HOVON 100 ALL/EORTC 06083 protocol (nonexperimental arm), 24 (37.5%) with the Hyper-CVAD protocol and 6 (9.4%) with CLG/EORTC 58951 protocol. In cases of Philadelphia chromosome–positive (Ph+) ALL, all patients received tyrosine kinase inhibitors (TKI) (in addition to multiagent chemotherapy). Sixty-two (96.9%) patients achieved complete remission (CR) after either one (90.6%, n=58) or two (6.3%, n=4) courses of induction therapy. Four patients (6.25%) died during consolidation treatment (2 related to toxicity of chemotherapy and 2 due to early relapse). Among 58 (90.6%) patients who completed consolidation therapy, 30 (51.7%, n=30/58) received allo-SCT (matched related donor, n= 23 and unrelated donor, n=7) and 28 (48.3%, n=28/58) were treated with maintenance chemotherapy. In patients receiving allo-SCT, 12 (40%, n=12/30) had ≤40 years and 19 (63.3%, n=19/30) were Ph+ ALL. The mortality rate in patients submitted to allo-SCT was 56.7% (n=17/30) and the majority (52.9%, n=9/17) of patients died in CR. In patients treated with maintenance chemotherapy, the mortality rate was 42.9% (n=12/28) and the main (75% n=9/12) cause of death was disease relapse.
The median follow-up was 27 months [1-108]. The disease-free survival (DFS) and overall survival (OS) rates at 3 years of the all cohort were 41.1% (+/-6.7%) and 48.4% (+/-6.7%), respectively. Comparing Hyper-CVAD protocol with HOVON 100 ALL/EORTC 06083 protocol, no differences statistically significant were found for RC vs NR (P=0.270), DFS (P=0.741) or OS (P=0.411). According to allo-SCT vs maintenance chemotherapy comparisons, no significant differences were observed for DFS (P=0.167) and OS (P=0.259) even including only Ph+ ALL patients in the analysis (DFS for Ph+ ALL allo-SCT vs Ph+ ALL chemotherapy-P=0.539; OS for Ph+ ALL allo-SCT vs Ph+ ALL chemotherapy-P=0.857). Additionally, when patients with ≤40 years were analyzed as a separate group, we failed to observe any statistical difference between the two post-remission therapies in terms of DFS (P=0.575) and OS (P=0.780).
Summary and Conclusions: Our study does not demonstrate any advantage for allo-SCT compared with maintenance chemotherapy as post-remission

therapy in adult B-ALL even in those patients with Ph+ or ≤40 years. In our analysis the non-relapse treatment-related mortality in patients submitted to allo-SCT was very significant. Although our series was small, it represents the results of daily clinical practice, where graft-*versus*-host-disease and serious infections are major challenges.

PB1601

A PEDIATRIC TREATMENT REGIMEN FOR PHILADELPHIA NEGATIVE ACUTE LYMPHOBLASTIC LEUKEMIA IN ADOLESCENTS AND YOUNG ADULTS IS FEASIBLE IN THE REAL-LIFE SETTING – SAFETY ANALYSIS IN A SINGLE-CENTER SERIES

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Background: There is considerable evidence that pediatric (PED), high intensity chemotherapy protocols may improve clinical outcome in adolescents and young adults (AYA) with Acute Lymphoblastic Leukemia (ALL). Toxicity, which is more frequent and severe in older patients (pts), is a key issue, limiting the potential benefits and becoming more evident outside clinical trials.
Aims: We evaluated the feasibility and safety of a PED regimen – the Dana-Farber Cancer Institute Consortium Protocol (DFCI) 05-01-in Ph- AYA ALL pts receiving treatment (Tx) in an adult Hematology reference center.
Methods: We conducted a retrospective analysis of all adverse events (AE) occurring during the DFCI 05-01 regimen in a series of consecutive AYA pts treated at our institution, with a focus on Grade 3-5 CTCAE toxicities and special interest side effects with at least a possible relationship to Tx.

Table 1.

Characteristics	Total N (%)	With Thrombotic event N (%)	Without thrombotic event N (%)	P
Patients	31 (100)	10 (32)	21 (68)	
Male/Female	12/19	4/6	8/13	NS
Age in years: median (range)	34 (17-56)	34 (25-47)	32 (17-56)	NS
Largest mass diameter in mm: median (range)	98 (50-150)	78 (50-150)	106 (58-150)	NS
Extranodal involvement	8 (27)	2 (20)	6 (28.5)	NS
SVCS	16 (52)	5 (50)	11 (52)	NS
Complete response after first line treatment	23 (74)	7 (70)	16 (76)	NS
4-year Overall survival: % (IC95%)	80 (64 - 96)	83 (53-100)	78 (59-97)	NS
4- year Progression-Free Survival: % (IC 95%)	71 (54 - 88)	85 (59 - 100)	64 (43 - 85)	NS

NS: No statistically significant.

Results: 22 Ph- ALL pts (median age 18 yo, range 15-42, 68.2% males), diagnosed between 2007 and 2014, were treated according to the DFCI 05-01 protocol. 54.6% were<20 yo, 13.6% 20-29 yo, and 31.8% were 30-42 yo. B-ALL was diagnosed in 11 (50%), T-ALL in 8 (36.4%), and T Lymphoblastic Lymphoma in 3 (13.6%). Only 1 case had detectable blast cells in diagnostic spinal tap (<5/ul). At the end of induction (IND) 20 (91%) pts achieved CR, 2 had refractory disease, being excluded from the analysis after this point, and 4 B-ALL cases had high MRD levels (≥0.001) assessed by RQ-PCR. Based on cytogenetics and molecular response at day 32, 7 (31.8%) pts moved to the very high-risk group and received intensified Tx according to protocol. 2 relapses occurred before maintenance and 2 pts died of refractory disease shortly after starting salvage regimens. 2 Tx-related deaths were observed. With a median follow up of 37 months, 16 pts (72.7%) are alive in CR1: 5 completed the protocol, 8 had an allogeneic stem-cell transplant after Consolidation II, and 3 are still under Tx. Table1 depicts the grade 3-5 toxicities and asparaginase (ASP) specific related toxicities observed during a median of 11 months of Tx per pt. No deaths were observed during IND and infectious complications were the most frequent AE: 9 pts (40.9%) developed febrile neutropenia with 7 confirmed bacteremias. Overall, infections were the most relevant reported AE during Tx, with 2 grade 5 events. 2 episodes of reversible AKD (not requiring renal-replacement therapy) during high dose MTX Tx were seen. 10/14 evaluable pts (71.4%) who initiated the 30-week ASP course were able to receive 26 or more IM *E. coli* ASP equivalent doses, with one of the ASP formulations – native *E. coli* ASP, PEG-L-ASP, or *Erwinia* L-ASP. 4 musculoskeletal AEs were observed, including 3 cases of avascular necrosis, and 1 bone fracture, possibly related to corticosteroid therapy.
Summary and Conclusions: Increased toxicity in AYA is the limiting factor for the use of intensified therapeutic regimens in ALL. We did not observe unexpected grade 3-4 AE, nor an unacceptable high incidence of these events, compared to literature. Tx-related mortality was also equivalent (9%). In this series ASP tolerance was acceptable: the incidence of major ASP-related toxicities was similar to older children, with a proportion of pts completing at least 26 weeks of high-dose ASP only slightly lower than described in PED populations (71.4% VS 88%). The application of tolerable, PED protocols may improve the outcome of adult ALL.

PB1602

THE EFFECTS OF THIOPURINE METHYLTRANSFERASE POLYMORPHYSMS (TPMT*2, TPMT*3B, TPMT*3C, TPMT*3A) AND ITS CYTOPLASMIC ENZYME LEVELS ON MYELOSUPPRESSION IN CHILDREN WITH ALL TREATED WITH 6-MERCAPTOPURINE.

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Background: Thiopurine Methyltransferase (TPMT) prevents the toxic effects of thiopurine drugs such as 6-mercaptopurine and neutralizes its side effects such as myelosuppression. This enzyme methylates toxic metabolites and leads to their inactivation.
Aims: The aim of this study was to evaluate *TPMT* gene polymorphisms (*TPMT*2, TPMT*3B, TPMT*3C, TPMT*3A)* and detection its cytoplasmic enzyme levels and myelosuppression and hepatotoxicity effects *in* children with acute lymphoblastic leukemia (ALL) treated with 6-MP.
Methods: 98 patients with standard risk paediatric ALL in maintenance phase were selected. PCR-RFLP method was performed to detect mutations in exon7 (460G>A; *TPMT*3B*), and in exon 10 (719A>G; *TPMT*3C*). *TPMT*2* (238G>C) allele, in exon 5 was analyzed by allele-specific PCR method. Exons 7 and 10 in 19 patients and exone 5 of 2 patients were also sequenced to confirm the results obtained by PCR-RFLP and AS-PCR methods. The enzyme level of 70/98 patients was assessed by ELISA. Then Correlation between genotype, phenotype and side effects such as myelosuppression and hepatotoxicity was analyzed by statistical methods.
Results: Frequency of *TPMT*1*3B* genotype was 1.1% and the frequency of *TPMT*3B* allele variant was 0.71%. No allele variant was detected in *TPMT*2* and *TPMT*3C,*3A, *4, *7, *8, *10* (4 allele variants *(TPMT *4, *7, *8, *10*) were analyzed by sequencing). The concentration of enzyme was low in 8 (11.4%) individuals. There was no meaningful association between phenotype and genotype of TPMT enzyme. A significant correlation was found between low concentration of TPMT enzyme and myelossuppresion *(P=0.04*, leucopenia*, P=0.02* neutropenia *)*. There was no statistical difference between low and normal concentration of TPMT enzyme and hepatotoxicity *(P=0.12)*.
Summary and Conclusions: The results of this study showed that there is a relationship between low enzyme concentration and myelosuppression in some patients who were treated with 6-MP. Therefore, to avoid some side effects of 6-MP such as myelosuppression, studying common and rare *TPMT* gene variant as well as measurement of TPMT enzyme level is recommended.

PB1603

MULTICOLOR FLOW-CITOMETRY IS A CHEAP AND RELIABLE PREDICTOR OF RELAPSE RISK IN ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS, RETROSPECTIVE DATA FROM REAL LIFE EXPERIENCE.

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Background: Many studies have shown the prognostic value of minimal residual disease (MRD) evaluation in Acute Lymphoblastic Leukemia (ALL). Nowadays, most of the modern therapeutic protocols include risk-oriented and MRD-driven consolidation program. MRD is usually evaluated by multicolor-flow cytometry (MFC) and RQ-PCR for VDJ rearrangements. The latter method is significantly more sensible but is quite expensive and complex, and usually requires a great amount of time. MFC is cheaper and quicker, and it is currently applied in most induction protocols, where is performed at fixed timepoints. However, few data are available on MFC MRD value outside clinical trials, where often response assessment timing greatly varies between different induction schedules and different patients.
Aims: The aim of the present study is to evaluate if MFC-MRD maintains prognostic significance in the real life therapeutic experience, outside clinical trials and with different induction schedules.
Methods: We retrospectively analyzed outcome of 132 consecutive ALL patients, treated in our centre in the last 10 years. Median age was 44.5 years (range 15-82 years). Induction regimens included Hyper-CVAD, standard three or four drug induction, with or without L-Asparaginase, and TKI with or without chemotherapy for Philadelphia positive ALL. Relapse-free survival (RFS) was calculated from the time of diagnosis until last follow-up or documented leukemic relapse. Patients were censored upon allogeneic transplantation.

MFC MRD levels were evaluated on bone marrow samples after first induction cycle, at a median of 36 days after diagnosis (range 28-52). A positive MFC MRD was defined by the presence of no less than 25 clustered leukemic cells/10^5 total events (threshold of $2.5x10^{-4}$ residual leukemic cells) at four-color flow-cytometry.
Results: Sixteen patients (12,1%) died during induction, mainly because of infectious events. Of remaining 116 patients, 101 (84%) achieved complete remission (CR). None of the analyzed variables significantly influenced CR probability. After a median follow-up of 58 months, 63 relapses were observed. The probability of disease relapse was significantly increased by leukocytosis at diagnosis, omission of L-Asparaginase and MFC MRD positivity after first cycle (P=0.003, P=0.047, P=0.048, respectively, Fig.1). Specifically, relapse rate in MFC MRD negative patients were very low (5/25 MFC-MRD negative patients, 25%), when compared to the whole cohort. MFC value resulted independent from ALL lineage, induction schedule, sex and age. High dose Methotrexate administration conferred a borderline advantage on RFS (P=0.066). Multivariate RFS analysis disclosed low WBC count and inclusion of L-Asparaginase as the strongest predictor of longer RFS, however without reaching statistical significance (P=0.061).

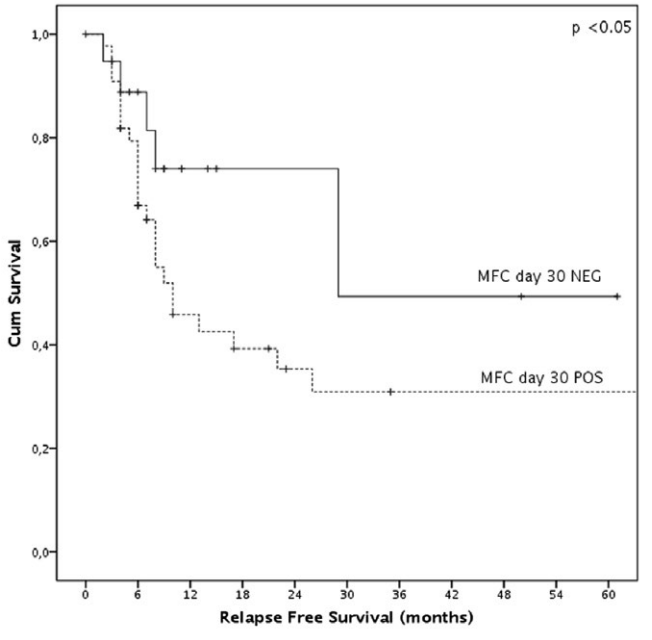

Figure 1. RFS according to MFC MRD.

Summary and Conclusions: Risk adapted consolidation and transplant timing has recently shown high efficacy in many MRD-driven clinical trial. Our data confirm that evaluation of MRD by MFC on bone marrow samples is a cheap, relatively simple and a reliable predictor of relapse risk, even outside clinical trials. Basing on those findings, MFC MRD evaluation could be a valid surrogate of RQ-PCR for VDJ rearrangement, with potential clinical utility especially in those setting where access to expensive molecular techniques is difficult, allowing to apply risk adapted consolidation even if molecular MRD methodic are not available.

PB1604

PREGNANCY IS NOT AN EXCLUSION CRITERIA FOR THE TREATMENT ACCORDING TO THE ADULTS ACUTE LYMPHOBLASTIC LEUKEMIA PROTOCOL ALL-2009: RESULTS OF THE RUSSIAN ALL STUDY GROUP (RALL)

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Background: Acute lymphoblastic leukemia ALL accounts for about a third of acute leukemias diagnosed during pregnancy. Pregnancy is a common exclusion criteria for any clinical trial. RALL study group decided to include the pregnant women with ALL in the multicenter clinical trial ALL-2009 (ClinicalTrials.gov public site; NCT01193933). The protocol is based on non-intensive but non-interruptive treatment.
Aims: To evaluate efficacy and toxicity the ALL-2009 protocol for pregnant women with ALL.
Methods: From Jan 2009, till Dec 2014, 30 centers enrolled 263 Ph-negative ALL pts, among whom there were 13 pregnant women (3-in 1st; 3- in the 2nd and 7-in 3rd trimester of pregnancy) from 3 hematological centers. The median age was 28 (18-32) years. B-cell ALL was diagnosed in 6 (46%) and T-cell ALL-7 (54%) patients. 10 patients (77%) were attributed to a high-risk, 3 (23%)-to a standard risk group. The treatment according the protocol ALL-2009 was carried out without deviations except the shift of L-asparaginase for later phases of treatment (after delivery) and applying intrathecal injections without methotrexate for those in whom chemotherapy was started during pregnancy. Medical abortion was performed in 3 patients at the 1st trimester (10-11 weeks of gestation) during the prephase (7 days with prednisolone 60 mg/m^2). 3 patients at 36-40 weeks of gestation were delivered before treatment and prephase was initiated after 2-3 days after delivery. All others (n=7) were started with chemotherapy during pregnancy (at 16, 19, 25, 28, 29, 31 and 34 weeks of gestation).
Results: All pregnant women were refractory to prednisolone defined by more than 25% of bone marrow blasts after 7 days of prephase. So dexamethasone was used for all further treatment. One patient is on the induction now, so CR rate was evaluated in 12. CR was achieved in 10 of 12 (83,3%), in 7 after the 1st phase and in 3 – after the 2nd induction phase. 2 patients had refractory ALL and died from the disease. There were no differences in the frequency of infections, duration of neutropenia, transfusion support during induction in comparison with common ALL pts. In 3 pts of those who were treated during pregnancy delivery was carried out after the 1st induction phase, in 2 – during the 2nd phase, in 1 – after the 2nd phase. Gestational age at delivery was 34 weeks (33-39 weeks). At time of delivery 4 pts were and 2 were not in CR. One pregnant woman is still on the induction treatment. The median interval between the last administration of cytostatic drugs and delivery was 6 days (1-20 days). The median time from delivery to continuation of chemotherapy was 16 days (11-44 days). Chemotherapy duration during pregnancy constituted 5-9 weeks. Totally 9 children were born at a median gestation time – 36 weeks (33-40). All are alive at a median 9 months (8-60 mo) and healthy. Allo-HSCT was performed in 2 pts, auto-HSCT-2. There were 2 relapses and 1 death in CR. The OS and DFS constituted 62% and 53% at 3 years, and these results did not differ from the other pts treated according ALL-2009 protocol (60% and 57%, respectively).
Summary and Conclusions: Our data demonstrate that ALL in pregnant women is characterized by higher frequency of T-cell phenotype and high risk disease, most cases occurred in the 2nd and 3rd trimester, CR rate is 83%. All born children are well. Long-term survival does not differ from that of the other patients on this protocol. So pregnancy should not be considered as an exclusion criteria for our protocol that is non-intensive, non-interruptive and easily reproducible.

PB1605

FOURTEEN YEARS EXPERIENCE WITH THE EORTC 58951 PROTOCOL FOR THE TREATMENT OF CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA IN THE SOUTH OF TUNISIA

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Background: Childhood acute lymphoblastic leukemia (ALL) is an hematologic malignancies with high rate of cure. We report the experience of the department of clinical hematology of Sfax for the treatment of childhood ALL with the EORTC 58951 protocol.
Aims: we report the results of fourteen years treatment of childhood acute lymphoblastic leukemia with the EORTC 58951 Protocol
Methods: From January 2000 to December 2013, we retrospectively studied the outcome of all childhood ALL treated with the EORTC 58951 pediatric protocol. For those patients we studied the leukemia characteristics (sex ratio, white blood cell counts WBC, blast's phenotype, cytogenetic abnormalities) and response to treatment: response to prophase, remission rate, risk group stratification, treatment related mortality (TRM) (induction and post induction death) and survival (overall survival OS, event free survival EFS and disease free survival DFS: calculated for patients in complete remission). We complete the analysis by a comparison between two periods P1 and P2 (P1: 2000-2006 and P2: 2007-2013).
Results: From January 2000 to December 2013, 160 children were treated with the EORTC 58951 protocol. Median age was 6 years (range: 13 months to 15 years). Sex ratio M/F was 1.4. WBC counts less than 10 G/l, from 10 to 100 G/L and more than 100 G/L were observed respectively in 42, 42 and 16% of cases. The blast's phenotype was B in 70% and T in 30%. Cytogenetic abnormalities were noted in 48% of cases. A good response to prophase was noted in 84% and complete remission in 97% of cases. The EORTC risk group stratification was Low Risk (LR) in 6.5%, Average Risk1 (AR1) in 45%, Average Risk2 (AR2) in 25.5% and Very High Risk (VHR) in 23%. TRM was 9%: induction rate death was 7% and post-induction rate death was 2%. Seven patients from VHR group with familial donor underwent allograft. The relapse rate was 23% of all patients in remission; 10% for LR group, 22% for AR1 group, 17% for AR2 group and 37.5% for VHR group. At 5 years of follow up, OS, EFS and DFS were 67, 64 and 74% respectively. We compared two periods, we noted less TRM and less relapse in P2 with respectively (2% vs 14%) and (19% vs

28%), so we have a good survival at 5 years in P2 (OS: 75% vs 58%, EFS: 73% vs 55% DFS: 79% vs 68%).
Summary and Conclusions: Childhood ALL in our institut were characterized by a poor presentation at diagnosis: male sex, leucocytosis more than 100 G/L, T phenotype and bad response to prophase are frequent compared to the occident reports. EFS are acceptable in our study but still less than observed in literature (85-90%). We have noted an improvement in the survival rates during the second period P2 this could be explained by improved support treatment

PB1606

WHAT IF YOU FAIL? IMPACT OF UNSUCCESSFUL CYTOGENETIC ANALYSIS ON TREATMENT OUTCOMES IN ACUTE LYMPHOBLASTIC LEUKEMIA (ALL): A TERTIARY CARE SINGLE CENTRE ANALYSIS FROM INDIA

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Background: Cytogenetic findings are important in predicting prognosis; and are an obligatory tool in stratifying patients for treatment assignment in ALL. The large studies in ALL have reported between 10-30% of cytogenetic failure. The impact of cytogenetic failure on treatment outcomes in this group with ALL has not been sufficiently explored.
Aims: To determine the differences in clinical features and treatment outcomes in patients with ALL based on the success of karyotype analyses.
Methods: We undertook a retrospective study to evaluate the impact of cytogenetic failure on treatment outcomes in patients with a diagnosis of ALL (Burkitt-excluded) treated at our tertiary care center from January 2009 till December 2014. Cytogenetic failure was used to define analyses that could not be performed in the laboratory due to no mitoses or non-informative morphology. Risk stratification and treatment was based on the BFM95 protocol for patients' ≤15 years and GMALL in the older patients. Standard of care diagnostic tests and supportive care was administered to all patients. The cytogenetic data was retrieved from the original reports of the laboratory performing the analyses at our centre. Only cytogenetic studies sent to the laboratory were included for analyses. Clinical details were ascertained through patient admission records and discharge cards. The collected data was analyzed using SPSS.

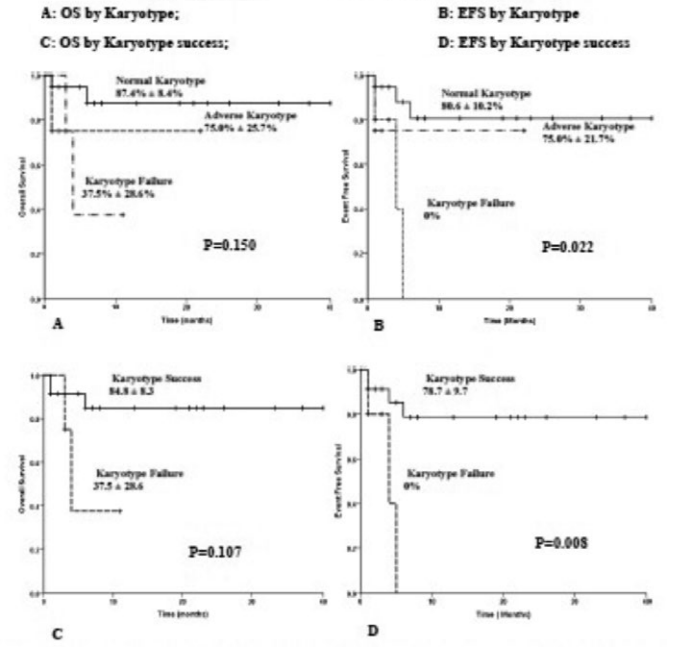

Figure 1.

Table 1. Socio-demographic characters, clinical features and laboratory parameters in newly diagnosed patients (N=54).

Variable	Cytogenetic Success (N=26) n (%)/Median (Range)/Mean ±SD	Cytogenetic failure (N=7) n (%)/Median (Range)/Mean ±SD	P value
Age (years)	16 (2-65)	17(11-30)	0.604
Sex (male)	16 (61.5)	3 (42.5)	0.203
ALL type (B-ALL)	20 (76.9)	3 (42.9)	0.092
Philadelphia (positive)	5 (20.8)	0 (0)	0.252
Steroid response (day8)	17 (89.5)	2 (40.0)	0.044
Post Induction Marrow (remission)	16 (80)	2 (50)	0.232
Infection in induction	7 (26.9)	1(14.3)	0.469
Treatment duration (Induction)	28 (27-41)	29 (28-35)	0.278
Haemoglobin (g/L)	82.0(24.0-137.0)	73.0 52.0-153.0)	0.706
WBC count (x10 ^{9}/ L)	68.2 (0.6-360.0)	33.2 (0.2-124.0)	0.327
Platelet count (x10 ^{9}/ L)	43(5.4-344.0)	84 (3.0-307.0)	0.589
Absolute Blast count(x10 ^{9}/ L)	24.1(0.12-229.4)	26.5(0-111.6)	0.912

Results: A total of 76 admitted patients were diagnosed with ALL. Of these 54 (71.1%) continued with treatment. Bone marrow was sent for cytogenetic analyses in 33 of these patients. There were no significant differences observed in terms of socio-demographic and baseline laboratory parameters among the two groups based on the success of cytogenetic analyses (table1).The Event Free Survival (EFS) and Overall Survival (OS) were lesser in patients with cytogenetic failure than in those who had a successful karyotype analyses (Figure 1). With a mean follow up of 32 months, the EFS at one year in those with cytogenetic failure was significantly lower than those with a successful karyotype analyses (P=0.008). When compared against the outcomes of patients with adverse karyotype (t (9; 22) or >3 abnormalities), those with cytogenetic failure had a significantly lower EFS (P=0.022).
Summary and Conclusions: Risk stratification in ALL has not defined the optimal strategy in patients with cytogenetic failure. Unsuccessful cytogenetics likely predicts a poor outcome. This needs to be further explored by facilitating the inclusion of these patients as a group in clinical trials.

PB1607

THE FREE RADICAL OXIDATION ROLE IN THE DEVELOPMENT OF ANTHRACYCLINE-INDUCED CARDIOTOXICITY IN PATIENTS WITH ACUTE LEUKEMIA IN THE PRESENCE OF CONCOMITANT ISCHEMIC HEART DISEASE

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Background: Anthracycline antibiotics (AA) are included into the most modern acute leukemia (AL) treatment regimens. The AA assignment in polychemotherapy (PCT) programs contributes to the clinical-hematological remission percentage growth, and the improvement of pts survival. However, the formation of anthracycline-induced cardiotoxic effects can be significant limiting factor of PCT full dose conducting, which certainly leads to reduced effectiveness of anticancer therapy. In this aspect particularly important becomes the assessment of the heart tissue injuries potential risks. The incidence of cardiotoxicity depends on the cumulative dose (CD) of AA. The generally toxic AA CD is 550 mg/m^2 for doxorubicin. An additional risk factor for anthracycline cardiotoxicity is considered to be the concomitant ischemic heart disease (IHD). The imbalance between generation and inactivation mechanisms of aggressive free radicals as a risk factor of heart tissue AA-induced injury in patients with AL with concomitant ischemic heart disease remain insufficiently studied.
Aims: To assess the prooxidant-antioxidant imbalance status in patients with AL in the dynamics of AA treatment taking into account concomitant IHD.
Methods: The study involved 41 patients with acute leukemia (acute lymphoblastic leukemia-13 pts, acute myeloid leukemia-28 pts), aged 16–72 years, 23 (56%) men and 18 (44%) women, which PCT included AA. Patients were divided into two groups according to the presence of concomitant IHD: I (n=24) – without concomitant IHD; II (n=17) – with the concomitant IHD. The general condition assessment of pts of the both groups was performed twice: before specific therapy and after reaching AA cumulative dose from 100 to 200 mg/m^2. The POL processes activity was determined by the malondialdehyde (MDA) level, the antioxidant protection (AOP) – the serum catalase concentration.
Results: Before treatment in patients of group I without concomitant IHD the MDA concentration in serum exceeded the upper limit of normal in 1.2 times, the catalase level – in 1.1 times. In patients of group II with concomitant IHD the MDA level was increased in 1.46 times with the simultaneous tendency to reduce the catalase concentration in serum compared to normal, indicating that the exhaustion of antioxidant protection on the IHD background. Upon reaching the AA CD of 100-200 mg/m^2 MDA concentration in serum was in 1.54 times higher in pts of group II compared with pts of group I (4.81±0.38 mmol/l vs 3.12±0.28 mmol/l; P<0.05). Simultaneously, with the presence of concomitant IHD in pts of group II the catalase level in serum was in 2.1 times lower in comparison with pts of group I (72.5±8.7 mkkat/l vs 33.8±3.2 mkkat/l; P<0.05).
Summary and Conclusions: Therefore, concomitant ischemic heart disease in patients with AL during the treatment AA is an additional risk factor for cardiotoxicity, due to exhaustion antioxidant protection system and deepening imbalance between the formation and inactivation of free radicals.

PB1608

OUTCOME OF ADOLESCENTS AND YOUNG ADULTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA TREATED WITH PEDIATRIC PROTOCOL: 62 CASES

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Background: Several retrospective studies have confirmed that adolescents and young adults (AyA) with acute lymphoblastic leukemia (ALL) treated with pediatric protocols have better outcomes than similarly aged patients treated with adult protocols. We reported results and feasibility of a pediatric-based protocol (EORTC 58951) in adolescents and young adults.

Aims: Pediatrics protocols improve the outcome of adiolescents and adults acute lymphoblastic leukemia
Methods: From January 2000 to December 2013, 62 patients aged 16 to 30 years with newly diagnosed ALL were treated, in the department of clinical hematology of Hedi Chaker Hospital, according to the pediatric protocol EORTC 58951. Further leukemia characteristics (Sex, White Blood cell count, Blasts phenotype, Cytogenetic results), we studied the protocol results: response to prophase, risk group stratification (average: AR1 and AR2, very high: VHR), treatment related mortality (TRM), remission rate, relapse rate and 5 years survivals (overall OS, event free EFS and relapse free survival RFS).
Results: Sixty two AyA ALL were treated with the pediatric protocol. The patients were 38 males and 24 females (SR=1.58). 34% had a WBC>100 G/l. A T blast phenotype was noted in 53% of cases. Nineteen patients (30.5%) had poor response to pophase. Fifty two patients (86%) received AR2 (52%) or VHR (34%) arm induction. Fifty seven patients (97%) achieved CR. Two patients failed to achieve complete respond after 2 courses of chemotherapy. Induction death was noted in 8%. Consolidation death was noted in 17%. Relapse was observed in 25%. Five years OS, EFS and RFS were respectively 48, 48.5 and 65%.
Summary and Conclusions: This study showed that pediatric protocol can offer good results concerning CR and DFS to adolescent and young adult ALL. However OS and EFS, sure better than adult ALL treated during the same period by adult protocol (OS= 14%, EFS=14% and DFS= 40%) was not satisfactory because the high toxic mortality rate.

PB1609

HIGH RISK CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA GROUP TREATED WITH THE EORTC58951 PROTOCOL: RESULTS AND OUTCOME

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Background: More than 80% of children with acute lymphoblastic leukemia (ALL) can be cured, but subsets of patients have significantly worse outcomes. In this study we analyzed the clinical, biological features and therapeutic results of patients treated with high risk group of the EORTC protocol.
Aims: Report the outcome of high risk childhood acute lymphoblastic leukemia group
Methods: From January 2000 to December 2013, 160 patients with ALL aged less than 16 years were treated by the EORTC protocol in the Hematologic department of Hedi Chaker Hospital, from those 37 patients were treated by high risk group (VHR). The VHR group includes patients with poor prednisone response at day 8 (more than 1000 blasts/mm^3 on the blood smear), those with defavorable cytogenetic abnormalities (t (9,22), t (4,11)...) and those with complete remission after two courses of chemotherapy. From those patients we analyzed the clinical and biological features (Age, sex, blood count, cytogenetic abnormalities and blasts phenotypes) and therapeutic results (remission rate, rate relapse, overall survival (OS), event free survival (EFS) and disease free survival (DFS) at 5 years follow up).
Results: In our study, thirty seven children were treated with VHR group of the EORTC protocol (23%). The median age of patients was 9 years (ranged 2-15 years). Sex ratio H/F=1,64. The median WBC count at diagnosis was 55.8 G/L (ranged from 2 to 400 G/L). There were 51% with B-cell ALL and 49% with T-cell ALL. Cortico-resistance was the only high risk factor in 23 patients, absence the remission after induction in 8 patients and only cytogenetic abnormalities in 4 patients. The cortico-resistance at day 8 was observed in 65% of patients. Five children (13.5%) were treatment failure after two courses of chemotherapy. The complete remission was obtained in 32 children (86%), among them 12 relapsed (37.5%). The post induction death was noted in 2 patients. Only 7 patients (16%) underwent allograft from familial donor. At 5 years follow-up, the OS, EFS and DFS were respectively 46%, 43% and 54%.
Summary and Conclusions: The frequency of high risk group in our study is more than those observed in the literature (12 to 16%). The poor 5 years survivals (OS=46%, EFS=43%) are related to the high rate of relapse. Improvement of outcome can be obtained by enlargement of donor source: unrelated and cord blood.

PB1610

CLINICAL CHARACTERISTICS AND CYTOGENETIC ABNORMALITIES OF CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS: SINGLE INSTITUTE EXPERIENCE FROM SAUDI ARABIA

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Background: Childhood Acute Lymphoblastic Leukemia are generally characterized by recurrent molecular and cytogenetic abnormalities; the identification of those abnormalities is clinically important because they are considered significant risk-stratifying markers.
Aims: Currently, no sufficient data exist regarding cytogenetic abnormalities and treatment outcome in Saudi pediatric ALL patients. Ninety-two cases of childhood ALL were examined to determine cytogenetic profile, and did correlations to other biologic factors.
Methods: Patients. From 2004 to 2014, we reviewed all cases with established diagnosis of childhood ALL. Of the 92 patients, 82 were B-lineage ALL, and 10 T-cell ALL. All patients were treated by UKALL 2003 protocol and risk stratified according previously published criteria. Cytogenetic Analysis. Chromosome banding analysis and fluorescence *in situ* hybridization (FISH) were used to detect genetic aberrations. Analysis of *FLT3* mutations. Bone marrow or blood samples were screened for *FLT3* mutations (internal tandem duplications, ITDs and point mutations, D835) using polymerase chain reaction methods (PCR).
Results: Figure 1 and Table 1. Summarize patient's characteristics and patient's outcome. Cytogenetic analysis showed chromosomal anomalies in 59 out of 92 cases with overall incidence 64.1%.The most frequent chromosomal anomalies in ALL were trisomy 21, t(9;22), and t(12;21). Our data are in accordance with those published showed that *FLT3* mutations not common in ALL patients (4.7%) and have no prognostic relevance in pediatric ALL patients while t(9;22) and MLL gene rearrangements were signs of a bad prognosis in childhood ALL, with high rate of relapse, shorter overall survival; (P=0.031) and event-free survival (EFS) was also worse(P=0.040**)** compared to standard risk group.

Tabella 1. Cytogentic abnormalities detected in our study.

Available for Karyotype	28 normal 11 abnormal
Available for Fish	n=83
t (9;22)	10 (12%)
t (12;21)	8 (9.6%)
MLL	6 (7.2%)
MYC	2 (2.4%)
t (1;19)	1 (1.2%)
+21	11 (13.3%)
Del 12p	5 (6%)
+6	1 (1.2%)
+9	3 (4.6%)
+8	1 (1.2%)
-19	1 (1.2%)
Hyperdiploid	6 (7.3%)
Pseudotriploidy	2 (3.1%)
Tetraploidy	1 (1.5

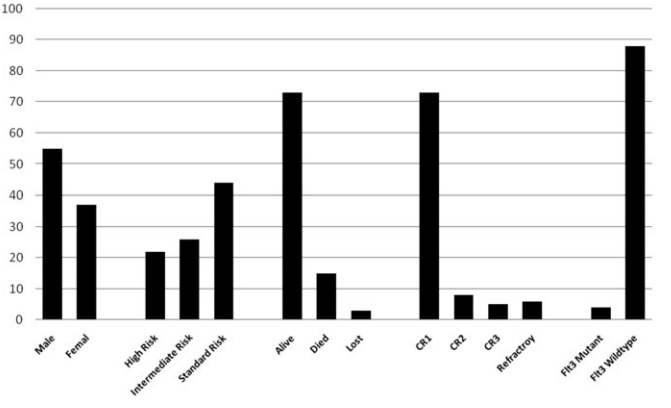

Figure 1. Patient characteristics and *FLT3* mutations.

Summary and Conclusions: Our data are in accordance with those published and confirm that the frequency of cytogenetic abnormalities and their prognostic relevant were comparable to those reported in the literature. FLT3 mutations not common among Saudi ALL and occur in low percentage and had no prognostic relevance as in AML and did not affect clinical outcome.

PB1611

IMPACT OF DURATION TO COMPLETE INDUCTION IN ACUTE LYMPHOBLASTIC LEUKEMIA: A SINGLE CENTER EXPERIENCE FROM INDIA

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Background: The improvement in event free survival in Acute Lymphoblastic leukemia(ALL) corresponds to advances in chemotherapy and supportive care. However despite these breakthroughs, the intensity of the chemotherapy predisposes patients to cytopenias and infections. These result in a break from the protocol and delay in induction completion. In a resource restricted setting where a major constraint to therapy remains the cost, it is important to analyze the factors to optimize treatment. The impact of duration to complete the initial intensive induction on survival needs analysis.
Aims: To evaluate the clinical characteristics and outcome of patients with a diagnosis of ALL (Burkitt- excluded) admitted at our tertiary care center.
Methods: We undertook a retrospective study on patients with ALL (Burkitt-excluded) admitted at our tertiary care center. Induction chemotherapy consisted of standard induction as in the BFM95 protocol for patients' ≤15 years and GMALL in the older patients. In addition to the chemotherapy; standard of care diagnostic tests and supportive care was administered to the patients. The data was retrieved from the patient admission records and discharge cards. The collected data was analyzed using SPSS.
Results: A total of 76 admitted patients were diagnosed with ALL during this period. Of these 54 (71.1%) continued with treatment (Fig 1A). The median age of newly diagnosed patients was 20 years (range: 1-65) and there were 53 (69.7%) males. In the treated patients, B-ALL was seen in 37(68.5%).The other baseline features are as depicted in Table1. The mean duration of induction chemotherapy was 30.4(± 5.3) days. A microbiologically documented infection was seen in 11(20.4%) and the induction was delayed more than the mean duration in 14 (37.8%). With a mean follow up of 15 months, the Event Free survival at one year in those with delay in completion of induction was 68.2%±15.4 and not statistically significant from those without a delay (Fig 1B). A similar conclusion was seen in the pediatric subgroup analysis (Data not shown).

Table 1. Descriptive baseline demogrphic characteristics in newly diagnosed patients with ALL.

Variable	PATIENTS (N=74)** n (%)/ Median (Range)/Mean (±SD)
Age (years)	20 (1-65)
Sex (Male)	53 (69.7)
Decision to continue treatment (Yes)	54 (71.1)
ALL subtype (B-ALL) n=54	37 (68.5)
Philadelphia positive (yes) n=37	6 (16.2)
Duration of induction	30.4 (± 5.3)
Haemogram N=43	
Haemoglobin (g/L)	79.6 (±32.5)
White blood cell count (x10 ^{9}/ L)	10.2 (0.2-360.0)
Platelet count (x10 ^{9}/ L)	38.0 (3.0-398.0)
Blasts in bone marrow (%)	82.2 (±19.6)
**(% derived from n)	

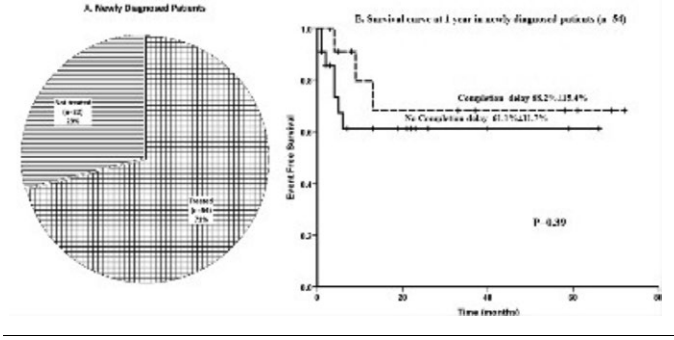

Figure 1. Newly diagno

Summary and Conclusions: In conclusion there are challenges in the management of ALL in India. One fourth of the patients decline treatment in view of financial constraints. In those who proceed with induction, there occurs a delay in one third. These delays do not appear to impact the event free survival in ALL. More analysis of prevalent practices will help tailor therapy in resource restricted settings.

PB1612

COST EFFECTIVE APPROACH IN FLOWCYTOMETRY BASED IMMUNOPHENOTYPE OF ACUTE LYMPHOBLASTIC LEUKEMIA. A TERTIARY CENTRE EXPERIENCE FROM AIIMS, NEW DELHI, INDIA

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Background: The diagnosis of acute lymphoblastic leukemia is based on morphology, cytochemistry, cytogenetics and immunophenotyping. In an under resource setting and sparsely insurance covered nation like India, the cost of investigation and treatment is one of the major barrier between disease and its curative management. With primary objective of establishing, the cost effective diagnostic panels without compromising the quality, we used primary screening antibodies panel comprising of major pan-lineage markers. This was followed by extensive secondary antibodies panel comprising of more lineage specific markers to reach a final diagnosis. In this study, we evaluated, the cost effective role of five-color primary screening antibodies to reach to the final conclusive diagnosis without compromising the quality of analysis.
Aims: Evaluation of the cost effective role of five-color primary screening antibodies panel in flowcytometry based immunophenotyping of Acute Lymphoblastic Leukemia.
Methods: A total of 234 newly diagnosed cases of Acute Leukemia from Jan 2013 to Jan 2015 were included in this study. All these cases were stratified to their respective lineages with the panel of primary screening antibodies comprising of cytoplasmic myeloperoxidase (MPO), cytoplasmic 79a (c79a), CD34, cytoplasmic CD3 (c3) and CD45.Once the lineage has been screened, then extensive secondary panel of antibodies with more specific marker of B-cell, T-cell and myeloid lineage were used to conclude for final diagnosis
Results: Of all the cases (234) evaluated 206(88%) were successfully identified on primary screening panel only, 28/234(17%) cases failed to show any specific lineage in primary screening panel. These 28 cases included 23/187(12%) B-ALL and 5/47(10%) cases of T-ALL
Summary and Conclusions: The five-color primary screening antibodies panel was very cost effective in identifying correct lineages in majority *i.e.* 88% of all cases. The absence of lineage specific marker was the most common reason for failure of the predictive power of the primary panel

PB1613

OVERALL-SURVIVAL, DISEASE-FREE SURVIVAL AND COMPLETE RESPONSE RATE IN ACUTE LYMPHOBLASTIC LEUKEMIA IN ADULTS IN COLOMBIA

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Background: The ASR incidence of acute lymphoblastic leukemia (ALL) in U.S. is 1.6 cases per 100,000, with 6,050 de novo cases per year and 1,440 deaths estimated during 2012. ALL accounts for 80% of all acute leukemias in children, with 3-years overall-survival (OS) around 80%. In adults, ALL is responsible of 20% of acute leukemias, with 3-year OS around 40%. In Colombia, the statistics are poor. Globocan and National Cancer Institute of Colombia estimated approximately 2,500 cases of leukemias in 2012, with an ASR incidence of 6.3 per 100,000 and an ASR mortality of 4.8 per 100,000. Taking into account these dates, approximately 73% of patients diagnosed with any kind of leukemia in Colombia die. Treatment response rates and OS data in Colombia are even scarcer; the study of the National Cancer Institute found a complete response (CR) rate of 64% and 3 years OS of 0%.
Aims: To determine the CR rate to treatment, disease-free survival (DFS) and (OS) in adults with ALL treated with different chemotherapy protocols in Hospital Militar Central (HMC) (Bogotá, Colombia) between 2006-2012.
Methods: A retrospective study was made on newly diagnosed ALL patients who came to HMC from January 2006 to December 2012. Descriptive statistics and cross-tabulation were used to describe patient characteristics. Mann-Whitney analyses were used to determine between-group differences. OS and DFS were analyzed with Kaplan- Meier method.
Results: Thirty-four patients older than 15 years were diagnosed with de novo ALL. Mean age was 28.5 years-old; 55.9% of cases had between 15 to 24 years-old. 23.5% of patients were older than 35 years- old, of which 25% had t(9;22). Mean leukocyte count was 38,490/uL (1500-367.000/uL). Anemia was present in 86.7% and 20.6% of patients had normal platelet count.
Twenty-six patients (76.5%) showed B-cell lineage, while T-cell lineage was found in 5 cases (14.7%). Ten patients (38.5%) with B-cell lineage had leukocyte count ≥ 30,000/µL (avg. 82,318/µL). All patients with T-cell lineage had leukocyte count less than 100,000/µL (avg. 17,536/µL). Karyotype was normal in 22 cases (64.7%). 80% of patients with abnormal karyotype had t(9;22), of which 66.7% had hyperleucocytosis. Twenty patients (58.8%) were at high risk for systemic relapse, (presence of one or more of the following: hyperleucocytosis ≥ 30,000/ul in B-cell phenotype, hyperleucocytosis ≥100.000/uL in T-cell phenotype, age ≥ 35 years, CD20 positivity >20% and t(9;22). The other molecular risk factors are not available in Colombia. The most frequent chemotherapy protocol used was GMALL (35%) followed by FRALLE 93 (29%). Two patients (5.9%) died during induction phase. Twenty-eight cases (82.3%) entered CR after induction therapy. Minimal residual disease was evaluated by flow cytometry in 17 patients (60.7%), being positive in 10 (58.8%) and negative in 7 (41.2%). From 28 patients who entered CR after induction therapy, 9 cases relapsed; 88.9% of relapses occurred during first 12 months. Mean time to relapse was 7.4 months. All patients who relapsed received second-line protocol

(R HyperCvad, HyperCvad, BFM Retz, IDA-FLAG and ALL R3) and only 3 responded (42.8%). OS and DFS at the first year was 92% and 84.8%, respectively. The OS and DFS to 3 years was 29.5% and 32.4%, respectively.
Summary and Conclusions: Comparing data previously obtained by National Cancer Institute between 2001-2005, this study shows improved CR rates and OS in ALL in Colombia during recent years. However these data are still less favorable than those obtained in studies from developed countries.

PB1614

SUPPRESSION OF ADRENAL AXIS FUNCTION AFTER HIGH-DOSE STEROID THERAPY FOR CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA IN IRAN

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Background: A 4 weeks course of high-dose glucocorticoids (GCs) may cause prolonged adrenal suppression even after a 9 days tapering phase.
Aims:.In this study, adrenal function and signs and symptoms of adrenal insufficiency were prospectively assessed in children with acute lymphoblastic leukemia (ALL) after induction treatment with high-dose prednisone.
Methods: In 42 children with newly diagnosed ALL, a baseline serum cortisol level was assessed and after receiving a 28 days of high dose prednisone according to the BFM 2009 protocol ad a 9 days tapering phase, serum cortisol level was assessed again and thosel was normal underwent low-dose adrenocorticotropic hormone (LDACTH) stimulation 24 h after the last tapered steroid dose. Signs and symptoms of adrenal insufficiency were recorded during the observation period. All patients except one who was excluded had normal basal cortisol values at diagnosis.
Results: Twenty-four hours after last GC dose, morning cortisol was reduced in 15 (36.5%) patients. LDACTH testing showed adrenal suppression in 17 (41.4%) patients.
Summary and Conclusions: High-dose GC therapy in ALL children may cause adrenal suppression even after a tapering phase. Laboratory monitoring of cortisol levels and steroid coverage during stress episodes may be indicated.

PB1615

EVALUATION OF BONE MINERAL DENSITY IN YOUNG ADULT ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) PATIENTS: A SINGLE CENTER EGYPTIAN EXPERIENCE

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Background: Bone health and the loss of bone density are important clinical concerns for patients with cancer who may be at risk for primary osteoporosis because of aging and other risk factors. They may have the added risk for cancer treatment-induced bone loss (CTIBL), which also could be termed secondary osteoporosis related to therapy and cancer as in acute lymphoblastic leukemia (ALL).
Aims: To assess bone mineral density in young adult patients with ALL at presentation and after induction therapy, to determine whether disease and/or chemotherapy can affect bone density
Methods: 25 Adult patients aged 20-45 years, with newly diagnosed ALL, presenting to Ain Shams University hospitals were recruited to this cross-sectional prospective study if they were eligible for induction therapy.All patients were evaluated according to published international guidelines for assessment of new acute leukemia patients. In addition, bone mineral density (BMD) was evaluated by using dual-energy X-ray absorptiometry (DXA) for all studied subjects at presentation and at D28 for evaluable patients. Measurements were performed at the lumbar spine (L2 to L4) and the left femoral neck using a Lunar DPX-L scanner. Bone mineral density was expressed in grams per square centimeter (g/cm2). Lumbar spine and femoral neck BMD was evaluated in all patients at diagnosis and after receiving induction chemotherapy. T-score was used to describe BMD (normal, osteopenia or osteoporosis) according to WHO classification.All patients except those who had mature B cell ALL received induction according to Holtzer protocol, while Patient with mature B-cell ALL received hyperCVAD chemotherapy (cycle A).
Results: 25 patients with newly diagnosis of ALL were recruited into this prospective study. Mean age was 30.32 (20-45 years), male: female ratio was 3:2. Presenting features include anemia (80%), bleeding tendency (52%), fever (52%) and hyperleucocytosis (32%). Extra medullary infiltration was detected in 3 patients, testis (n=1) and Central nervous system (n= 2).Sixteen patients (64%) had Pre-B ALL, one patient (4%) had Pro-B ALL, two patients (8%) had mature B ALL and six patients (24%) had T ALL. Clonal chromosomal abnormalities was detected in 4 patients (complex chromosomal abnormalities in one patient and t (9; 22) in 3 patients). 21 were evaluable at day +28. Seventeen patients (68%) were in complete remission and four patients (16%) had refractor leukemia.None of the patients had osteoporosis either in the pre or post treatment evaluation (T-score<-2.5). Seven patient (28%) fulfilled the WHO criteria for osteopenia in the lumbar spine at diagnosis (T- score -1 to -2.5). At post-treatment evaluation, ten patients (40%) were found to have osteopenia as assessed at the lumbar spine. Yet the difference in bone density at the lumbar spine did not reach statistical significance (p-value >0.05). There was statistically significant reduction in the BMD at the left femoral neck, in the post treatment evaluation as compared to the pre treatment evaluation, with p-value<0.001.We have tried to correlate the bone density (BMD,T score, Z score) in femoral neck to several clinical variables in a multivariate analysis (age, sex, extra medullary disease, and Ph chromosome). However, we did not find any statistically significant correlation with any of the previous factors.

Table 1. Bone mineral density BMD pre- and post tratment values at the lumber spine and the left femor neck.

Left Femur Bone Density			Paired Differences		t-test	
	Mean	±SD	Mean	±SD	t	p-value
Lt.femoral BMD pre treatment	1.33	0.15	0.11	0.10	5.384	**<0.001**
Lt.femoral BMD - post treatment	1.22	0.14				
Lt.femoral T score pre treatment	2.25	0.96	0.81	0.68	5.441	**<0.001**
Lt.femoral T score post treatment	1.44	1.05				
Lt.femoral Z score pre treatment	2.02	0.99	0.77	0.69	5.107	**<0.001**
Lt.femoral Z score post treatment	1.25	1.10				
Lumbar spine BMD pre-treatment	1.14	0.10	0.057	0.231	1.134	0.270
Lumbar spine BMD post-treatment	1.08	0.26				
Lumbar spine T score pre-treatment	-0.50	0.77	0.205	0.765	1.227	0.234
Lumbar spine T score post-treatment	-0.70	1.18				
Lumbar spine Z score pre-treatment	-0.92	0.86	0.005	0.762	0.029	0.977
Lumbar spine Z score post-treatment	-0.92	1.18				

Summary and Conclusions: Skeletal morbidity, characterized by bone pain, osteonecrosis, fractures, loss of mobility, bone deformation, or osteopenia, is frequently encountered in patients affected by ALL. Clinically important sites for evaluation of osteopenia/ osteoporosis in adult are the lumbar spine (L2-L4), and femoral neck. Larger sized studies are required to draw more firm conclusions and to design screening and prophylactic programs.

PB1616

ANALYSIS OF THE EFFICACY OF CCLG-ALL2008 PROTOCOL ON THE ADOLESCENTS AND YOUNG PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Acute lymphocytic leukemia is a kind of heterogeneous disease, the prognosis of the pediatric patients was significantly better than that of adults. Adolescent and young adult (AYA) patients could adopt pediatric or adult chemotherapy regimens. In recent years, some studies showed pediatric like regimens could improve the prognosis of AYA ALL patients.
Aims: Analysis of efficacy and safety of the pediatric ALL chemotherapy regimen CCLG-ALL2008 on adolescents and young ALL.
Methods: 15 to 30 years old ALL patients treated with CCLG-ALL 2008 regimen from January 2008 to December 2013 in our cancer center was retrospectively analyzed. CR rate on day 14 and day 28, relapse-free survival, overall survival and the relativity between the clinical characteristics and RFS were analyzed. The chemotherapy related toxicity was assessed with WHO criteria. The informed consents of each patient was obtained before the treatments.
Results: 21 cases of adolescent ALL included 13 males and 8 females were analyzed, with a median age of 18 years old and 18 cases with median-risk and 3 cases with high-risk according to regimen CCLG-ALL2008. 14 days CR rate was 90.5% and 28 day CR rate was 100%, median CR time were 14 days (14 to 28d), median cytogenetic remission time was 2 months, 17 patients reached molecular biology remission with a median time of one month. Three cases relapsed and 1 patient was lost in follow-up, five cases underwent hematopoietic stem cell transplantation. 2-year OS rate was 100.0% and 2-

year RFS rate was 66.6%; Univariate and multivariate analysis showed that the presence of extramedullary infiltration was unfavorable factors of 2-year RFS. Neutropenia was observed in 17 patients (81%), neutropenia fever occurred in 12 cases (57.1%) and were documented with infection, including 2 (9.5%) pneumonia and 5 sepsis (23.8%). 4 degree of thrombocytopenia occurred in 13 patients (61.9%) and coagulation abnormalities occurred in 16 patients (76.2%), Non-fatal bleeding occurred. 1-2 grade toxicity occurrence rates in heart, liver and kidney and gastrointestinal were 33.3%, 61.9%, 23.8% and 71.4% respectively.
Summary and Conclusions: The regimen of CCLG-ALL2008 has obtained good curative efficacy in AYA ALL patients, and the toxicity was easily tolerated.

PB1617

EVALUATION OF ENDOCRINE LATE COMPLICATIONS IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA SURVIVORS

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Background: Improvement in long-term survival in patients with acute childhood leukemia has led to the need for monitorization of chemotherapy related morbidity and mortality.
Aims: This study aimed to evaluate long-term endocrine compliations in acute lymphoblastic leukemia survivors.
Methods: Sixty patients diagnosed with acute lymphoblastic leukemia between December 2003 and May 2009 at Pediatric Hematology Clinic, Ankara Children's Hematology and Oncology Education and Research Hospital, who were in remission for at least two years, were evaluated retrospectively for endocrine complications.
Results: There were 31 male and 29 female patients, while 55 of them were diagnosed with precursor B-cell ALL and 5 of them with T-cell ALL. Median age of the patients at the time of diagnosis, at the time of chemotherapy completion and at the time of study was 5 years (range:3-7,8 years), 8 years (6,2-10,8 years) and 11.7 years (range: 10-14.9 years), respectively and median duration of remission was 4 years (range: 2.5-5 years). At least one complication was observed in 81,6% of patients. Vitamin D insufficiency / deficiency (46,6%), overweight / obesity (33,3%) and dyslipidemia (23,3%) were the most three frequent endocrine complications. Other complications seen in our patients were hyperparathyroidism secondary to vitamin D deficiency (15%), insulin resistance (11,7%), hypertension (8,3%), failure to thrive (6,7%), thyroid function abnormality (5%), precocious puberty (3,3%) and decreased bone mineral density (1,7%), respectively. There were no statistically significant correlation between endocrine complications and age, sex and radiotherapy.
Summary and Conclusions: A high frequency of endocrine complications were observed in the current study. It is estimated that about two-thirds of cancer survivors will experience at least one late adverse effects and more than 40% may have a severe, disabling or life-threatening condition or may die 30 years after cancer is diagnosed as a result of their cancer and it's therapy. The high frequency of late effects necessitates long-term surveillance of this population to better understand the incidence of late-occurring events and defining high-risk features that can facilitate developing intervention strategies for early detection and prevention.

PB1618

ANALYSIS OF THERAPY RESULTS IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKAEMIA. A SINGLE CENTER EXPERIENCE IN TURKEY

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Background: Prognosis of children with acute lymphoblastic leukemia (ALL) which the most common cancer in childhood, has improved in the last years.
Aims:
Analysis of treatment outcome and identification of prognostic factors in children treated for acute lymphoblastic leukaemia (ALL).
Methods: The patients diagnosed for ALL in our Hospital of Kayseri-Turkey between 2000-2014 were evaluated retrospectively. Children were treated according to the protocol of BFM-90 (Berlin-Frankfurt-Münster). Patients were classified into standart-risk (SR), medium-risk (MR) and high-risk (HR) group according to initial leukaemic burden, early treatment response, genotype of leukemia.
Results: A total number of 341 children aged 4 monts -17 years (median age 5.45 years) were diagnosed for ALL in our Hospital of Kayseri-Turkey between 2000-2014. Children were treated according to the protocol of BFM-90 (Berlin-Frankfurt-Münster).Duration of the chemotherapy was two years. Treatment results were evaluated in 341 children. With a median follow-up of 7 years, event-free-survival (EFS) was 85.4% and overall survival 86.4%. Relapse was diagnosed in 14.6% of the patients.
Summary and Conclusions: Large progress has been made in the treatment of acute lymphoblastic leukaemia of childhood and adolescence over the analyzed period of 14 years. Our results for children with acute lymphoblastic leukaemia are similar to the results in the other BFM study-Group.

PB1619

IS CD45 EXPRESSION LEVEL RELATED TO HIGH RISK DEFINITION IN CHILDHOOD PEDIATRIC B ALL?

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Background: Two report shows CD45 brightnes of childhood ALL blast is associated with poor prognosis. Borowitz et al showed that bright CD45 expression was associated with a poor prognosis in BCP-ALL patients treated according to theprotocols of the Pediatric Oncology Groupstudy group and Cario et al also in ALL-BFM-2000 protocol.
Aims: In the present study, we assessed the relation of brightness of CD45 expression in pediatric ALL patients treated according to he ALL-IC-BFM 2009 protocol.
Methods: The brightness of CD45 expression was compared in ten patients of high risk group and ten patient of standart and intermediate groups. In the ALL-IC BFM 2009 trial, the high-risk group was defined by poor prednisone response on treatment day 8, FCM blast ratio >%10 at day 15 and BCR-ABL OR MLL positivity. The brightness of CD45 expression was evaluated as mean flouresence index (MFI).
Results: CD45 MFI was 710,2 for blast, 13784,6 for normal lymphocytes, 0,056 for blast/lymphocytes in hr group, CD45 MFI was 590 for blast, 7904,4 for normal lymphocytes, 0,082 fr blast/lymphocytes in hr group. The differenceb between groups was not significantly different.
Summary/Conclusion: In conclusion, the CD45 brightnes of childhood BCP-ALL did nod have any additional contribution to high risk stratification. This result is different from previous reports. New reports is required to solve this debate.

PB1620

T315I CLONE SELECTION IN A PHI+ ALL PATIENT UNDER MAINTENANCE PONATINIB

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Background: Ponatinib is a third generation tyrosine kinase inhibitor (TKI) approved for the treatment of chronic myeloid leukemia (CML) or Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph+ ALL) with T315I or resistant/intolerant to other TKIs. Serious vascular adverse events have been reported at the dosage of 45 mg once daily suggesting that a reduction in the dosage of ponatinib may be advisable.
Aims: We present here the case of a Ph+ ALL in 2nd relapse who achieved a molecular remission with ponatinib 30 mg/day. To decrease the risk of vascular complications, ponatinib dosage was reduced to 15 mg/day during maintenance therapy. We examined whether this ponatinib dosage is sufficient to prevent molecular relapse.

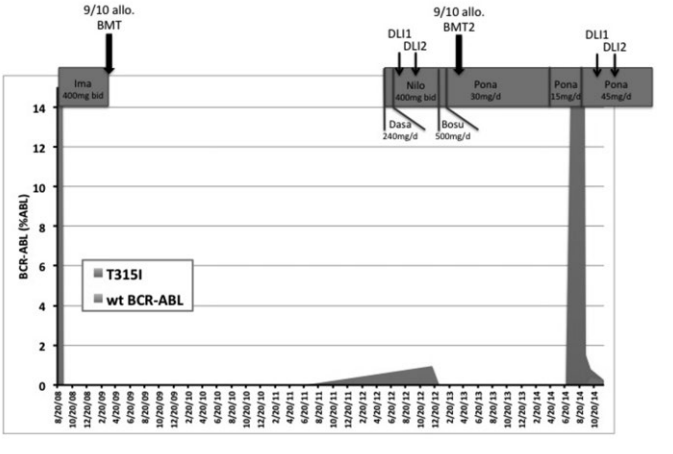

Figure 1.

Methods: Residual disease was measured by RQ-PCR and BCR-ABL mutations were detected by Sanger sequencing.
Results: This 39-year-old patient was diagnosed in 2008 with a Ph+ ALL (Figure 1). He was first treated with imatinib (GRAALL/GRAAPH 2005 trial) and

then successively with dasatinib, nilotinib and bosutinib because of intolerance or resistance and finally with ponatinib. Due to liver toxicity, ponatinib was given at 30 mg/day as maintenance therapy. One year after the second transplant, while the patient was still in CR, an unexpected high rate of ponatinib-induced vascular events was reported. According to recommendations made for CML in major molecular response, the maintenance therapy was reduced to 15 mg/day. Six months later, a molecular relapse was identified in bone marrow and Sanger sequencing revealed a T315I mutation in BCR-ABL kinase domain (KD). The patient was treated with steroids, a single dose of vincristine (1,4 mg/m^2) and ponatinib 45mg once daily. Fifteen days later, MBCR-ABL level was reduced by 1 log and sequencing showed the concomitant presence of wild-type and T315I BCR-ABL KD. Donor lymphocyte infusions were started. Three months later, the MBCR-ABL/ABL ratio was 0,025%. Presently, 6 months after the 2nd relapse, the patient is in complete molecular remission, without graft *versus* host disease.
Summary/Conclusion: We report here the clinical course of a Ph+ ALL patient who was treated with ponatinib 15mg/day, as maintenance therapy, and developed a BCR-ABL T315I mutation leading to leukemia molecular relapse. This clonal evolution was reversed, without adverse effects, by increasing ponatinib dosage to 45mg/day suggesting that ponatinib dose reduction may not be appropriate in Ph+ALL maintenance therapy.

PB1621

A RETROSPECTIVE ANALYSIS OF COMPLICATIONS OBSERVED IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA DURING CHEMOTHERAPY
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Background: ALL is the most common cancer in the paediatric age group. In recent years, through the use of intensive chemotherapy together with improved supportive care, the 5-year survival rate has increased above 85%. Despite this high survival rate, completing the entire therapy regimen is challenging because of the severe complications that interrupt the chemotherapeutic process. A minor proportion of patients can be expected to complete chemotherapy without experiencing serious complications during chemotherapy.
Aims: We aimed to evaluate the complications that we observed in children with acute lymphoblastic leukaemia (ALL) during the remission induction, consolidation, and reinduction phases of chemotherapy retrospectively.
Methods: We analysed the clinical records of 128 patients with ALL who were diagnosed and treated in the Department of Pediatric Hematology of Istanbul Medeniyet University Goztepe Training Hospital between August 2009 and April 2014.
Results: Of these documented complications, 53.05% were in males, 46.95% in females; 32.26% were in standard-risk, 45.52% in medium-risk, 22.22% in high-risk group of patients. Common documented events were pneumonia (25%), therapy-induced hyperglycemia (16.40%) therapy-related hepatitis (15.62%), generalized tonic-clonic seizures (14.84%), anaphylaxis to asparaginase (14.06%), hypertension (13.28%), varicella zoster infection (13.28%), renal tubulopathy (12.5%). Time of complications was induction phase in 32.62%, consolidation in 19.35%, HR blocks in 18.28%, reinduction 29.75% during the phases. Mortality rate due to complications was 13.28%.
Summary and Conclusions: Therapy-related complications can limit the survival rates in children with ALL. To minimize the treatment burden, even very rare complications must be considered and treated promptly with a multidisciplinary approach.

Acute myeloid leukemia - Biology

PB1622

BONE MARROW MESENCHYMAL STROMAL PRECURSOR CELLS FROM ACUTE MYELOID LEUKEMIA PATIENTS ARE DAMAGED BY LEUKEMIC CELLS AND THE PROCEDURE OF ALLOGENEIC BONE MARROW TRANSPLANTATION
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Background: Leukemic cells and high dose chemotherapy affect both hematopoietic and stromal precursor cells. Changes in the hematopoiesis occurring in acute myeloid leukemia (AML) are well characterized, however, the mechanisms of alterations in stromal microenvironment during the debut of AML and pre-transplantational conditioning are still obscure.
Aims: This study aimed to analyze the alterations in the characteristics of human multipotent mesenchymal stromal cells (MSC) and their more differentiated progeny – fibroblastic colony forming units (CFU-F) derived from the bone marrow (BM) of AML patients.
Methods: 18 newly diagnosed and 20 patients who underwent allogeneic bone marrow transplantation (alloBMT) were included in the study after informed consent. BM was aspirated prior to any treatment in the newly diagnosed group, before the conditioning and at 6 time points during 1st year after alloBMT in the corresponding group. MSC were cultured in aMEM with 10% fetal calf serum. Cumulative MSC production was counted after 3 passages. CFU-F concentration was analyzed in standard conditions. The relative expression level (REL) of different genes was measured by TaqMan RQ-PCR. As a control MSC and CFU-F from 88 healthy donors were used.
Results: The CFU-F concentration in the BM of newly diagnosed AML patients was 1/3 of the donor's (8.7±3.7 *versus* 25.8±3 per 10^6 nucleated cells, P=0.0008). There were no correlation between CFU-F concentration and blast count in the patients BM samples. So the decrease in CFU-F concentration could not be explained by mere substitution of stromal cells by blasts, but rather it reflects the action of leukemic cells on the stromal microenvironment. In the remission BM CFU-F concentration restored (26.7±7.2 per 10^6 BM nucleated cells). After the alloBMT CFU-F concentration in patients' BM decreased 3-9 fold during the next year of observation. The decrease at each time point was highly significant comparing to donors. Similar picture was observed in MSC features. Cumulative cell production in cultures of newly diagnosed AML patients was half to donor's (5.03±0.8x10^6 *versus* 7.6±0.8x10^6, P=0.07). MSC from the BM of patients before alloBMT were also slightly and insignificantly lower than from healthy donors (5.9±1x10^6). In patients after alloBMT cumulative MSC production decreased 1.3-5.2 fold during the next year. The decrease at almost each time point was significant comparing to donors. Gene expression analysis revealed that in MSC of new diagnosed patients the expression of FGF2 and VEGFA significantly decreased, while REL of CSF1, FGFR1, JAG1, PPARG, PDGFRA and PDGFRB-increased. In remission REL of FGF2 and LIF were twice as high as donor's, after alloBMT the expression of FGF2 was mainly elevated. However, REL of CSF1, PDGFB, VEGF and VCAM1 was significantly decreased at the most time points after alloBMT. It seems that expression profile of MSC at manifestation of AML reverses in the remission but still does not achieve normal levels.
Summary and Conclusions: During the AML development leukemic cells alter the stromal precursor cells leading to the decrease in their proliferative ability, in the level of expression of some regulatory genes and in the number of CFU-F in the BM. Chemotherapy used for induction the remission restores the stromal precursor cells incompletely. Conditioning regiments used for the alloBMT significantly damage both types of studied stromal precursors, and the effect lasted at least for 1 year. Thus, both AML cells and chemotherapeutic treatment affect BM hematopoietic microenvironment.

PB1623

CHARACTERIZATION OF KMT2A (MLL) CHROMOSOMAL BREAKPOINTS
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Background: The *KMT2A* gene (*MLL*, mixed lineage leukemia) is located at chromosome region 11q23. Chromosomal rearrangements involving the human *MLL* gene are associated with development of childhood, adult and therapy-related acute leukemia. The presence of certain *MLL* rearrangements is an

independent prognostic factor and patients are usually treated according to high-risk protocols. Minimal residual disease (MRD) detection provides an objective assessment of treatment response and enables risk stratification of patients. However, MRD assays using *MLL* fusion transcripts as molecular markers usually do not provide sufficiently sensitive detection of residual leukemic cells.

Aims: Identification of the unique chromosomal breakpoints of the *MLL* gene, which is necessary for the design of a quantitative real-time PCR (qPCR) DNA-based MRD assay.

Methods: For the identification of patient-specific *MLL* breakpoint sequences we used two different technical approaches. The first approach was long-range PCR followed by sequencing of the PCR products. The second approach included a combination of conventional chromosome microdissection, amplification of the microdissected material, next-generation sequencing, long-range PCR and sequencing of the final PCR products. Patient-specific sequences of the chromosomal breakpoint were used to develop MRD assays and enabled us to perform sensitive monitoring of MRD using qPCR in six acute leukemia patients.

Results: We identified unique breakpoint sequences in five patients with acute myeloid leukemia and one patient with acute lymphoblastic leukemia at the time of diagnosis. Using first approach (*i.e.* long-range PCR followed by sequencing of the PCR products) we detected *MLL/AF6*, *MLL/AF9* (two patients) and *MLL/ELL*. A combination of conventional chromosome microdissection, amplification of the microdissected material, next-generation sequencing, long-range PCR and sequencing of the PCR products was used for identification of *MLL/AF10* and *MLL/AF4* breakpoints. Identification of unique breakpoint sequences was followed by the design of sufficiently sensitive qPCR MRD assays. The MRD levels of residual leukemic cells correlated with clinical outcome.

Summary and Conclusions: *MLL* breakpoints could be identified by various methods (*e.g.* inverse PCR, panhandle PCR). Our results show other approaches for identification of unique *MLL* breakpoint sequences, which can be utilized for the design of the leukemia-specific assay for DNA-based MRD monitoring in patients with acute leukemia.

PB1624

MUTATIONAL PROFILE STUDY OF ADULT DE NOVO ACUTE MYELOID LEUKEMIA BY HIGH-DEPTH NEXT GENERATION SEQUENCING (NGS)

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Background: AML is a highly heterogeneous pathology with a variety of subtypes stratified function of cytogenetic and molecular markers. To date, the major prognosis criteria is cytogenetics, however the 40-50% of *de novo* patients have normal karyotype. Recent sequencing techniques such as NGS allow us to identify the mutational profile, to decide the diagnosis and risk stratification of patients and to personalize therapy.

Aims: To identify the mutational profile of AML and to correlate with the patient's survival data.

Methods: A cohort of 65 AML patients who were treated with intensive chemotherapy was selected. Mediam age at diagnosis was 52 years (range: 18-79), male:female ratio 30:35; 53 patients had de novo AML, 10 s-AML following MDS or MPN, and 2 t-AML; median leukocytes was $36.8x10^9/L$. Cytogenetic results were available in 64 cases and categorized according to MRC (Blood 2010): favorable in 6 (9.4%), intermediate in 44 (68.8%), and adverse in 14 (21.9%). We performed targeted gene sequencing by NGS (*Ion Torrent Proton System–Life Technologies*) using a panel of 33 genes implicated in leukemia prognosis; in addition, FLT3 internal tandem duplication (ITD) was detected by GENSCAN and NPM1 mutation was detected by qPCR.
The discrete variables of patients with and without analyzed gene mutations were compared using the X^2 test. Kaplan-Meier survival curves and Long-rank test were used for estimation of survival and difference between groups

Results: On average, 90,8% of the target sequence showed a mean depth coverage around 1300. We discovered 140 non-synonymous mutations which 118 were somatic single nucleotide variants (SNVs) and 22 small insertions and deletions (Indels) in coding regions. No mutations were detected in 5 samples, 14 samples presented 1 mutation, and the others 46 samples present 2 or more mutations. TET2 was the most frequently mutated gene (33,8%), followed by DNMT3A (26,2%), NPM1 (26,2%), FLT3 (21,5%), NRAS (20%), FLT3-ITD (18,5%), KMT2A (16,9%), IDH2 (13,8%), RUNX1 (13,8%), EPOR (12,8%), KRAS (12,8%), ASXL1 (9,2%), IDH1 (9,2%) and the others genes had a frequency below 8%. Mutations in ASXL1 (P=0.005), KRAS (P=0.033), JAK2 (P=0.061) and ZRSF2 (P=0.019) were associated with secondary AML. DNMT3A (P<0.001) and ZRSF2 (P=0.005) mutations with NPM1 mutations. DNMT3A and ETV6 mutations with FLT3 ITD AML (P=0.041 and 0.029 respectively). AML patients who present TP53 or TET2 mutations showed a statistically non-significant correlation with death (P=0.103 and 0.062 respectively), in contrast IDH2 mutations were more frequent among alive patients (P=0.061). RUNX1 mutations were associated with female gender (P=0.023), and these and TP53 mutations were associated with refractoriness (P=0.009 and P=0.001, respectively). EZH2 and TET2 mutations were associated to AML patients >55yrs (P=0.011 and 0.025 respectively). In contrast to KRAS mutations that were detected mainly in patients<55yrs (P=0.090). Median RFS was significantly longer in female (28.5 vs 10.8months, P=0.017) and also in patients<55 years (18.9 vs 10.8 months, P=0.026). Median OS was significantly longer in patients<55 years (18.9 vs 11.8 months, P=0.038). No significant differences in RFS in function of analyzed gene mutations were found, but we observed that the median OS was significantly shorter in mutated TET2 patients (8.4 vs 18.8 months, P=0.049).

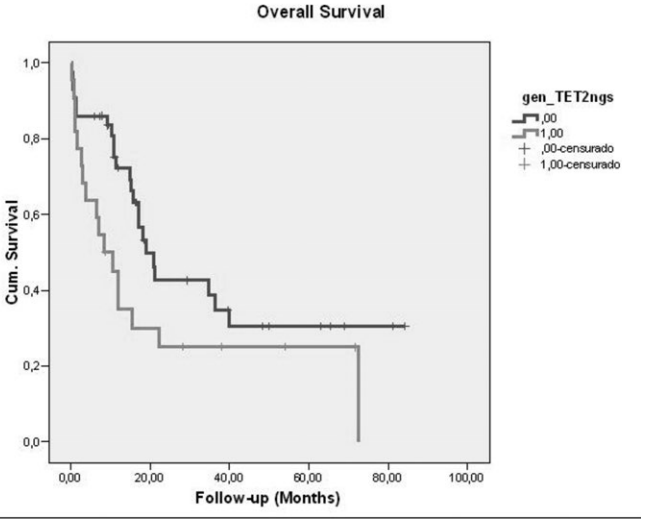

Figure 1.

Summary and Conclusions: NGS is a useful technique to classify groups in AML with biological and prognosis implications. These results support the role of TET2 as an important poor prognostic biomarker in AML. This study was funded by Instituto Carlos III (PI13/02387)

PB1625

CD81 AS NEW POTENTIAL MARKER IN ACUTE MYELOID LEUKEMIA

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Background: AML is a heterogeneous disease at both the phenotypic and molecular level with a variety of distinct genetic alterations giving rise to the disease. This heterogeneity extends to the leukemic stem cell (LSC), with this dynamic compartment evolving to overcome various selection pressures imposed upon it during disease progression. Since LSC are thought to be resistant to current chemotherapeutic regimens and mediate disease relapse, their study may have profound clinical implications. Various markers have been described to characterize the LSC. CD81 antigen belongs to the tetraspanin family (33 members in mammals) which are cell surface transmembrane proteins and may be involved in the re-entry of hematopoietic stem cells into quiescence.

Aims: In this project, we plan to investigate the role of CD81 in the heterogeneity of AML, its potential to induce tumor dormancy and if this is characterized by resistance to chemotherapy.

Methods: In order, to study CD81 expression on primary leukemic blasts, we designed a MFC panel (CD36, CD81, CD33, CD90, CD123, CD34, CD38, CD45).Cell surface marker expression was measured in fresh or thawed bone marrow samples from adult patients with AML at diagnosis (n=122) or at relapse (n=10) using the above mentioned panel. This is a retrospective study to test the prognostic value of CD81, in comparison with other markers of LSC/HSC, for survival (overall survival, event free survival, relapse free survival). AML was diagnosed between 2010 and 2013 at the CHRU of Lille. To further study the function of CD81, we will use AMLxenografts. Blasts obtained from newly diagnosed AML, were FACS sorted based upon high CD45 intensity and were then directly injected into NSG mice. After engraftment, the mice were sacrificed, and the bone marrow and spleen were analyzed by flow cytometry. Serial engraftment is performed by injecting one part of the blast cells into a subsequent NSG mouse.

Results: We performed the analysis on 122 diagnosis bone marrow samples and we found that 35% of the samples were positive for CD81 expression. Classification by CD81 expression predicts OS and EFS in AML (EFS: P=0.012; OS: P=0.0066). 23 primary AMLs have been injected and 8 of them produced

serial engraftments. Yet, we did not find any difference in phenotype between the AMLxenografts and the AML diagnosis. However, the phenotype of the blasts differ between bone marrow and spleen (P= 0.027).
Summary and Conclusions: Our preliminary results show that CD81 may have an important role in AML as their expression on blast cells predicts worse clinical outcome (OS and EFS) in this pathology. Furthermore, we have put in place and phenotypically validated AMLxenografts, and we will be able to study the functional role of CD81 in AML in the near future. Nevertheless, the impact ofxenografts in general and in particular with regard to CD81 on prognosis will be studied once we have sufficient follow-up time and numbers of patients analyzed and injected into NSG mice.

PB1626

DEOXYCYTIDINE KINASE IS DOWN-REGULATED UNDER HYPOXIC CONDITIONS AND CONFERS RESISTANCE AGAINST CYTARABINE IN ACUTE MYELOID LEUKEMIA

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Background: Leukemia initiating cells reside within specialized niches in the bone marrow where they undergo complex interactions with different stromal cell types. The bone marrow niche is characterized by a low oxygen tension resulting in high expression of hypoxia-inducible factor 1 alpha (HIF-1α). Expression of HIF-1α has been shown to represent a negative prognostic factor in acute myeloid leukemia (AML).
Aims: In the current study, we investigated the impact of hypoxic *versus* normoxic conditions on the sensitivity of AML cell lines and primary AML blasts to cytarabine.
Methods: AML cell lines HL60, Kasumi-1, MOLM-13, OCI-AML5, MV4-11 and KG-1 as well as primary blasts from AML patients were cultured under normoxic and hypoxic conditions (oxygen content of 20% vs. 6%, respectively). After an adaptation period of three days, cells were plated for proliferation and colony formation assays to investigate the susceptibility to cytarabine (Cell Pharm GmbH, Bad Vilbel, Germany). Proliferation and colony formation was determined after 3 and 7 days of cytarabine treatment, respectively. Using quantitative RT-PCR analysis, we investigated the mRNA expression of deoxycytidine kinase in AML cells cultured under normoxic vs. hypoxic conditions for 3 days. AML cells under hypoxic conditions were additionally treated with the HIF-1α inhibitor BAY87-2243 (Selleckchem, Houston, TX).
Results: AML cells cultured under 6% oxygen were significantly more resistant to cytarabine compared to cells cultured under normoxic conditions in proliferation assays. HL60 cells were the most susceptible cells to the drug-induced growth inhibition under hypoxic conditions for cytarabine concentrations between 10 nM and 1000 nM. Although less pronounced, Kasumi-1, MOLM-13, OCI-AML5 and MV4-11, also showed the hypoxia-induced resistance to cytarabine. Only in KG-1 cells, no significant differences in growth inhibition could be detected under normoxic vs. hypoxic conditions. Furthermore, increased sensitivity to cytarabine under normoxia could also be observed in primary AML samples (n=5). Response to cytarabine under normoxic vs. hypoxic conditions was also analyzed by colony formation assays for several AML cell lines using cytarabine concentrations ranging from 25 nM to 100 nM. Colony numbers at day 7 of cytarabine treatment were significantly higher at 6% oxygen than at normoxia. Interestingly upon cultivation under hypoxia, the expression of the cytarabine-activating enzyme deoxycytidine kinase was down-regulated in all analyzed AML cell lines and primary AML samples representing a possible mechanism for resistance to chemotherapy. Furthermore, the down-regulation of deoxycytidine kinase could directly be associated with HIF-1α as treatment with its inhibitor BAY87-2243 hampered the down-regulation of deoxycytidine kinase expression under hypoxic conditions.
Summary and Conclusions: In conclusion, our data reveal that the hypoxia-induced down-regulation of deoxycytidine kinase might represent one mechanism of drug resistance to cytarabine in acute myeloid leukemia.

PB1627

MUTATED REGIONS OF NUCLEOPHOSMIN 1 ELICIT CD8+ T-CELL RESPONSES IN PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background: Stimulating lymphocytes from healthy donors by DCs loaded with NPM1^{mut}peptides *in vitro* in order to induce specific cellular immune responses against the peptides.
Aims: Detecting the NPM1^{mut} peptide specific T-cell in peripheral blood of patients with NPM1^{mut} positive acute myeloid leukemia (AML), to provide a theoretical basis for immune therapy of AML.
Methods: 1. newly diagnosed patients of AML underwent gene mutations screening routinely. 2. peripheral blood mononuclear cells (PBMCs) were isolatedfrom healthy donors with HLA-A* 0201 or A*1101 and NPM1^{mut} positive AML patients in complete remission. 3. Inducing differentiation of PBMCs into DCs. 4. Generation of NPM1-specific cytotoxic T cells. 5. ELISPOT analysis and intracellular staining.
Results: 1. In the Han Chinese population, the most common alleles of HLA-A loci were A* 1101, A*2402, and A*0201 allele. 2. Through prediction of the aforementioned software, HLA-A*0201 restricted wild type NPM1 amino acid sequences (DLWQWRKSL), mutated NPM1-A/D amino acid sequences (AIQDLCLAV), and HLA-A*1101 restricted mutated NPM1-A/D amino acid sequences (AVEEVSLRK) were synthesized. There were no proper epitopes for either HLA-A*1101 restricted wild type NPM1 protein or HLA-A*2402 restricted wild type and mutated NPM1 protein. 3. Expression of surface antigens of DCs on day 7 were as follows: CD14(2.6%), CD80(43.4%), CD83(7.8%), CD86(99.9%), HLA-DR(67.1%), which was consistent with DCs phenotype.
4. About 10 days after DCs were co-incubated with their own PBMCs, the number of lymphocytes increased significantly, especially in the NPM1^{mut} peptide pulsed group. On day 7 of co-incubation, ELISPOT analysis results of all samples were negative. However, 3 cases' ELISPOT results were positive for the mutated peptide holes on day 14, in contrast, the results of the wild type peptide holes were negative. The NPM1^{mut} peptide-specific T lymphocytes positive rate [=(average number of spots in mutated peptide holes-the average number of spots in negative control holes)/total lymphocytes per hole] was about 1/2500. Intracellular staining showed that in the aforementioned 3 cases, the proportion of $CD8^{+}IFN\text{-}\gamma^{+}$ cells of the mutant peptide group was higher than either the wild type peptide group or negative control group, but there was no difference between the later two groups. 5. 6 peripheral blood samples of patients with NPM1^{mut+}AML were performed ELISPOT analysis, with only 1 case (16.7%) showing a approaching positive result.
Summary and Conclusions: NPM1^{mut} peptide pulsed DCs can stimulate their own PBMCs from healthy donors *in vitro* to produce mutated NPM1 specific T lymphocytes, and it is expected to be used as immune therapy of AML. The mutated NPM1 specific CTL in NPM1^{mut+}AML patients are almost undetectable that indicates immune system have been comprised because of chemotherapy and disease, and can not responses to antigens efficiently. Perhaps AML patients can accept CTL transfusion from their relatives. Our study provide an experimental basis for cellular immunotherapy of AML.

PB1628

PHENOTYPIC AND FUNCTIONAL ANALYSIS OF BONE MARROW MESENCHYMAL STEM CELLS OF PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background: Mesenchymal stem cells MSC are main cells found in bone marrow BM microenvironment. They play a major role in hematopoietic stem cell HSC niche. These niches support acute myeloid leukaemia AML cells proliferation and differentiation.Many studies showed the protection of AML cells from chemotherapy induced apoptosis by BM microenvironment. The mechanism of this protection is still unclear.
Aims: Our present work is to compare the phenotype and functional properties of AML BM-MSCs to normal MSCs from BM to identify new mechanisms could help to improve the treatment of AML.
Methods: MSC were obtained from the BM of patients with AML and healthy donors (n=5 per group). Following parameters were used for: cell morphology, cell proliferation test, cell cycle, immunophenotype, differentiation capacity into osteoblastic and adipogenic lineages. Gene expression profile was determined by Q-PCR.
Results: 4/5 of AML BM-MSC have heterogenous morphology and 1/5 has homogenous fusiform, fibroblast-like appearance. AML BM-MSC show, significantly (P<0.05), low proliferative ability than normal BM-MSC, which induces an increase of doubling time (62,8-/ + 7h and 44,5-/ + 8h for AML and normal BM-MSC, respectively; P<0,01). The analysis of cell cycle show that the number of cells in AML BM-MSC cultures in G2/M phase, significantly P<0.05, reduced by 50% compared to normal BM-MSC cultures. However, Both of Normal and AML BM-MSC have similar immunophenotype profile (positive: CD90, CD73, CD105, CD166 and CD146; negative: HLA-DR, CD34, CD45). Both of cell types have a potential to differentiate into osteoblastic and adipogenic lineages showed by specific stains. This result was confirmed by the expression levels of Runx2, BMP2 and ALP Alkaline phosphatase (osteoblasts); and PPAR-γ2 (adipocytes). However, expression of these genes in osteoblasts derived from AML BM-MSC remains, significantly P<0.01, lower than the expression in osteoblasts obtained in normal BM-MSC differentiated cells.
Summary and Conclusions: The proliferative capacity of MSC obtained from the BM of patients with AML are limited and have a heterogenous morphology although the expression of phenotypic markers remains unchanged compared to normal BM-MSC. More significantly, the capacity to differentiate into

osteoblasts is reduced in AML BM-MSC. This result suggest that, the bone quality obtained by AML BM-MSC is affected. Therefore, there is an influence on the endosteal microenvironment and that could affect the behavior of CD34 HSC in the endosteal niche.

PB1629

EVIDENCES OF A DNMT3A DEPENDENT CYTOSTATIC EFFECT OF S-ADENOSYLMETHIONINE ON OCI-AML3 CELLS

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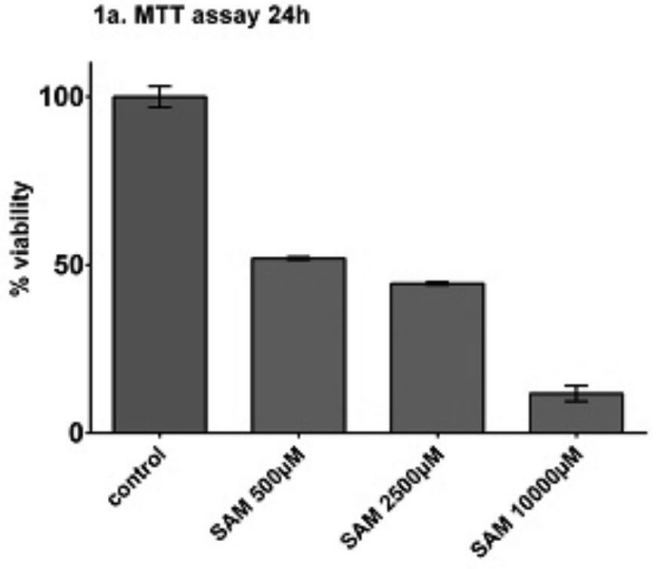
Background: AML with mutated NPM1 and its cell line OCI-AML3 carry the NPM1 mutation A and the heterozygous R882C mutation of the DNA (cytosine-5-)-4 methyltransferase 3 alpha (DNMT3A). The mutant DNMT3A protein exerts a dominant negative inhibition on the wild type protein resulting in focal hypomethylation. Of note, AML with mutated NPM1 harbors a distinct methylation profile among AML subtypes. S-adenosylmethionine (SAM) is a universal methyl donor and a coenzyme of DNMT3A. SAM has been used in the clinical practice as an antidepressant drug with very limited side effects. There are growing evidences of the antineoplastic activity of SAM *in vitro* and in murine models of solid cancers. Recently, the combination of SAM with demethylating agents showed an interesting *in vitro* synergistic cytotoxicity on breast cancer cells.



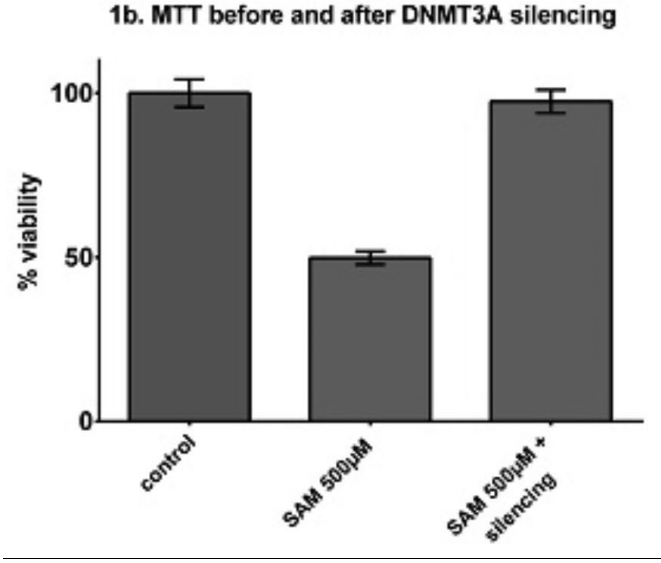


Figure 1.

Aims: To explore the effect of SAM on OCI-AML3 cells and its dependence on DNMT3A activity.
Methods: We used the MTT assay to test the cytostatic effect of SAM iodide (Sigma-Aldrich, St. Louis -MO) on OCI-AML3 cells (DSMZ Leibniz Institut, Braunschweig -Germany). The cells were treated with SAM for 24h. Untreated and treated cells were tested in triplicate for each SAM concentration. Results were expressed as percentage of cells viability compared to the control (*i.e.* OCI-AML3 untreated cells). The Apoptosis assays were performed after 24h and 72h of SAM treatment using the Tali® Apoptosis Assay Kit–Annexin V Alexa Fluor®488/Propidium Iodide (Invitrogen, Carlsbad, CA) for cells staining, and the Tali Image Based Cytometer (Invitrogen) for cells counting. In order to verify if the observed effect was mediated by DNMT3A, we repeated the MTT assay after DNMT3A silencing with a predesigned SiRNA directed against DNMT3A (SiRNA ID HSS176224, life technologies). Transfection was performed on triplicates cultures with lipofectamine RNAiMax (Invitrogen) and transfection efficiency calculated by BLOCK-IT AlexaFluor Red Fluorescent Oligo (Ambion-life Technologies). DNMT3A knock-down was verified by RTPCR (TaqMan Applied Biosystem ABI7500; Taqman primer/probes for DNMT3A, Hs01027166_m1).
Results: A significant dose dependent reduction of the cells viability was evident for SAM concentrations equal or superior to 500µM, with an IC50 of 500µM (Figure 1a). Since a Cmax of 211µM (SD 94) after single intravenous infusion of SAM was previously reported in healthy volunteers, we tested SAM concentations close to 211µM, and observed a significant reduction of the cells viability with SAM 200µM (62,74% viable cells) and SAM 300µM (53.32% viable cells), (data not shown). The apoptosis assay at 24h and 72h of SAM treatment, showed no significant increase in the amount of apoptotic cells, (data not shown), however a dose dependent decrease of the percentages of living cells was observed which was considered congruent with the results of the MTT tests. The MTT assay was repeated testing at the same time DNMT3A silenced and non-silenced OCI-AML3 cells. SiRNA transfection efficiency was 80%. Gene knock-down was confirmed by a DNMT3A expression of 39% compared to the non-silenced cells. In the MTT assay with SAM 500 µM (24h) the non-silenced cells showed a significant reduction of the cell viability (49,8% viable cells), while no effect was observed in the DNMT3A silenced cells (97,5% viable cells) (Fgure 1b).
Summary/Conclusion: SAM shows *in vitro* cytostatic activity on OCI-AML3 cells, at concentrations close to the blood concentrations achievable in humans. These preliminary results are in line with other studies using similar SAM concentrations in solid tumors cell lines. The MTT assay performed after DNMT3A knock-down demonstrated that the cytostatic effect of SAM on OCI-AML3 cells is specifically mediated by DNMT3A. This finding suggests that modulating the activity of DNMT3A with supraphysiological concentrations of its substrate can induce cell growth inhibition in AML cells with heterozygous DNMT3A mutation. SAM should be further investigated as a potential antileukemic compound in AML with NPM1 and DNMT3A mutations.

PB1630

CLINICAL SIGNIFICANCE OF DIFFERENT GATA-2 MUTATION TYPE IN ACUTE MYELOID LEUKEMIA

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Background: Cytogenetic and gene abnormalities are considered to be the most important prognostic factors of acute myeloid leukemia(AML) patients. Gene mutations such as internal tandem duplications (ITDs) or tyrosine kinase domain (TKD) of FMS-like tyrosine kinase 3 (FLT3), C-KIT, neuroblastoma RAS viral oncogene homolog gene (NRAS) have been considered to belong to class involving signal transduction, nucleophosmin gene (NPM1), the CCAAT/enhancer binding protein α gene (CEBPA) or the mixed-lineage leukemia (MLL)gene, which can be affected either through chromosomal translocation or via an intragenic partial tandem duplications (PTDs) to form a fusion gene to class those causing impaired differentiation. GATA-2 is a zinc-finger(ZF) transcription factor and play important roles in hematopoiesis in many cell lineages.
Aims: To evaluate the prognostic value of GATA-2 mutation in acute myeloid leukemia (AML) patients, we performed sequence analysis to see if GATA-2 mutation play important role in AML leukemogenesis.
Methods: We performed sequence analysis to detect GATA-2 mutation in 135 AML patients. We also performed luciferase assay and Western-Blot assay to analyze the biological function of mutant GATA-2.
Results: A total of 5 (3.7%) different *GATA-2* mutations were detected in 135 patients. The mutation types of *GATA-2* were *GATA-2* W10C(tgg>tgt, Trp→Cys), *GATA-2* S277N(agc>aac, Ser→Asn), *GATA-2* P304H(cct>cat, Pro→His), *GATA-2* R362Q(cga>caa, Arg→Gln), *GATA-2* V369A(gtc>gcc, Val→Ala). Luciferase assay showed the transcription activity of GATA-2 G320D was significantly lower than that of wt GATA-2(P<0.05), whereas the activity of GATA-2 P304H and GATA-2 R362Q was slightly lower than wtGATA-2 (*P*>0.05). The result of Western-Blot showed that the expression of GATA-2 protein in GATA-2 P304H(*P*=0.002) was significantly higher while GATA-2 R362Q or GATA-2 G320D mutants was lower than wt GATA-2 (*P*=0.02 and *P*=0.004, respectively).
Summary and Conclusions: GATA-2 mutation is a rare mutation in AML patients. The mutations located in zinc-finger domain may contribute to AML leukemogenesis.

PB1631

DETECTION OF TET2, KRAS, AND CBL VARIANTS BY NEXT GENERATION SEQUENCING IN CHILDHOOD ACUTE MYELOID LEUKEMIA

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Background: Acute myeloid leukemia (AML) is a heterogeneous clonal neoplasm characterized by accumulated genetic aberrations, which result in enhanced proliferation, block in differentiation, increased survival of the leukemic blast cells and variable response to therapy. During the past decades, a number of recurrent cytogenetic and molecular genetic abnormalities have been identified in AML such as t(8;21), inv(16), FLT3, NPM1, CEBPA, Ten-Eleven-Translocation 2 (TET2), Kirsten rat sarcoma viral oncogene homolog (KRAS), and Casitas B-cell lymphoma (CBL). Mutations in TET2 gene could contribute to leukemogenesis by altering epigenetic regulation of transcription via DNA methylation. The incidence of TET2 mutations is approximately 10-20% in AML. RAS mutations, especially KRAS, represent about 90% of cancer-associated mutations. RAS proteins play a major role in cell signaling pathway of cell proliferation, differentiation, and survival. KRAS mutations are the most frequently seen and found in 10-15% of these patients. *CBL* is a mammalian gene encoding the protein CBL which is an E3 ubiquitin-protein ligase involved in cell signaling and protein ubiquitination. These mutations have also been observed in 1% of AML.

Table 1. Demographic characteristics and TET2, KRAS, and CBL variants of children with AML

						TET2				CBL				KRAS			
No	Gender/ Age/	Diagnosis	FAB	Risk Group	Cytogenetic-Molecular abnormalities	Rs Number	Nucleotide Change	Localization	Amino asid change	Rs Number	Nucleo tide Change	Localizat ion	Amino asid change	Rs Number	Nucleotide Change	Localizatio n	Amino asid change
1	M/13	AML	M4	HR	46, XY, monosomy, Trisomy 14	Novel	5284 A>G	Exon 11	Leu/Val	-	-	-	-	-	-	-	-
2	M/13	AML	M1	HR	t(9;22)	Rs 12498609	86 G>C	Exon 3	Pro-Arg	Rs 17851045	182 A>T	Exon 3	Gln-Leu	-	-	-	-
										Rs 111836309	310 A>C	Exon 3	Tyr-Ser				
3	F/8	AML	M5	SR	46, XX	Rs 17253972	1088 C>T	Exon 3	Pro- Leu	-	-	-	-	Novel	1347 T del	Exon 8	Gly- Gly
						Novel	1842 G del	Exon 3	Gly-Gly								
						Rs 142173406	5162 T>G	Exon 11	Ser-Ser								
						Novel	46 A del	Exon 4	-								
						Novel	730 A del	Exon 3	Trp-His								
4	F/3	AML+ T-ALL	M4	HR	47, XX (+21) c 21 der(14) (14q1,2→q 3,2:: 1q21→q43),der(19)(19qter) →p13.3::11q13) 11qter (9). t(9,22)	Rs 200327850	2286 C>T	Exon 3	Pro- Leu	Rs 111836309	310 A>C	Exon 3	Tyr-Stop	Novel	1347 T del	Exon 8	Gly- Gly
						Rs 112576862	2386 G>C	Exon 3	Ser-Ser								
						Rs 17253972	1088 C>T	Exon 3	Pro- Leu								
						Novel	1842 G del	Exon 3	Gly-Gly								
						Rs 142173406	5162 T>G	Exon 11	Ser-Ser								
						Novel	46 A del	Exon 4	-								
						Novel	730 A del	Exon 3	-								
5	M/3	AML	M4	SR	46, XY, monosomy 7	Novel	1842 G del	Exon 3	Gly-Gly	Rs 111836309	310 A>C	Exon 3	Tyr-Stop	Novel	1347 T del	Exon 8	Gly- Gly
						Novel	46 A del	Exon 4	-								
						Novel	730 A del	Exon 3	-								
						Rs 144386291	2599 T>C	Exon 3	His- Arg								
						Novel	5343 A>G	Exon 11	His- Val								
						Rs 372499795	5164 C>T	3' UTR	Thr-Ile								
6				HR		Rs 17253972	1088 C>T	Exon 3	Pro- Leu	-	-	-	-	Novel	1347 T del	Exon 8	Gly- Gly
	M/7	AML	M4		47, XY,+ 22(12) inv (16:16), Fragile X syndrome	Novel	1842 G del	Exon 3	Gly-Gly								
						Rs 142173406	5162 T>G	Exon 11	Leu/Trp								
						Novel	46 A del	Exon 4	-								
						Rs 150298743	100 C>T	Exon 3	Trp-Ile								
						Rs 68431410	652 G>A	Exon 3	Val- Met								
						Novel	5333 A>G	Exon 11	His-Arg								
7	F/9	AML	M2	SR	46, XX	Novel	1842 G del	Exon 3	Gly-Gly	Rs 111836309	310 A>C	Exon 3	Tyr-Stop	Novel	1347 T del	Exon 8	Gly- Gly
						Novel	46 A del	Exon 4	-								
8	M/8	AML-MDS	M5	HR	46, XX	Rs 12498609	86 G>C	Exon 3	Pro-Arg	-	-	-	-	Novel	1347 T del	Exon 8	Gly- Gly
						Novel	1842 G del	Exon 3	Gly-Gly					Novel	1111 T>C	Exon 8	Try-His
						Novel	46 A del	Exon 4	-								

HR high risk; SR standard risk; FAB French- American- British classification

Aims: In this study, we aimed to screen whole TET2, (KRAS) and (CBL) genes by using Next generation sequencing (NGS) analysis to find new possible genetic markers in children with AML.
Methods: Study population consisted of eight patients aged between 1 and 15 years who were admitted to Lösante Hospital for Children with leukemia with the diagnosis of AML. The Patient characteristics of the cases are shown in Table 1. Blood samples were collected with EDTA-containing tubes and DNA was extracted from peripheral blood and bone marrow leukocytes with MagNA Pure automatic DNA isolation instrument (Roche Diagnostics, Manheim, Germany). We utilized NGS to study three candidate genes at *TET2, KRAS* and *CBL*. All coding exons of *TET2* (exons 3 and 11) were presented by 27 amplicons. Beside, two primer pairs were amplified known mutational hotspot regions to describe the RING finger domain and linker sequence for *CBL* (exons 8 and 9) and *KRAS* (exons 2 and 3).
Results: We detected 16 variants in *TET2*, 2 variants both *KRAS* and *CBL*. The most of *TET2* variants were described in the largest exon 3 and 11; 1842 G>- (6/8 patients, 75%) and 1088 C>T (5/8, 62,5%) variants, 5162 T>G(5/8, 62,5%) in exon 3 and exon 11 of *TET2*, respectively. 310 A>C (7/8 87,5%) variation was the highest among the variants in intron 2 of *KRAS*. 1347 T>- in exon 8 of *CBL* was detected 6/8 patients (75%).Table 1 summarizes the association of patients' characteristics and variants of *TET2, KRAS* and *CBL* profiles.
Summary and Conclusions: In this study, we screened the mutations of *TET2, KRAS* and *CBL* genes in pediatric AML patients. We used an amplicon based sequencing method to find possible new genetic markers for leukemia diagnosis. *TET2, KRAS* and *CBL* genes were selected based on recent studies on genetic abnormalities in AML and other hematologic malignancies. In 8 patients, we report novel mutations at *TET2* and *CBL* genes. Seven of 16 substitutions were missense mutations in the exon and UTR regions. These mutations may result in truncated translation of protein. In conclusion, we found that *TET2* mutations are more frequent than *KRAS,* and *CBL* mutations in pediatric AML in this study. The usage of NGS to search for *TET2, KRAS,* and *CBL* mutations might be fruitful, however, these results need to be confirmed by further studies on a larger number of patients.

PB1632

IMPACT OF LEUKEMIA STEM CELL FREQUENCY AT DIAGNOSIS ON SURVIVAL IN ADULT ACUTE MYELOID LEUKEMIA PATIENTS

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Background: Acute myeloid leukemia (AML) is a heterogeneous disorder with treatment results much inferior to acute lymphoblastic leukemia. Treatment failure is largely attributed to the persistence of leukemia stem cells (LSCs) which are less accessible and hence less responsive to chemotherapeutics. The classical LSC phenotype is CD34+/CD38-; however LSCs express other markers especially CD123 and CD133 which may be even earlier than CD34. We hypothesized that CD123 and CD133 may be better markers of LSCs and that higher frequency of LSCs at diagnosis may be associated with shorter survival.
Aims: To evaluate CD123 and CD133 as LSC markers as compared to the standard CD34+/CD38- and to study the impact of LSCs frequency at diagnosis on overall survival (OS) and disease free survival (DFS) in adult AML as well as correlate the LCS frequency with other findings at diagnosis.
Methods: The study was performed on 84 newly diagnosed AML patients including 51 Males and 33 females with an age range of 18-70 with a median of 31.5 years. Two 4 color panels of monoclonal antibodies were used: CD45/CD34/CD38/Cd123 and CD45/CD133/CD90/Cd33 and analyzed on Navios Coulter Flow Cytometer. Cell populations with different surface markers were calculated using the prism function of the soft ware. Results were correlated to other parameters at diagnosis. The median value for each marker alone and in different combinations (panels) was used to divide the cohort into high and low expressers. Patients were treated by (3+7) protocol; they were followed up for a period of 0.2-24 with a median of 5.5 months and evaluated for OS and DFS. The study was performed according to the guidelines of Helsinki declaration for studies performed on human beings and approved by the Institution Review Board (IRB) of the National Cancer Institute, Cairo University. An informed signed consent was obtained from all study subjects before enrollment.
Results: Single markers or panels expression were not correlated to age, gender, percentage PB or BM blasts, Hb level, TLC or platelet count**.**
Among the studied single markers CD123 and CD133 high expression was associated with shorter OS (P=<0.001 and<0.006 respectively) and DFS (P=<0.001). Among the panels, CD123+/CD34+/CD38- was the best discriminator for OS (P=0.005) followed by CD123+/CD34- /CD38+ (P=0.025) and CD34+/CD38- (P=0.025). For DFS CD123+/CD34+/CD38- and CD123+/CD34- /CD38+ were both associated with shorter DFS (P=<0.001); CD34+/CD38- population level at diagnosis had no significant impact on DFS.
Summary and Conclusions: The classical phenotype of CD34+/CD38- is not inclusive; LSC may be present among CD34- and/or CD38+ populations. CD123 and CD133 are sensitive markers, even better than CD34+/CD38-, for the detection of LSC. Higher frequency of leukemia stem cells at diagnosis is associated with shorter OS and DFS.

PB1633

NOVEL TETRACYCLINE TIGECYCLINE EFFECTS ON HEMATOLOGICAL MALIGNANCIES VIA CASPASE 3,9 PATHWAY AND NFKB PATHWAY

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Background: Novel tetracycline derivative tigecycline has an antibiotic effect against a wide range of microorganisms including an activity in multi-resistant drug infections and modulates the production of cytokines and chemokines. FDA approved it for the treatment of complicated infections in 2005. Besides it might affect hematological malignancies. Skrtic *et al.* reported tigecycline's anti-leukemia activity in TEX cell lines and M9-ENL-1 cell lines. The phase I clinical trial has undergone in USA.
Aims: We examined the anti-leukemic effect and elucidated the mechanism of tigecycline. Furthermore, we confirmed tigecycline is also effective on cytarabine resistant (AraC-R) HL60 cell line and clofarabine resistant (CAFdA-R) HL60 cell line. Two cell lines showed 200-fold and 100-fold resistant to parental HL60 cells, respectively.
Methods: To assess the effects of tigecycline on cell viability in acute myeloid

leukemia HL60 cell line, acute lymphocytic leukemia CEM cell line, multiple myeloma U266 cell line and chronic myeloid leukemia K562 cell line, XTT assay was performed. Annexin-PI assay, sub G1 analysis in cell cycle were performed using by flowcytometry in HL60 and CEM cell line. Apoptosis related protein (caspase 3, 8, 9, Bax, Bad, Bclxl, Bcl2) and the phosphorylation of IKKα/β, NFκB were detected by Western Blotting. The effectiveness of tigecycline on AraC-R HL60 cell line and CAFdA-R HL60 cell line, those have been established in our laboratory, were evaluated. Sensitivities on Bcl2 inhibitor (ABT737) and NFkB inhibitor (BAY11-7821) in HL60, CEM, AraC-R HL60 and CAFdA-R HL60 cells, were also examined.

Results: 1) The cell viability was measured at 72 hours by XTT assay. The values of Inhibition Concentration 50% (IC_{50}) of tigecycline in HL60, CEM, K562 and U266 cells were 5.0μM, 2.5μM, 2.7μM and 21.2μM respectively. It was shown that tigecycline was cytotoxic to leukemic cells. 2) Tigecycline induced apoptosis in a dose- and time- dependent manner. In addition, sub G1 analysis showed that tigecycline suppressed S phase in cell cycle. 3) Tigecycline suppressed the expression Bcl2 in a time-dependent manner and inhibited the phosphorylation of NFκB in HL60 cells and CEM cells. Tigecycline activated caspase3, 9. 4) We examined the effectiveness of tigecycline on AraC-R HL60 cells and CAFdA-R HL60 cells. The values of IC_{50} of tigecycline were 6.7 μM (AraC-R) and 2.0 μM (CAFdA-R). ABT737 and BAY11-7821 displayed more effective on resistant cells compared to HL60 parental cells. The values of IC_{50} of ABT737 were 881nM (Parental), 73.3nM (AraC-R), 68.4nM (CAFdA-R). Those of BAY11-7821 were 2.4 μM (Parental), 1.1 μM (AraC-R), 1.5 μM (CAFdA-R).

Summary and Conclusions: Tigecycline showed an anti-leukemic effect on HL60, CEM and K562 cell lines. Tigecycline is presently used for the treatment of certain infections. Pharmacological concentration of tigecycline is useful for treatment of cancer and leukemia. Targeting caspase3, 9 pathway and NFκB pathway, tigecycline induced apoptosis. Furthermore, tigecycline were cytotoxic to both resistant cells. That means tigecycline could be used as the new strategy for treating the recurrent and resistant leukemia. ABT737 and BAY11-7821 also could be new agents, however, they have not been approved for the clinical use. Interestingly, ABT737 showed collateral sensitivity to the resistant cells. Tigecycline mechanically has both effects on leukemic cells. In short, tigecycline might give a marked improvement in anti-leukemic chemotherapy.

PB1634

GENE EXPRESION MARKERS PANEL IN POSTTRANSPLANT RELAPSES EVALUATION

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Background: It is known that up to 50 percent of cases of acute myeloid leukemia (AML) does not have informative genetic markers. At the same time, the studies of donor chimerism do not fully indicate the degree of tumor cells elimination and not always allows to estimate the risk of post-transplant relapse. Thus, finding of universal markers allowing for adequate therapy in post-transplant period, is quite important. *WT1, BAALC, EVI1* and *PRAME* gene expression analysis is one of the possible approaches is this field.

Aims: Evaluation of WT1, BAALC, EVI1, PRAME gene expression markers significance in assessment of posttransplant relapse likelihood of acute myeloid leukemia.

Methods: Our study included 63 patients with AML (1 to 60 years old) (M0-2pts, M1-11pts, M2-13pts, M3-2pts, M4-21pts, M5-11pts, M6-1pt, M7-2pts) who underwent allogeneic transplantation of hematopoietic stem cells (allo-HSCT). In 24 patients myeloablative conditioning regimen were used, 39 – received reduced toxicity protocols. Assessing the levels of *WT1, BAALC, EVI1, PRAME* gene expression and the level of chimeric transcripts was performed by means of RQ-PCR with normalization for ABL gene expression. For the donor chimerism monitoring a panel of STR-markers was used.

Results: As based on gene expression in healthy donors, we have established cut-off overexpression (gene exp. / *ABL* exp. X100) values for the genes: *WT1* – 250, *EVI1*-10, *BAALC* – 20, *PRAME*-200. For the present patient setting, we found no statistically significant differences in *WT1, BAALC, EVI1, PRAME* genes expression between the patients with different FAB variants of AML. However we were able to identify a trend to higher values of *PRAME* and *BAALC* gene expression in patients with M1 variant, and *WT1* gene values among patients with M4 variant of AML. In patients who underwent transplantation in relapse state, we have noted a significant overexpression of *EVI1* (P=0.006), *WT1* (P<0.001), *BAALC* (P<0.001). A similar trend was observed for *PRAME* gene (P=0.08). *EVI1* gene overexpression was revealed for 6 patients (33%), *WT1* in 13 cases (72%), *BAALC* in 10 patients (55%) and *PRAME* in 4 patients (22%). When comparing the data on chimeric transcripts expression, we detected a correlation between expression levels of the studied genes and chimeric transcripts (*PML-RARa* and *RUNX1-RUNX1T1*, P<0.05) as well as with donor chimerism levels. At the same time, we did not find this relationship, when comparing with data about of expression of a *CBFB-MYH11* chimeric gene. When evaluating the test sensitivity and specificity analysis we revealed a bellow-cutoff value of the expressed genes in presence of the chimeric transcript less than 2%.

Summary and Conclusions: Evaluation of universal gene marker expression is an attractive approach for assessing efficiency of therapy in patients with AML. Thus, their use in early diagnostics of relapse in post-transplant period is quite promising. However, due to low specificity caused by basal expression in normal cells, futrher application of these markers is limited, when detecting minimal residual disease levels.

PB1635

PROGNOSTIC VALUE OF HEMATOGONES IN PATIENTS WITH ACUTE MYELOID LEUKEMIA IN FIRST COMPLETE REMISSION

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Background: Hematogons (HGs) are normal bone marrow cells; that may reflect the quality of the bone marrow response to chemotherapy. Many studies have focused on the role of HGs in acute leukemia.

Aims: In our study, we investigated the prognostic value of HGs expression in patients with AML in first complete remission.

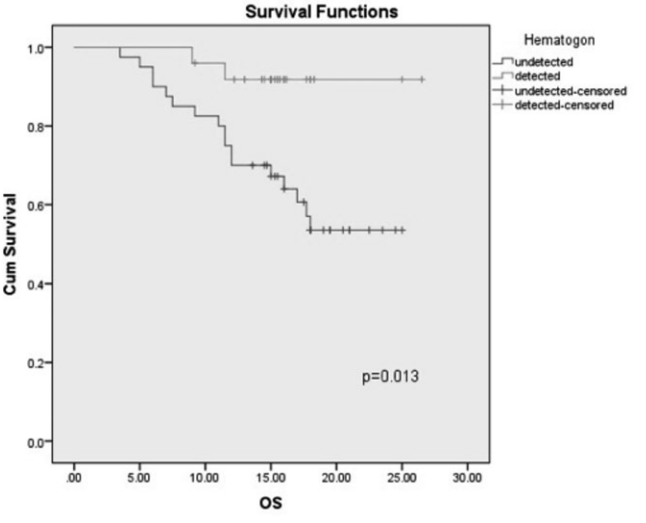

Figure 1. Overall-survival according to HGs status.

Methods: A total of 65 patients with non- promyelocytic AML, in first complete remission were enrolled in this study, and four color flow cytometry was used to quantify Hematogones. We identify the HGs detectable group as those who had more than or equal to 0.01% HGs in bone marrow aspirated sample.

Results: HGs were detectable in 25 patients' marrow samples, and they were significantly associated with cytogenetic risk (P=0.01). After a median follow-up of 17.6 months, patients with detectable HGs had better DFS and OS than those with undetectable levels (P=0.013 and<0.001; respectively) and only 3 patients with detectable HGs in marrow remission samples experience relapse. On multivariate analysis, the HG ≥0.01% is an independent predictive value for DFS (P< 0,0001), and OS (P<0,007), but number of chemotherapy cycles to achieve CR and poor cytogenetic had significant prognostic effect on DFS but not on OS.

Summary and Conclusions: we can concluded that AML patients in first complete remission with HGs ≥0.01% have better DFS and OS.

PB1636

THE MICROARRAY GENE PROFILING ANALYSIS OF ACUTE PROMYELOCYTIC LEUKEMIA CELLS IN RESPONSE TO FISETIN AND HESPERETIN

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Background: Flavonoids are reported to prevent the initiation, promotion and progression of cancer by making changes in various signaling pathways. Although several treatment options exist for acute promyelocytic leukemia (APL), it is not cured effectively due to the development of drug resistance and intolerance and its heterogenic nature. Thus, it is important to develop novel natural treatment approaches in order to improve outcome. Fisetin and hes-

peretin, bioactive flavonoids, found in fruits and vegetables have been reported to be promising novel antioxidants with their potentials as chemopreventive/chemotherapeutic agents in several cancer types such colon, breast and prostate cancers. However, there is no information about the precise mechanisms by which fisetin and hesperetin exert their antileukemic effects.
Aims: We intented to explain the molecular mechanisms and global gene expression patterns modulated by fisetin and hesperetin using genome-wide microarray analysis in APL cells.
Methods: Illumina Human HT-12v4 beadchip microarrays (San Diego, CA) were used to assess global gene expression. Poly-A tail mRNA isolated from fisetin and hesperetin treated HL60 APL cells was converted to biotin labelled c-RNA. The data obtained after hybridization of c-RNA to beadchips was analyzed using Illumina GenomeStudio software to cluster genes. The list of differentially expressed genes constrained by p-value<0.05 and at least 2.0 fold change was obtained. Affected genetic networks were determined by using the Kyoto Encyclopedia of Genes and Genomes (KEGG) and Ingenuity Pathway Analysis (IPA).
Results: A total of 54 and 1608 genes were significantly regulated (P<0.05) in 20 and 50 μM fisetin treated HL60 cells, respectively. Fold change analysis displayed that TXNIP, TFPI, miRNA1974, ID1 and ID3, HSPA1B and IDH1 were altered genes in both 20 and 50 μM fisetin-treated HL60 cells. On the other hand, MAP3K1, Caspase 4 and LASS6 were the examples of upregulated genes while LONP1, STAT5A and STAT3 and JAK1 were some of specifically downregulated genes in 50 μM fisetin treated HL60 cells. KEGG and IPA analysis displayed that MAPK, JAK/STAT and PI3K/AKT signaling pathways and ID signaling pathway were examples of the most altered networks. Moreover, HL60 cells treated with 100 and 150 μM hesperetin changed the expression of 130 and 691 genes (P<0.05), respectively. SASH1, MT1F and SPRR2D were common upregulated genes while TUBB1, ID3, ID1, NMU, FGFR3 and S100P were common dowregulated genes in both 100 and 150 μM hesperetin treated HL60 cells based on fold change analysis. Futhermore, 150 μM hesperetin induced more genes that were either upregulated or downregulated as compared to 100 μM hesperetin. TXNIP, MT1A, MAP3K1 and SPRR2F were some of upregulated genes while RPS25, C-MYC, TUBA1C were the examples of downregulated genes. ID signaling pathway, translation-, gluconeogenesis- and mitosis-related networks, TGF-β and MAPK pathways were the affected signaling networks based on KEGG and IPA analysis.
Summary and Conclusions: Our data show that fisetin and hesperetin trigger growth inhibitory and apoptotic effects by modulating the expression of genes involved in cellular processes. The genetic networks identified in this study enlight some important biological pathways that are altered by fisetin and hesperetin while giving a list of candidate genes that could be targeted for APL therapy. In conclusion, we determined the molecular mechanisms by which fisetin and hesperetin exert pleiotropic effects on APL cells.

PB1637

CHARACTERISTICS OF PATIENTS WITH ACUTE MYELOID LEUKEMIA AND MYELODYSPLASTIC SYNDROMES WITH MONOSOMAL KARYOTYPE

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Background: It has been shown previously that monosomal karyotype (MK) is associated with bad prognosis in patients with acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS). At the same time there are ambiguous data about relationship of MK with other clinical and biological features of AML and MDS.
Aims: To characterize clinical, morphological and cytogenetic signs of AML and MDS patients that are associated with MK.
Methods: A retrospective analysis of 44 patients with AML and 33 patients with MDS was done. To diagnose AML and MDS the criteria of WHO classification was used. Karyotype was studied by standard method. The cases with t(8;21), t(15;17), and inv(16) were excluded from the analyses.
Results: The median age of AML and MDS patients was 64 (17-84) y and 63 y (28-81), accordingly. Number of patients with therapy-related AML and MDS was 11.4% and 12.1%, accordingly. In the MDS group there were 3 (9.1%) patients without excess of bone marrow (BM) blasts and 30 patients had ≥5% BM blasts. So the ratio was 1:10. The most frequent monosomies were -5, -7, and -18 regardless of the diseases. The number of monosomies in individual patients was ranged from 1 till 7. In the AML group the number of cases with ≥3 monosomies was more frequent event in patients ≥60 y than in patients<60 y: 46.4% vs 18.7%; P=0.02. There was correlation between the number of monosomies and AML patients' age: r=0.308; P<0.05. Specific CD expression different from the typical myeloid blasts' phenotype was not found. Median overall survival (OS) of AML and MDS patients were 6 and 8 months, accordingly. Nevertheless there were some long-liver patients: 4 with AML (12-171 mo) and 2 with MDS (20, 38 mo). Three of the AML patients were treated with chemotherapy only and the period of follow-up without relapse were 52, 119 and 171 months. Their karyotype did not characterize by any iterant chromosomal aberration. AlloSCT was realized to the fourth AML patient. Long-liver MDS patients had variant without excess of BM blasts and died after AML transformation or pancytopenia's intensification.
Summary and Conclusions: Monosomal karyotype is the cytogenetic finding that is not associated with patients' age, negative influence of the previous therapy or specific CD expression on the BM blast. But it may be more often event in the group of advanced MDS. AML patients with monosomal karyotype have to be treated aggressively as there is a chance of long relapse-free and OS after standard intensive chemotherapy.

PB1638

MORPHOFUNCTIONAL STATE OF U-937 TREATED WITH PROTEIN KINASES INHIBITOR MALEIMIDE DERIVATIVE IN COMBINATION WITH PHORBOL-12-MYRISTATE-13-ACETATE

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Background: Maleimide derivative (MI-1, 1-(4-Cl-benzyl)-3-Cl-4-(CF_3-phenylamino)-1H-pyrrole-2,5-dione synthesized in Taras Shevchenko National University of Kyiv, Ukraine) is a competitive inhibitor of PDK1, VEGF16, SW-620) *in vitro* and in vivo decreases the number of colon tumors and normalizes an increased number of monocytes and platelets in blood of rat with 1,2-dimethylhydrazine-induced colon carcinogenesis. MI-1 inhibits proliferation and mitotic activity, activates apoptosis, causes shift of cells from G_2/M+S to Go/G_1 stage of human monocytic cells U-937.
Aims: The aim of this study was to investigate the effects of MI-1 on the morphofunctional state of U-937 in combination with agonist protein kinase C phorbol-13-miristat-13-acetat.
Methods: Cells U-937 were incubated (5% CO_2, 100 humidity, 37 ºC) in 96 well plates in RPMI-1640 («Sigma», USA) with 10% FBS («Sigma»), 2 mM Glutamine and 40 μg/mL gentamicin. MI-1 at final concentration of 0.008 mM or 0.016 mM in combination with 100 nM phorbol-12-myristate-13-acetate (PMA) were added to cell cultures and incubated for 24 or 48 hours. The number of live and dead cells was calculated in hemocytometer with 0.1% trypan blue staining. Percent of apoptotic, mitotic or necrotic cells was calculated per 1000 cells in cytospin prepared specimens after Pappenheim's stained. SPSS 16.0 One-Way ANOVA followed by Hochberg and DunnettT3 tests were used. Mean and SD are presented.
Results: MI-1 at 0.008 mM with 100 nM PMA tended to reduce proliferation of U937 by 22% (0,67±0,18; P=0.064), at 0.016 mM and PMA reduced proliferation by 35% (0,56±0,12; P=0.004) in comparison with PMA only (PMA-control 0,86±0,13) after 24 hours exposure. Number of dead cells didn't differ between the indicated groups (0,08±0,03, P=0.944; 0,12±0,03, P=0,12 vs. PMA-control 0,07±0,05). Extension of treatment for 48 hours with 0.008 mM of MI-1 and PMA reduced proliferation by 40% (0,57±0,13; P<0.001), with 0.016 mM-by 62% (0,36±0,09; P<0.001) vs. PMA-control (0,95±0,13). Number of dead cells at 0.008 mM of MI-1 (0,08±0,04; P=0.94) didn't differ from the control (0,10±0,04), at 0.016 mM-increased (0,32±0,09; P=0.008). MI-1 at 0.008 mM and PMA tended to increase by 26% (2,57±0,32%; P=0.245) and at 0.016 mM increased the number of apoptotic cells in 8,5 times (17,17±2,52%, P=0.018) vs. PMA only (2,03±0,31%). The number of apoptotic cells was increased after 48 hours exposure of MI-1 and PMA (10,17±0,15%, P=0.001; 10,73±0,64%, P<0.001 respectively) vs. PMA-control (2,00±0,53%).MI-1 at both investigated concentration with PMA decreased the number of mitotic cells (1,90±0,10%, P=0.005; 1,60±0,10%, P=0.001 respectively) vs. PMA-control (2,50±0,10%) after 24 hours exposure. 48 hours exposure of MI-1 at 0.008 mM and PMA reduced by 20% (1,07±0,15% P=0.526), at 0,016 mM-by 47% of the mitotic cells (0,70±0,10%, P=0.125) vs. PMA-control (1,33±0,31%). The number of necrotic cells after 24 exposure of MI-1 at 0.008 mM and PMA (2,63±0,15%, P=0.001) lower from the PMA-control (5,10±0,36) and tended to reduce at 0.016 mM (4,27±0,31%, P=0.060) but after 48 hours exposure tended to increase (1,90±0,36% P=0.592, 2,17±0,40% P=0.060) vs. PMA (0,87±0,31%).
Summary and Conclusions: MI-1 in combination with PMA reinforces oppression proliferation activity of U-937, reduces mitotic activity and activates apoptosis through inhibiting of VEGFR-, EGFR- and of non-receptor PDK1-, Src- and Syk- kinases that are involved in hematopoiesis.

Acute myeloid leukemia - Clinical

PB1639

CLINICAL CHARACTERISTICS OF PATIENTS AFFECTED BY ACUTE MYELOID LEUKEMIA RESISTANT TO PRIMARY INDUCTION CHEMOTERAPY: AN ANALYSIS OF 385 CASES FROM A SINGLE INSTITUTION

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Background: patients (pts) affected by acute myeloid leukemia (AML) resistant to a first line treatment are a relatively small group with a very poor prognosis. There are few data about clinical and molecular features predictive of primary induction failure (PIF) at baseline. Ravandi observed in a series of 1597 AML patients that higher age, unfavourable cytogenetic, high white blood cells (WBC) count at presentation were associated with PIF while they failed to identify any association with molecular abnormalities such as FLT3 mutations.
Aims: aim of our work was to evaluate clinical and molecular features of PIF AML patients at baseline in order to identify any possible clinical characteristics predictive of PIF.
Methods: Clinical and molecular data at baseline of 385 pts affected by AML fit for standard chemotherapy from 2000 to 2013 were collected in a prospective manner in our institution. The distribution of patients' characteristics were summarized using percentiles, for continuous variables, and percentages and frequencies for categorical variables. In order to test difference in distribution of patients' characteristics according to PIF we performed the Wilcoxon rank sum test for continuous variables and the χ^2 test for categorical variables. We performed a logistic regression analysis to identify risk factors associated of failure to achieve complete remission after induction treatment.
Results: 104 (27%) out of 450 pts resulted refractory to the first line treatment. Clinical characteristics at baseline were as follows: median age 58 (± 19) years, 207 out of 385 pts were male. 25,8% of patients who achieved complete remission showed organomegalies *versus* 28,6% of PIF pts. High WBC count at presentation was evident in 22% of PIF *versus* 23,9% of controls. Median platelets count at presentation was similar in the two subgroups: 60 in controls *versus* 48 in PIF pts. Cytogenetic risk was as follows: 9% of controls and 2,3% of PIF pts were low risk, 23,8% of controls and 39,1% of PIF patients were high risk, the remaining pts were intermediate risk. The majority of the pts (64%) were treated by standard dose anthracycline based induction regimen, while 26% were treated by fludarabine based regimen and 10% were treated by high dose chemotherapy. At univariate analysis age, unfavourable cytogenetic risk and fludarabine based regimen were predictive factors of induction failure (OR 1,02, 2.05 and 1,77 respectively with 1,01-1,04, 1,22-3,45 and 1,09-2,87 95% CI) while NPM mutated status, high dose ARA-C regimen and favourable cytogenetic risk were associated with complete remission (OR 0,33, 0,4 and 0,23 with 0,1-1,07, 0,17-0,99 and 0,05-1,02 95% CI). We failed to identify a statistically significant association with WBC, platelets count and FLT3 status and PIF status.
Summary and Conclusions: In our experience the only predictive factors of PIF in adults affected by AML are higher age and unfavourable cytogenetic at baseline. Flt3 status is confirmed not to be a molecular marker of treatment failure while NPM mutation seems to have a positive predictive value but the data need to be confirmed in a larger series of cases. Even if our study is only observational, our data showed that pts treated by conditioning regimen based on high dose ARA-C showed a better outcome than pts who underwent fludarabine based induction chemotherapy. Further studies are needed in order to identify more clinical and biological factors predictive of primary treatment failure at baseline.

PB1640

EFFICACY OF DECITABINE IN 10-DAY REGIMEN IN ELDERLY PATIENTS WITH ACUTE MYELOID LEUKEMIA INELIGIBLE FOR INTENSIVE CHEMOTHERAPY

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Background: Currently the hypomethylating agents (azacitidine, decitabine) are safe and effective alternative treatment for patients with acute myeloid leukemia (AML) that do not fit for intensive chemotherapy treatments. Several studies have been described the modification of the treatment regimen with hypomethylating agentes. We analyzed the effectiveness of decitabine in patients with acute leukemia not eligible for intensive therapy.
Aims: The objective of this study is to analyze the safety and efficacy of using decitabine in 10-day regimen.
Methods: We reviewed 11 patients with AML treated with DEC between June 2010 and January 2015. The schedule was DEC 20 mg/m2x 10 days in induction therapy. Patients who achieved remission cytological (defined as<5% blasts in the bone marrow aspirate) received consolidation therapy with DEC 20 mg/m2x 5 days until progression and the remaining patients received a re-induction before start consolidation therapy.
Results: 36.4% were de novo AML 63.6% were secondary AML (2 myelofibrosis, 4 MDS and CMML 1). All patients were male with a median age of 82 years old (range 69-86) and 36.4% patients had cytogenetic abnormalities in diagnosis according to European Leukemia Net (ELN): 6 patients were favorable risk, intermediate risk 1 and 3 high-risk. 54.5% patients had>30% blasts and 45.4% received treatment previously. 91% of patients had some comorbidities. A median of 6 cycles (range 1-10) were administered in total 57 cycles of decitabine. All patients had adverse effects during treatment, the most frequent febrile neutropenia (63%) and there was an early death (<8 weeks) associated with liver failure in a patient previously diagnosed. According to ELN, with induction in 10 days, we had a global response rate of 72.7% (2 CR with cytogenetic remission; 2 CR, 4 CR with incomplete hematologic recovery, 1 PR and 3 patients with refractory disease. Secondary AML, transfusion dependence and number of blasts>30% at diagnosis, had no effect on treatment response induction. In our study 6 patients died because disease progression infection. Median follow-up of 340 days (range 148- 1506 days) with OS of 63% in 1-year and 45.5% during the follow up with a DFS of 45.5%.
Summary and Conclusions: In our experience, decitabine in 10-day regimen in elderly patients is safe and very effective, even in poor prognosis patients.

PB1641

INTENSITY OF CHEMOTHERAPY INFLUENCING AN OVERALL SURVIVAL OF PATIENTS WITH ACUTE MYELOID LEUKEMIA OLDER THAN 70 YEARS

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Background: Acute myeloid leukemia (AML) is common disease in people aged >70 years. While the best management of AML is based mainly on intensive chemotherapy (CT) in younger patients, are still remains a matter of controversies in the elderly. In elderly AML patients, intensive CT is often poorly tolerated and gives scanty results.
Aims: The aim of this study was to identify the patients with AML not eligible for CT and who could be suitable only for supportive care.
Methods: This single-center study involved 68 patients aged >70 years (range 71-85) with nonpromyelocytic AML during follow-up of 5 years. Patients were treated by three type of therapeutic regimen with various intensity: CT (induction therapy with daunorubicin 30 mg/m^2 on day 1 and 3 and cytarabine 100 mg/m^2 given for 5 days; or mitoxantrone 10 mg/m2 and etopozide 100 mg/m2 given for 5 days), paliative therapy (Hydroxiurea, Etoposide or 6-mercaptopurine per os) and supportive therapy (transfusions). Comorbidities were evaluated by using the hematopoetic cell transplantation-specific comorbidity index (HCT-CI). Performance status (PS) was evaluated by Eastern Cooperative Oncology Group (ECOG), ranged 0-4. Cytogenetic risk group was assessed by recomandation of Europian LeukemiaNet (ELN). The following parameters were estimated as risk factors for treatmement with CT: age >75 years, leukocytopenia (white blood count<4x10^9/L), thrombocytopenia (platelet count<50x 10^9/L), higher absolute peripheral blasts (>5x 10^9/L), ECOC PS (<2 vs ≥ 2), ELN cytogenetic risk group, and HCT-CI (<3 vs ≥ 3). Risk factors were identified using the univariate and multivariate analysis.
Results: In this group of patients, 16 pts treated with CT, 24 pts treated with palliative and 28 pts with supportive therapy. In group of patients treated with CT, univariate analysis showed that the following risk factors were significant for OS: leukocytopenia (P=0.039), thrombocytopenia (P=0.043), higher absolute peripheral blasts (P=0.002), ECOG PS ≥ 2 (P<0.001) and HCT-CI ≥ 3 (P< 0.001). Multivariate analysis indicated HCT-CI ≥ 3 as the most important risk factor for poor OS of patients treated with CT: P<0.001, relative risk (RR)=3.689; 95% confidental interval (CI)=1.817-7.489. Patients with leukcytopenia and HCT-CI ≥ 3 were separated in single group and in this group (17 pts) most benefit for OS had patients treated only with supportive therapy: P=0.043, RR=0.382, CI=0.131-1.115.
Summary and Conclusions: Elderly AML patients with cytopenia and comorbidities are not suitable for intensive CT. In these patients supportive care need to be concidere as a choise of therapy because the intensive CT has a poor influence on its OS.

PB1642

MYELOID SARCOMA: CLINICAL FEATURES AND OUTCOMES AFTER INTENSIVE TREATMENT, TEN YEARS EXPERIENCE AT A SINGLE CENTRE.

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Background: Myeloid Sarcoma (MS) is a rare neoplasm composed of immature myeloid cells potentially occurring in any extramedullary organ, most commonly in the lymph node and soft tissues. MS develops before, concurrently or after the diagnosis of acute myeloid leukemia (AML), but can rarely occur without bone marrow infiltration by leukemia. MS is associated with poor outcome. The rarity of this presentation has been an obstacle to characterize its clinical features and prognosis.
Aims: The aim of the study was to analyze characteristics, treatments and overall survival of all patients presenting with MS.
Methods: We retrospectively reviewed medical record of patients who presented with MS to our institute from 2004 through 2015. In total 28 patients with MS were identified.
Results: Thirteen patients had a MS at disease onset, 2 as primary sarcoma and 11 associated with AML, 15 developed MS at relapse of AML, 9 patients had only extramedullary relapse (EM), 6 also bone marrow involvement (table 1). The median age of patients at MS presentation was 45 years (range 22-76). The most commonly involved site for patients with MS at disease onset was abdomen (gastrointestinal tract and kidney.) Six out of 13 patients had cytogenetic abnormalities involving chromosomes 8, 21, 16, Y; karyotype was normal in 3/13 and complex in 2/13. Cytogenetic was not available in 2/13 cases. Eleven out of 13 patients with MS at diagnosis received allogeneic stem cells transplantation (alloSCT) as post-remission treatment and 2 patients were treated with palliative chemotherapy due to old age. After a median follow-up of 560 days (range 108-3715) 8/13 patients (61%) are alive and 6/13 (46%) are disease free. Among the 15 patients who have developed a MS at AML relapse the most frequent location was paravertebral (table 1) The median age at presentation was 46 years (range 22-65). Seven out of 13 available cytogenetic analysis were normal, 5 complex and 1 was t(8;21) plus delY. The median time from AML diagnosis to EM relapse was 657 days (range 308-2312) and in 14/15 cases EM occurred after alloSCT, with a median time to relapse of 444 days (range 254-3195). Salvage treatment of these patients: 6 alloSCT, 3 immunotherapy, 4 chemotherapy, 2 palliative radiotherapy. At last follow up three out of 15 patients (20%) are alive and in complete remission from AML and MS (median follow-up was 350 days, range 39-1847).

Table 1. Sites of Myeloid Sarcoma.

sites of MS	MS at disease onset	MS at relapse of AML
mediastinic	1	
addomen	5	2
paravertebral	1	4
mammary	1	1
nodes	1	
multiple (at least 3 sites)	3	3
retrorbital	1	
nasopharynx		1
mucosal		1
testis		1
bone		2

Summary and Conclusions: With this study we provide a single institution experience of MS management and outcome. Our Institution is a referral centre for alloSCT thus explaining the high rate of alloSCT in this series. Our data support alloSCT as part of first line treatment of MS after chemotherapy. EM relapse of AML after first line therapy has a poor prognosis even after intensive treatment. The high rate of EM relapse after alloSCT (14/15) suggests that MS may represent a form of post alloSCT AML immune escape not necessary related to sarcomatous presentation at onset. Larger multicenter studies are needed to better and more fully assess outcomes of these patients.

PB1643

PRELIMINARY RESULTS OF ACUTE LEUKEMIA EPIDEMIOLOGICAL ESTIMATIONS BASED ON CLINICAL REGISTIRY DATA IN RUSSIA

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Background: The incidence of acute leukemia (AL) in US and European countries is about 5 per 100000 according data from cancer registries. Russian Federal Cancer Register reported AL incidence rate across the country as about 3 per 100000. Russian Hematology Society in 2013 initiated the AL population based registry study in which participated 8 regions of Russia Federation. The primary goal was to pre-registry patients for clinical studies, and secondary-to evaluate the epidemiology of AL. 5 regions were included into analysis of epidemiology statistics. The criteria were the fullness and reliability of AL cases registration. Also, only regions with one big hematological center accumulating all AL cases were chosen. All new cases were recorded on-line into special Web based data capture system.

Table 1.

	Adult population (10^6)			Registered cases			Duration of registration (y)	Crude incidence		
Region	All	M	F	All	M	F		All	M	F
Kirov	1.15	0.52	0.63	41	19	22	1.96	1.82	1.87	1.78
Mordovia	0.72	0.33	0.40	44	20	24	1.88	3.24	3.26	3.22
Ryazan	1.01	0.45	0.56	37	23	14	1.48	2.48	3.45	1.69
Kaluga	0.88	0.39	0.48	19	13	6	0.87	2.50	3.80	1.44
Tambov	0.95	0.43	0.52	27	19	8	1.56	1.82	2.85	0.98

Aims: To evaluate the age-gender specific incidence, profile and other epidemiologic characteristics AL in Russian regions.
Methods: The registry was started at 1st April of 2013 and is ongoing.
Results: 169 new ALL cases from Kirov, Ryazan, Kaluga, Tambov regions and Mordovia republic (4,7 M of population) were included into analysis. The distribution by AL subtypes was following: AML-109 (74,6%), ALL - 31 (21,2%), APL-5 (3,4%), other-1 (0,7%). Median age was for AML-59 (17-85), ALL - 38 (18-80), APL 54 (38-60) years. Gender female/male proportions in AML were – 18/13, in ALL - 54/55, in APL - 4/1. 105 (62,5%) patients are alive; 58(34,5%)-died, 5 (3%) were withdrawn. The incidence rate estimates by region are listed in Table1. Two regions (Kirov region and Mordovia) with longest duration of registration were taken for age specific incidence calculations. Incidence of AL by age is following: in 15-19 age group – 0.90; in 20-39 age group – 1.13; in 40-59 age group – 2.45; in 60-79 age group – 3.54; in 80-99 age group – 2.18. Total crude incidence in this two regions is 2.09, standardized by WHO population – 1.87.
Summary and Conclusions: The first estimations of AL epidemiology in selected Russian regions was made based on direct registration data from hematological centers. The incidence rate estimations are less than expected. Some explanations may be: incomplete registration of cases, under -diagnostics of AL, non-admission of AL patients to hematological center.

PB1644

AML-PATIENTS FROM A SOCIALLY DISADVANTAGED ENVIRONMENT SHOW POORER THERAPEUTIC OUTCOME

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Background: The socioeconomic status (SES) is strongly associated with the development and clinical course of several malignant and non-malignant diseases. Only few studies examined the clinical outcome of AML patients in relation to indicators of the social environment at their area of residence, showing divergent country-specific results. So far, no evaluation of area level social differences for survival in AML-patients has been conducted in Germany.
Aims: The aim of the study was to investigate if the social environment in the area of residence is related to complete remission and overall survival of individual AML-patients in the city of Aachen.
Methods: All patients who were treated with a confirmed diagnosis of AML from 1996-2014 at the University Hospital in Aachen were assessed retrospec-

tively (n=139). Clinical and prognostic parameters like age at diagnosis, sex, cytogenetic risk group, primary or secondary AML, curative or palliative intention of treatment were recorded. Patients were assigned to geographical areas using administrative area codes. We classified the social status at the area of residence using area-specific socioeconomic indicators (*e.g.* proportion of unemployed, crime rate). Based on this index we divided the sample into patients living in high or medium SES areas and those living in low SES areas. Survival analyses were performed using Kaplan-Meier estimator and Log-rank test. Furthermore univariate and multivariate Cox-regression analyses were conducted.

Results: First of all, the distribution of prognostic factors (age at diagnosis, cytogenetic risk group, therapeutic intention) was assessed for the two social environments. No significant differences were detected. Survival analyses for all patients and defined subgroups (stratified by age and therapeutic intention) were performed. Younger patients (≤65 years) who received induction therapy with a curative intention showed a significant difference in overall survival (P=0.0084) between the two social spaces (Figure 1).

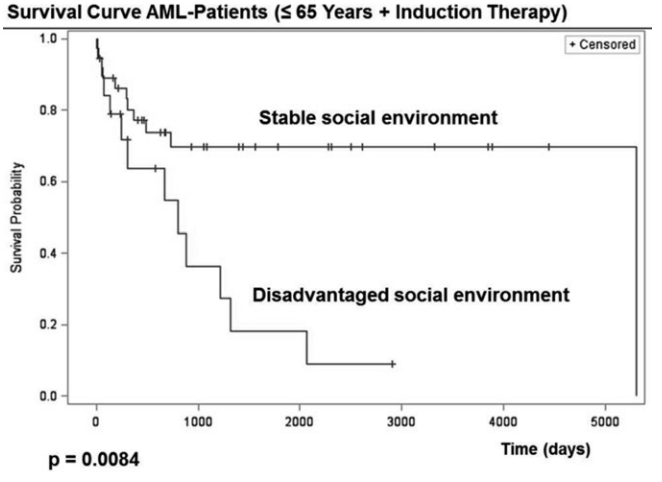

Figure 1.

In the assessment of the clinical course, patients from a social disadvantaged environment reached significantly less often a complete remission (23.1% vs. 12%, P=0.0039).

Summary and Conclusions: Similar to several solid tumors the socioeconomic environment in the area of residence was significantly related to the clinical course in younger patients with AML. A detailed evaluation of the underlying mechanisms is needed for the improvement of this unsatisfactory situation. As these are findings from analyses not specified in advance confirmation with independent data is needed.

PB1645

FAVORABLE EFFECT OF PRIMING WITH GEANULOCYTE COLONY-STIMULATING FACTOR IN REMISSION INDUCTION OF HYPOCELLULAR ACUTE MYLOID LEUKEMIA

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Background: The hypocellular variant of acute leukemia is very rare atypical leukemia which mainly occurs in older patients. It ussually has a myeloid phenotype. Survey of Pub Med have shown that there are no guidelines in the world literature for the treatment of such subtype of AML. The effect of G-CSF priming has not been investigated as a key constituent of remission induction chemotherapy in this cohort of patients. We point out the need for such contribution of world centers to show the best way for good outcome of these rare subtype of acute myloid leukemia.

Aims: The aim was to evaluate the results of treatment with different protocols.

Material and methods. We retrospectively analyzed all hypocellular acute myeloid leukemias which were treated in ten years period, between january 1990 and december 2010 at the Clinic of Hematology, Clinical Center Belgrade. All diagnostic criteria were met: pancytopenia with blasts in peripheral blood, less than 20% of bone marrow cellularity and more than 20% of blastas in bone marrow which were myeloperoxidase positive responding to myelod phenotype. There were 30 patients, 18male 12female. The median age of the patients was 58,9 years (ranging from 32-76 years) and median cellularity of bone marrow of this group of patients was 16%. Only one patient had myelodysplastic syndrome as antecedent disorder. No one of presented patients received prior chemoradiotherapy for non-hematopoietic neoplasms.

Results: All patients at presentation had leucopenia ranging from 0.3-2.0 (with SD 0.4). The distribution according to FAB Classification were as follows: 5 patients(pts) had M0 (16%), 11 pts M1 (36%), 6 M2 (20%), M4 1pts(3%), M5a 2 pts(6%) and M7 1 pts (3%) and 4 patients had unclassified acute myeloid leukemia. The ECOG performance status was: 17 patients had favorable performance status (1 PS was in 4 pts and 2 in 13 pts – 56,6%), status 3 in 8 (26,6 %) and 4 in 5 (1.3%). Sixteen patients had a normal diploid karyotype (or –Y in one), in seven patients cytogenetic analyses were inadequate, in four patients it was not done, in one 47XY, der(13)/46,XY, and another 46,XX, t(8;20) ad finally 46,XX,add(9)(p24)[6]/46,XX[14] was found. AML M1 was the most common subtype. Followed by M2 and M5a. Twelve patients received priming with G-CSF followed by standard 3+7 regimen, 5 patients GCSF plus small doses of cytosine arabinoside, 8 patients only support and 5 patients hydoxiurea. Thirteen patients (43%) achieved a complete remission, 11 treated with G-CSF followed by standard induction chemotherapy and 2 patients treated with GCSF and low doses of cytosine arabinoside. Disease free survival was from 4 months to 72 months (median 14 months). On multivariate analysis overall survival, remission duration and event free survival were comparable to other patients with acute myeloid leukemia.

Summary and Conclusions: This report describes a cohort of patients with hypocellular AML characterized by prominent cytopenias, older age, low bone marrow cellularity, cytogenetic findings and distribution of morphologic subtypes similar to normocellular AML. We found that patients treated with priming G-CSF achieved the best CR rate and OS which were statistically significantly longer(Log rank P<0.0001) (P<0.008) then the rest of the cohort patients.

PB1646

MOLECULAR ANALYSIS OF FUSION POINTS AND RELATIVE EXPRESSION OF MEIS1, EVI1, HOXA5 AND FLT3 IN MLL-MLLT10 HARBORING AML PATIENTS

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Background: A frequently occurring chromosomal aberration in acute myeloblastic leukemia (AML) is a translocation involving the mixed lineage leukemia (*MLL*) gene at 11q23 with one of more than 70 partner genes described to date. Although, in general, *MLL* rearrangements are related with a poor outcome, there are great differences based only on the translocation partner of *MLL*. Up to 15% of all *MLL* rearrangements in AML are due to *MLLT10* fusions. *MLL*-rearrangements result in an upregulation of *HOX* transcription factor genes. Moreover, *EVI1* and *MEIS1* upregulation are significantly associated to a poor prognosis in AML and not all *MLL*-rearranged leukemia cases overexpress these genes.

Aims: In this work we report on the molecular analysis and gene expression profiling for *MLL-MLLT10* AML.

Methods: *MLL* gene fusion was detected by cytogenetics; FISH with LSI *MLL* dual color, Break Apart probe, and WCP 10 and 11 probes; reverse transcription PCR (RT-PCR) and sequencing. Gene expression was analyzed by real-time PCR in a reaction with specific primers and SybrGreen. Relative quantification was performed by 2^{-DDCt} method. We include analysis of two *MLL-MLLT10* harboring leukemias of the FAB-M5 subtype. Both patients obtain complete remission (CR) with positive MRD and disease relapse 4 months after diagnosis. Patient 1 die 3 months after transplantation and second one is currently receiving rescue chemotherapy.

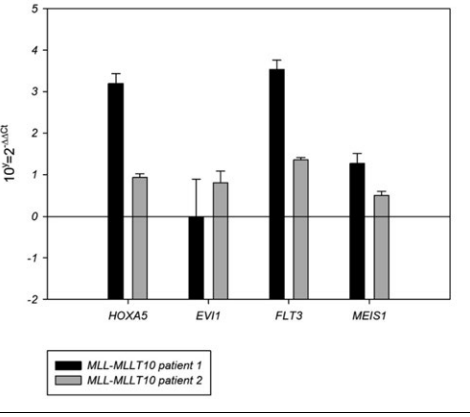

Figure 1. Gene upregulation in MLL-MLLT10 harboring AML.

Results: Patient 1 cytogenetic analysis revealed 46,XY,del(10)(p12),add(11)(q23)[20]. FISH analysis using *MLL* locus specific probe showed a 73% of an uncertain pattern with two nearby split signals. Metaphase FISH with WCP confirmed that chromosome 10 material was present at 11q and that the chromosome 11 material had not been transferred elsewhere in the genome. Patient 2 presented a 46,XX[20], and the same *MLL* FISH pattern that patient 1. Molecular analysis for the *MLL-MLLT10* fusion demonstration was performed based

on the presence of NG2 by flow cytometric analysis and a FISH pattern suggesting a gene insertion in 11q23. The cDNA was used in multiplex PCR reactions for detection of the MLL-MLLT10 fusion, and PCR product was sequenced. In patient 1, a mRNA fusion between exon 7 of 5' MLL mRNA and exon 15 of 3' MLLT10 mRNA was detected, with frame conservation and the whole sequence of both exons (GenBank ID: KF778381). The breakpoint described here is unusual in 10;11 rearrangements and the same fusion at mRNA level was previously described in only one case. In the second case, the same RT-PCR analysis revealed an mRNA fusion between exon 8 of 5' MLL mRNA and exon 16 of 3' MLLT10 mRNA. This MLL-MLLT10 fusion was first described in patient C from Chaplin, *et al.* 1995. Gene expression analysis showed overexpression of *HOXA5, FLT3* and *MEIS1* in both cases but *EVI1* was only overexpressed in patient 1.
Summary and Conclusions: Molecular studies are required for *MLL-MLLT10* detection in cryptic rearrangements and, as illustrated in this work, for the identification of new *MLL-MLLT10* fusion points. Gene expression analysis shows overexpression of *MEIS1* in both *MLL-MLLT10* patients, previously related to poor outcome, and *EVI1* only in patient 1.

PB1647

PREVALENCE AND PROGNOSTIC IMPACT OF IDH1 AND IDH2 MUTATIONS AMONG CYTOGENETICALLY NORMAL AML EGYPTIAN PATIENTS

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Background: Acute myeloid leukemia (AML) is a clonal malignant disease of haematopoietic tissue caused by somatic mutations in genes that control normal cell proliferation and differentiation.The molecular genetic alterations is one of the most important prognostic factors that have been identified in AML and the role of this genetic alterations has been emphasized by the 2008 revised WHO classification of AML like FLT3, NPM1, and CEBPA. The isocitrate dehydrogenases (IDHs) are enzymes that catalyze oxidative decarboxylation of isocitrate into ketoglutarate by using NAD or NADP as a cofactor to yield NADH or NADPH, respectively.
Aims: This study aimed to evaluate the clinical impact and prognostic relevance of IDH1 and IDH2 mutations among cytogenetically normal AML patients.
Methods: Two hundred and eleven patients were assessed for the presence of IDH1 and IDH2 mutations by sequencing.
Results: IDH1 mutation was detected in 18 out of 211 AML patients (8.5%) and IDH2 mutation was detected in 22 out of 211 AML patients (10.4%). The IDH1 mutant cases were more older age, female, with high platelet count (P 0.01), high bone marrow blast cells count (P 0.002) and the NPM1 mutation was more common in wild *versus* mutated groups (81.1% vs 18.9% respectively with P<0.001). The IDH2 mutation was more common in older age, normal karyotyping (P 0.001), with low WBCs count (P 0.003) and high platelets count (P<0.001). The induction of remission rate wasn't significantly associated with neither IDH1 nor IDH2 mutation status. Patients with mutant IDH1 showed poor OS *versus* patients with wild type (6 vs 9 months respectively; P 0.07), however there was no statistically significant relation between IDH2 mutation and OS. In this study, there was 24 patients with CN-AML patients (ie: NPM1 mutation and without FLT3 mutation), IDH1 mutation in this group showed significantly shorter overall survival *versus* patients with IDH1 wild (5 vs 13 months respectively; P 0.02).
Summary and Conclusions: IDH1 and IDH2 mutations occur in 8.5% and 10.4% respectively in AML patients which is a minor subset of the newly diagnosed cases and these mutations have an association with molecular and cytogenetic status. We recommend molecular testing for *IDH1* and *IDH2* mutations to confirm the role of these mutations as novel genetic markers in risk stratification of AML patients in a larger study.

PB1648

ACUTE ANTHRACYCLINE CARDIOTOXICITY IN ACUTE MYELOID LEUKEMIA DURING INDUCTION CHEMOTHERAPY: ROLE OF ECHOCARDIOGRAPHY

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Background: Anthracyclines are used in induction treatment of acute myeloid leukemia**.** However, they cause dose-dependent cardiac dysfunction through generation of reactive oxygen species, mediated by topoisomerase -IIb in cardiomyocytes and forming complexes with intracellular iron, leading to lipid peroxidation and DNA damage. Due to low potential for regeneration in cardiac myocytes, these changes are likely irreversible. Early detection of cardiotoxicity is important before changes become clinically overt. Left ventricular ejection fraction (LVEF) has been the main indicator of cardiac dysfunction but impairment in LVEF is detected only after considerable myocyte loss has occurred. So, more sensitive tools to detect these alterations are warranted.
Aims: To study acute cardiac toxicity of anthracycline in acute myeloid leukemia (AML) patients by serial echocardiographic parameters during induction chemotherapy.
Methods: The prospective study included newly diagnosed AML patients. Patients with history of previous anthracycline exposure were excluded. AML induction therapy consisted of daunorubicin 60 mg/m^2/day for 3 days and cytarabine 100 mg/m^2/day for 7 days. All patients were assessed by echocardiography at start and end of induction chemotherapy and after 3 days of daunorubicin. Parameters on M mode, Doppler and tissue Dopper modes at mitral valve (lateral) and septum were recorded.

Table 1.

				P value		
Parameter	Baseline(B) Mean+SD (n= 32)	Mid (M) Mean+SD (n= 29)	Post (P) Mean+SD (n=16)	B vs M	B vs P	M vs P
E vel (cm/s)	94.7+20.7	83.4+21.0	73.4+19.5	0.2	0.02	0.6
A vel (cm/s)	63.5+19.2	57.1+20.1	67.1+17.6	0.09	0.9	0.5
E/A	1.6+0.6	1.6+0.6	1.1+0.4	0.6	0.4	0.2
Sm (lateral)(cm/s)	11.6+2.4	10.6+2.6	10.4+2.3	0.7	0.1	0.9
Sm (septal)(cm/s)	8.9+1.4	8.6+1.6	8.3+1.5	0.9	0.9	0.9
Em (lateral)(cm/s)	16.4+4.6	14.4+3.8	12.9+4.1	0.6	0.002	0.2
Em (septal)(cm/s)	11.4+3.5	9.6+2.8	9.1+2.5	0.5	0.04	0.9
Am (lateral)(cm/s)	8.9+2.6	7.7+2.2	6.7+2.0	0.4	0.001	0.2
Am (septal)(cm/s)	8.5+2.4	8.0+1.9	7.8+2.1	0.9	0.4	0.9
LVEF (%)	65.2+5.9	63.7+7.1	63.6+9.0	0.2	0.5	0.9

Results: A total of 32 patients of AML were enrolled with median age of 21 years (range 7-57 years) and 18 males (56.2%). Median leucocyte count was 6300/uL (range 300-380,000). 16 patients (50%) had fungal infection during induction and 14 patients expired (44%). Cardiac chamber dimensions were not altered during induction. Doppler parameters: E velocity decreased in AML patients (P value 0.023). A velocity did not changed significantly. E/Ashowed overall decreasing trend (P value 0.09). Tissue Doppler parameters: Em lateral decreased (P value 0.005) in AML patients. Em septal decreased from baseline to post chemotherapy echo (P value 0.04). Am lateral dropped from baseline to post chemotherapy echo in AML patients (P value 0.001). Sm lateral and septal were not altered significantly. Isovolumetric contraction time was significantly increased from baseline to post chemotherapy echo (P value 0.019). Ejection fraction did not show significant changes during induction.
Summary and Conclusions: Significant changes occur in various Doppler and tissue doppler parameters during induction chemotherapy reflecting underlying diastolic dysfunction due to anthracycline induced cardiotoxicity. Ejection fraction is relatively insensitive tool to ascertain these early changes. It is suggested that these patients be followed up with repeated ECHO to find improvement or worsening of these changes before any overt clinical symptoms due to heart failure develop. Early interventions can be planned before overt heart failure in consultation with cardiologists especially while planning treatment for patients with relapsed disease or transplantation.

PB1649

ELLIPTICINES DRIVE LEUKAEMIA CELL DEATH VIA MITOCHONDRIAL ROS

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Background: Ellipticine is an anticancer agent which induces cell death by multiple mechanisms of action[1]. Unfortunately, this compound and its analogues have limited solubility and have been associated with several toxic effects which has hampered their clinical development. A panel of novel ellipticine isomers was designed and synthesised with the aim of evaluating their cytotoxic effects[2]. A preliminary NCI 60-cell screen demonstrated that these compounds display promising anti-tumour activity across a number of different cancer cell types, particularly leukaemia cell lines.
Aims: We examined the effect of these derivatives in detail on the acute myeloid leukaemia (AML) cell line, MV4-11. The most cytotoxic compound, 7-formyl-10-methylisoellipticine was identified from this panel. The mechanism by which this compound induces its cytotoxic effects was investigated.
Methods: Cell cycle was monitored by flow cytometry. Cell number and viability was measured by dye exclusion. Confocal microscopy was used to visualise compound accumulation. Protein levels were evaluated by Western blotting. Reactive oxygen species (ROS) levels were measured by flow cytometry using the ROS probes dihydroethidium and MitoSOX.
Results: Cell cycle analyses revealed that the compounds had a range of distinctive cell cycle effects[3]. 7-formyl-10-methylisoellipticine showed the most promise with respect to cytotoxic activity. We demonstrated that this compound killed cells by apoptosis which is mediated by an induction of reactive oxygen species (ROS). In a cell, mitochondria and NAPDH oxidase (NOX) are the

major sources of ROS. Previous research in our laboratory has shown that NOX derived ROS is critical for cell survival[4]. ROS inhibitors were employed to reveal the origin of the ROS. Our study suggests that the compound generates ROS in mitochondria which ultimately leads to its cytotoxic effect. Interestingly synergistic effects are seen when 7-formyl-10-methylisoellipticine is used in combination with a NOX inhibitor as well as the clinically used AML chemotherapeutic, daunorubicin.

Summary and Conclusions: This study provides detailed cellular information on the potential use of isoellipticines as chemotherapeutic agents. By probing the mechanism of action of this novel compound class we have uncovered a potential clinical application in the field of adjuvant therapy. We are currently investigating the effects of the compound in a pre-clinical AML mouse model.

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PB1650

TREATMENT OUTCOME OF CHILDREN WITH DE NOVO AML GROUPED BASED ON RISK FEATURES AND TREATMENT RESPONSE

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Background: Approximately 80-100 children are newly diagnosed as AML in Korea. The survival rate of pediatric AML has improved due to intensified well-designed chemotherapy, better supportive care, risk-based treatment adaptation and allogeneic HSCT. However, relapse still remains the leading cause of events, which accounts for 30 to 40%. Because pediatric AML is a heterogeneous disease and only some subgroup of patients benefits from allogeneic transplantation in 1st CR, risk-adapted treatment strategy is needed.

Aims: This multicenter pilot study was aimed to evaluate the feasibility of new treatment protocol based on risk stratification for newly diagnosed pediatric de novo acute myeloid leukemia (AML).

Methods: Patients with acute promyelocytic leukemia and AML of Down syndrome were excluded. Risk features were defined as follows: 1) low risk features (LRF): inv(16)/t(16;16), t(8;21) without c-kit mutation, or with normal karyotype in the presence of NPM1 or CEBPA mutation without FLT3/ITD mutation; 2) high risk features (HRF): -5, 5q-, -7, 3q abnormalities, t(8;16), t(6;9), t(16;21), t(6;11), t(10;11), AMKL without t(1;22), complex karyotype, or FLT3/ITD mutation; 3) standard risk features (SRF): all the others. A new chemotherapy regimen was developed which consisted of 2 induction and 4 consolidation cycles. Treatment responses were determined according to the marrow response at the end of each induction as good response (CR-CR), delayed response-1 (PR [blast 5~15%]-CR), delayed response-2 (NR [blast>15%]-CR), refractory (PR/NR-PR/NR), and early relapse. Patients were allocated into either favorable (FG), intermediate (IG), or poor (PG) prognostic group considering both risk features and treatment response. Patients in FG were not allocated to transplantation.

Results: Fifty-seven patients were enrolled. There were 13 patients (22.8%) with LRF, 22 (38.6%) with SRF, and 22 (38.6%) with HRF. The overall CR rate following 2 cycles of induction was 87.5%. One patient (1.8%) died during induction therapy. Eight patients relapsed of whom 6 were in PG, 1 in IG, and 1 in FG. Twenty-three patients underwent allo-HSCT. With a median follow-up of 13 mo, the estimated 2-y overall survival and event-free survival of all patients were 91% and 71%, respectively.

Summary and Conclusions: Although follow-up duration is short, our new treatment protocol based on risk stratification seems effective.

PB1651

PROGNOSTIC SIGNIFICANCE OF INTRACELLULAR SURVIVIN IN MYELOID BLAST CELLS AS AN INHIBITOR OF APOPTOSIS IN EGYPTIAN ADULT ACUTE MYELOID LEUKEMIA (AML) PATIENTS

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Background: The balance between cell death and cell viability is important in tissue homeostasis. Abnormalities in the control of apoptosis play an important role in tumorigenesis (Altieri Cancer 2013). Survivin is one of eight members of the inhibitor of apoptosis protein family (IAP) that, regulates, integrates cell division and suppresses apoptosis. Over expression of several IAPs has been detected in various hematological malignancies, including acute leukemias, myelodysplastic syndrome, chronic myeloid leukemia, and many types of lymphoid malignancies, such as chronic lymphocytic leukemia and diffuse large B-cell lymphoma. Many publications revealed significant correlation between a high level of IAPs, especially of XIAP and survivin, and tumor progression contributing to leukemogenesis due to deregulated apoptosis. The expression of survivin may be a general feature of cancer and alone or with other antiapoptosis genes such as Bcl-2, survivin may extend the viability of transformed cells and regulate their susceptibility / resistance to apoptosis-based therapy. For this reason survivin may provide an ideal therapeutic target for its selective expression in neoplasia. Survivin shuttles between the nucleus and the cytoplasm, it effectively inhibits apoptosis, by binding to second mitochondrial activator of caspase.Expressed during embryonic development and by many cancer cell types,but not in the differentiated normal tissue,survivin is implicated in control of cell survival and regulation of mitosis in cancer(Coumar *et al*. Cancer 0013).

Aims: To assess expression of survivin in Egyptian AML patients, and its correlation to outcome, impact on progression, and survival.

Methods: 120 adult Egyptian patients with AML (52 females and 68 males) were recruited and followed up for 2 years, (mean age:42.1±13.1years), 16 patients had AML-M0, 32 AML- M1,32 AML- M2, 12 AML- M3, 16 AML -M4 and 12 AML-M5 (FAB classification). A control group of 60 age and sex matched normal healthy volunteers was included.All enrolled patients were treated according to our unit ongoing induction and consolidation regimens(NCCN guidelines). Patients were followed up for two year and outcome was designated as either favorable or unfavorable.Detection of intracellular Survivin antigen in myeloid blast cells was done by flow cytometry on bone marrow samples at D0, D28 and then every 6 month (Kim et al Ann Lab Med 2013).

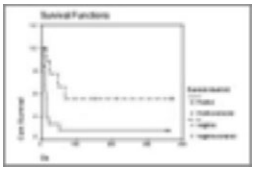

Figure 1.

Results: Survivin expression was higher in AML patients at D0 and D28 compared to healthy controls (P=0.001),highest survivin level were seen in AML M5 (FAB subtypes) followed by AML M4,then by AML M1, AML M0, AML M3 and AML M2 subtype. A statistically significant positive correlation was found between age of patient at diagnosis and Survivin expression, also between survivin level at D0 and at D28 among responders patients (P=0.001), also between D0-survivin level of AML patients with favorable response compared to patients with unfavorable response (P=0.005), survivin expression positively correlated with CD15, CD14 and CD11c expression. No statistically significant correlations were found between survivin expression and sex, or with results of cytogenetic studies,nor with AML FAB subtypes. There was statistically significant negative correlation between survivin level and with Complete remission (CR),overall survival (OS) Figure -1. EFS, and PFS. CR and post induction response to chemotherapy occured in patients with negative survivin expression more than in patients with positive survivin expression (P=0.005),and after 2 year follow up patients with positive survivin expression relapsed more (P=0.015).

Summary and Conclusions: Survivin is important factors involved in control of apoptosis in malignant cells. Survivin expression was found to be higher in elderly AML patients compared to younger patients group, A statistically significant negative correlation was detected between survivin level at D0 and CR,OS,EFS, and PFS. Being preferentially and highly expressed in cancer cells, with little expression in normal tissues,makes it an attractive therapeutic target to inhibit cancer growth by inhibiting extrinsic and intrinsic apoptotic pathways and confers resistance to apoptosis by directly suppressing caspase activity.

PB1652

EVALUATION OF MINIMAL RESIDUAL DISEASE IN ACUTE MYELOID LEUKEMIA WITH NPM1 MARKER

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Background: Minimal residual disease (MRD) tests provide early identification of hematologic relapse and timely management of acute myeloid leukemia(AML)patients. Approximately 50% of AML patients do not have clonal chromosomal aberrations and categorize as a cytogenetically normal acute

myeloid leukemia (CN-AML). Molecular markers are useful in the dissection of this heterogeneous group of AML patients into prognostically different subgroups. About 60% of adult CN-AML have a mutationin exon 12 of the NPM1 gene. This mutation is specific for malignant clone and potentially is a good marker of MRD.
Aims: In this retrospective study, we set up a NPM1quantitative test and then AML patients carrying $NPM1^{mut}$ were followed up for MRD monitoring.
Methods: In this study, we prepared plasmids containing a cDNA fragment of the NPM1 and ABL genes by PCR cloning. ABL served as a control to compensate for variations in the quality of the RNA and for differences in the efficiency of the reverse transcription reaction the plasmids were used to construct standard curves quantitation of each of the target and control genes. Eleven patients were analyzed using established method. Serial PB /and or BM samples (71 samples) were taken in1-3 month intervals (mean 1.5-month intervals) and median follow up duration after chemotherapy was 11 months (5- 28.5 months).
Results: In this study, we developed RNA-based RQ-PCR to quantitation of NPM1 mutation A with sensitivity of $10^{(-5)}$. NPM^{mut} transcript levels were expressed as a percentage ratio of NPM^{mut} to ABL transcripts. The percent of NPM^{mut}/ ABL level showed a range between 132 and 757 with a median of 383.5 in samples at diagnosis. The median NPM^{mut} transcript level log reduction was 3 log.Relapse occurred in 45% of patients (5 cases), All cases at diagnosis demonstrated the same mutation at relapse. In patients who experienced relapse, Log reduction levels of NPM1 mRNA transcript after therapy were 4(2 patients),3(two patients) and 1 log(1 patient). Totaly NPM^{mut} level showed less than 5 log reduction in all of them, Whereas this reduction was 5-6 log in other patients.
Summary and Conclusions: Despite the limitations of this study in terms of sample size and duration of follow up, it showed the accuracy of set up for detection of mutation and this marker has worth for following up at different stages of disease. Because of high frequency, stability, specificity to abnormal clone and high sensitivity, NPM1 is a suitable marker for monitoring of NPMc+ AML patients.

PB1653

DECITABINE AS THE FIRST-LINE TREATMENT FOR ELDERLY PATIENTS WITH ACUTE MYELOID LEUKEMIA; A SINGLE CENTER EXPERIENCE IN KOREA

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Background: Decitabine, a DNA hypomethylating agent, was approved by the European Union for the treatment of elderly patients with acute myeloid leukemia (AML) who are not candidates for standard induction chemotherapy in 2012, but the role of decitabine for remission induction is still controversial. Recently, Korean Food and Drug Administration also approved decitabine as the first-line treatment for as same indication as of EU.
Aims: Through this study, we want to share our recent experience of decitabine for the treatment of elderly AML patients.
Methods: Among patients who were diagnosed with AML except acute promyelocytic leukemia from Jan. 2014 at Severance hospital in Korea, 16 patients treated with decitabine as the first-line therapy for remission induction were evaluated retrospectively. All patients were treated with at least one course of decitaibine at an initial dose of 20 mg/m^2 intravenously daily for 5 days in 28-day cycles. Patients showing complete response (CR) or partial response (PR) could continue to decitabine therapy, and those showing no response (NR) or loss of response (LOR) could be considered treatment failure and received second-line treatment.
Results: The median age of total patients was 73.5 (range, 68-82) years, with 9 patients 65–74 years and 7 over 75 years. Cytogenetic risk assignment based on the National Comprehensive Cancer Network criteria was favorable in 3 patients (18.8%), normal karyotype or other intermediate-risk in 11 (68.8%), poor-risk in 1 (6.3%) and unknown in one. The median follow-up duration after diagnosis was 138 (range, 39-310) days. Median and mean number of cycle was 3 and 3.75 (range, 1-8), respectively, and 2 patients received total 8 cycles. Fifteen (93.8%) patients completed the second cycle, and 9 were evaluated for response. CR was achieved in 2 patients (22.2%) after the second cycle and in additional 2 patients after the fourth cycle, for an overall response rate (ORR) of 33.3%. LOR was shown in 2 patients. Eight patients discontinued decitabine treatment due to NR or LOS in 5 (62.5%) and side effects in 3 (37.5%). Grade 3-4 febrile neutropenia was developed in 7 (43.8%), grade 2-3 fatigues in 3 (18.8%), and grade 3-4 heart failure in 1 (6.25%). The estimated median overall survival (OS) was 302 (95% CI, 83.5-520.5) days. The pretreatment characteristics that differed significantly between responders and non-responders was age (P=0.042) and serum ferritin level (P=0.016). Age and serum ferritin level were younger and lower in responder than in non-responder.
Summary and Conclusions: In our experience, 5-day decitabine treatment has efficacy in elderly patients with AML considered unfit for conventional chemotherapy with 33.3% of CR rate and 10 months of median OS. Further study will be objected to discover good clinical determinants and biomarker to select patients who may drive greater benefit from decitabine.

PB1654

THE EFFECT OF SERUM IRON TESTS AND IRON OVERLOAD ON TRANSPLANTATION-RELATED COMPLICATIONS AND PROGNOSIS IN ACUTE LEUKEMIA

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Background: Many studies have investigated impacts of serum iron parameters and/or radiological evidence of systemic iron overload on stem cell transplantation in acute leukemia.Some of them aimed to discover if iron overload might have any detrimental effect on the transplantation. On the other hand some other studies were interested in negative prognostic sign of hyperferritinemia irrespective of body iron status. Unfortunately, some of the studies evaluating iron overload in transplant setting did not precisely show the patients with iron overload, mainly due to inadequacy of radiological methods in relatively mild hemosiderosis and ignoring consideration of transferrin saturation along with hyperferritinemia for elimination of non-iron overload etiologies of hyperferritinemia.
Aims: Herein, we aimed to investigate the the effect of serum iron tests and iron overload on transplantation related complications and prognosis in acute leukemia with adequate methodology.
Methods: Patients who undergone allogeneic stem cell transplantation for acute leukemia in Hacettepe University Medical School Department of Hematology were screened retrospectively in order to find cases with serum iron tests within 9 months before transplant. Serum iron (SI), total iron binding capacity (TIBC) and ferritin levels had been measured. Transferrin saturation was calculated by dividing serum iron level to TIBC.
Results: There were 84 patients suitable for inclusion. Important baseline characteristics and rates of the endpoints are presented in Figure 1. When various ferritin plus TS cut-off values presented in Table 1 were investigated for a possible relationship with major transplant results only ferritin>2000 + TS>45% was found to have an association with VOD at borderline significance (P=0.067) (Table 1).

Table 1. The relationship between iron overload and major transplant results

	Ferritin >2000 and TS >45%P value		
	Yes	No	
aGVHD	2	12	0.410
cGVHD	3	11	0.813
VOD	2	12	0,067
OS	69.6±12.5	69.5±7.3	0,603
DFS	61.5±13.4	62.5±7.2	0.729

Table 2. Baseline characteristics and rates of the endpints.

Age (Range)	35 (17-66)
Gender (M/F)	47/37
Type of transplant (Myeloablative/Non-myeloablative)	17/67
Iron, ug/dl	82 (25-277)
TIBC, µg/dl	260 (81-418)
TS, %	34 (7-98)
TS > 45% (Yes/No)	22/40
TS > 60% (Yes/No)	17/45
Ferritin, ng/ml	1104 (12-15000)
Ferritin > 500 and TS > 45% (Yes/No)	39/45
Ferritin > 1000 and TS > 45% (Yes/No)	29/55
Ferritin > 1500 and TS > 45% (Yes/No)	19/65
Ferritin > 2000 and TS > 45% (Yes/No)	14/70
Ferritin > 2500 and TS > 45% (Yes/No)	0/84
aGVHD (Yes/No)	17/70
cGVHD (Yes/No)	19/68
VOD (Yes/No)	4/80
DFS (% at 5 years)	%49.8
OS (% at 5 years)	%51.7

Summary and Conclusions: There are many studies reporting about the impact of iron overload on stem cell transplantation. Unfortunately only ferritin level was used to detect iron overload in these studies. There are many conditions associated with increased serum ferritin level (eg. Inflammation, infection, macrophage activation, liver injury, etc...) Therefore an increased ferritin level should not be considered as iron overload by itself. The laboratory sign of iron overload is ferritin elevation in the presence of an increased transferring saturation.Iron overload was investigated by radiological tests in some studies. Although this method is an optimal one in apparent iron overload, it might miss iron overload in early stages. There are some studies which investigated the association between iron overload and post-transplant prognosis using an acceptable method to define iron overload. Some of them found a relationship between iron overload and post-transplant survival. On the other hand some others did not. Interestingly the relationship between iron overload and VOD, probably the most expected specific transplant complication in patients with liver injury, was not reported in any of these studies considered to have adequate methodologies for detection of iron overload. In conclusion, we observed a possible relationship between iron overload and post-transplant venoocclusive disease. We did not confirm other post-transplant complications reported in the literature. It must be noticed that although many studies intended to investigate the relationship between iron status and transplant outcomes, only a few of them really looked for the effect of iron overload.

Aggressive Non-Hodgkin lymphoma - Clinical

PB1655

IS THERE A PLACE FOR TOTAL TUMOR RESECTION OF PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA IN PATIENTS WITH GOOD PERFORMANCE STATUS?

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Background: The outcome of the patients with primary diffuse large B cell lymphoma of central nervous system (PCNSL) still remains poor. The prognostic value of available prognostic scores need to be validated as well as the surgical approach since the improvements made in the field of neurosurgery.
Aims: The aim of this study was to evaluate treatment approach and the validity of the International Extranodal Lymphoma Study Group (IELSG) score of the patients with PCNSL.
Methods: This study involves 30 patients (13 males/17females) with a median age of 53.5 years (range 29-72 years). The patients presented usually with more than two different symptoms which mostly reflected tumor location, and were as following: headache (13 patients), hemiparesis (13 patients), neuropsychiatric (11 patients), dysarthria (8 patients), nausea and vomiting (5 patients), visual symptoms and vertigo (5 patients), and other symptoms occasionally. Five patients (16.7%) had B symptoms, 5 patients (16.7%) had bone marrow infiltration and 13 patients (43.3%) had deep tumor location. According to the Eastern Cooperative Group performance status (ECOG), 9 patients (30%) had ECOG 1–2 and 21 patients (70%) had ECOG 3–4. Regarding the International Prognostic Index (IPI), low score was present in 44.4% of patients, low intermediate in 26%, high-intermediate in 22.2% and 7.4% of patients had high IPI score. The prevalence of the IELSG score was as following: low score had 17.4% of patients, intermediate had 65.2% and 17.4% had high IELSG score. Treatment options included total tumor resection in 13 patients (43.3%), partial tumor resection in 10 patients (33.3%) and biopsy only in 7 patients (23.3%). A customized de Angelis protocol based on high doze methotrexate with/without whole brain irradiation was applied in all patients.
Results: The median overall survival (OS) was 41 months. Median event free survival was 36 months. Overall treatment response was achieved in 22 patients (73.4%), 8 patients (26.6%) had primary resistant disease with lethal outcome within 5 months. Statistical analyzes showed that the patients with ECOG≥3 had worst prognosis than the patients with ECOG 1–2 (Log Rank $\chi^2$11.722, p<0.01). Median OS in the group of patients with ECOG 1-2 was 62 months and 23 months in the group of patients with ECOG 3-4 (95% CI 3.118-42.882). The IPI didn`t show prognostic significance in the patients with PCNSL (Log Rank χ^2 0.274, P=0.601), however the IELSG score significantly predicted survival in these patients (Log Rank χ^2 12.755, P<0.01). The patients who underwent an open surgery with total tumor reduction had significantly longer OS (median 62 months) in comparison to the patients who had partial tumor reduction or biopsy only (Log Rank χ^2 5.692, P=0.017) and whose median OS was 23 months (95% CI 1.634-44.366).
Summary and Conclusions: Despite that total tumor resection is not standard procedure in the patients with PCNSL, according to our data, it should be considered in patients with good performance status and surgically accessible lesions for resection. The IELSG score should be used as prognostic index in patients with PCNSL.

PB1656

Abstract withdrawn

PB1657

UNRECOGNIZED RENAL INSUFFICIENCY AMONG NON-HODGKIN LYMPHOMA PATIENTS TREATED WITH R-CHOP: EVALUATION OF PREVALENCE, CHARACTERISTICS AND EFFECT ON REGIMEN RELATED TOXICITY

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Background: Estimated glomerular filtration rate (eGFR) is more accurate than serum creatinine (SCR) in assessing renal function. The IRMA study demonstrated a 50% prevalence of decreased eGFR despite normal SCR, coined as “unrecognized renal insufficiency (RI)”, among patients with solid malignancies. Unrecognized RI potentially may have an impact on treatment toxicity. In a recent study, breast cancer patients with unrecognized RI receiving cyclophosphamide and doxorubicin had an adjusted OR of 3.56 for regimen-

related toxicity. However, little is known regarding its prevalence and clinical implications in hematologic malignancies.
Aims: To determine the prevalence of unrecognized RI and its association with treatment toxicity in non-Hodgkin lymphoma (NHL) patients treated with rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone (R-CHOP).
Methods: Patients ≥ 18 years with normal pre-treatment SCR (<1.3 mg/dl) and NHL treated with R-CHOP every 21 days at our institute's hemato-oncology unit (1/1/2005 – 31/8/2013) were included in this retrospective cohort. Patients who had previous chemotherapy or total bilirubin ≥ 3mg/dl were excluded. The eGFR was calculated from pre-treatment SCR, using Cockcroft-Gault (primary analysis), aMDRD and CKD-EPI equations. Joint primary outcomes were neutropenic fever and premature treatment termination, while dose reduction after cycle 1 (>20%), dose delay (≥7 days), any hospital admission and death, were secondary outcomes. Rates of treatment toxicities were compared between eGFR groups as defined by NKF-KDOQI and KDIGO guidelines, using Kendall's Tau.

Table 1. Baseline characteristics of study partecipants with a comparison between e GFR groups (based on Cockcroft Gault formula).

		All patients* (N=94)	eGFR ≥ 90* (N=28)**	eGFR 60-89* (N=31)**	eGFR 30-59* (N=35)**	p value***
Age (yrs, median[min;max])		67.0 [36.0;87.0]	55.5 [36.0;85.0]	64.0 [36.0;85.0]	77.0 [38.0;87.0]	0.134
Female sex		50 (53.2%)	19 (67.9%)	16 (51.6%)	15 (42.9%)	0.14
BMI (mean ± SD)		26.6 ± 4.27	28.4 ± 3.74	26.9 ± 3.49	25 ± 4.74	0.01
Creatinine (mg/dl; mean ± SD)		0.96 ± 0.19	0.90 ± 0.16	0.98 ± 0.20	1.00 ± 0.19	0.07
Type of NHL****	Indolent lymphoma	18 (19.1%)	8 (28.6%)	5 (16.1%)	5 (14.3%)	0.31
	Aggressive lymphomas	76 (80.9%)	20 (71.4%)	26 (83.9%)	30 (85.7%)	
Ann Arbor stage	I	12 (12.9%)	3 (11.1%)	3 (9.68%)	6 (17.1%)	0.53
	II	10 (10.8%)	3 (11.1%)	3 (9.68%)	4 (11.4%)	
	III	22 (23.7%)	10 (37.0%)	5 (16.1%)	7 (20.0%)	
	IV	49 (52.7%)	11 (40.7%)	20 (64.5%	18 (51.4%)	
B-symptoms		33 (47.1%)	7 (31.8%)	11 (52.4%)	15 (55.6%)	0.22
Dose reduction of first cycle ≥ 10%		28 (29.8%)	2 (7.14%)	6 (19.4%)	20 (57.1%)	**<0.001**
Dose reduction of first cycle ≥ 33%		11 (11.7%)	0 (0.00%)	1 (3.23%)	10 (28.6%)	**<0.001**
No. of treatment cycles planned (median [min;max])		6.00 [3.00;9.00]	6.00 [3.00;8.00]	6.00 [6.00;9.00]	6.00 [4.00;8.00]	0.19
No. of treatment cycles administered (median [min;max])		6.00 [3.00;8.00]	6.00 [3.00;8.00]	6.00 [4.00;8.00]	6.00 [3.00;8.00]	0.36
Prophylactic GCSF		47 (50.0%)	11 (39.3%)	13 (41.9%)	23 (65.7%)	0.06
Diabetes mellitus		19 (20.2%)	6 (21.4%)	7 (22.6%)	6 (17.1%)	0.85

* eGFR units: ml/min/ 1.73 m2

** N (% of total number of patients in same eGFR group), unless specified otherwise

*** p value for difference between the eGFR groups, in univariate analysis

****Indolent lymphomas: Follicular lymphoma (n=11), Marginal cell lymphoma (N=1), Mucosa-associated lymphoid tissue lymphoma (N=2), small lymphocytic lymphoma (N=3) ; Aggressive lymphoma: Diffuse large B-cell lymphoma (N=74), Mantle cell lymphoma (N=2)

BMI, body mass index; eGFR, estimated glomerular filtration rate; GCSF, granulocyte colony stimulating factor; NHL, non-hodgkins lymphoma

Results: 94 patients fulfilled the study criteria, reaching the planned sample size. Seventy percent had unrecognized RI distributed evenly among CKD stage 2 and 3. CHOP dose reduction ≥10% from cycle 1 was associated with lower eGFR groups (57% of patients with CKD3 vs 7% in CKD1), as was lower BMI. These were the only statistically significant differences in patient characteristics between CKD stages (Table 1). Patients with CKD 3 were older than patients in CKD 2 and CKD 1, although the difference was not statistically significant (P=0.134). There was no difference in rates of neutropenic fever (overall 29.8%, P=0.68) or other regimen-related toxicities between eGFR groups. Premature treatment discontinuation was most frequent in the lowest eGFR group (P=0.03) in univariate analysis using Cockcroft Gault equation (but not with aMDRD OR EPI_CKD), but lost statistical significance after multivariate analysis. Thirty percent of patients had baseline dose reductions ≥ 10%. They were older (P<0.001) and with a lower eGFR (70% vs 22.7% CKD3, P<0.001), but had similar outcomes.
Summary and Conclusions: Most NHL patients treated with R-CHOP have unrecognized RI. The absence of an association between eGFR and treatment toxicity among these patients is in contrast to findings from solid malignancies. This may indicate that relatively subtle changes in renal excretion of cyclophosphamide do not carry clinical significance in this potent multidrug regimen. This could also be a false negative result, reflecting the much more common preplanned dose reductions in older patients with lower eGFR. Thus, in this population, unrecognized RI is common and had no effect on treatment toxicity when chemotherapy doses were reduced in older patients with lower eGFR, at the hematologist's discretion. Further research is needed to determine whether lower eGFR is indeed associated with treatment toxicity in a large cohort of older patients receiving full R-CHOP dose intensity, or whether it is merely a marker of older and frailer NHL patients with normal SCR.

PB1658

OCCURRENCE OF DERMOPATHY BY TREATMENT WITH ANTI-CCR4 ANTIBODIES FOR ADULT T-CELL LEUKEMIA

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Background: Human T-cell leukemia virus type I (HTLV-I) is a human retrovirus that is an etiologic agent of adult T-cell leukemia/lymphoma (ATL/ATLL). The poor outcome of adult ATL is mainly because of an intrinsic resistance of the leukemic cells to conventional or high doses of chemotherapy and severe immunosuppression. The CC-chemokine receptor 4 (CCR4) is expressed in almost ATLL cells. Thus, anti-CCR4 antibodies can be used as a treatment strategy for ATLL. Mogamulizumab (MOG), which is a defucosylated anti-CCR4 monoclonal antibody, showed good results even in patients with recurrent ATLL in phase I or II studies. MOG has strong antibody-dependent cellular cytotoxicity (ADCC). The common adverse events in the phase II study were infusion reaction and skin rashes. In our study, MOG inhibited the growth of ATL cells and induced remission. CCR4 is expressed not only in ATL cells but also in non-ATL regulatory T cells (Treg). After treatment with MOG, the population of Treg decreased significantly, which boosted the antitumor activity. MOG treatment was associated with the risk of viral infection as an opportunistic infection and skin disturbance (dermopathy).
Aims: In this study, we observed the effects of MOG as CCR4-specific ADCC against CCR4-positive ATL cells. At the same time, frequency of opportunistic viral infection including active CMV infection and severe skin disturbance (dermopathy) have been observed.
Methods: Patients were eligible for enrollment in the study if they were at least 18 years of age, had previously untreated ATL, and recorded a World Health Organization (WHO) performance status (PS) of 0-3. Fourteen patients diagnosed with acute type ATL were selected for this study. In the present study, we treated 14 patients with ATL who were resistant to chemotherapy using MOG monotherapy.
Results: All patients showed CR with a marked decrease in the number of ATL cells. These results suggested that MOG was effective in chemotherapy-resistant ATL patients. No rearrangement of TCRαβ gene was observed, which indicated molecular CR. Skin disturbance (dermopathy) was found 9 cases of 14 ATL patients treated with MOG monotherapy. The skin rash spread throughout her body with bulla and skin biopsy was performed, and diagnosed as Stevens–Johnson syndrome (SJS)(Figure) in 2 patients of them. Administration of steroid and intravenous immunoglobulin was performed, and SJS gradually improved. Further, in other patients, grade 2 and 3 dermopathy was found, and the patients were treated with glucocorticoid steroid. CMV infection was detected using C7-HRP methods. CMV infection was diagnosed in 5 patients of 14 MOG treated ATL patinets. One of the patients died because of severe CMV infection despite of adequate treatment
Summary and Conclusions: Thus, physicians should be aware of CMV infection and development of immunocompromised condition after MOG monotherapy. In that case, specific anti-CMV treatment for CMV reactivation should be recommended. Moreover, We have to find out the skin rash as soon as possible after treatment with MOG and it should be recommended quick administration with glucocorticoid. Further study for the dermopathy should be considered.

PB1659

THE ROLE OF PROGNOSTIC AND COMORBIDITY INDEXES IN PATIENTS WITH DIFFUSE LARGE B CELL LYMPHOMA TREATED WITH IMMUNOCHEMOTHERAPY

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Background: The influence of comorbidities in patients with diffuse large B-cell lymphoma (DLBCL) in the rituximab era has been modestly investigated. Different comorbidity scoring systems are available for cancer patients.
Aims: In order to evaluate the impact of comorbidities on the overall survival (OS) we have performed retrospective analyses of clinical records of the patients with DLBCL treated with R-CHOP, R-EPOCH and R-CVP protocol. The following comorbidity scores were used: Cumulative Illness Rating Scale (CIRS), Charlson Comorbidity Index (CCI), age adjusted CCI (aaCCI) and Hematopoietic-Cell-Transplantation Comorbidity Index (HCT-CI)
Methods: A total of 378 patients (195 female/183 male) were included in the study. Median age was 58 years (range 18-80). Elderly population (70 years and older) represented 20.2% of analyzed group. According to the Ann Arbor classification, stage I, II, III and IV had 51patients (13.5%), 129 (34.1%), 72 (19%) and 129 (33.3%), respectively. Bulky disease was present in 88 patients (23.3%) and B symptoms in 248 patients (65.6%). Regarding Eastern Coop-

erative Group (ECOG) performance status (PS), 23.7% of patients had ECOG PS ≥ 2. The most frequent extranodal localization were bone marrow (27.2% of patients) and gastrointestinal tract (28.8% of patients). Revised International Prognostic Index (R-IPI) was represented as low score in 70 patients (18.5%), intermediate in 203 (53.7%) and high in 105 (27.8%). Approximately 55% of patients didn`t have any comorbidity. Regarding comorbidity indexes (CI), CIRS ≥ 17 had 4% of patients (minimal score was 14), CCI ≥ 3 had 3.2%, aaCCI ≥ 5 had 34.1%, and HCT-CI ≥ 2 had 31.2% of patients. The patients were treated with R-EPOCH protocol (13 patients, 34.4%), R-CVP (22 patients, 5.8%), and R-CHOP (343 patients, 90.7%).

Results: Complete and partial remission were achieved in 334 (88.4%) of patients and 44 patients (12.6%) had primary resistant disease. The prognostic value of R-IPI was highly statistically significant (Log Rank χ^2 53.569, P<0.01). The patients with ECOG PS ≥ 2 had poorer outcome than the patients in good PS (Log Rank χ^2 33.075, P<0.01). In patients with CIRS ≥ 17 median OS was 30 months (95% CI 10.147-49.859) comparing to the patients with CIRS 14-16 whose median OS was not reached (Log Rank χ^2 10.046, P<0.01). The patients with CCI ≥ 3 had poorer outcome (median OS of 24 months, 95% CI 0.00-50.877) than the patients with CCI 0-2 (Log Rank χ^2 12.659, P<0.01). The patients with HCT-CI≥2 had significantly poorer outcome than the patients with low CI (Log Rank χ^2 8.041, P<0.01). OS in patients with HCT-CI≥2 after 1, 2 and 5 years was 69%, 63% and 49% respectively, and 89%, 81% and 72% for those with HCT-CI 0-1. The patients treated with R-CHOP and R-EPOCH protocol had statistically significant better OS than the patients treated with R-CVP protocol (Log Rank χ^2 8.754, P<0.01). Multivariant analysis (CIRS, CCI, aaCCI, HCT-CI, therapeutic approach, R-IPI) revealed that the most predictable factor for OS was R-IPI (P<0.0001).

Summary and Conclusions: Comorbidities are an independent prognostic parameters for worse OS in patients with DLBCL treated with immunochemotherapy. Omission of the antracyclines due to the frailty, older age or high comorbidity score leads to significantly poorer outcome. Finally, in the rituximab era prognosis is appreciably influenced with the value of initial R-IPI.

PB1660

THE COMBINATION OF IFOSFAMIDE, CARBOPLATIN AND ETOPOSIDE (ICE) WITH OR WITHOUT RITUXIMAB IS SAFE AND EFFECTIVE SALVAGE THERAPY FOR ELDERLY PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA

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Background: Elderly patients are under-represented in clinical trials, especially in studies evaluating intensive salvage combinations for relapsed or refractory diffuse large B-cell lymphoma (DLBCL). Yet, the risk of developing non-hodgkin lymphomas increases with age, with approximately 50% of patients are diagnosed above the age of 65 years. This leads to uncertainty regarding the risks and benefits of these treatments, and can have a substantial impact on treatment decisions.

Aims: To evaluate a 10-year single-center experience and results of therapy using the ifosfamide, carboplatin and etoposide±rituximab (ICE±R) as a salvage regimen in elderly patients with DLBCL.

Methods: This a retrospective single-center study evaluating the efficacy and tolerability of ICE±R regimen used for the treatment of elderly patients (>70 years) with relapsed or refractory DLBCL. The ICE±R regimen consisted of etoposide (100 mg/m^2) on day 1-3, carboplatin (dose=5x [25+creatinine clearance], capped at 800mg) and ifosfamide (5000 mg/m^2, mixed with an equal dose of MESNA administrated by continuous infusion over 24 hours) on day 2±rituximab (375 mg/m^2) given on day 0, as reported previously.

Data were collected from medical records. Adverse events (AEs) graded according to the Common Terminology Criteria for Adverse Events version 4.0. Disease response was defined according to the revised response criteria for malignant lymphoma. Clinical endpoints were defined according FDA guidance. The study was approved by the local institutional Helsinki ethics committee.

Results: Between October 2003 and June 2014, a total of 32 patients (21 women and 11 men) with DLBCL older than 70 years, were treated in our institute with the ICE±R regimen. Median age of the entire cohort was 75.6 years (range 70.6-87.1). Most of the patients had an Ann Arbor stage IV disease (56%, n=18), an intermediate-high to high sIPI (63%, n=20), a low Carlson comorbidity index (0-1 in 81%, n=26), and an excellent ECOG score (0-1 in all cases). In 27 patients (84%) the dosage of the chemotherapy was reduced, with a median dose reduction of 25% (range 0-50%). ICE±R was administered in all, except one case, as an in-patient therapy and all received G-CSF as primary prophylaxis. The overall response rate observed was 53.1% with a complete response of 40.6%. After median follow-up of 12 months, the median progression free survival (PFS) and overall survival (OS) were 3.9 months and 17.0 months, respectively. Patients who responded to ICE±R (including cases followed by autologous stem-cell transplantation) achieved median PFS of 47.2 months and OS of 78.9 months. Previous response to first-line therapy appeared to be the strongest predictor of response, PFS and OS to second-line treatment. Following treatment with ICE±R, an attempt to harvest peripheral blood stem cells was performed in 8 patients. Harvesting was successful in seven patients (>2.5x10^6 of CD34 cells/Kg) of which six (19%) proceeded to autologous stem-cell transplantation (ASCT). Patients ineligible for ASCT that responded to ICE±R (n=11) were treated with extended cycles of ICE±R (median of 4 cycles per patient), and achieved median PFS of 18.9 months and OS of 21.7 months. ICE±R was generally well tolerated and major toxicity related mostly to hematological adverse events.

Summary and Conclusions: ICE±R is a safe regimen and achieves high response rates in elderly patients with relapsed or refractory DLBCL. Response to first-line therapy is the strongest predictor of response to ICE±R. Patients with chemosensitive disease, who are not transplant-eligible, should be considered for extended treatment with this regimen.

PB1661

SUBCUTANEOUS PANNICULITIS-LIKE T-CELL LYMPHOMA: CLINICAL CHARACTERISTICS AND SURVIVAL STUDY OF 21 THAI PATIENTS IN A SINGLE INSTITUTION

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Background: Subcutaneous panniculitis-like T-cell lymphoma (SPTCL) is a rare lymphoma subtype which is commonly associated with hemophagocytic syndrome (HPS) and had an aggressive clinical course. The incidence of SPTCL is high in Asian countries including Thailand. Due to the rarity of the disease, there are limited data on the clinical characteristics and optimal therapeutic approaches of this entity.

Aims: The aim of this study was to characterize the clinical features, treatment outcomes, and survival of SPTCL in Thai patients at a single institution.

Methods: Twenty-one patients diagnosed as SPTCL according to WHO classification 2008 were enrolled from January 2001 to December 2014 in Songklanagarind Hospital. All tissue specimens were reviewed by hematopathologist. The demographic, clinical, treatment, and outcomes data were collected for analyses.

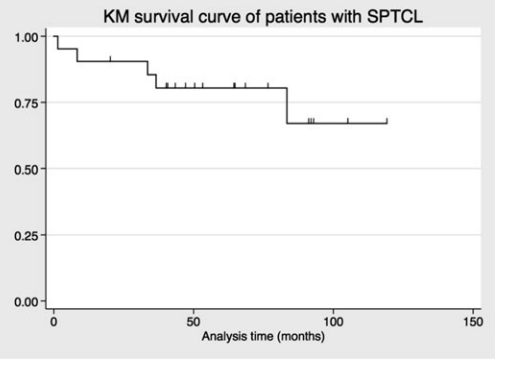

Figure 1.

Results: The median age was 33 years (range 22-64 years) and 12 patients were female. Lesions usually presented as multifocal subcutaneous nodules or masses in 90%, commonly at lower extremities and trunk. Nine patients had extra-cutaneous manifestations. The common sites of involvement were liver, spleen, and lymph nodes. None of the patients had HPS in this study. Most patients had a good ECOG performance score (90%) and low prognostic scores (IPI low to low-intermediate in 71% and PIT score 0-1 in 90%). Fifty-seven percent of the patients had limited stage of disease. B symptoms occurred in 57% and elevated LDH level in 86%. Nineteen (90%) patients were treated with chemotherapy, CHOP chemotherapy was used in 85%. Only one patient was treated with radiotherapy. Of the 20 patients evaluated, the overall response rate was 85% with 75% complete remission. Nevertheless, progressive disease occurred in 8 patients, including 5 patients who had obtained complete remission. With median follow-up time of 52 months, the median OS and RFS were unreached. The 5-year OS and RFS were 80% and 68%, respectively. No significant prognostic factors were identified in this study.

Summary and Conclusions: SPTCL frequently affected young females. Lesions typically presented as multifocal subcutaneous nodules or masses on trunk and lower extremities. Patients responded well to CHOP chemotherapy with long-term survival outcomes.

PB1662

CLINICAL FEATURES AND TREATMENT OUTCOMES IN NON-HODGKIN'S LYMPHOMA IN CHILDHOOD: 24 YEARS EXPERIENCE OF A SINGLE CENTER

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Background: Non-Hodgkin's lymphomas (NHL) of childhood and adolescence are a heterogeneous group of malign diseases originating from the lymphoid cells. NHL usually responds to the polychemotherapy and survival rates in NHL have increased significantly in the last decades.
Aims: This study aims was to evaluate and compare the demographic data and treatment results of childrens with NHL treated and therapeutic efficacy of modified NHL German Berlin Frankfurt Munster (BFM) protocols in our center retrospectively.
Methods: 115 children (79 male, 36 female) from January 1990 to September 2014, new diagnosed with NHL were enrolled to the study. The patients were stratified by risk factors and treated either with a modified B-nonB NHL BFM-90 (before 2004) or BFM-95 (after 2004) protocols. (Until September 1993, lymphoblastıc patients recived LSAL2, non lymphoblastıc NHL patients recived COMP) and the use of 1 or 3 g/m2 of methotrexate instead of 5 g/m2/24 h was the only important modification in BFM-90 protocol.
Results: Demographic results: the median age 7 years(range: 3-14.5years) were treated in the center with median 81 months follow-up. Histopathologic subtypes were:24 lymphoblastıc, 11 anaplastic large cell, 80 nonlymphoblastic Burkitt/diffüz B cell. 3 patients (3%) were in stage I, 19 patients (17%) in stage II, 64 (56%) in stage III, and 29(24%) in stage IV. The most common initial primary tumor location sites were abdomen (58%), head and neck (18%) and thorax (15%).The median LDH level at diagnosis was 790U/L(224-10300). Treatment results: 32 patients (24 progressive disease, 6 toxicity, 2 seconder neoplasm)died.7 patients were follow-up. The 10-year overall survival (OS) for all patients was 72%, and event-free survival (EFS) was 67% respectively. 10 year survival was 100,89,76,51% in stage I,II,III, and IV. The 10-year OS rates in modified BFM-90 and in BFM-95 protocols were 66% and 84%; the 10-year EFS rates in these 2 protocols were 60% and 84%, respectively (P=0.061 for OS, P=0.049 for EFS).Survival rates were significantly higher in patients receiving modified BFM regimen, than in ones COMP and LSA2L2
Summary and Conclusions: Survival rates in the whole group are in parallel with advances attained in the world in NHL.The significantly higher survival rates achieved in patients with advanced stage non lymphoblastic patients receiving modified BFM(1 g/m2MTX) may be due to the decreased toxicity seen in this group and to the advences in supportive care in the last decade.

PB1663

FAVORABLE PROGNOSTIC PARAMETERS IN TREATMENT OF AIDS RELATED LYMPHOMA PATIENTS; TEN YEAR SINGLE CENTRE EXPERIENCE

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Background: AIDS-related lymphomas (ARL) are frequent complication of HIV infection, due to progressive CD4+ T-lymphocytes depletion and subsequent immune impairment. HIV-positive patients (pts) have up to 200-fold increased incidence for non-Hodgkin lymphomas (NHLs). Most ARLs are aggressive forms of B-NHLs. Usually pts presents with advanced disease, and prognosis is generally poor. Previous studies reported International prognostic Index score (IPI) ≤2 as significant predictive factor for overall survival (OS). Current standard in ARL patient's treatment is concomitant combined antiretroviral therapy (ART) and chemotherapy.
Aims: The aims of this study were to detect prognostic factors and estimate benefit of concomitant ART and chemotherapy for ARL patients OS.
Methods: We enrolled 37 ARL patients in our study. All were treated at the Clinical Centre of Serbia, Belgrade in period 2004-2014. Thirty-two pts had an aggressive NHL. Indolent NHL and Hodgkin disease were diagnosed in 2 cases each, unclassified lymphoma in 1 case. Concomitant ART (same in all cases) and chemotherapy was applied in 30 cases, chemotherapy only in 4, neither ART nor chemotherapy in 3 cases.
Results: We found that use of concomitant ART and chemotherapy (P=.009, DF=1), IPI risk score ≤2 (P=.031, DF=1) and LDH level ≤400IU (P=.028, DF=1) were significant for OS (10 months, 2-102). Same parameters were confirmed as positive OS predictors in univariate survival analysis (P=.020, 95.0%CI for RR 0.141-0.847; P=.046, RR 1.013-4.813; P=.097, RR 0.875-4.25 respectively). In multivariate analysis concomitant ART and chemotherapy remained significant for OS (P=.012, RR 0.120-0.774).
Summary and Conclusions: Our results suggested necessity for prompt use of concomitant ART and chemotherapy in ARL treatment, in order to improve OS. Most ARLs are very aggressive, both in terms of histology and clinical course, but pts without advanced disease seems to have better OS rate when treated this way.

PB1664

TOXICITY AND EFFICACY OF LMBA-02 PROTOCOL IN THE TREATMENT OF BURKITT'S LYMPHOMA AMONG THE MOROCCAN ADULT POPULATION

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Background: The geographical position of Morocco, straddling Europe and sub-Saharan Africa gives the Burkitt lymphoma equitable distribution between the endemic and the sporadic form.
Aims: The objective of this study is to evaluate the toxicity and the efficacy of LMBA02 Protocol on the Moroccan population.
Methods: This is a retrospective study including all patients managed in our training for Burkitt's lymphoma and treated using the LMBA02-Mabthera protocol.
Results: A total of 18 patients were included. The median age was 44 years, with a range of 16-70 years. The rate of men was more likely higher than women with a sex ratio [M/F] of 1.6. Diagnosis delay was 3 months average. At diagnosis, 39% of patients had abdominal localization, 33.3% had a mandibular one, 16.6% had a neurological localization and 11% had a marrow infiltration. Two patients (11%) were infected with human immunodeficiency virus. 39% were in stage III and 39% in stage IV Murphy. 50% of patients had systemic symptoms. All patients were treated according to the protocol LMBA02- Mabthera. The overall response rate (OR) was 83.2% with a complete response rate (CR) of 66.6% and a partial response (PR) to 16.6%. The treatment-related mortality (TRM) was 16.6%. The overall survival rate was 62% at 6 months, 50% at 1 year and 36% at 2 years; when analyzing it in terms of age, there is a 2-year survival of 75% among patients under 40 years and 14% in patients over 40 years (P<0.05). The main causes of death are dominated by treatment-related toxicity (37.5%), resistance to first-line treatment (37.5%) and relapse of the disease (25%). Both mortality risk factors are age and cure spacing due to the toxicity of treatment.
Summary and Conclusions: Despite its effectiveness, the treatment-related mortality of LMBA02-Mabthera protocol in patients over 40 years is considered high.

PB1665

ALLOGENEIC STEM-CELL TRANSPLANTATION FOR ADVANCED MYCOSIS FUNGOIDES/SEZARY SYNDROME

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Background: T-cell lymphomas are heterogeneous group of diseases that account for<10% of non-Hodgkin lymphomas. The prognosis for patients with most forms of T-cell lymphoma is generally poor. Mycosis fungoides (MF) and Sezary Syndrome (SS) are cutaneous T cell lymphomas. Several studies have suggested that allogeneic hematopoietic stem cell transplantation (HSCT) may improve survival in advanced MF and SS.
Aims: We aim to present the outcome of our patients with MF or SS who underwent HSCT
Methods: We retrospectively evaluated totally 8 patients, 5 advanced stage MF and 3 SS who underwent peripheral HSCT rescue at Ankara University Department of Hematology from Oct 2012 to Oct 2014. Three MF patients had large cell transformation at pre-transplantation period. All of the patients received at least three lines of therapy prior to HSCT (Table). Two out of 8 patients were transplanted from unrelated HLA one antigen or allele mismatch donors whereas other 6 patients had fully matched siblings. All of the patients had refractory disease prior to HSCT, 6 of 8 patients received reduced intensity conditioning regimen (Fludarabine-Cyclophosphamide-TBI +/-ATG) while 2 patients received myeloablative conditioning regimens including Cyclophosphamide plus total body irradiation. Peripheral blood was the stem cell source in all the patients. For graft-*versus* host disease prophylaxis, patients received cyclosporine plus methotrexate or mycophenolate mofetil except one patient in whom metal-prednisolone was used due to renal toxicity.

Table 1. Patient characteristics.

Patient	Age/Sex	Disease	Disease onset to HSCT (years)	Clinical Stage	Diseases tatus at HSCT	Largecell transformation	Number of priortreatments	HSCT/HLA mismatch	Conditioning Regimen (MA/RIC)	Acute GVHD / Grade	Chronic GVHD/ location	GVHD prophylaxis	Engraftment ANC >0.5X10..4/ (Day)	Outcome
1	53/M	MF	11	T4N2M1 B0 IVB	Active disease	-	4	U-PBSCT (9/10)	RIC (Flu+Cy+TBI+ATG)	+/2	NA	CSA+MMF	9	Ex,fungal infection,23 th day posttransplant
2	50/M	MF	2	T4N2M0 B2 IVA1	Active disease	+	8	R-PBSCT (10/10)	RIC (Flu+Cy+TBI)	+/3	Limited	CSA+MMF	Never below	Relapsedafter 10 months, ECP
3	61/M	MF	4	T3N1M0 B0 IIB	Active disease	+	4	U-PBSCT (9/10)	RIC (Flu+Cy+TBI+ATG)	+/2	Extensive	CSA+MMF	12	VGPR (8months)
4	55/M	SS	3	T4N2M0 B1 IIIB	Active disease	-	3	R-PBSCT (10/10)	RIC (Flu+Cy+TBI+ATG)	0	-	CSA+MMF	11	Relapsed after 4months
5	56/F	MF	3	T4N1M0 B0 IIIA	Active disease	+	5	R-PBSCT (10/10)	MA (Cy+TBI)	+/2	-	CSA+MTX	16	Relapsed 4 months
6	50/F	SS	1	T4N2M0 B2 IVA1	Active disease	-	6	R-PBSCT (10/10)	RIC (Flu+Cy+TBI)	NA	-	CSA (bac)+MMF+PREDNOL	No	Ex, fungalinfection, 16th daypostransplant
7	49/M	MF	4	T4N0M0 B0 IIIA	Active disease	-	7	R-PBSCT (10/10)	RIC (Flu+Cy+TBI)	0	-	CSA+MTX	14	Ex, acinetobacter sepsis 16th day posttransplant
8	49/M	SS	3	T4N2M0 B1IIIB	Active disease	-	3	R-PBSCT (10/10)	MA (Cy+TBI)	0	Limited	CSA+MMF	22	CR (24 months)

Results: The median age of the group was 59 years (range 49-61). The median time from diagnosis to HSCT was 3 years (range 1-11 years). The Sorror HCT-CI scores prior to HSCT were listed as: 2/8 score of 0, 3/8 score of 1, 3/8 score of 2. The patients achieved engraftment at a median of 12 days after HSCT (range 0-22). We evaluated the disease response in only 6 patients because two patients died of the early transplant-related mortality in 16th day of the transplantation due to fungal or Acinetobacter infection. Disease responses after the transplantation could evaluated in only six patients as follows: 2 complete remissions, 2 very good partial remission (VGPR), and 2 minimal responses. We obtained complete remission in one patients with VGPR after developing acute GvHD. Acute GvHD developed in 57.1% of the patients (4/7). Chronic GvHD was detected in 3 out of 5 patients surviving longer than 100 days after the transplantation. Early transplant-related mortality was seen in 25%. Our follow-up was changing between 16 days and 769 days. The possibility of two-year progression free and overall survival was 37.5%±1.7% and 62.5%±1.7%, respectively.
Summary and Conclusions: Our results in a small patient group suggest that allo-HSCT is an effective treatment in the patients with advanced stage refractory MF/SS.

PB1666

INCIDENCE OF THROMBOTIC EVENTS IN PRIMARY MEDIASTINAL B-CELL LYMPHOMA AND ITS INFLUENCE ON THE OUTCOME OF THESE PATIENTS: EXPERIENCE OF SINGLE CENTER

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Background: Primary Mediastinal B cell Lymphoma (PMBL) is a lymphoid neoplasm that accounts for 2% to 4% of non-Hodgkin lymphomas (NHL). Because of its particular features (fibrosis, large mediastinal mass, pericardial and/or pleural effusion) is included in the Large B-cell lymphomas (LBCL) with special features category. PMBL presents usually with superior vena cava syndrome (SVCS) and thrombotic events of the mediastinal circulation although the real prevalence of these events have not been accurately reported in the literature.
Aims: The main objective of the study was to determine the incidence of these thrombotic events and how this complication influences the outcome of patients diagnosed with PMBL.
Methods: Thirty-one patients consecutively diagnosed with PMBL in a single center between 1995 and 2014 were included. After reviewing the pathology, the demographic and clinical characteristics of the series were recorded and evaluated, paying particular attention to the thrombotic events.

Table 1.

Characteristics	Total N (%)	With Thrombotic event N (%)	Without thrombotic event N (%)	P
Patients	31 (100)	10 (32)	21 (68)	
Male/Female	12/19	4/6	8/13	NS
Age in years: median (range)	34 (17-56)	34 (25-47)	32 (17-56)	NS
Largest mass diameter in mm: median (range)	98 (50-150)	78 (50-150)	106 (58-150)	NS
Extranodal involvement	8 (27)	2 (20)	6 (28.5)	NS
SVCS	16 (52)	5 (50)	11 (52)	NS
Complete response after first line treatment	23 (74)	7 (70)	16 (76)	NS
4-year Overall survival: % (IC95%)	80 (64 - 96)	83 (53-100)	78 (59-97)	NS
4- year Progression-Free Survival: % (IC 95%)	71 (54 - 88)	85 (59 - 100)	64 (43 - 85)	NS

NS: No statistically significant.

Results: The median age at diagnosis was 34 years (range: 17 to 56) and 61% were female. The median of the largest diameter of the mediastinal mass was 98 mm (range: 50-150 mm), 26% had extranodal involvement (most frequently associated with adjacent structures) and 52% had SVCS. Ten patients (32%) had thrombotic events, 90% detected at diagnosis. There were no differences between the clinical characteristics (sex, age, larger diameter of the mediastinal mass, SVCS presence or extranodal involvement) between patients with or without thrombotic events. With a median follow-up of 71 months (range: 1-183) we did not find any difference between patients with or without thrombotic events regarding the response to therapy and the outcome (PFS and OS), Table 1.
Summary and Conclusions: The prevalence of thrombotic events in PMBL reported in our study is higher than the one described in other types of lymphomas. Nevertheless, response to therapy and outcome seems not to be influenced by this complication.

PB1667

ALCOHOL INTAKE AND SMOKING HABITS HAVE NO MAJOR IMPACT ON THE SHORT TERM SURVIVAL IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA

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Background: Individual lifestyle factors such as smoking and alcohol intake are common in the Western world and modify the risk of several cancer types, including non-Hodgkin lymphoma (NHL). However, less is known about the impact of these lifestyle factors on survival once the lymphoma has arisen.
Aims: To examine the prognostic impact of smoking and alcohol intake on overall survival (OS) in patients with newly diagnosed DLBCL treated with immunochemotherapy.
Methods: This retrospective study included adult patients with newly diagnosed DLBCL seen at the hematology centers of Aalborg (2003-2010), Holstebro (2006-2012), Odense (2006-2012), and Copenhagen (Rigshospitalet, 2010-2012). All patients had been treated with R-CHOP/CHOP-like regimens. Patient lists were obtained from the Danish Lymphoma registry (LYFO). The LYFO contains detailed information on a wide range of clinico-pathological features as well as complete follow-up data. Medical records were retrieved and reviewed for information on smoking habits, alcohol consumption, and comorbidities. Overall survival (OS) estimates were made using the Kaplan-Meier method. The impact of lifestyle and comorbidity on OS was evaluated with simple and multiple Cox models with inclusion of the standard international prognostic index (IPI) risk groups and comorbidities.
Results: A total of 502 DLBCL patients had available information on smoking and alcohol intake and were included in the present study. Patients were categorized as low-risk (26%), low-intermediate (24%), intermediate-high (26%) and high risk (18%), according to the IPI. Never smokers accounted for 43% of the patients, former smokers 31%, and current smokers 26%. A low alcohol intake (<3 standard drinks per week) was noted in 33% of the patients, a moderate intake (3-14 standard drinks per week) in 55%, and heavy drinking (>14 standard drinks per week) in 11%. The Table shows the impact of lifestyle on outcome. In male patients, both current and former smoking were associated with a lower 3-year OS. Smoking habits did not influence survival in female patients. However, the associations between smoking and OS were attenuated after adjusting for comorbidities, which suggest that the effects of smoking were at least partially mediated by excess comorbidity in these patients. Participants with a moderate alcohol intake had the highest 3-years OS (71%), whereas heavy drinkers had the lowest 3-years OS (60%), but alcohol consumption did not have impact on survival in univariate or multivariate Cox analyses.

Table 1.

	3-year OS (95% CI)			Crude HR (95%CI)			Adjusted HR (95%CI)*		
	All	Men	Women	All	Men	Women	All	Men	Women
Never smokers	73% [66-78]	73% [63-81]	72% [62-79]	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
Former smokers	66% [57-74]	60% [48-70]	77% [62-86]	1.23 [0.83-1.81]	1.47 [0.88-2.46]	0.84 [0.42-1.68]	1.12 [0.75-1.67]	1.13 [0.66-1.95]	0.83 [0.41-1.67]
Current smokers	66% [57-73]	60% [47-70]	75% [61-85]	1.27 [0.85-1.90]	1.66 [0.96-2.86]	0.85 [0.42-1.68]	1.09 [0.70-1.68]	1.34 [0.74-2.41]	0.80 [0.40-1.59]
Low alcohol intake	67% [58-74]	60% [47-71]	73% [61-82]	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
Moderate alcohol intake	71% [65-76]	70% [62-77]	72% [63-80]	0.92 [0.64-1.32]	0.77[0.48-1.24]	1.10 [0.64-1.90]	0.91 [0.62-1.32]	0.70 [0.42-1.18]	1.06 [0.61-1.87]
Heavy drinkers	60% [43-74]	51% [31-67]	-	1.07 [0.62-1.84]	1.13 [0.62-2.07]	-	0.91 [0.51-1.61]	0.94 [0.49-1.79]	-

*Adjustment for IPI score and comorbidity

Summary and Conclusions: Overall, smoking habits and alcohol intake had no significant impact on the short term survival in this large cohort of uniformly treated DLBCL patients. However, current and former smoking were associated with a strong trend toward inferior OS, which is possibly mediated by excess comorbidity in this subset of patients.

PB1668

UPFRONT AUTOLOGOUS STEM CELL TRANSPLANTATION OVERCOMES A NEGATIVE IMPACT OF MALE GENDER IN HIGH-RISK DIFFUSE LARGE B-CELL LYMPHOMA PATIENTS TREATED WITH R-CHOP

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Background: Combining rituximab to combination chemotherapy (R-CHOP) has significantly improved overall survival (OS) of patients with diffuse large B-cell lymphoma (DLBCL), but high-risk patients stratified according to the international prognostic index (IPI), non-germinal center B cells (non-GCB) subtype, and male patients have shown inferior treatment outcomes. Although the first-line treatment of patients with DLBCL is R-CHOP irrespective of the risk-group, there have been few studies evaluating the role of upfront ASCT as consolidative treatment strategy for high-risk DLBCL patients.
Aims: To investigate the role of upfront ASCT as consolidation after R-CHOP chemotherapy according to the gender in newly diagnosed advanced stage DLBCL patients.
Methods: We performed a multicenter retrospective analysis of 204 newly diagnosed DLBCL patients between January 2008 and December 2013. Patients who were younger than 65 years of age with advanced stage (Ann Arbor stage III or IV) and elevated serum lactate dehydrogenase were included. All the patients completed 4 to 8 cycles of planned R-CHOP chemotherapy and achieved at least PR after completion of planned R-CHOP chemotherapy.
Results: The median age at diagnosis was 50 (range 22-64) years and 111 (54.4%) patients were male. Median follow-up for surviving patients was 42 (range 4-115) months. Seventy-five (36.8%) patients underwent upfront ASCT as consolidation. Fifteen (11.6%) patients in the non-ASCT group (n=129) underwent ASCT after relapse. The 3-year OS and PFS rate for all patients were 78.8% and 68.4%, respectively. In multivariate analysis, non-CR after R-CHOP chemotherapy and non-upfront ASCT was associated with shorter OS (hazard ratio (HR) 2.57; 95% CI 1.34-4.95; P=0.005 and HR 2.97; 95% CI 1.38-6.40; P=0.005, respectively). Non-ASCT was also associated with shorter PFS in multivariate analysis (HR 2.26; 95% CI 1.25-4.07; P=0.014). Among the male patients, upfront ASCT showed superior OS and PFS compared to the non-ASCT group (P=0.016 and P=0.024, respectively). GCB subtype compared to the non-GCB subtype showed better PFS in male patients (*P*=0.026), but there were no difference in OS and PFS in male patients who received upfront ASCT. In female patients, non-CR after completion of chemotherapy was associated with shorter OS on multivariate analysis (P=0.024). However, survival differences in OS and PFS according to the disease status after the completion of chemotherapy were not observed in female patients who received upfront ASCT.

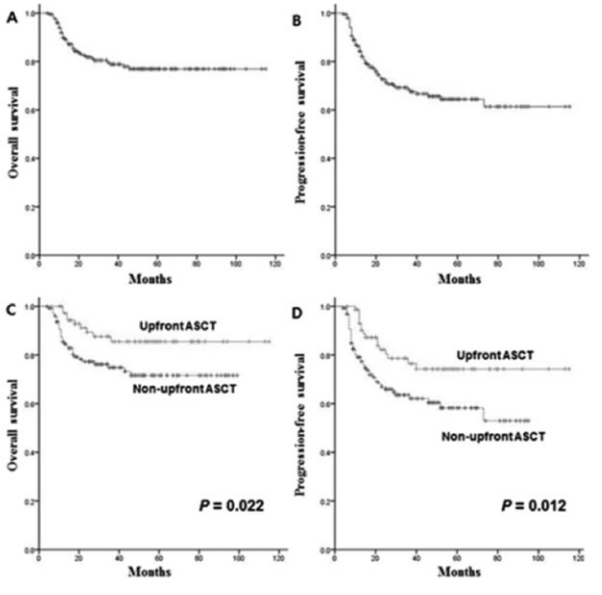

Figure 1.

Summary and Conclusions: Upfront ASCT as consolidation after R-CHOP chemotherapy overcomes a negative impact of male gender and non-GCB subtype in male patients with high-risk DLBCL. Among female patients with high risk DLBCL, upfront ASCT after R-CHOP chemotherapy may overcome a negative impact of non-CR response to initial therapy. Additional data comparing with new regimens such as additional use of rituximab are necessary to find out the exact role of upfront ASCT.

PB1669

HIGH NEUTROPHIL LYMPHOCYTE RATIO AT DIAGNOSIS IDENTIFIES DIFFUSE LARGE B-CELL LYMPHOMA PATIENTS WITH POOR CLINICAL OUTCOME

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Background: Diffuse large B-cell lymphoma (DLBCL) is the most common type of non-Hodgkin lymphoma (NHL) and is a biologically heterogeneous disease. At present, the stratification of DLBCL patients is performed by the revised international prognostic index (R-IPI). However, some preliminary data suggest the important role of inflammation in cancer biology. It has been assumed that systemic inflammatory response has prognostic significance in a wide range of different cancer types including NHL. The neutrophil to lymphocyte ratio (NLR) in the peripheral blood has been proposed as a prognostic factor in cancer patients. However data regarding the prognostic significance of NLR in DLBCL patients is quite limited.
Aims: To evaluate the prognostic significance of baseline NLR in a cohort of newly diagnosed DLBCL patients treated with chemoimmunotherapy.
Methods: Data from 254 DLBCL patients, at a median age of 58.6 years (range, 19-82 years), diagnosed between 2007 and 2014, were evaluated retrospectively. The patients were treated with R-CHOP. The NLR cutoff value for survival analysis determined by receiver operation characteristics (ROC) curve in the whole patients' cohort was 3,01. The prognostic influence of the NLR and other factors including age, lactate dehydrogenase (LDH), β_2 microglobulin (β_2 M), R-IPI and Ann Arbor stage at diagnosis on 5-year overall- (OS) and disease-free (DFS) survival was studied by Kaplan–Meier curves. To evaluate the independent prognostic relevance of NLR, univariate and multivariate Cox regression models were applied.
Results: A significantly higher proportion of patients with NLR above 3,01 had elevated levels of LDH and β_2 M (64% vs 28.8% and 75% vs 53.1%, respectively), advanced disease stage (III – IV) (48.2% vs 31.1%), high risk (R-IPI 3-5) disease (9.3% vs 32.2%) in comparison to patients with NLR below the cutoff value. Patients with high NLR at diagnosis experienced an inferior 5-year OS (38.5% vs 74.4%, P<0.001) and 5-year DFS (61.8% vs 77.7%, P<0.001). By multivariate analysis, an independent significant association between high NLR and poor clinical outcome in terms of OS (harard ratio [HR]=1.99, 95% confidence interval [CI], 1.07-3.71, P=0.03) and DFS (HR=2.58, 95% CI, 1.04-6.43, P=0.04) was identified.
Summary and Conclusions: Our data suggest that NLR at diagnosis provides useful independent prognostic information to assess clinical outcomes in DLBCL patients treated with R-CHOP. Further studies are required to confirm these results.

PB1670

COMBINATION OF CHEMOTHERAPY AND RADIATION IMPROVE THE PROGNOSIS OF PRIMARY DIFFUSE LARGE B-CELL LYMPHOMA OF THE TONSIL

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Background: Non-Hodgkin lymphoma of the Waldeyer's ring is a relatively rare entity and the tonsil is the most frequently involved site. The majority of tonsil lymphomas are of B-cell origin and the most common histological type is diffuse large B-cell lymphoma (DLBCL). Treatment approaches that have used include chemotherapy (CTx) alone, radiation (RT) alone, and combination of both.
Aims: We reviewed our data and evaluate treatment outcome of patient with primary diffuse large B-cell lymphoma of the tonsil.
Methods: Retrospective review of 114 stage I-II DLBCL patients treated with curative intent between 1995 and 2010. Forty-five (39.5%) patients had stage I disease and systemic symptoms (B-symptoms) were present in only 7 (6%) patients. Seventy-two (65.5%) patients were treated with CTx alone, whereas the remaining 38 (34.5%) received treatment with a combination of CTx and RT. Chemotherapy was CHOP-based, with R-CHOP in 80 patients (70%). Median involved-field RT dose was 3,960 cGy, with 96% receiving more than 3,000 cGy.
Results: The median age was 59 years and the majority of patients (61%) were males. Low risk disease: low to low intermediate by International prognostic index (IPI) was 97.3% and only 10 (8.8%) patients had high serum lactate dehydrogenase (LDH) level. Overall CR rate was 73.5% and seven (13.5%) of the patients who had achieved CR had recurred. The median follow up was 28 months (34 months for CHOP-treated, 23 months for R-CHOP). Disease-free survival (DFS) and overall survival (OS) were 43.9% and 42.5%, respectively. Significant prognostic factors included: age ≥60 year-old (OS: 93.1% vs. 74.1%, *P*=0.011; DFS: 83.3% vs. 80.6%, *P*=0.160), LDH >upper normal limit (OS:

88.2% vs. 72.9%, *P*=0.003; DFS: 86.8% vs.20.0%, *P*<0.001), IPI>0 (OS: 90.0% vs. 55.9%, *P*=0.007; DFS: 86.5% vs. 64.0%, *P*=0.034) and combination of CTx and RT (OS: 79.6% vs. 48.2%, *P*=0.025; DFS: 72.6% vs. 48.5%, *P*=0.038). Germinal center (GC) and non-GC phenotype were not predictors of outcome in localized primary DLBCL of the tonsil. Combination chemotherapy-treated patients with rituximab did not show a significantly better OS and DFS than the combination chemotherapy-treated patients without rituximab. On multivariate analysis; LDH >upper normal limit (DFS: hazard ratio [HR], 14.958; 95% CI, 2.474-90.432, *P*=0.003; OS: HR, 9.341; 95% CI, 1.635-53.361, *P*=0.012), and combination of CTx and RT (DFS: HR, 0.088: 95% CI, 0.009-0.834, P=0.034; OS: HR, 0.112; 95% CI, 0.014-0.918, *P*=0.041), retained statistical significance.

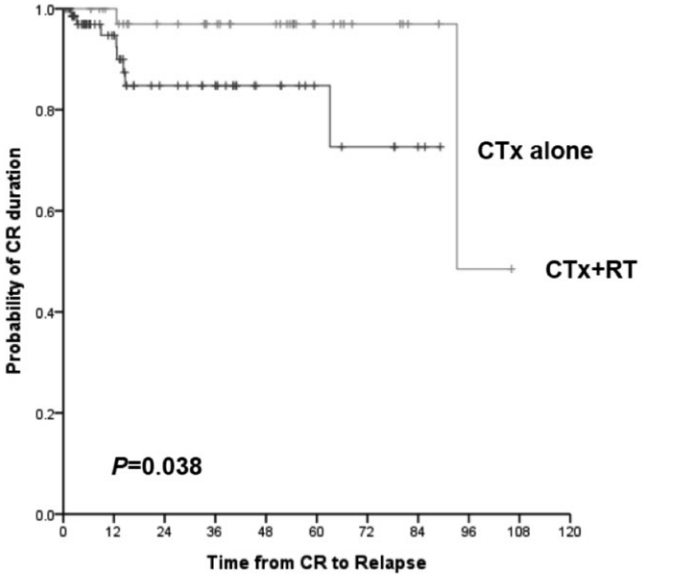

Figure 1.

Summary and Conclusions: The DFS and OS rates were significantly better for patients receiving combination of CTx and RT. A combined treatment, consisting of CTx and RT results in a satisfactory outcome in patients with localized primary DLBCL of tonsil.

PB1671

TREATING HIV-RELATED NON-HODGKIN LYMPHOMA IN SEVERELY IMMUNOCOMPROMISED PATIENTS, IN THE CART ERA. A SINGLE CENTER EXPERIENCE.

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Background: Low CD4+ lymphocyte count at diagnosis, in patients (pts) with HIV-related non-Hodgkin's lymphoma (NHL), is associated with poor clinical outcome, mainly due to low treatment tolerability. Severely immunocompromised pts are frequently excluded from clinical trials. Moreover, several authors claim that Rituximab should be held in pts with very low CD4 count due to the increased risk of infections
Aims: To define the outcome of severely immunocompromised HIV positive (+) pts with NHL in the real-life, in terms of possibility to receive treatment with curative intent, treatment-related mortality (TRM), complete remission (CR) rate and survival
Methods: All consecutive HIV pos pts diagnosed at our Institution from Jan 1997 to Dec 2013 (cART era) with aggressive systemic NHL and CD4+ lymphocyte count<100/mcl (CD4<100) at lymphoma diagnosis were considered. Histology included DLBCL, Burkitt, plasmablastic or other aggressive lymphomas. Pts'characteristics, treatment response and long-term outcome were compared with the concomitant series of HIV-related NHL pts with CD4+ lymphocyte count >100/mcl (CD4>100). Each pt was treated with standard state-of-the art in lymphoma and HIV care at the time of diagnosis, or enrolled in prospective trials. After 2001 all pts with CD20+ NHL received Rituximab combining chemotherapy (CT)
Results: Since 1985 to 2013 198 HIV pos pts were diagnosed at our Institution with NHL, 84 in the pre-cART era (1985-1996) and 114 in the cART era (1997-2013). The proportion of pts with CD4<100 during the cART era was 35% vs 63% in the pre-cART era (P=.0002). The clinical characteristics of the 2 groups (CD4>100 vs CD4<100) are shown in table 1. Significant differences included worse performance status (PS) and more pts with B symptoms in CD4<100 group and a greater proportion of pts on cART at NHL onset in CD4>100 group. The proportion of pts with CD4<100 we treated with curative intent was 63% vs 97% in pts with CD4>100 (P<0.0001). Pts were excluded from curative treatment for poor PS, major infections and/or severe comorbidities. After a follow-up of 60 ms (1-204), the 5y-overall survival (OS) of the 2 groups of pts was 31% (CD4<100) and 60% (CD4>100) (P=0.003). However, analysing pts who underwent CT, CR rate was similar (54% for CD4<100 and 64% in CD4>100; P=0.6). TRM was respectively 21% and 9% and included mainly infectious events. Overall, 14 pts (58%) died in CD4<100 (cause of death: NHL 7 pts,TRM 5,HCV cirrosis 1 and unknown 1) and 29 (41%) in CD4>100 group (NHL 19,TRM 6,lung cancer 1,car crush 1,suicide 1 and drug overdose 1). Response duration was not different (85% and 84% at 5 years). A trend towards worse OS and PFS were seen in pts treated with CD4<100 (38% vs 62% and 39% vs 52%) (P=NS). Fifteen pts with CD4<100 (DLBCL 11 and Burkitt 4) received Rituximab combining CT, while 6 pts (DLBCL 5 and Burkitt 1) did not. The CR rate for pts who received Rituximab was higher (60% vs 33%, P=NS) with similar TRM (20% vs 17%), and median OS and PFS of 19 vs 4.5 ms and 17 vs 4.5 ms (P=NS).
Summary and Conclusions: After the advent of cART the proportion of severely immunocompromised pts with HIV-related NHL has significantly decreased. In our single center experience, a substantial proportion of them could not receive adequate treatment and OS remained poor. However, if properly treated with standard immunochemotherapy, the CR rate was similar to pts with less immunocompromise, with chance of favourable long-term outcome. TRM was not negligible. Rituximab appears feasible in this setting and it seems to improve CR rate and possibility of long-term survival.

Table 1. Comparison between characteristcs of patients with CD4+ limphocyte count more or less than 100 at lymphoma diagnosis.

	CD4 < 100 pts 39	CD4 > 100 Pts 73	P
Histology: -BDLCL	25 (64%)	36 (49%)	0.1
- Burkitt	7 (18%)	18 (25%)	0.4
- Plasmablastic	2 (5%)	11 (15%)	0.2
- Others/NOS	5 (13%)	8 (11%)	1.0
Median age ys (range)	44 (27-64)	42 (26-73)	0.8
Stage III-IV	82%	81%	1.0
LDH > N	76%	77%	1.0
B symptoms	74%	55%	0.001
PS >1	79%	59%	0.03
IPI int-high	84%	80%	0.7
cART pre dx	31%	55%	0.01
Median CD4 count (range)	50 (1-99)	279 (100-1047)	< .0001
Detectable HIV-viremia	79%	63%	0.1
Prior AIDS	26%	17%	0.3
HCV+	50%	41%	0.4

PB1672

CLINICAL OUTCOMES OF PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA IN PARTIAL RESPONSE AFTER FIRST-LINE R-CHOP CHEMOTHERAPY: THE PROGNOSTIC VALUE OF SECONDARY IPI

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Background: Rituximab in combination with cyclophosphamide, vincristine, doxorubicin and prednisolone (R-CHOP) has been a standard treatment for patients with diffuse large B cell lymphoma (DLBL). Although complete response (CR) rate is up to 60~70%, 10~20% patients remained in PR after R-CHOP. Patients with PR are relatively chemo-sensitive compared to primary refractory diseases, but predictive factors and treatment strategy for these patients are in controversy.
Aims: We investigated the characteristics and prognosis for the patients who achieved PR after the first-line R-CHOP to analyze the survival of these patients and to define the prognostic factors affecting the clinical outcome of this specific patient group.
Methods: We performed a retrospective multicenter study on behalf of the Consortium for Improving Survival of Lymphoma (CISL). Patients who achieved PR by Cheson response criteria, and who had available PET scans at the end of R-CHOP were enrolled. Clinical parameters before and after R-CHOP were obtained to evaluate whether the patients' dynamic condition after treatment could predict the outcome. For survival analysis, PFS2 (from the date PR was

documented to disease progression or death) and OS2 (from the date PR was documented to death) were used.
Results: A total of 88 patients were enrolled. Median age was 53.5 years (21-88), and the number of patients with initial stage III-IV was 64 (72.7%). The International Prognostic Index (IPI) score at diagnosis was >2 in 46 (52.3%). At the time of PR documentation, secondary IPI scores (IPI2) assessed after R-CHOP were 0 in 33 (37.5%), 1 in 27(30.7%), and >1 in 28 (31.8%). The Deauville scores of PET scans after R-CHOP were ≤2 in 24 (28.2%), 3 in 26 (30.6%), ≥ 4 in 35 (41.2%), and unknown in 3 cases. As a second-line treatment, local radiation to residual lesion without any systemic treatment was performed in 33 (38.6%) patients. Intensive chemotherapy such as salvage chemotherapy or upfront ASCT was performed in 42 (47.7%) patients. Four (4.5%) patients received less intensive therapy such as rituximab or other oral agents. With a median follow up of 47.8 months, 3-year PFS2 and OS2 rates were 57.9% and 68.1%. In the univariate analysis, age, bone marrow involvement, lymphopenia, and high IPI score at diagnosis were significantly associated with poor PFS2 and OS2. High IPI2 score (≥1) and the Deauville score (≥4) were significantly associated with worse PFS2 (P=0.001 and P=0.029, respectively). In the multivariate analysis, high IPI2 was an independent predictive factor for PFS2 (HR 2.40, 95% CI 1.08-5.269, P=0.031) after adjustment with initial IPI (P=0.163), bone marrow involvement (P=0.005), lymphopenia (P=0.124) at diagnosis and the Deauville score after R-CHOP (P=0.338). For overall survival, high IPI2 score was also associated with poor OS2 in univariate analysis (P=0.013), but not the Deauville score (P=0.134). Only the initial IPI score >2 (P=0.041) and bone marrow involvement at diagnosis (P=0.001) remained significant in the multivariate analysis model.

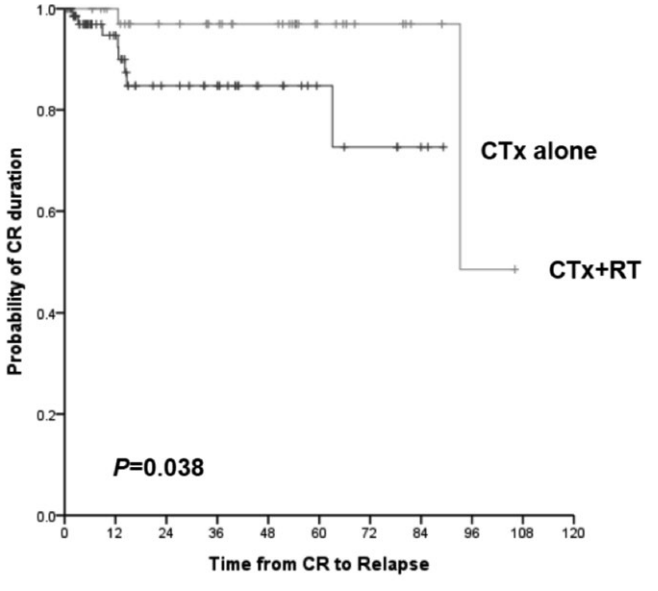

Figure 1.

Summary and Conclusions: IPI2 in patients with DLBL who achieved PR after R-CHOP was an important predictive factor for further survival outcomes. This suggests that the patient's condition and residual tumor burden might be helpful when we are planning the next treatment.

PB1673

SURVIVAL BENEFIT OF HEMATOPOIETIC STEM-CELL TRANSPLANTION FOR PTCLS: A RETROSPECTIVE COMPARISON STUDY BETWEEN CONVENTIONAL CHEMOTHERAPY AND STEM CELL TRANSPLANTATION FOR PTCLS FROM A CHINESE CENTER

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Background: Peripheral T-cell lymphomas (PTCLs) are rare malignancies with poor outcome after conventional chemotherapy. The role of stem cell transplantion in the treatment of PTCLs is still unclear.
Aims: Here, we present the result of a comparison study between conventional chemotherapy and stem cell transplantation for PTCLs in our centre.
Methods: From July 2004 to July 2014, 104 cases were analyzed retrospectively, including age, IPI score and morbid state before transplantion. 52 patients underwent HSCT including angioimmunoblastic T-cell Imphoma (n=15), anaplastic large cell lymphoma (n=14), PTCL not specified (n=12), NK/T cell lymphoma (n=11). In this group, 43 patients were IPI≥3-4). Thirty-three patients (63.5%) received autologous stem-cell transplantion (autoSCT) and nineteen patients (36.5%) received allogeneic stem-cell transplantion (allo-SCT). Before stem-cell transplantation, 43 Patients were in complete remission (CR), 2 patients were in partial remission (PR), 7 patients were not-remission(NR). In conventional chemotherapy group, 52 patients (median age, 49.5 years, IPI≥3-4: 40 patients) only received conventional chemotherapy. Primary outcomes of progression-free survival (PFS), and overall survival (OS) rates were estimated by using the Kaplan-Meier method.
Results: After a median follow-up of 23.5 months, K-M analysis showed that the 5-year PFS for HSCT and chemotherapy were 60% and 30%(P=0.006), the 5-year OS were 65% and 33% (P=0.007) (Fig 1), respectively. These results suggested that frontline stem cell transplantation for high-risk PTCLs may improve treatment outcome. The 5-year PFS for autoHSCT and alloHSCT were 60% and 58%(P=0.074). The 5-year OS were 62% and 61% (P=0.074) (Fig 2). There were no significant survival differences between the auto-HSCT and allo-HSCT.

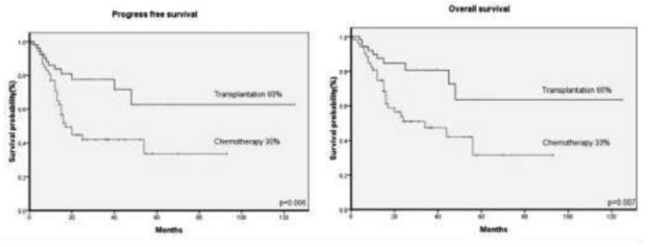

Figure 1. Progression-free survival an overall survival by transplantation and conventional chemotherapy for PTCLs.

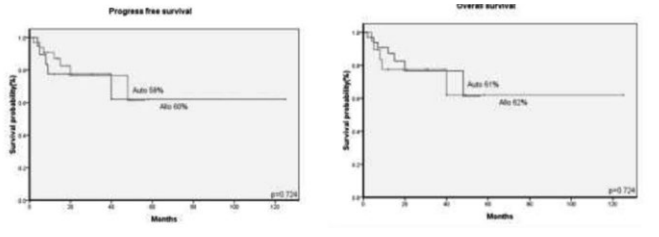

Figure 2. Progression-free survival an overall survival by auto-HSCT and allo-HSCT fot PTCLs.

Summary and Conclusions: The results of this retrospective study suggest that HSCT have great advantange copared to conventional chemtherapy on long-term survival in peripheral T-cell lymphomas. However, the resullt of auto-HSCT and allo-HSCT seems to have no difference. Therefore, autoHSCT should be considered to be the first -line therapy in peripheral T-cell lymphomas, especially for those patients with high risk factors.

PB1674

PERIPHERAL ABSOLUTE MONOCYTE AND LYMPHOCYTE COUNTS AT DIAGNOSIS PREDICT SURVIVAL IN PATIENTS WITH PRIMARY NODAL DIFFUSE LARGE B-CELL LYMPHOMA

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Background: Diffuse large B-cell lymphoma (DLBCL) is a biologically heterogeneous disease with primary nodal and extranodal origin. At present, the stratification of DLBCL patients is performed by the revised international prognostic index (R-IPI). However, some preliminary data suggest the important role of blood monocytes and lymphocytes in cancer biology. It has been assumed that baseline levels of peripheral absolute lymphocyte (ALC) and monocyte (AMC) counts have prognostic value in solid tumors. However, data regarding their impact on clinical outcomes of DLBCL patients are limited.
Aims: To evaluate the prognostic significance of baseline ALC and AMC in a cohort of newly diagnosed DLBCL patients treated with chemoimmunotherapy.
Methods: Data from 255 DLBCL patients, at a median age of 58.6 years (range, 19-82 years), diagnosed between 2007 and 2014, were evaluated retrospectively. Primary nodal and extranodal lymphoma were detected in 60.4% (154/255) and 39.6% (101/255), respectively. The patients were treated with R-CHOP. Values<1.0x 10^9/L for ALC and >0.8x 10^9/L for AMC were considered abnormal and applied to stratify patients in the present study. The prognostic influence of the ALC and AMC, and other factors including age, lactate dehydrogenase (LDH), β_2 microglobulin (β_2 M), R-IPI and Ann Arbor stage at diagnosis on 5-year overall- (OS) and disease-free (DFS) survival was studied by Kaplan–Meier curves. To evaluate the independent prognostic relevance of

ALC and AMC, univariate and multivariate Cox regression models were applied.
Results: The median ALC at diagnosis was 1.74x 10^9/L (range 0.28–7.16x 10^9/L) and abnormal ALC were detected in 16.8% (42/255) of the patients. A significantly higher proportion of patients with ALC<1.0x 10^9/L had elevated levels of LDH and β_2 M (76.2% vs 36.1% and 86.8% vs 57.6%, respectively), advanced disease stage (III – IV) (60.9% vs 34.9%), high risk (R-IPI 3-5) disease (43.9% vs 15.8%) in comparison to patients with ALC ≥ 1.0x 10^9/L. The median AMC at diagnosis was 0.59x 10^9/L (range 0.28–7.16x 10^9/L) and AMC >0.8x10^9/L were detected in 24.3% (62/255). Similarly, a significantly higher proportion of patients with abnormal AMC had elevated levels of LDH and β_2 M (59.7% vs 37.3% and 76% vs 60.1%, respectively), advanced disease stage (III – IV) (52.4% vs 34.9%), high risk (R-IPI 3-5) disease (36.8% vs 15.5%) in comparison to patients with AMC ≤0.8x 10^9/L. Patients with abnormal ALC at diagnosis experienced an inferior 5-year OS (18% vs 64.7%, P<0.001) and 5-year DFS (48.9% vs 78.2%, P<0.001). Patients with abnormal AMC at diagnosis experienced an inferior 5-year OS (60% vs 79.2%, P=0.002) and 5-year DFS (53.6% vs 80.4%, P=0.001). However, regarding nodal and extranodal origin of lymphoma the analysis revealed that only primary nodal DLBCL patients with abnormal baseline ALC and AMC experienced inferior 5-year OS and 5-year DFS. Moreover, an independent significant association between abnormal ALC and poor clinical outcome in terms of OS (hazard ratio (HR) 2.14, 95% confidence interval (CI) 1.13-4.06, P=0.019) and DFS (HR 5.88, 95% CI 2.00-17.25, P=0.001) was identified by multivariate analysis only in primary nodal DLBCL patients.
Summary and Conclusions: Our data suggest that ALC and AMC in peripheral blood at diagnosis predict survival in primary nodal DLBCL treated with R-CHOP. However only ALC is independent, poor prognostic factor for DFS and OS, and can be used in combination with other prognostic features to better predict the outcome of these patients.

PB1675

PROGNOSTIC FACTORS AND INTERNATIONAL PROGNOSTIC INDEX VARIANTS IN PATIENTS WITH B-LARGE CELL LYMPHOMA-AN OBSERVATIONAL STUDY OF KROHEM, THE CROATIAN COOPERATIVE GROUP FOR HEMATOLOGIC DISEASES

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Background: B-large cell lymphoma (B-LCL) is the most common form of NHL. 5-year survival rates vary between 40 and >90% depending on prognostic factors but the importance of many of them is disputed. Those found most important and reproducible in the pre-rituximab era were included in the IPI. Since the original description, various variants of this index have been published.
Aims: To reassess the value of the IPI, revised IPI (R-IPI), age-adjusted IPI (aaIPI), stage-adjusted IPI and different possible clinical prognostic factors in an unselected population of patients with B-LCL receiving rituximab containing front-line therapy.
Methods: 371 patients diagnosed with B-LCL during 2007 and 2008 and treated with rituximab plus chemotherapy in 16 Croatian hematology departments were included in this study. Patients were registered at the time of treatment start, and data on demographics, clinical features and laboratory parameters collected. Follow-up was performed yearly. The study was approved by the Croatian Central Ethics' Committee. Prognostic values of IPI, R-IPI, aaIPI, stage-adjusted IPI, individual factors used in indices (age, PS, LDH, stage, number of extranodal organs involved), bulk, gender, anemia, bone marrow infiltration and the presence of B symptoms were evaluated with respect to overall survival (OS) and progression-free survival (PFS) were evaluated. Survival analyses were performed using the Kaplan-Meier method and comparisons using the log-rank test. Multivariate analysis is ongoing.
Results: 5-year OS and PFS of the whole cohort were 50% and 49.5%. Significant negative prognostic factors in univariate analyses for OS and PFS were: age>65, LDH high, PS>1, stage>2, Hb<120 g/l, male gender, bone marrow infiltration and presence of B symptoms. Number of involved extranodal sites and presence of bulky disease did not influence prognosis. Regarding prognostic indices, conventional IPI was most useful, distinguishing 4 categories with reasonable proportions of patients. R-IPI was less useful; the differences in PFS between the three prognostic categories were significant, but there was no difference in OS between patients with scores 0 and 1-2. aaIPI distinguished only two categories; patients with score 0 had excellent prognosis, while there was no difference in outcomes between those with scores 1 and 2-3. Stage adjusted IPI distinguished three prognostic groups, but very few patients had a score 0.
Summary and Conclusions: Our study suggests that conventional IPI remains the most useful prognostic index. Bulky disease does not seem to be of prognostic importance, probably because of widespread use of adjuvant radiotherapy to initial bulky sites after immunochemotherapy. As seen in some other studies, men have a worse prognosis, possibly related to differences in rituximab metabolism. Additional negative prognostic factors include anemia, B symptoms and bone marrow infiltration, possibly as markers of aggressive systemic disease.

PB1676

CLINICAL IMPACT OF EARLY RECOVERY OF POST-TRANSPLANT PERIPHERAL BLOOD ABSOLUTE LYMPHOCYTE COUNT ON THE OUTCOME OF FRONTLINE AUTOLOGOUS STEM CELL TRANSPLANTATION FOR DIFFUSE LARGE B-CELL LYMPHOMA

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Background: Several studies have shown that lymphopenia, which is considered a surrogate marker of immunological incompetence, has considered as an adverse prognostic factor in Non-Hodgkin`s lymphoma (NHL). Recently, it has been suggested that early recovery of an absolute lymphocyte count (ALC) at 2-3 weeks following therapy has been associated with superior progression-free survival (PFS) and overall survival (OS) in patients who received autologous stem cell transplantation (ASCT) for NHL,. However, the prognostic significance of early recovery of peripheral ALC following frontline ASCT in diffuse large B-cell lymphoma (DLBCL) remains unclear.
Aims: The purpose of this study was to investigate the prognostic role of early recovery of peripheral ALC after ASCT in patients with DLBCL who underwent frontline ASCT.
Methods: We retrospectively evaluated 51 patients who underwent ASCT for DLBCL at Yonsei University Severance Hospital between January 2006 and 2014. All patients were treated with R-CHOP (rituximab-cyclophosphamide, doxorubicin, vincristine, and prednisone) every 3 weeks for 3 to 8 cycles as first-line therapy and received frontline ASCT as consolidation. Most patients (n=40) received intravenous busulfan-based conditioning chemotherapy. The ALC at the time of D+14 after ASCT was obtained. Receiver operating characteristics (ROC) analysis was performed to determine the optimal cut-point for the ALC.

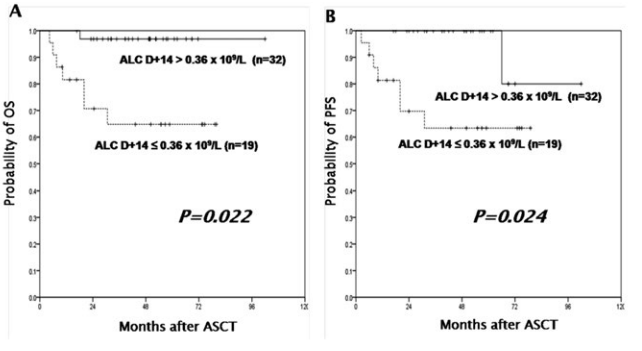

Figure 1.

Results: The study population included 51 patients with a median age of 53 years (range, 19-66 years). Fourty-six (90.2%) had stage IV disease according to the Ann Arbor staging system. Most patients (87.3%) were younger than 60 years. Thirty-five (68.8%) patients had B symptoms. International prognostic index (IPI) was low in 2 (4%), Low-intermediate in 15 (30%), high-intermediate in 25 (50%) and high in 8 (16%). Pre-transplant disease status was complete remission (CR) in 30 (58.8%) patients and partial remission (PR) in 21 (41.2%) patients. The median ALC at D+14 after ASCT was 0.43x 10^9/L (range, 0.03-1.57x 10^9/L). The ROC curve analysis identified 0.36x 10^9/L as the cutoff value of ALC at D+14 for predicting relapse with an area under curve of 0,759 (95% CI, 0.628-0.890, P=0.020). When comparing the baseline clinical characteristics of patients with an ALC at D+14 of ≤0.36x 10^9/L (low ALC group, n=19) and >0.36x 10^9/L (high ALC group, n=32), no significant difference was found between two groups, except for a female dominance and presence of B symptoms at diagnosis in the low ALC group. The median survival of patients following ASCT was 49.4 months (range, 4.4-101.9 months). In a univariate analysis from the time of ASCT, it appears that high ALC at D+14 was associated with a better OS (HR=0.083; 95% CI 0.010-0.694, P=0.022) and PFS (HR=0.086; 95%CI 0.010-0.720, P=0.024) and event-free survival (EFS) (HR=0.283; 95% CI 0.082-0.971, P=0.045). Multivariate analysis revealed that high ALC at D+14 was a good prognostic factor for OS (HR=0.086; 95%CI 0.008-0.979, P=0.048).
Summary and Conclusions: The early recovery of ALC at D+14 after ASCT can be regarded as a good prognostic marker in patients with DLBCL who underwent frontline ASCT.

PB1677

INVESTIGATION OF SEROUS EFFUSIONS WITH FLOW CYTOMETRY

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Background: In current clinical practice serous effusions are evaluated by cytopathologic, chemical and microbiologic examinations. As well as infections and inflammatory diseases, serous body fluids can also be observed in hematopoietic and solid neoplastic diseases. Flow cytometry(FC) is well established as a critical and quick diagnostik tool used routinely in the diagnosis of hematopoietic neoplasms by fine-needle aspiration and an alternative diagnostic method for the evaluation of serous body fluids.
Aims: The aim of this study was to investigate immunophenotypic analysis of serous body fluids by FC.
Methods: During the time period of 2010-2014, a total of 79 consecutive serous effusion specimens (45 pleural, 33 peritoneal, 1 pericardial) of 76 cases (40 female, 36 male, 24-84 years of age) were investigated with the six-color FC (FACSCanto II, BD Biosystems, San Jose, USA) in the Division of Hematology Laboratory at Akdeniz University Hospital. We retrospectively analyzed these datas.
Results: FC was positive for definitive immunophenotypic evidence of a hematopoietic malignancy in 3 of 45 pleural fluid specimens (Diffuse Large B-cell lymphoma(1 case), Burkitt's lymphoma(1 case), T-cell Acute Lymphoblastic Leukemia / Lymphoma(1 case)) and 3 of 33 peritoneal fluid specimens (T-cell prolymphocytic leukemia(1 case), diffuse large B-cell lymphoma(1 case), Burkitt's lymphoma(1 case)(Figure 1)). Subsequent tissue studies confirmed the diagnosis of a hematopoietic neoplasm in these seperate six cases.

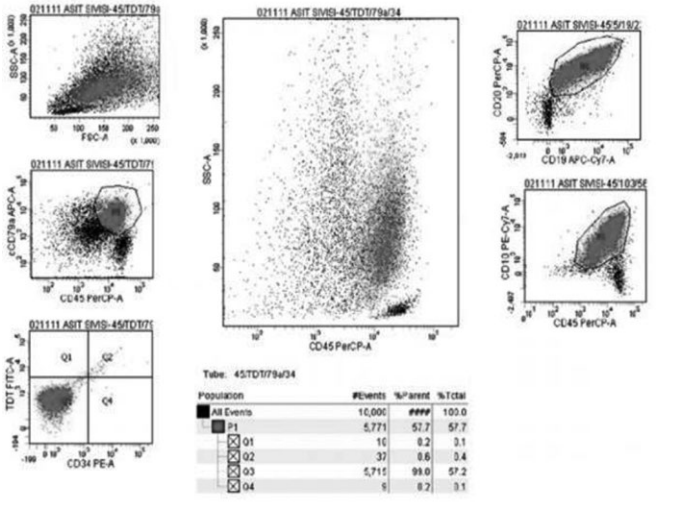

Figure 1.

Summary and Conclusions: Cytopathologic evaluation may lay long especially in the diagnosis of aggressive lymphomas which requires urgent evaluation and treatment. Immunophenotypic analysis of serous body fluids by FC can provide better access to early diagnosis and also accurate clinical staging.

PB1678

EVALUATION OF MINIMAL RESIDUAL DISEASE AND USAGE OF TARGETED THERAPY IN PEDIATRIC PATIENTS WITH ANAPLASTIC LARGE CEL LYMPHOMA

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Background: Anaplastic large cell lymphoma (ALCL) is an aggressive T cell non-Hodgkin lymphoma, characterized by an increased risk of relapse. Its presentation maybe cutaneous or systemic. It is classified on the basis of the expression of anaplastic lymphoma kinase (ALK) protein, which carries the translocation t(2;5)(p23;q35) and results in the specific fusion gene Nucleophosmin (*NPM-ALK*). In 50% to 60% of patients with NPM-ALK–positive ALCL, *NPM-ALK*–expressing cells can be detected in peripheral blood and bone marrow samples by qualitative reverse-transcriptase polymerase chain reaction (RT-PCR). ALCL is designated by the uniform expression of CD30, making this surface antigen a target for immunotherapeutic approaches.
Aims: The purpose of the present report was the evaluation of minimal residual disease in children with ALCL and its usage in guiding treatment with targeted therapy, namely the anti-CD30 antibody-drug conjugate, Brentuximab vedotin.
Methods: RT-PCR for *NPM-ALK* was performed in bone marrow or peripheral blood samples in two pediatric patients with NPM-ALK–positive ALCL, at regular time points.
Results: The first patient is an 11-year-old girl with systemic NPM-ALK–positive (lymphadenopathy, skin, orbit and central nervous system involvement). She achieved remission and completed chemotherapy based on the AEIOP protocol. Three months after treatment completion she presented with disseminated relapse and required ventillatory support. She started targeted treatment with Brentuximab vedotin. She promptly responded to the treatment and she was able to be weaned off ventillatory support within 3 days after the infusion. The patient entered clinical and radiological remission (MRI and PET scan) with Brentuximab vedotin given at dose of 1.8 mg/kg IV every 3 weeks. She received a total of 6 cycles and subsequently underwent mega-therapy with autologous bone marrow transplantation rescue. Six months after transplantation, RT-PCR for *NPM-ALK* became positive, while the patient remained in clinical remission and the imaging examinations were negative for disease. Brentuximab vedotin was reinstituted and she entered molecular remission with negative minimal residual disease after the 5th cycle. The second patient is a 9-year-old boy with systemic NPM-ALK–positive ALCL, presenting with an abdominal primary mass. He completed chemotherapy and is monitored by radiological examinations and RT-PCR for *NPM-ALK* at regular time points. Minimal residual disease in this patient remains negative six months after the end of therapy.
Summary and Conclusions: Recent advances in the treatment of adult patients with NPM-ALK–positive ALCL have led to better follow up and expanded options of therapeutic modalities, especially for patients who relapse. Early detection of relapse by assessing minimal residual disease with molecular techniques seems to be superior to classical clinical risk features, identifying a high risk for overt relapse group of patients, which can be amenable by alternative therapeutic approaches. In conclusion, the potential use of minimal residual disease detection and second-line treatment like the novel antibody-drug conjugate, Brentuximab vedotin, warrant further evaluation for pediatric patients with CD30-expressing ALCL.

PB1679

PERFORMANCE OF THE NGS-BASED LYMPHOTRACK® IGK ASSAY: IDENTIFYING AND MONITORING CLONALITY IN LYMPHOPROLIFERATIVE DISEASE

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Background: During B-cell development functional immunoglobulin genes are assembled from individual V, D, and J gene segments to generate V-D-J combinations of length and sequence that are unique for each cell. Rearrangements within IGH occur first, followed by IGK. Along with the Vk and Jk segments, there are other elements in the IGK loci that can be involved in recombination. The Kde can rearrange to Vk gene, but also to an isolated RSS in the Jk-Ck intron (INTR). If IGK rearrangements that produce functional IgH/IgK molecules are not successful, rearrangement proceeds within the other immunoglobulin light chain (IGL) locus. Leukemia and lymphomas originating from the malignant transformation of individual lymphoid cells generally share one or more of these cell specific or "clonal" gene rearrangements. Assays that identify clonal lymphocyte populations in clinical specimens are used on a routine basis to assist in the diagnosis of lymphoproliferative disease.
Currently, most IGK clonality assays use multiplex PCR followed by capillary electrophoresis. However, these methods often do not provide sufficient analytical sensitivity and are incapable of identifying the specific rearranged DNA sequence data required to track clones in follow up testing. The emergence of cost-effective, next-generation sequencing (NGS) platforms and development with associated bioinformatics tools have resulted in powerful new approaches for clonality detection and monitoring.
Aims: To establish the performance of a sensitive, robust and reliable NGS assay formatted for the Illumina® MiSeq instrument that includes the bioinformatics tools to identify clonal IGK gene rearrangements and the corresponding specific V-J DNA sequence information.
Methods: LymphoTrack® IGK Assay (Invivoscribe, Inc., San Diego, CA USA) uses a single PCR multiplex master mix to amplify each sample (24 indices,5 reactions each, are included in each kit).The amplicons were purified using the AMPureXP system and purified equimolar amounts of amplicons were pooled to generate a library. A portion of the library was sequenced on a MiSeq instrument using the MiSeq v2 Reagent kit (500 cycles). The MiSeq data was analyzed using LymphoTrack® bioinformatics software, which generated frequency distributions and identified the rearranged DNA sequences.
Results: Data generated with the LymphoTrack® IGK Assay and bioinformatics software identified clonality and corresponding DNA sequences of IGK Vk-Jk, Vk-Kde, and INTR-Kde gene rearrangements. The results obtained generally were concordant with results of testing with capillary electrophoresis. The assay demonstrated excellent linearity, sensitivity, and reproducibility using contrived samples. This assay was used to test genomic DNA from peripheral blood, bone marrow and FFPE specimens. In addition, this NGS testing approach identified the specific break point sequence of the INTR element.

Summary and Conclusions: A comprehensive NGS assay has been developed for the Illumina MiSeq platform that identifies clonal IGK Vk-Jk, Vk-Kde and INTR-Kde rearrangements and the associated specific DNA sequences. The assay can be used both to detect and monitor clonal populations.

PB1680

PRIMARY CENTRAL NERVOUS SYSTEM POST-TRANSPLANT LYMPHOPROLIFERATIVE DISORDER (PTLD-CNS) IS AN EBV-RELATED DISEASE WITH GOOD RESPONSE TO ISS REDUCTION AND RADIOTHERAPY: SINGLE-CENTER STUDY OF 16 CASES

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Background: Post-transplant lymphoproliferative disorders are a heterogenous group of diseases occurring in the setting of post-transplant immunossupression. Clinically, extranodal involvement is common, and it occurs in the CNS in approximately 7-15% of cases. Most data on PTLD-CNS are based on case series/reports, with an exception of a multicenter study of 84 patients. In this group, PTLD-CNS showed to be a poor prognosis, late-ocurring EBV-associated disease. We retrospectively analyzed 16 cases of PTLD-CNS in Hospital do Rim, Sao Paulo, Brazil.
Aims: To analyze the cases of PTLD with CNS involvement diagnosed on our center over the past 17 years.
Methods: From 1998 to 2014, a total of 11,284 kidney transplants were performed at Hospital do Rim e Hipertensão/UNIFESP (Federal University of São Paulo). We retrospectively analyzed cases of PTLD with CNS involvement diagnosed over the past 17 years. Only confirmed cases of PTLD with available clinical and epidemiological data were included.
Results: From 1999 to 2014, a total of 20 patients were diagnosed with PTLD-CNS. Four patients were excluded from the analysis due to conflict data. Among 16 patients, the median age at time of diagnosis was 40, with a male:female ratio of 0,77:1. 56% of patients received ATG at the time of transplant. Regarding immunossupression (ISS), all patients received Prednisone, 37,5% received FK, 43,7% received MMF, and 56,2% received CSA. The median time of transplant to PTLD was 93 months, with 25% patients with polymorphic PTLD and 75% with monomorphic PTLD (DLBCL). Regarding EBV positivity, 14/16 were EBV+, with 1 EBV- and 1 inconclusive. All patients had ISS reduction, 43% of patients received brain radiotherapy. Only 1 patient received high dose Methotrexate. 75% of patients responded to treatment. No death related to lymphoma progression was observed: four patients died, all of them due to infectious complications, and none of them during therapy.
Summary and Conclusions: In our population, CNS-PTLD was an EBV-related disease with good response to ISS reduction and radiotherapy, with most patients achieving responses. No death due to disease progression was observed. Late effects of brain radiation, however, should be taken in account when deciding the best therapy for this rare complication of transplant.

Bleeding disorders (congenital and acquired)

PB1681

Abstract withdrawn

PB1682

CIRCUMCISION AND COMPLICATIONS IN ADOLESCENT AND ADULT PATIENTS WIITH HEMOPHILIA IN SOUTHERN PART OF TURKEY

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Background: Circumcision is the oldest and most frequent surgical procedure in the world and especially in Turkey as is seen in the other Islamic countries because of religious and traditional pressures. The world health organization defines an adolescent as any person between ages 10 and 19. The social pressures are very strong in adolescent patients and especially adult patients. In this study, we aim to report the experience of circumcision at Çukurova University in total 36 adolescent and adult patients with hemophilia between 1994 and 2013.
Aims: We retrospectively reviewed medical records of 33 hemophilia patients without inhibitors and 3 hemophilia patients with inhibitors who had been circumcised. Before the year 2000, factor concentrates were given before and after circumcision for 6-7 days. After 2000, we used fibrin glue together with factor concentrates for only 3 days. By-passing agents were used for circumcision in hemophilia patients with inhibitors.
Methods: We retrospectively reviewed medical records of 33 hemophilia patients without inhibitors and 3 hemophilia patients with inhibitors who had been circumcised. Before the year 2000, factor concentrates were given before and after circumcision for 6-7 days. After 2000, we used fibrin glue together with factor concentrates for only 3 days. By-passing agents were used for circumcision in hemophilia patients with inhibitors.
Results: 33 patients with hemophilia were circumcised in our centre under general anesthesia except for 3 patients who were given local anesthesia. Eight of 33 hemophilia patients (24,2%) without inhibitors had 5 mild and 3 moderate bleeding complications. A few patients had significant bleeding despite adequate factor replacement. Two of three hemophilia patients with inhibitors had 2 mild bleeding complications.
Summary and Conclusions: Our experience showed that circumcision for patients with hemophilia should be carefully performed by surgeons together with pediatric hematologist under appropriate conditions in hemophilia centers which has comprehensive coagulation lab.

PB1683

MONITORING OF TREATMENT WITH BYPASSING AGENTS IN PATIENTS WITH ACQUIRED AND CONGENITAL HAEMOPHILIA WITH INHIBITORS USING ROTEM: A SINGLE-CENTRE EXPERIENCE

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Background: Acquired and congenital haemophilia with inhibitor against FVIII or FIX are usually managed with bypassing agents, such as recombinant factor VIIa (rFVIIa) and activated prothrombin complex concentrates (APCC). To be able to evaluate and predict the clinical effects of treatment with bypassing agents, global coagulation assays could be useful monitoring tools. One of the currently leading candidate methods for this purpose is thromboelastography/rotational thromboelastometry (TEG/ROTEM).
Aims: Our purpose is to evaluate the changes in ROTEM parameters during treatment with bypassing agents and its correlation with the clinical effect.
Methods: In this retrospective study 11 haemophilia patients were included (age 5-85 years). Four (n:4) were patients with congenital severe haemophilia A with inhibitors and seven (n:7) were patients with acquired haemophilia A. Recorded total 15 bleeding episodes, acute or associated to surgical procedures and all of the patients treated with APCC. Monitoring of global hemostasis was performed with ROTEM assay, before and after bypassing agent administration.
Results: Excellent and good haemostatic effect was demonstrated in 87% of patients. One patient had adverse effect complicated with thrombosis. According to ROTEM analysis in NATEM the average of clotting time (CT) before and after APCC administration was 2110 sec and 888 sec respectively. Furthermore the clot stability was evaluated with maximum clotting firmness (MCF) parameter and that was 11 mm and 62 mm before and after APCC administration respectively.
Summary and Conclusions: The results of this single-centre report demonstrates, in a limited number of patients, improvement in ROTEM parameters (CT and MCF in NATEM) after APCC administration. In the most of the cases these results accompanied with good or excellent clinical response (87%) in

bypassing agent treatment. According to our data the ROTEM method could be a useful monitoring tool to predict the efficacy of treatment with bypassing agents in haemophilia patients with inhibitors. Undoubtedly further studies should be performed with adequate number of patients in order to reinforce our conclusion.

PB1684

RARE BLEEDING DISORDERS IN OMANI CHILDREN: A SINGLE CENTER EXPERIENCE

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Background: Rare bleeding disorders (RBDs) are autosomal recessive heterogeneous group of inherited coagulation factor deficiencies, with a prevalence varying between 1:500,000 and 1:2,000,000. Remarkably, there is a paucity of data on RBDs from the Middle East. Despite education and social modernization, Oman still has a high prevalence of consanguineous marriage, reaching more than 50% of all registered marriages. Being mostly autosomal recessive, RBDs are expected to be relatively high in such community compared to western countries.
Aims: The aim of the current work is to study the demographic characteristics, clinical presentations and management of RBDs in Omani pediatric patients.
Methods: Retrospective data analysis of all children diagnosed with inherited coagulation factor deficiencies in Pediatric Hematology Unit, Sultan Qaboos Universsity Hospital, Muscat, Oman from January 2009 till December 2014.
Results: Deficiencies of fibrinogen, FV, FVII, FX and FXIII were diagnosed in 16 pediatric patients (7 males and 9 females), accounting for 9.5% (16/169) of all children with inherited coagulation factor deficiencies. The age ranged from 3 days to 6 years and consanguineous marriages were found in 13/16 cases (81.3%). The clinical spectrum varied from mild mucocutaneous bleeding to serious sight-threatening retrobulbar and intraocular hemorrhage with subsequent unilateral enucleation. As an initial presentation, intracranial hemorrhage occurred in 5/16 cases (31.3%). Two patients (12.5%) suffered from global developmental delay due to severe intracranial hemorrhage in early infancy. Four patients (3 with FV deficiency and 1 with FXIII deficiency) are on regular prophylaxis. The detailed demographic data, clinical presentations, complications and management of all patients are shown in Table 1.

Table 1. Demographic data and clinical characteristics of pediatric patients with rare bleeding disordes.

Data	Afibrinogenemia	Hypofibrinogenemia	FV def	FVII def	FX def	FXIII def
Number	3	4	4	3	1	1
M:F	1:2	1:3	1:1	1:2	1:0	1:0
Age range	3 d-6 m.	6-18 m.	2 m-6 y.	2-6 m.	9 m.	5 d.
Consanguinity %	100%	75%	75%	66.6%	100%	100%
Intracranial *	1	-	2	1	-	1
Umbilical stump*	2	1	-	-	-	-
Musculoskeletal*	-	-	1	1	-	-
Post circumcision*	1	1	-	-	1	-
Muco-cutaneous*	3	4	1	2	1	-
Intraocular*	1	-	-	-	-	-
Developmental delay	-	-	1	-	-	1
Treatment	Cryoppt	Cryoppt	FFP	rFVIIa	APCC	Cryoppt
Prophylaxis	-	-	3 cases	-	-	1 case

* bleeding site, def:deficiency, d: days, m: months, y: years, cryoppt: cryoprecipitate, APCC: Activated prothrombin complex concentrate, FFP: fresh frozen plasma, rFVIIa: recombinant activated FVII.

Summary and Conclusions: In conclusion, children with RBDs constitute almost one tenth of cases of hereditary coagulation factor deficiencies in our center. They have some unique features in terms of severity, clinical profile and the need for prophylaxis early in life. We recommend establishing a national/regional registry of RBDs in collaboration with other centres in Oman and the Middle East. This will serve to identify the epidemiology, clinical presentations, genotype-phenotype correlation and therapeutic options of such rare, yet significant disorders in this part of the world.

PB1685

COMBINED FV AND FVIII DEFICIENCY (F5F8D) IN A CHINESE FAMILY WITH A NOVEL MISSENSE MUTATION IN MCFD2 GENE

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Background: Mutations in LMAN1 or MCFD2 genes cause a rare autosomal recessive bleeding disorder, which was named combined FV and FVIII deficiency (F5F8D). Encoded proteins by MCFD2 and LMAN1 genes which may form a Ca2+-dependent cargo receptor complex have been confirmed to participate in the transport of FV and FVIII from the endoplasmic reticulum (ER) to the Golgi. We made a diagnosis F5F8D by findings of decreased FV and FVIII levels in a Chinese family.
Aims: Try to study the molecular mechanism of the disease and observe the clinical features and treatment in this family.
Methods: We do a molecular genetic analysis in the family. All the exons of LMAN1 and MCFD2 genes were PCR amplified and sequenced.
Results: We found a novel missense mutations p.Asp81Ala in MCFD2 gene in a Chinese family. The patient was homozygous missense mutation for Asp81Ala in exon 3 of MCFD2 gene, while his father and one of his sisters were heterozygous. LMAN1 gene in this family was revealed no additional mutations.
Summary and Conclusions: The presence of consanguineous marriage in the family played an important role in the cause of this disease. The Asp81 missense mutations reported in MCFD2 to date was carried out from the many patients, like Asp81Tyr and Asp81Asn, while the mutations 'Asp81Ala' have not previously been reported. Therefore, It seems likely that the mutation in the Asp 81 is a hot region, The Asp81 residue replacement by Ala most likely disrupts the MCFD2–LMAN1 cargo receptor complex.

PB1686

SURGICAL MANAGEMENT OF ELECTIVE SURGERY IN PATIENTS WITH MILD HAEMOPHILIA FROM A SINGLE INSTITUTION

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Background: There is a spectrum of severity of haemophilia, defined as 'mild', 'moderate' or 'severe' according to the plasma levels of FVIII or FIX activity. Mild haemophilia may not be diagnosed until a minor surgery causes prolonged bleeding. Although general surgery in patients with 'mild' haemophilia has become more common, the surgical literature concerning these patiens remains limited.
Aims: The purpose of this single centre retrospective study was to investigate perioperative management and outcome of mild haemophiliac patients undergoing different surgical procedures.
Methods: For this study, we revised 12 mild hemophilia patients, who underwent 31 surgical procedures between 2008 and 2014 at our institution. Classification into minor/major surgery was carried out according to the BUPA system. We analized the hematologic diagnosis, type of surgery, genetic mutation linked to haemophilia, treatment undertaken to correct the bleeding disorders, the dose and duration of clotting factor administrated and post-surgical complications.
Results: We revised a total of 31 surgical procedures performed in 12 mild haemophilia patients, 9 Haemophilia A (HA) and 3 Haemophilia B (HB). Median age at surgery 37 years (range: 20-54). No patients received an anticoagulatory prophilaxys for perioperative management. Among this haemophiliac group, 5 patients (41.6%) were hepatitis C seropositive who did not receive antiretroviral therapy. We didn´t found any seropositive for HIV or other viral disease. Molecular study was available in 7 patients (6 HA and 1 HB). 6 patients showed missense mutations: exon 14 (p.Arg698Trp), Exon 7 (p.Val 266Ala), Exon 2 (c.6374G>A), exon 12 (p.Arg593Cys), Exon25 (p.Asp2267Gly) and Exon 3 (p.Asp82Glu) and exon 2 (c.6374G>A) in 1 HB patient. Data about surgery, pre and post procedure treatment are seen in Table 1, 23 (74.2%) were minor and 8 (25.9%) were major surgery. Loco-regional anaesthesia was performed in 22 (70.9%) procedures. 8 (66.7%) patients (6 HA/2 HB) underwent more than one surgical procedure. Pre and post-procedure dose of factor replacement treatment were adjusted according WFH guidelines. Plasma derived FVIII was employed in 2 patients during 4 procedures and recombinant FVIII or FIX were employed in 9 patients. Desmopressin alone and tranexamic acid alone were employed in 2 patients who underwent dental extraction (no wisdom tooth). Regarding minor procedures, 20 patients received Tranexamic Acid IV on postoperative days 1, 2 and 3. Before surgery, 1 patient who underwent 1 surgical procedure (dental extraction) showed a baseline factor level 5%>10%, 6 patients (12 procedures) showed factor levels 10%>20%, 5 patients levels 21%>30% (16 procedures) and 2 patients (2 procedures) 31%>40%. One HB patient (3.2% of all procedures) with a baseline factor level of 7.6% developed local bleeding after dental extraction although preoperative dose of recombinant FIX was administered, and he required one additional dose of recombinant FIX. No inhibitor development was observed.

Table 1.

SURGICAL PROCEDURES

Dental procedures	Dental implants Dental extractions	17 1
Orthopedic procedures	Arthroscopies Osteosynthesis Osteosynthesis material extraction	2 3 2
Urological surgery	Testicular torsion	1
Another surgeries	Inguinal repair Haematoma muscle drainage Fibroma extirpation Skin suture Foot surgery	1 1 1 1 1

Summary and Conclusions: Surgical procedures in patients with mild haemophilia can be performed with low hemorrhagic morbidity rates when there is good haematological support and appropriate hemostatic treatment. There was no association between the type of product used in perioperative period and inhibitor development in our group of patients. It is important that minor surgery should be scheduled to use desmopressin analogs +/- antifybrinolytics agents instead of recombinant clotting factor VIII/IX to minimize the risks of therapies and reduce overall treatment costs.

PB1687

CHANGES IN THE HEMOSTATIC SYSTEM IN PATIENTS WITH APLASTIC ANEMIA

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Background: Introduction. Since the first description by Paul Arliam acquired aplastic anemia (AA) over 100 years ago, but today this disease remains in the spotlight hematologists and physicians in adjacent fields. AA-a serious disease of the blood system, resulting from damage to the stem cells (precursors of all blood cells), resulting in a profound inhibition of normal hematopoiesis with the development of pancytopenia. One of the main clinical manifestations is hemorrhagic syndrome caused by thrombocytopenia. Moreover, the clinically significant hemorrhagic syndrome is observed usually only with severe thrombocytopenia. In connection with this, is of great interest the study of the compensating functions of the system of hemostasis in AA – patients.
Aims: The search for diagnostic markers hypercoagulation status in patients with AA.
Methods: The research material was venous blood 25 patients (17 men and 8 women, mean age 38±0,5), with a diagnosis of aplastic anemia". Plasma level of hemostasis was evaluated in the following coagulation tests: activated partial thromboplastin time (APTT), prothrombin time Quick (PT), thrombin time (TT), the concentration of fibrinogen (Fg), the activity of the factor VIII activity of antithrombin (AT). The control group consisted of 40 clinically healthy men and women of similar age.
Results: Compared to the normal performance of screening tests (APTT, PV, TV, FG) in 18 (72%) of 25 patients reported a significant increase in the activity of factor VIII, which is usually regarded as an indicator hypercoagulation state of hemostasis (189,8%±96,5% against normal value 119,0%±30.5%, P<0,001). However, in 8 (44%) of 18 patients with elevated factor VIII revealed reduced levels of antithrombin, which amounted to 68% (fluctuations from 74% to 62%). However, clinical manifestations hypercoagulation syndrome (thrombosis and thromboembolism) single patient not were observed.
Summary and Conclusions: Thus, in patients with AA showed increased activity of factor VIII and decreased levels of anti-thrombin. Whether these indicators markers hypercoagulation state, or is it a manifestation of compensatory functions, which prevent severe hemorrhagic complications in this category of patients. Research in this direction continues.

PB1688

FACTOR VII DEFICIENCY IN WEST ALGERIA

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Background: Factor VII deficiency is one of the rare inherited bleeding disorders, the frequency is 1/500000. His molecular study is very interesting because of its rarity.
Aims: The objectif of this study was to describe the bleeding tendencies and the variation of factor VII level.
Methods: Retrospective study coducted for 7 years (2008 to 2014) in the west Algeria. 63 patients has been tested for diagnosis of rare bleeding disorder.
we use the assigned categories of clinical bleeding severity of European Network of Rare Bleeding Dsorders: 1. Asymptomatic (no document bleeding episodes). 2. Grade I bleeding (bleeding that occured after trauma or drug ingestion). 3. Grade II bleeding (spontaneous minor bleeding, bruising, ecchymosis, minor wounds, oral cavity bleeding, epistaxis and menorrhagia). 4. Grade III bleeding (spontaneous major bleeding, hematomas, hemarthrosis, central nervous system, gastrointestinal and umbilical cord bleeding).
Results: 18 patients (28.57%) diagnosed with factor VII deficiency, 06 patients have factor level<10% classified with severe form, 01 patients had factor level between 10 and 20% classified with moderate form and 11 patients have factor level >20%, according the classification of Europen Network of Rare Bleeding Disorders. Regarding the clinical severity 10 patients are asymptomatic (factor level >30%), 07 patients classified on grade II (factor level between 01 and 40%) and 01 patients on grade III (factor level<5%). The oral cavity bleeding (25%) and hematoma (17%) are the frequent bleeding symptom in our patients. The molecular study performed for 05 patients from the same family revealed a mutation c.430+78 G>A and polymorphism IVS7H7 in the homozygous state (02 patients), in the heterozygous state (03 patients).
Summary and Conclusions: Not corelation found between factor VII level and the clinical severety. This results are according the literature database.
The diagnosis of factor VII deficiency is depending the laboratory testing (level factor depending to the reagent) and the bleeding severety define the disease. The mutation c.430+78 G>A and polymorphism IVS7H7 are described in the literature and involved in the modulation rates of factor VII.

PB1689

ANGIODYSPLASIA IN A CHILD AS A CAUSE OF LOWER GASTROINTESTINAL BLEEDING FOLLOWING HEMATOPOIETIC STEM CELL TRANSPLANTATION, WHICH WAS SUCCESSFULLY CONTROLLED BY ANGIOEMBOLIZATION

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Background: Gastrointestinal complications are common following hematopoietic stem cell transplantation (HSCT); however angiodysplasia is not well characterized in terms of its association with HSCT and only a few reports of angiodysplasia in transplant recipients are available, all of them are adults.
Aims: Here we report a 7 years old child with a massive lower gastrointestinal bleeding, caused by angiodysplasia (vascular ectasia) following HSCT. To our knowledge, this is the first reported pediatric case demonstrating the association of angiodysplasia with HSCT and successfully controlled by angioembolization.
Methods: The patient was a 6-year-old boy who underwent HSCT for relapsed acute lymphoblastic leukemia. He was in remission at the time of transplant. Preparative regimen consisted of 12 Gy total body irradiation and 60 mg/kg etoposide. He received $2{,}4x10^6$/kg CD34+ stem cells from his well-matched father (one antigen mismatched at HLA-C). Cyclosporin was administered for GVHD prophylaxis. Full engraftment was achieved with neutrophil, platelet and erythrocyte recovery being 13, 20 and 27 days respectively. On the 14th day of transplantation grade IV acute graft-*versus*-host disease (GVHD) was developed (skin stage III, liver and gastrointestinal tract stage IV). Skin manifestations were resolved, but liver and gastrointestinal GVHD went on depite prednisolone therapy and the increase of cyclosporin. However, thrombotic microangiopathy (TMA) was developed on the 19th day and resolution was not achieved despite the switch of cyclosporin to mycophenolate mofetil. He underwent therapeutic plasma exchange sessions on alternate days. The resolution was achieved after 14 sessions, liver GVHD was also resolved, but gastrointestinal symptoms went on with recurrent massive gastrointestinal bleeding despite therapies including octreotide, recombinant human coagulation Factor VIIa and third party mesenchymal stem cells. The localization of the gastrointestinal bleeding couldn't be identified despite nuclear medicine scan, endoscopy and colonoscopy. The localization of the bleeding couldn't be identified with transcatheter arteriography also, but there were seen angiodysplastic lakes in branchs of celiac, gastroduodenal and superior mesenteric artery (Figure1). With the suspicion of these lakes may be the origin of bleedings, superselective embolization was performed and gastrointestinal bleeding episodes were resolved completely without any complication. Occult gastrointestinal bleeing was also became negative after ten days of the embolization and he was discharged on the 130th day of HSCT.
Results: In the literature on the subject, it is observed that HSCT associated gastric vascular ectasias are with a prior TMA episode and suggested that local endothelial injury and subsequent local thrombosis in TMA may obstruct submucosal blood vessels and eventually lead to development of vascular ectasia. The fibrin thrombi and/or ectasia in mucosal vessels observed histologically in these patients supported their hypothesis
Summary and Conclusions: In conclusion, angiodysplasia is a rare but important cause of recurrent intestinal bleeding in children and should be kept in mind as a diagnostic possibility especially in HSCT patients who have a history of TMA. Early diagnosis of this rare lesion is important to avoid a possible fatal outcome and thus the physician should be aware of this lesion as a rare cause of intestinal bleeding in children.

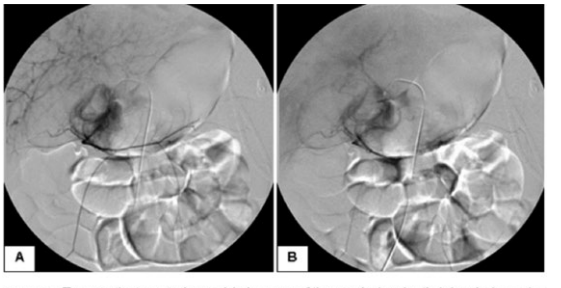

Figure 1: Transcatheter arteriographic images of the angiodysplastic lakes in branchs of gastroduodenal artery before (A) and after (B) superselective embolization

Figure 1.

PB1690

RELATION BETWEEN INHIBITOR PRODUCTION AND BCLI/INTRON 18 AND VNTR ST14 POLYMORPHISMS IN A IRANIAN POPULATION WITH HEMOPHILIA A

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Background: According to several reports, 20-30% of severe hemophilia A and 1-3% of severe hemophilia B patients develop inhibitors. There is a collection of mutations related to production of inhibitor in hemophilia A.
Aims: In factor VIII gene that is associated hemophilia A, the most useful polymorphisms are bi-allelic that are formed by a single nucleotide substitution. Identifying these mutations can help to diagnosis the susceptible people for this disorder. Here, two of these mutations were studied in Iranian Hemophilia with inhibitor production.
Methods: We have 150 patients with hemophilia and rare bleeding disorders in our center. It was studied all of patients with hemophilia A from our center. Inhibitor was tested for all of them and cases with inhibitor >0.5 BU determined. 20 patients from 80 had inhibitor. BclI/intron 18 and VNTR st14 polymorphisms of factor VIII gene were analyzed in 20 patients of hemophilia A with inhibitor. DNA was extracted from whole blood. RFLP and usual PCR techniques were done for BclI/intron 18 and VNTR st14, respectively. Results: In this population, 55% had a restriction site on BclI. All of patients were homozygote for VNTR st14 and just one allele was observed.
Results: In this population, 55% had a restriction site on BclI. All of patients were homozygote for VNTR st14 and just one allele was observed.
Summary and Conclusions: The observed BclI/intron 18 was quite high in our cases in comparison other hemophilias without inhibitor. But, the heterozygosity rate for VNTR st14 was different in our study in comparison with other hemophilias without inhibitor. Therefore BclI/intron 18 can increase risk of production of inhibitor in Iranian population.

PB1691

SEQUENTIAL THERAPY WITH ACTIVATED PROTHROMBIN COMPLEX CONCENTRATES AND RECOMBINANT FVIIA IN PATIENTS WITH SEVERE HAEMOPHILIA A, INHIBITORS AND LIFE THREATENING BLEEDING

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Background: Haemophilia patients with inhibitors can develop bleeding episodes refractory to monotherapy with either recombinant factor VIIa (rFVIIa) or activated prothrombin complex concentrates (APCC). These bleeds are often very difficult to treat and can result in significant morbidity. For such patients, therapies with sequential administration of standard doses or concomitant administration of lower dose of rFVIIa and APCC have been suggested. However, treatment with a combination of these agents is not widely practiced.
Aims: To present our experience of sequential therapy with rFVIIa and APCC in patients with severe haemophilia A, inhibitors and life threatening bleeding.
Methods: We reviewed the medical records of all the inhibitor patients for whom sequential therapy was prescribed, defined as having received both APCC and rFVIIa within 6 h of each other.
Results: Case 1) A 19 year's old patient with sever haemophilia A and previously detected inhibitor (11 BU) developed intracranial hemorrhage. He was treated with rFVIIa in dose 90 µg/kg/2 h. Due to very bad venous access central venous catheter was inserted with rVIIa (120 µg/kg every 2 hours, 2 dose). Four hour after surgery massive neck oedema and breathless developed. CT scan showed diffuse large neck muscle and soft tissue hematoma. Therapy with APCC 100 U/kg/12h and rFVIIa 90 µg/kg every 2 to 3 hours resulted in bleeding control. After 12 h of sequential usage, therapy was continued with rFVIIa. Case 2) A 35 year's old patient with sever haemophilia A and previously detected inhibitor (220 BU) developed spontaneous mediastinal and pericardial bleeding, without sings of tamponade. He was treated with APCC 100 U/kg/12h. Two days after admission haemoglobin level decreased from 134 to 78 g/L. CT showed massive mediastinal bleeding complicated with pleural effusion. Therapy was continued with rFVIIa 90 µg/kg every 2 hours, APCC 100 U/kg/12 h and blood transfusion. After 24 h of sequential usage therapy was continued with APCC. Case 3) A 19 year's old patient with sever haemophilia A and previously detected inhibitor (20 BU) was prepared for tooth extraction with rFVIIa (90 µg/kg). Extraction was complicated with massive subconjuctival, infraorbital and buccal hematoma with difficult swallowing. Dose of rFVIIa was increased to 120 µg/kg every 2 hours, without effect. Sequential therapy with rFVIIa 90 µg/kg/2 hours and APCC 100 U/kg/12 h was continued and bleeding was stopped. After 24 h of sequential therapy treatment was continued with APCC. Symptoms and sings of thrombosis and disseminate intravascular coagulopathy, as well as d dimer elevation were not seen in our patient's during sequential therapy.
Summary and Conclusions: Our report confirms efficacy and the safety of short course sequential rFVIIa and APCC for the management of life threatening bleeding refractory to monotherapy with APCC or rFVIIa. However, controlled trials with a larger number of patients are required to further assess the safety, efficacy and cost benefit of this treatment.

PB1692

PREOPERATIVE SCREENING FOR BLEEDING DISORDERS: OUTCOME OF EVALUATION IN CHILDREN WITH ABNORMAL TESTS

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Background: Children prior to invasive procedures undergo screening tests with platelet count and basic ciagulation studies like prothrombin time (PT), Partial Thromboplastin Time (PTT) to identity children with bleeding disorders
Aims: This study aims at studying the outcome of these children with abnormal screening tests or positive bleeding history
Methods: Case records of children who satisfy the inclusion criteria and evaluated in the last 5 years (2009-2014) were analyzed for the final diagnosis and surgery details.
Results: Twenty seven children were evaluated during this five years period due to abnormal screening tests or positive bleeding history. Twenty three children tested to have a bleeding disorder with variable severity. Three children did not have any bleeding disorder on evaluation. Two had positive lupus anticoagulant and therefore prolonged PTT. The above five children underwentrequired surgery without excess bleeds. Te diseases picked up during evaluation of children with abnormal screening tests or positive bleeding history were hemophilia (5), von Willebrand disease (type3>type1) (5), mild factor 7 deficiency, mild platelet function disorder(2), Bernard Soulier syndrome (2) and Glanzmann's thrombasthenia. One third of these cjildren underwent surgery with appropriate component replacement.
Summary and Conclusions: These inexpensive screening tests coupled with a good bleeding history is an essential tool to screen children for bleeding risk. This is especially important in children who would have never been challenged till date and this will definitely prevent critical bleeding which might be life threatening.

PB1693

SUBSTITUTION WITH RECOMBINANT ACTIVATED FACTOR VII (NOVOSEVEN) IN A PATIENT WITH CONGENITAL COMBINED DEFICIENCY OF FACTOR VII AND FACTOR X DURING SURGERY OF LUNG TUMOR-CASE REPORT

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Background: Combined deficiency of coagulation factors is considered as an extremely rare bleeding disorder (RBD) inherited in an autosomal recessive pattern. Combined FX et FVII deficiency appear also associated with coagulation-unrelated abnormalities (carotid body tumors, mitral valve prolapse, atrial septal defect, ventricular septal defect, thrombocytopenia absent radius- TAR syndrome, mental retardation, microcephaly and cleft palate).
Aims: The purpose of our case report was to evaluate therapy options for prevention of bleeding in patient with hereditary combined deficiency of FVII and FX who underwent surgery.
Methods: Application recombinant activated factor VII (NovoSeven) in patient with congenital combined deficiency of factor VII and factor X during surgery of lung tumor.
Results: A 19-year- old woman admited to the hospital due to preparation treatment before teeth extraction. Combined deficiency of factors VII and X has been diagnosed when she was 14 years old (27% FVII, FX 50%). In early childhood was noted the delay in physical development, with spastic paraparesis and brachydactyly on foot. Cytogentics analysis showed a normal female karyotype. Her sister also has a combined deficiency of FVII and FX with mental retardation. Patient had surgery operation of echinococcus cyst at age of 15 years, and teeth extraction on several occasions. Prior to teeth extraction patient received fresh frozen plasma (FFP), recombinant activated factor VII (rFVIIa) and antifibrinoyitic. Intervention proceeded without complications. Few months later, she was admited for teeth extraction (15 and 16) in general anesthesia, due to mental retardation. During the administration of FFP she developed severe allergic reactions with intensive urticaria, and anesthesiologist has not consented the intervention done. After 15 days the patient received rFVIIa 20µg/kg b.w. (2mg i.v.) and tranexamic acid imidiately before teeth extraction. It was performed without complications (25% FVII, FX 43% before therapy). Two days after the intervention, the patient had chast pain. ECG, cardiospecific enzymes and D-dimer were in the reference values. X-rays and CT of the chest showed a tumor in the left lung diameters 55x51x43 mm, which could corresponded as tumor or echinococcus cyst. In December 2014, underwent resection of lung tumor with preoperative application of rVIIa in a dose of

2 mg i.v (20µg/kg b.w) The therapy was continued at the same dose at 12 h following 5 days to remove the drain. The levels of FVII were from 80% to 130% during the treatment. Histopathology examination showed hamartoma. During and after the surgical intervention she had not unexpected bleeding complications.
Summary and Conclusions: Therapy options for prevention of bleeding in patients with hereditary combined deficiency of FVII and FX, who underwent surgery, can be concentrate of rFVIIa, in dosage 20µg/kg, as the treatment of choice.

PB1694

PREVELANCE OF H PYLORI INFECTION AND RISK OF GIT BLEEDING IN EGYPTIAN HAEMOPHILIC PATIENTS
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Background: : H pyelori is endemic in Egypt and present a main cause of gastrointestinal bleeding.
Aims: Aim of this study was to evaluate the prevalence of Helicobacter pylori infection in hemophilic patients, and to assess its impact on gastrointestinal bleeding associated with this infection in such patients.
Methods: Methods: we prospectively investigated the prevalence of H. pylori infection in 40 Egyptian patients with hemophilia A,B and von Willebrand syndrome and 20 male subjects were included. Every patient and control subject in the study was tested one time for H. pylori stool antigen by ELISA. All patients and control subjects were tested for occult blood using Guaiac-based fecal occult blood test.
Results: Twenty eight out of 40 patients were H. pylori positive (70%); and 12 out of 20 control subjects were H. pylori positive (60%). The odds ratio is 1.55, 95% CI (0.6162 to 3.9269), Significance level P=0.3497. Among 28 H.pylori positive patients, 5 patients tested positive for occult blood (17.9%). Among the 12 H.pylori positive subjects in the control group, only one tested positive for occult blood (8.3%). Odds ratio for Occult bleeding in H pylori positive patients and control was 2.39: P=0.4504. None of the H. pylori negative patients or control subjects had a positive occult blood disease.
Summary and Conclusions: Conclusion: We concluded that in patients with hemophilia, H. pylori should be considered as an important cause of GI bleeding. The recurrence of the infection and GI bleeding can be prevented with eradication of H. pylori. Screening test for H. pylori would be needed in patients with hemophilia in endemic areas.

PB1695

HEMOPHILIC PSEUDOTUMOR OR TUMOR? DIAGNOSTICS AND TREATMENT CHALLENGES
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Background: Hemophilic pseudotumor is characterized by slowly expanding, encapsulated mass. It develops as a result of extra-articular musculoskeletal hemorrhage, in 1-2% of hemophiliacs. Hemophilia treatment has significantly improved, bringing the opportunity for age-related morbidities development, including malignancies. Sometimes after imaging diagnostics, surgery is necessary for differential diagnostics.
Aims: To present hemophiliac patient with large tumor mass, initially suspected for hemophilic pseudotumor. Pathohistological finding of tumor biopsy revealed HCC metastasis with HCV infection. We discuss treatment option for such patient.
Methods: Single case report.
Results: Hemophilia B with F IX 1% was diagnosed at the age of 15. Forty years later, our patient was diagnosed with HCV infection and he received subsequent antiviral therapy. After four years, following abdominal CT finding (liver necrotic lesions) and presence of elevated serum alpha-fetoprotein (AFP, 174 ng/mL), HCC diagnosis was established. Trans-arterial chemoembolization (TACE) was opted as treatment choice but prior to intervention, right forearm painful swelling occurred. Since MRI finding of right arm and shoulder showed large tumor mass in humeroscapular region with severe osteolytic lesions of the right humerus, hemophilic pseudotumor was highly suspected. F IX concentrate substitusion was applied but with no obvious therapy response. Subsequently, tumor biopsy was performed and it revealed HCC metastasis. PET scan finding confirmed this metastasis as solitary. Several treatment options were discussed. Since HCC is known to be poor radiotherapy responder, and it would only be applied to metastasis, we excluded this treatment. Second generation tyrosin kinases inhibitors that would have a certain degree of systemic effect were not available in our country. Radical surgery that included right hand amputation was also excluded, due to a very high perioperative risk and present comorbiditiy (Diabetes mellitus type 2). Patient was subsequently treated with palliative therapy. Five months later, lethal outcome occurred, after severe bleeding in humeroscapular region.
Summary and Conclusions: A leading cause for HCC in hemophilia is HCV chronic infection. Extra hepatic metastases occur in approximately half of HCC patients, and in our case tumor biopsy revealed very unusual HCC metastasis. If HCV infection is diagnosed it has to be treated. Due to the high risk for HCC in hemophilia, regular ultrasonography should be performed. When pseudotumor is suspected, HCC possibility should also be investigated. HCC should be surgically treated in its early stage.

PB1696

ASSESSMENT OF EFFICIENCY OF DIFFERENT PLASMA DERIVED FVIII CONCENTRATES IN BLEEDING CONTROL-HEMOPHILIA PATIENTS' PERSPECTIVE
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Background: Patients in Serbia are usually switching between different plasma derived FVIII concentrates (pdFVIII), dependent on their market availability. We observed that some of the patients expressed a personal impression that there is a difference in efficiency of different factor concentrates in achievement of bleeding control.
Aims: To investigate is there any difference regarding efficiency in achievement bleeding control in hemophilia A patients between different pdFVIII concentrates, based on analysis of subjective assessments recorded in patients diaries
Methods: We extracted the data from a personal patient diary of eight adult patients, average age of 29, with severe form of hemophilia A about spontaneous joint bleeding episodes, while they were on a low dosage tertiary prophylaxis. Over a 4-year observation period from January 2010 to March 2014, our patients received, twice a week, the same dosage (15U/kg) of pdFVIII. During this time they switched between five different pdFVIII, dependent on the availability at that specific point of time. For every bleeding episode we recorded the type of pdFVIII applied, pain intensity, graded from 1 to 10, time necessary for the bleeding to stop measured in hours and overall subjective assessment of the therapy efficiency (OSA). OSA was graded from 1, meaning that patient considered therapy completely ineffective, to 10, meaning that bleeding stopped immediately after treatment.
Results: Eight patients received first plasma derived FVIII concentrate (pdFVIII1) during altogether 88 months, second (pdFVIII2) 83 months, third (pdFVIII3) 68, fourth (pdFVIII4) 79 and fifth (pdFVIII5) during 82 months. For pdFVIII1 average bleeding frequency per month was 0.53, for pdFVIII2 0.52, pdFVIII3 0.52, pdFVIII4 0.59, pdFVIII5 0.58. Mean time for bleeding to stop while using different pdFVIII was 6.15, 5.88, 6.08, 5.91, 6,02 hours. Average OSA was 6.0, 7.38, 6.67, 6.0, 6.9, for each pdFVIII respectively. In multivariate logistic regression analysis, OSA, was highly influenced by the time necessary for the bleeding to stop (OR=0,108 CI 0,068-0,147), but not with used pdFVIII concentrate.
Summary and Conclusions: According to our results, the type of pdFVIII applied didn't have any influence on OSA as well as the pain intensity. The OSA was dependent only on the length of bleeding episode.

PB1697

FACTOR XIII DEFICIENCY WITH INTRACRANIAL HEMORRHAGE
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Background: Congenital factor XIII (FXIII) deficiency is a rare autosomal recessive coagulation disorder. This congenital disorder is associated with poor wound healing, spontaneous abortion, life-long tendency towards spontaneous bleeding and a high risk of potentially life-threatening intracranial hemorrhage.
Aims: Routine prophilaxis with FXIII concentrate is recommended in all individuals with FXIII levels of<1 IU/dl from the time of diagnosis and in some severely affected patients with FXIII levels of 1-4 IU/dl.Therefore reliable assays for FXIII are necessary not only for the diagnosis of deficiency state but also to guide prophilaxis and replacement therapy in patients during times of increased risk. In Iran, ureaclot lysis test (FXIII screen) is the most available test and reliable assays are not accessible because of limitations of laboratory equipment. Hence, we recommended prophilaxis in any cases with abnormal FXIII screen and history of intracranial hemorrhage.
Methods: In here, it is reported the clinical course and outcome of 38-month old boy with intracranial hemorrhage that successfully managed and received prophilaxy with fibrogammin® P. He had a history of recurrent spontaneous bleeding or bleeding after trauma in extremities from birth. He admitted at 27 month of age in hospital due to gluteous hematoma after muscle injection. He was tested for PLT, PT, PTT, INR, fibrinogen (clauss method) and FXIII screen (ureaclot lysis test). He had abnormal FXIII screen test and other tests were normal. He managed with fresh froze plasma before diagnosis and recommended for on demand therapy after diagnosis
Results: He admitted at 30 months of age due to headache and vomiting. Brain CT scan revealed intracranial hemorrhage. He was treated with fibrogammin® P. The prophylactic substitution therapy with fibrogammin® P 20 IU/kg every 4 weeks performed and he is free from bleeding episodes. Nugent *et al*, 2012, studied on 41 patients with congenital FXIII deficiency. Fibrogammin® P was administrated

intravenously at an initial dose of 40 IU/kg every 4 weeks, with dosing adjusted to maintain a trough FXIII activity level of 5-20%. No spontaneous bleeding episodes requiring FXIII treatment were reported during the study.
Summary and Conclusions: According to inaccessibility to reliable assays of FXIII in Iran we recommended prophilaxy with fibrogammin® P in all cases with intracranial hemorrhage.

PB1698

FACTOR II DEFICIENCY IN WEST OF ALGERIA : SINGLE CENTER EXPERIENCE

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Background: Factor II deficiency is one of the rare inherited bleeding disorders the frequency in world is 1/ $2*10^6$. But in algeria there is high consanguinity rates have increased the number of patients with Rare Bleeding Disorders (RBDs)
Aims: It was established a clinical and biological classification of patients with rare deficiency disorders. It was explored the relationship between coagulation factor activity level and bleeding severity for this patients.
Methods: A retrospective study conduct from 2008 to 2014. It was concerned 63 patients with a mean age 29 years (range1 year to 73 years) and Sex ratio ♂/♀=1.17. We use assigned categories of clinical bleeding severity of The European Network of Rare Bleeding Disorders (EN-RBD): 1. Asymptomatic : No documented bleeding episodes. 2. Grade I : bleeding Bleeding that occurred after trauma or drug ingestion (antiplatelet or anticoagulant therapy). 3. Grade II : Spontaneous minor bleeding:bruising, ecchymosis, minor wounds, oral cavity bleeding, epistaxis and menorrhagia. 4.Grade III : bleeding Spontaneous major bleeding: hematomas*, hemarthrosis, central nervous system,gastrointestinal and umbilical cord bleeding.
Results: 06 patients (09.5%) of RBDs were diagnosed with Factor II deficiency, Clinical bleeding showed that the most common symptoms in patients with FII deficiency were oral cavity bleeding(34%), followed by hematomas(17%), epistaxis (17%) .
Summary and Conclusions: Any association between bleeding manifestations and residual FII coagulant activity in plasma was found, but is very difficult to ascertain this association because the small number of reported cases. However, 03 patients with FII coagulant activity<10% reported to be associated with severe bleeding manifestations grade II and III.and a weak direct correlation between laboratory severity and the age at first bleed was noted.

PB1699

RESISTANT THROMBOSIS WITH STANDARD HEPARIN TREATMENT: ANTI-THROMBIN III DEFICIENCY

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Background: Sinus vein thrombosis is a type of stroke anda multifactorial disease that usually manifested by non-specific symptoms in children.In most cases there is an underlying prothrombotic risk factors or a combination of many clinical reasons. The prevalence of antithrombin III deficiency in patients with thrombosis incidence is 0.02%, as this rate in normal populations is 1%.In this article, we will discuss a case with antithrombin III deficiency that he do not receive the standard heparin treatment. A twenty years old male patient with sudden onset of headache, dizziness, nausea and vomiting was admitted to our clinic.His physical examination and vital findings were stable. His cranial MRI showed thrombosis of superior sagittal sinus and the left transverse and sigmoid sinuses.Clexane(enoxaparin) started 2x1 mg/kg/daily and contunied for 15 days.There was not any recanalization in the thrombosis veins on his repeated cranial MRI after the 15 days treatment.The patient's anti-thrombin III activity was 34.8% as other normal thrombophilia tests. Antithrombin III deficiency was diagnosed. Coumadin(warfarin) treatment was started due to ineffective low molecular weight heparins. The possibility of antithrombin III deficiency should be considered when a low molecular weight heparin therapy is not effective in the follow up patients with the diagnosis of thrombosis. Sinus vein thrombosis is a type of stroke anda multifactorial disease that usually manifested by non-specific symptoms in children.In most cases there is an underlying prothrombotic risk factors or a combination of many clinical reasons.
Aims: The prevalence of antithrombin III deficiency in patients with thrombosis incidence is 0.02%, as this rate in normal populations is 1%.In this article, we will discuss a case with antithrombin III deficiency that he do not receive the standard heparin treatment.
Methods: A twelve years old male patient with sudden onset of headache, dizziness, nausea and vomiting was admitted to our clinic.His physical examination and vital findings were stable. His cranial MRI showed thrombosis of superior sagittal sinus and the left transverse and sigmoid sinuses. Clexane(enoxaparin) started 2x1 mg/kg/daily and contunied for 15 days. There was not any recanalization in the thrombosis veins on his repeated cranial MRI after the 15 days treatment. The patient's anti-thrombin III activity was 34.8% as other normal thrombophilia tests. Antithrombin III deficiency was diagnosed.
Results: Antithrombin III deficiency was diagnosed. Coumadin(warfarin) treatment was started due to ineffective low molecular weight heparins.
Summary and Conclusions: The possibility of antithrombin III deficiency should be considered when a low molecular weight heparin therapy is not effective in the follow up patients with the diagnosis of thrombosis.

PB1700

LUPUS ANTICOAGULANT-HYPOPROTHROMBINEMIA SYNDROME IN A 7-YEAR OLD GIRL: A CASE REPORT

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Background: Lupus anticogulant (LA) can be associated with bleeding as a consequence of hypoprothrombinemia.
Aims: We present the report of a patient with hemorrhagic symptoms in combination of hypoprothrombinemia and LA.
Methods: A 7 year-old girl was admitted with epistaxis for 5 days. On physical examination, she had scleral icterus, echimosis and hepatomegaly. The complete blood count was normal expect hemoglobin of 47 g/l. The coagulation screening showed a prolonged prothrombin time (PT) (60.8 s) and activated partial thromboplastin time (aPTT) (118.6 s). Although a packed fresh frozen plasma was administered to the patient, the prolonge of PT and aPTT continued. Therefore, the mixing study was performed but both PT and aPTT did not improve.
Results: Factor assay revealed a low prothrombin level (4,46%). The lupus anticoagulant and anti nuclear antibody were found positivity but anti DS-DNA was negativity in advanced laboratory examination of patient respectively. Further evaluation revealed the presence of immunoglobulin (Ig) G an M anticardiolipin antibodies, IgG and IgM anti-β-2-glycoprotein antibodies, IgG and IgM anti phosphotydilserin antibodies. After 5 days of mega dose methylprednisolone (30 mg/kg/d) was administered to the patient, PT(13.6 s) and a PTT (38.4 s) were measured normal and directly Coombs test was found negatively. Therefore, mega dose methylprednisolone treatment was stopped. One month after stopping of the treatment with mega dose methylprednisolone she had again both prolonged PT and aPTT, and the positivity of directly Coombs test. In this period, it was revealed the presence of immunoglobulin (Ig) G an M anticardiolipin antibodies, IgG and IgM anti-β-2-glycoprotein antibodies, IgG and IgM anti phosphotydilserin antibodies. Hence, the patient re-treated with steroid. Her family asked the follow-up in another hospital.
Summary and Conclusions: The low dose immunosuppresive treatment in the patients with LA and hypoprothrombinemia is necessary to continue for the long time.

PB1701

NEW BIRTHS OF CHILDREN WITH HEMOPHILIA: REPORT FROM A SINGLE CENTER

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Background: Great progress has been reported during recent years on prenatal diagnosis of several congenital diseases, including hemophilia. In addition, pre-implantation techniques have enabled couples at risk to give birth to healthy babies. In Greece, genetic counseling is offered in specialized centers to all women carriers of hemophilia who opt to seek advice.
Aims: The aim of the present study was to report on new births of hemophilic children in Central-Northern Greece during the last 5 years (January 2010-December 2014).
Methods: Patient records of the Pediatric and Adolescent Center for Hemorrhagic Diseases were retrispecively studied. The Center is one of the 2 pediatric hemophilia centers in Greece, following all patients aged under 18 years, living in the area of Northern and in parts of Central Greece. New births in the given 5 year period were recorded, as well as data regarding age of diagnosis, disease severity, family history and prenatal counseling.
Results: During the study period 12 new patients with hemophilia A were born, 8/11 with severe hemophilia, 2/11 with moderate hemophilia and 2/11 with the mild form of the disease. Overall, 8/11 had a positive and 3/11 a negative family history. In none of the cases was prenatal screening performed, even though 6/8 cases with a known family history were associated with severe hemophilia and in one case death due to the disease in a very young age was reported. Mean age of diagnosis in the a positive history group was 9.8 months (3 days – 3 years), while in 3/8 cases bleeding episodes preceded laboratory investigation. In the negative family history group diagnosis was reported at an earlier mean age (8.5 months, 7 months-12 months), in all cases diagnosis following

a bleed. In one of the 3 patients with a negative family history, the mother was pregnant to a second baby-also a hemophiliac- when she was informed of the diagnosis of the first child, but the family did not opt for prenatal screening.
Summary and Conclusions: New births of hemophilia are still recorded, even in cases with known-even severe-family history. This could be attributed to the knowledge of progress in the management of hemophilic children, as well as to religious issues. Weaknesses in the health system's provision of information regarding availability of prenatal counseling cannot be ruled out.

PB1702

SUCCESSFUL ABDOMINAL OPERATION WITHOUT REPLACEMENT THERAPY IN A PATIENT WITH COMBINED FV AND FVIII DEFICIENCY DUE TO NOVEL HOMOZYGOUS MUTATION IN LMAN1

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Background: Surgical operations in congenital coagulation factor deficiencies' patients will increase risk of perioperative bleeding if without adequate replacement therapy. After adequate replacement therapy, patients with hemophilias can have successful experience of surgical operation. Patient with congenital combined FV and FVIII deficiency (F5F8D) can also have successful percutaneous coronary intervention after replacement therapy. There are rare reports about successful surgical operations in patients with F5F8D without adequate replacement therapy.
Aims: We reported that a patient with combined FV and FVIII deficiency had successful abdominal operation without replacement therapy and analyzed the molecular mechanism of the disease for the patient.
Methods: Before the diagnosis of the combined FV and FVIII deficiency, a 35 years old female patient was admitted for abdominal operation due to hydrosalpinx and chocolate cyst of ovary. We do a molecular genetic analysis in the family. Peripheral blood DNA was extracted. All the exons of LMAN1 and MCFD2 genes were PCR amplified and sequenced.
Results: Although with prolong activated partial thromboplastin time and prothrombin time, the patient underwent successful abdominal operation without replacement therapy. She tolerated well for abdominal operation therapy without increase risk of perioperative bleeding. Molecular analysis showed that the patient has a novel homozygous deletion mutation in exon 12 of LMAN1 (1456delGTG).
Summary and Conclusions: Our results suggest that a F5F8D patient with homozygous deletion mutation in exon 12 of LMAN1(1456delGTG) can safely undergo abdominal operation therapy for hydrosalpinx and chocolate cyst of ovary without fresh frozen plama and recombinant FVIII replacement therapy.

Bone marrow failure syndromes incl. PNH - Clinical

PB1703

SERUM FERRITIN IN PATIENTS WITH APLASTIC ANEMIA RECEIVING COMBINED IMMUNOSUPPRESSIVE THERAPY.

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Background: Iron metabolism disorders in patients with AA are not only related to disease pathogenesis but to regular red blood cells (RBC) transfusions.
Aims: Analyze iron parameters before immunosupressive therapy, in time of it (meaning RBC transfusions dependence retained) and in remission phase.
Methods: A total of 85 patients from 18 to 65 years old were enrolled: 41 males and 44 females at age from 18 to 65 years old. Patients were followed up from 1 month to 3 years. Severe forms of AA were detected in 59 patients, non-severe in 26 pts. All patients received immunosupressive therapy (ATG + cyclosporine). Serum ferritin level (SF) was applied as the main iron overload (IOL) indicator (N 11-160 ng/mL). Depending on obtained results all patients were divided into 3 groups. Group 1 patients had SF evaluation prior to combined immunosupressive therapy (n=47), these patients were split into subgroups 1A and 1B: 1A subgroup patients (n=16) received<20 transfusions in 1-4 months prior to the study, 1B subgroup patients (n=31) received >20 transfusions in 2-19 months prior to the study. Group 2 patients (n=20) retained long term RBC transfusion dependence in spite of continued immunosupressive therapy. Group 3 patients (n=20) demonstrated good immunosuppressive therapy response, therefore developed significant hematological improvement and became transfusion independent.
Results: In patients of 1A subgroup SF median was 303 ng/mL (range 40-1483 ng/mL), in 1B Group-722 ng/mL (range 105-4950 ng/mL) with significant difference between SF levels received (P=0.025). Group 2 patients had SF median of 1018 ng/mL (range 250-6300 ng/mL), and Group 3 patients were notable for lower SF levels with SF median of 480 ng/mL (range 15-1500 ng/mL). Significant differences recorded when comparing Group 1 SF levels of and Group 2 patients data (P=0.004) as well as Group 2 and Group 3 patients SF levels (P=0.014).
Summary and Conclusions: It was discovered that iron metabolism disorders have been detected in almost all AA patients prior to immunosupressive therapy start and then progressed in time of ongoing RBC transfusions. Notable high SF levels of maintained for a long time after patients became transfusion independent. These data suggest chelation therapy is valuable not only for transfusion dependent AA patients but may also be useful in remission patients with retained laboratory signs of iron overload.

PB1704

DIAGNOSTIC, THERAPEUTIC AND EVOLUTIVE ASPECTS OF ACQUIRED APLASTIC ANEMIA: ABOUT A RETROSPECTIVE STUDY OF 107 CASES

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Background: The acquired aplastic anemia (AAA) is a rare but serious and severe disease. Evolution could be fatal by medullary isuffisance 's complications.
Aims: We found it useful to carry out a retrospective study over a period of 16 years and to analyze the diagnostic, therapeutic and evolutive characteristics of myelosuppression in southern Tunisia.
Methods: Our study is retrospective, it has enrolled the acquired aplastic anemia patients diagnosed and monitored in the hematology department of Sfax hospital over 16 years from january 1998 to december 2013. 107 cases were collected.The date of point was fixed to January 2015. The etiological diagnosis contained a careful history, a study of medullary and constitutional karyotype, a search for HPN clone, viral serology....overall survival was carried out according to Kaplan Meier method.
Results: 107 cases of acquired aplastic anemia were collected, they were 60 men and 47 women with a median age of 27 years (range 2-81 years). The circumstances of discovery were hemorrhagic syndrome, anemic syndrome and fever respectively in 7%,% and11% of cases. Our patient's distribution according to Camitta) score showed 38% AAA moderate, 31% severe AAA and 31% very severe AAA. The etiological investigation has revealed negative in 85 patients (79%) and labeled idiopathic. It showed a toxic agents or viral infections as in postseronegative hepatitis respectively in 7% and 11% of patients. Two patients have had AM / HPN.In addition to symptomatic treatment, specific treatment concerned 99 patients. It involved the allogenic bone marrow (allo-

graft), ciclo associated with SAL, ciclo only and androgen.The therapeutic results according to the used therapeutics are detailed in the following table.

Table 1.

therapy	Patients number	Death rates(%)	5years Overall survival	Terapeutic response(%)
Allogeneic bone marrow	33	15	80	RC=79 RP=6
SAL Ciclo	10	20	71	RC=40 RP=40
Ciclo	40	67	40	RC=25 RP=8
androgens	6	50	50	RC=33 RP=17
Total	99	37	60	RC=52 RP=11

Summary and Conclusions: The acquired aplastic anemia is a rare and serious disease. It is a therapeutic emergency requiring a carefull etiological investigation and early management with effective therapeutic.

PB1705

CONTRIBUTION OF FLOW CYTOMETRY IN THE BALANCE SHEET OF ACQUIRED APLASTIC ANEMIA

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Background: Flow cytometric immunophenotyping has become the gold standard for the diagnosis and monitoring of Paroxysmal hemoglobinuria(PNH). PNH results from acquired somatic mutation of the phosphatidylinositol glycan complementation class A (PIG-A) gene, leading to partial or complete absence of the glycosylphosphatidylinositol (GPI) anchor that is responsible for linking a large number of proteins to the cell membrane.
Aims: The aim of our work is to detect and quantify by flow cytometry (FC) PNH clones in aplastic anemia (AA) patients.
Methods: From January 2001 to december 2013,we have evaluated 103 patients with aplastic anemia. The presence of a PNH clone was assayed by peripheral blood flow cytometry on granulocytes(CD16) and monocytes(CD14). The diagnosis of PNH was retained by the presence of a deficit clone greater than or equal to 50% of white blood cells. The PNH clone is considered small if >5%, and major if >30%.
Results: Among all AA patients, 15% patients had the PNH clone. Ham's test was performed in ¾ of cases. Anemia was present in all patients with PNH-AA syndrome. Among them,80% had severe aplastic anemia. Bone marrow transplantation (BMT) concened 21% of patients with PNH-AA syndrome. three patients with PNH-AA syndrome died by severe thrombotic accident, progression to acute leukemia and severe anemia.

Table 1.

	Patients number	Percentage
Major HPN clone	5	5%
Small HPN clone	10	10%
No clone HPN	88	85%
Total	103	100%

Summary and Conclusions: The flow cytometry on granulocytes is a useful method to diagnose and characterize PNH. This test is good for early detection of PNH clones in AA patients at initial diagnosis. PNH clone search using specific erythroid markers (CD 55 and CD59) should improve the specificity of our results.

PB1706

CLINICAL MANIFESTATIONS AND TREATMENT OUTCOME OF FANCONI ANEMIA: RESULTS OF A SINGLE CENTER

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Background: Fanconi anemia is an inherited disease characterized by congenital malformations, pancytopenia, cancer predisposition, and sensitivity to cross-linking agents. The molecular diagnosis of Fanconi anemia is relatively complex for several aspects including genetic heterogeneity.
Aims: The aim of this study is to characterize clinical manifestations and treatment of Turkish patients with Fanconi anemia (FA) at our center.
Methods: The medical records of 16 FA patients diagnosed at Istanbul University, Cerrahpasa Medical Faculty from 2006 to 2014 were retrospectively reviewed.
Results: The median age at diagnosis was 6,5 years (6 months-12 years). Nine of them were male and 7 of them were female (M/F:). Two of them were brother and sister. All patients showed evidence of marrow failure and one or more physical stigmata. Most common findings were cafe-u-lait, hypopigmented lesions, thumb anomaly and renal anomalies. Chromosome breakage tests were positive in 12 out of 12 available patients and 2 of them had mosaism in the chromosome breakage tests. The median follow-up duration was 63 (7-112) months. None of the patients underwent stem cell transplantations (SCTs). All patients were alive by the end of the study. None of the patients developed leukemia. Androgens were used in 7 patients for treatment. Two of the patients received erythrocyte suspension and 1 of them received thrombocyte suspension.
Summary and Conclusions: We provide information of FA patients at our center. A nation-wide FA registry that includes information of the genotypes of is required to further characterize ethnic differences and provide the best standard of care for FA patients.

PB1707

CHRONIC LIVER DISEASE WITH HYPERSPLENISM PRESENTING AS PANCYTOPENIA

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Background: Hypersplenism is one cause of pancytopenia. Chronic viral hepatitis and liver cirrhosis are prevalent in Asian populations and often result in splenomegaly and hypersplenism.
Aims: In this study, we reviewed and analysed the diagnostic process of chronic liver disease associated with hypersplenism initially presenting as pancytopenia.
Methods: Patients with chronic liver disease with hypersplenism who initially presented with bicytopenia or pancytopenia from January 2002 to December 2013 at Chungnam National University Hospital were enrolled. The patients' characteristics and laboratory investigation findings at the time of diagnosis were analysed retrospectively.
Results: Nineteen patients with a median age of 61.5 years (range, 43–77 years) were enrolled, accounting for about 3% of patients presenting with bicytopenia or pancytopenia during the study period. All patients were first seen by a haematologist. In 12 patients (63.2%), bicytopenia or pancytopenia was incidentally found during a health check-up at a local clinic. A history of liver disease was limited to six patients (31.6%), and one patient (5.3%) was a carrier of the hepatitis B virus. Hepatitis B surface antigen and anti-hepatitis C antibody were detected in four (21.1%) and seven (36.9%) patients, respectively. Eighteen (94.7%) patients exhibited one or more abnormalities among the aspartate transaminase level, alanine transaminase level, alkaline phosphatase level, total bilirubin level, albumin level, and prothrombin time. All patients exhibited hepatic dysfunction and splenomegaly on Tc-99m phytate liver scintigraphy; therefore, all were diagnosed with liver cirrhosis accompanied by hypersplenism. The causes of the cirrhosis were hepatitis B in four patients (20.0%), hepatitis C in seven (36.9%), alcohol consumption in two (10.5%), chronic heart failure in one (5.3%), and an autoimmune disorder in one (5.3%). No clear aetiology was found in four (20.0%) patients. Of 15 patients with liver cirrhosis who underwent a bone marrow study, 2 (13.3%) were confirmed to have aplastic anaemia in addition to hypersplenism. Myelodysplastic syndrome and megakaryocytic hyperplasia were reported in three (20.0%) and two (13.3%) patients, respectively, and were later confirmed to be reactive changes of hypersplenism.
Summary and Conclusions: Chronic liver disease accompanied by splenomegaly and hypersplenism should be highly suspected when bicytopenia/pancytopenia and abnormalities in chemistry coexist in the same patient, even in those with no history of liver disease or who are negative for viral hepatitis markers. The Tc-99m liver scan is a simple but highly efficient investigational tool for these patients.

Chronic lymphocytic leukemia and related disorders - Biologyl

PB1708

IMPLEMENTATION OF MINIMAL RESIDUAL DISEASE IN CHRONIC LYMPHOCYTIC LEUKEMIA ON REUNION ISLAND

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Background: Emerging data strongly indicate that quantification of Minimal Residual Disease (MRD) in Chronic Lymphocytic Leukemia (CLL) appears to be a powerful predictor of progression-free and overall survival, regardless of the clinical response, type of therapy and known biological markers. Two methods are currently available for assessing complete remission according to the international consensus and IWCLL guidelines, *i.e.* allowing to detect one malignant cell in 10.000 leukocytes. These approaches are either based on specific immunoglobulin heavy chain rearrangement in each clone (RQ-ASO IgH-PCR) or on the unique immunophenotype profile of CLL cells using multiparameter flow cytometry.
Aims: Our aim was to assess complete remission in CLL on Reunion Island. The assay had to be not as complex, expensive and time-consuming as previously published techniques. This study describes the different steps in implementing and optimising an eight-colour flow cytometry MRD assay in peripheral blood.
Methods: Our work is based on an antibody panel designed by the ERIC expert group (www.ericll.org) including the following combination: CD81/CD79b/CD22/CD19/CD43/CD20/CD5/CD3. The malignant clone is identified among normal B cells thanks to 15 scattergrammes combining six CLL highly characteristic markers. Tumor cells typically express CD5, dim CD22, dim CD20, dim or negative CD79b, CD43, dim or negative CD81. In order to reach a higher specificity and sensibility for detection of CLL cells, we have been working on three different approaches: modification of sample preparation procedure, introduction of a new brilliant fluorochrome for the violet laser line and optimisation of data analysis strategy. Once the implementation of the assay had been carried out, we studied performance criteria according to learned societies, considering clinical relevance and practical feasibility.
Results: After modifying the eight-colour protocol, we achieved a precise estimation of the number of total leukocytes events by excluding unlysed cells (SSC/CD43 dot plot), erythrocytes debris (CD81/CD19 dot plot) and doublets (FSC-A/FSC-H dot plot). This new gating algorithm allows us to calculate the number of CLL cells events as a percentage of total leukocytes, according to international guidelines. The intersection of 16 regions of interest contribute further to clearly define the CLL population by minimizing the error of inclusion risk. Analysis of ten healthy donor blood has been carried on to assess the limit of detection, which is 5.3. The application of this new data analysis strategy achieves a sensitivity of $5x10^{-6}$ after acquiring one million leukocytes. Performance criteria proved mostly satisfying: imprecision, accuracy, carryover and specimen stability meet the standards we set.
Summary and Conclusions: We have developed on Reunion Island a flow cytometric technique reliable and sensitive enough to detect the presence of less than one CLL cell in 100.000 leukocyte. This 8-colour assay reduces subjectivity, sample requirements and time taken for acquisition and analysis, compared with 4 and 6-color assays. It also presents the advantage of requiring less specialized equipment and being not as expensive as the sensitive PCR methods.

PB1709

THE EFFECT OF THE RETINOIC ACID DERIVATIVE, ACITRETIN, ON CHRONIC LYMPHOCYTIC LEUKEMIA CELL VIABILITY, MIGRATION AND APOPTOSIS.

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Background: Retinoids modulate the activity of a large number of genes through binding to the retinoic acid receptor (RAR/RXR) resulting in a variety of molecular and biochemical consequences. The role of retinoids in different malignancies has been extensively explored. However little is known about their effect on lymphoid malignancies.
Acitretin is a synthetic retinoid which has been shown to induce cell differentiation, and apoptosis in squamous cell carcinoma, where it was shown to induce apoptosis of malignant squamous cells without effecting normal epithelial cells.
Aims: We aimed to investigate the effects of Acitretin on the CLL like cell line MEC-1 and on primary CLL cells.
Methods: Peripheral blood samples were obtained from 28 CLL patients who gave written informed consent (14 treatment naïve, 14 with relapsed disease). The CLL like cell line MEC-1 was obtained and experiments were performed on these and on primary CLL cells, freshly isolated from whole blood using a density centrifugation technique. Viability assays were performed using the Celltitre96 AquousOne® MTS assay. Migration assays were performed on the Boyden chamber platform where migration of acitretin treated cells toward the chemokine CXCL12 was compared to untreated controls. Apoptosis was measured using FACS analysis of CLL cells treated with 10microM Acitretin for 24 and 72hrs. Treated cells and vehicle controls were labelled with Annexin 5 and Propidium Iodide to detect early apoptotic cells and dead cells.
Results: Acitretin significantly reduced proliferation of MEC-1 cells at drug concentrations of up to 25μM (28.6% mean reduction, P=0.0002).
Acitretin reduced the proportion of viable CLL cells in 5 out of 7 patients as compared to control in cells from treatment Naïve patients (18% mean reduction, P=0.0001), at drug concentrations up to 20 μM. Furthermore, one clinic patient with early stage CLL was commenced on acitretin for control of squamous cell carcinoma of the skin. His lymphocytosis was found to have resolved 3 weeks into treatment. His lymphocyte count continued to be normal 1 year into follow up. However, acitretin had no effect on viability of cells from relapsed patients. Acitretin also had a variable effect on cell migration whereby it reduced the ability of cells to migrate towards the chemokine CXCL12 in 2 out of 5 patient samples and did not appear to effect migration of the cells in 3 patient samples. Acitretin does not appear to significantly affect the rate of early apoptosis in the primary cells as measured by the proportion of Annexin V/Propidium Iodide labelled cells. Future analysis of additional primay cell lines will further substantiate these results.
Summary and Conclusions: The retinoid derivative acitretin reduced the proliferation rate of MEC-1 cells and the viability of primary CLL cells from treatment Naïve patients *in vitro*, but had a variable effect on cell migration and did not appear to effect early apoptosis.
Our findings show a potential effect of retinoids in early CLL however the mechanism and clinical significance needs to be further defined.

PB1710

MIMICKING THE TUMOR MICROENVIRONMENT OF CHRONIC LYMPHOCYTIC LEUKEMIA *IN VITRO* CRITICALLY DEPENDS ON THE TYPE OF B CELL RECEPTOR STIMULATION

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Background: The B cell receptor (BCR) plays a key role in the crosstalk between chronic lymphocytic leukemia (CLL) cells and the tissue microenvironment, which favors disease progression by promoting proliferation and drug resistance. In these protective niches in the bone marrow and secondary lymphoid organs, the BCR becomes activated and sets a signaling cascade in motion that contributes to the survival and expansion of the malignant clone. *In vitro* studies of this crosstalk investigating downstream signaling and functional effects of BCR ligation often report contradictory results, in part due to the lack of a standardized protocol for BCR stimulation in CLL.
Aims: Our aim was to define a biologically relevant and robust *in vitro* stimulation method with regard to cellular phenotypic and transcriptional responses.
Methods: We evaluated mRNA (*MYC, FOS, LPL*) and protein (CD54, CD19, CD62L, CD184) expression of genes modulated by BCR triggering in *IGHV* mutated and unmutated CLL cells, after stimulation using soluble or immobilized anti-IgM antibodies from different brands.
Results: The effect of BCR stimulation on gene and protein expression was comparable in all CLL patients, irrespective of *IGHV* mutation status, as previously reported by us. However, immobilized anti-IgM stimulation elicited clear and robust changes in gene and protein expression, whereas the response to soluble anti-IgM was far less obvious.
Summary and Conclusions: These results show that the method of BCR stimulation is of major importance regarding responsiveness of CLL cells in the context of the tumor microenvironment, while genetic differences in the BCR pathway are less critical.

PB1711

ANALYSIS OF MONOCYTES SUBPOPULATIONS WITH CYTODIFF™* IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: During last decade 3 sub-populations of monocytes have been described in the human blood: classical, intermediate and non-classical monocytes, which can be differentiated with flow cytometry based on the expression of CD14 and CD16. The involvement of monocyte subpopulations in different pathological processes also was described, including sepsis, HIV infection,

tuberculosis and cardiovascular disorders. Very limited information available about monocyte sub-populations in Chronic Lymphocytic Leukemia (CLL), but according to literature they seem to be important for tumor progression and immune suppression in CLL.
Aims: The aim of our study was to compare CD-16 negative and CD16-positive monocytes in CLL patients and in normal individuals. Also we aimed to compare the groups of CLL patients with bad and good prognosis.
Methods: 58 patients with confirmed diagnosis of CLL were included in the study at the time of diagnosis. The comparison group included 307 blood donors. EDTA-anticoagulated blood sample were analyzed with CytoDiff™* panel according to the recommendation of the supplier. The CytoDiff™* panel is a 5-color / 6-marker reagent that provides an extended 18-part white blood cell differential from whole blood specimens by flow cytometry (Beckman Coulter). Among other WBC sub-populations, CytoDiff™* is able to provide the count of CD16-positive and CD16-negative monocytes (@M+ and @M-) in peripheral blood. The expression of CD38 on lymphocytes was used as prognostic marker for CLL patients.
Results: The absolute number of classical CD16-negative monocytes (@M-) was similar in donors and in CLL patients (503 cells/mcl vs 510 cells/mcl respectively). When compared to normal individuals, patients with CLL were characterized by higher absolute count of CD16-positive monocytes (@M-, 40 cells/mcl vs 30 cells/mcl, P=0.0007), higher proportion of CD16-positive monocytes among all monocytes (@M+/Total Mono; 8.8% vs 5.8%, P<0.0001) and lower proportion of CD16-negative monocytes among all monocytes ((@M-/Total Mono; 94.2% vs 91.3%, P<0.0001). Within the group of 58 CLL patients we identified patients with unfavorable prognosis (n=16) based on the CD38 expression (>6%). In patients with bad prognosis CytoDiff™* results revealed higher proportional (0.24% vs 0.175%, P=0.1346) and absolute (63 cells/mcl vs 38 cells/mcl, P=0.166) count of CD16-positive monocytes, but the difference did not reach the statistical significance.
Summary and Conclusions: CytoDiff™* analysis is very efficient tool to monitor the monocyte subpopulations in various pathologies, including CLL. The exact role of CD16-positive monocytes in CLL and their significance remain to be investigated on a larger cohort of patients. *Not available in the United States and other geographies. For research use only. Not for use in diagnostic procedures. @-Research Use Only (RUO) parameters

PB1712

ASSOCIATION OF CD38, ZAP-70, CD49D AND CXCR4 EXPRESSION IN CHRONIC LYMPHOCYTIC LEUKEMIA
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Background: Chronic lymphocytic leukemia is disease with very heterogeneous clinical course, some patients have long survival without treatment, while others have short survival in spite of intensive treatment. Therefore is important to predict clinical outcome at the time of diagnosis and to plan adequate therapy. Classic prognostic parameters is based on tumor size and are not valid for prognosis in the early clinical stages. CD38, ZAP-70, CD49d and CXCR4 are involved in cell signalization and interactions with microenvironment. This molecules conduct proliferative and anti-apoptotic signals and contribute to the development of the disease and resistance to treatment. Expression of CD38, ZAP-70, CD49d and CXCR4 correlates with active subpopulation of leukemic clone and can predict course of the chronic lymphocytic leukemia at the time of diagnosis.
Aims: Aim of the study was to determine if there is a correlation of CD38, ZAP-70, CD49d and CXCR4 expression in chronic lymphocytic leukemia and if there is a correlation between expression of these antigens with clinical and laboratory parameters.
Methods: 40 patients with chronic lymphocytic leukemia was analysed before treatment. Expression of CD38, ZAP-70, CD49d and CXCR4 was detected by immunocytochemical staining. Correlation of expression of the CD38, ZAP-70, CD49d, CXCR4, and correlation between expression of these antigens and clinical and laboratory parameters were tested with standard statistical methods.
Results: Median age of patients was 61,8 years; 14 patients were in Binet stage A, 14 in Binet stage B and 12 in Binet stage C. Median total lymphocyte count was 38,8x10.9/l. Among all patients, CD38 positive were 45%, ZAP-70 positive 55%, CD49d positive 52,5% and mean percent of CXCR4 positive cells was 57,2% cells. There were statistically significant correlations between CD38 and ZAP-70 positivity, CD38 and CD49d positivity, and ZAP-70 and CD49d positivity. There were also correlation between percentage of cells which express CD38, ZAP-70 and CD49d, and correlation between percentage of cells which express CD49d and CXCR4. Expression of ZAP-70 correlate with advanced clinical stage and hepatomegaly, expression of CD49d with hepatomegaly, and expression of CD38 with number of lymph nodes and splenomegaly.
Summary and Conclusions: In our study, expression of CD49d in chronic lymphocytic leukemia cells strongly correlates with expression of CD38 and ZAP-70, and CD49d expression correlates with CXCR4 expression, suggesting cooperation of these molecules in leukemic cell functions.

Chronic lymphocytic leukemia and related disorders - Clinical

PB1713

CHROMOSOMAL ABERRATIONS BY FLUORESCENCE IN SITU HYBRIDIZATION IN JAPANESE CHRONIC LYMPHOCYTIC LEUKEMIA: CLLRSG-01 STUDY.
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Background: Chronic lymphocytic leukemia (CLL) is the most common leukemia in Western countries but is very rare in Asian countries, including Japan. There are few reports about immunophenotypes, chromosome aberrations and mutations of immunoglobulin heavy chain gene variable region (IGVH) in Japanese CLL patients.
Aims: Hence we have conducted a nationwide registry study (CLLRSG-01) from January 2011 to clarify the characteristics of Japanese CLL patinets. We present here the interim results of prospective study.
Methods: Seventy-five untreated patients with indolent B-cell neoplasms met the inclusion criteria: (i) absolute lymphocyte counts of 5000/ul or more in the peripheral blood; or (ii) abnormal lymphocytes of 20% or more in the peripheral blood. The morphological examination was carried out according to the WHO classification by diagnostic committee. “Atypical CLL” was determined according to the morphology by the FAB classification, but this term does not mean atypical expression of surface immunophenotype. Immunophenotyping was performed using flow cytometry with a panel of antibodies including CD5, CD19, CD20, CD21, CD22, CD23, CD38, CD69, FMC7 and ZAP-70. The immunophenotypic score was calculated based on the CLL scoring system proposed by Matutes *et al.* We investigated del(13q14), del(11q22), del(17p13), and trisomy 12 using interphase fluorescence *in situ* hybridization (FISH). Cases with mantle cell lymphoma were excluded by FISH with t(11;14) probe and/or the overexpression of CCDN1 by immunostaining.
Results: According to the morphologic review of peripheral blood smear, 41 patients (55%) were classified as typical CLL, 12 (16%) as atypical CLL and 22 (29%) as other indolent B-cell tumors (others) including 8 hairy cell leukemia. The immunophenotypic profile demonstrated that the frequency of expression of CD5, CD21, CD23 and CD69 in CLL (typical and atypical) group was higher than that of others. The Matutes’ CLL scoring system showed that 27 patients (51%) with CLL had higher values (5 or 4) whereas 21 patients (95%) with others had lower score values (0-3) (P=0.004). There was no significant difference between the typical and atypical CLL in this scoring system. Of the 75 patients analyzed by FISH, 44 (59%) had at least one aberration. The most frequent abnormality was del(13q14)(37%), followed by trisomy 12 (23%), del(11q22)(7%), and del(17p13)(7%). Del(17p13) was not detected in patients with CLL, but was detected in 5 patients (23%) with others. Among CLL patients, atypical morphology was shown to correlate with ZAP-70 expression and trisomy 12 by FISH analysis in comparison with typical CLLs.
Summary and Conclusions: The present study demonstrated immunophenotypic and cytogenetic characteristics of CLL in Japan. Our results have shown that Japanese CLL has atypical immunophenotypes and similar chromosomal aberrations as those in Western countries. Our results should be confirmed by further Japanese and Western collaborative study.

PB1714

AUTOIMMUNE HAEMOLYTIC ANAEMIA DURING BENDAMUSTINE TREATMENT IN CLL PATIENTS
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Background: Immune dysregulation with autoimmune phenomena, especially autoimmune haemolytic anaemia (AIHA), is a common complication over the lifetime of CLL patients. Therapy-related haemolysis in CLL was first described in 1966 in patients treated with radiotherapy or alkylating agents. Drug-induced haemolytic anaemia mostly related to the use of fludarabine has been often reported, even if the use of monoclonal antibody with chemotherapeutic agents could reduce the incidence of haemolytic episodes. Bendamustine is an alkylating agent composed by benzimidazole ring which is similar to some purine analogs; for these reasons the haemolysis generated by bendamustine should be similar to that induced by fludarabine. To date, this complication was rarely reported in association with bendamustine.

Aims: We reported the experience of 4 Italian haematological centres focusing on AIHA during treatment with bendamustine plus rituximab (BR) in patients affected by CLL.
Methods: We included in the study all CLL patients who underwent BR treatment as front-line or successive treatment for progressive disease. All patients who experienced AIHA during or after BR treatment were described. AIHA was diagnosed as reduction of haemoglobin level with positivity of haemolysis tests.
Results: One hundred-thirty CLL patients were treated with BR in our four centres. Of these, 61 patients were treated for progressive CLL as first-line therapy and 69 patients as second-line therapy. No patient experienced autoimmune phenomena when bendamustine was used with rituximab as part of front-line therapy. On the opposite in CLL patients treated with BR as second-line therapy, 5 episodes of AIHA were reported as shown in Figure 1. The prevalence of bendamustine related AIHA in patients who underwent to BR treatment as second line therapy was 7.2%. Three of five patients who experienced AIHA had positive DAT, AIHA or immunological autoantibodies at the onset of CLL. Three cases used fludarabine based regimens as front-line therapy. Three of five patients developed DAT positive AIHA; low median lymphocytes count (1000/mmc) and the use of rituximab could justified DAT negative AIHA in two patients. The onset of AIHA was observed during the BR treatment in 4 patients and at the end of planned therapy in 1 patient. The course of AIHA was mild, it responded to steroid or rituximab therapy and it was solved in a few days.

Table 1. Characteristics and biological profile of AIHA/CLL patients.

Patient number	1	2	3	4	5
Sex/Age	F/58	F/62	M/68	F/64	F/63
Binet/Rai stage	B/II	B/II	B/II	B/II	C/III
Zap-70/CD38	neg/pos	pos/pos	neg/pos	neg/neg	n.a/n.a.
IgVH	unmutated	unmutated	unmutated	unmutated	mutated
FISH	del13/del11	del13	del13	del17	del11/+12
First-line treatment	BR	CtxR	FCR	FCR	FCR
Autoimmunity at first-line treatment	AIHA thyroiditis	DAT+ thyroiditis	DAT+ thyroiditis	DAT-	DAT-
Response to 1st line	PR	PR	CR	SD	CR
Type of treatment prior AIHA	BR	BR	BR	BR	BR
Time of insurgence of AIHA respect bendamustine	30 days after the end of therapy	After 4th cycle	After 2nd cycle	After 3rd cycle	After 4th cycle
Type and specificity of DAT	4+/-	2+/ IgG	4+/-	neg	neg
Lymphocyte count at AIHA (mmc)	550	2510	1000	680	2500
Minimum Hb level during AIHA gr/dL	7.7	7.5	5.4	9.4	8.5
Type of therapy for AIHA	steroid	steroid	R-CVP	Rituximab	steroid

Summary and Conclusions: All 5 patients experienced bendamustine related AIHA during or after BR treatment as second line chemotherapy. Our experience confirms the literature data in which 9 of 10 cases of bendamustine related AIHA in CLL patients reported, appeared after second or further treatment. Patients who experienced previous autoimmune phenomena or underwent fludarabine as front-line therapy, probably for its similitude in the drug structure to bendamustine, showed an increase risk of AIHA. AIHA is probably due to depletion of CD4 cells which can lead to failure to control auto-reactive T-cells that are free to create autoimmunity. The course of bendamustine related AIHA was mild, it could be quickly solved in a few days with the use of immunosuppressive agents as steroid or monoclonal antibodies. In conclusion BR is safe and effective regimen in CLL patients, a care observation should be taken in patients in which BR was used as second or further line therapy. Previous autoimmune phenomena, prior use of fludarabine and CD4 depletion could explain the increased incidence of bendamustine related AIHA.

PB1715

SIMILAR EPIDEMIOLOGICAL TRENDS OF LYMPHOID MALIGNANCIES AND THEIR RESPECTIVE PRECURSOR LESIONS IN TAIWAN

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Background: Based on epidemiological studies, it is well known that Taiwan differs from the Western countries in terms of incidence of lymphoid malignancies with follicular lymphoma (FL), multiple myeloma (MM), and chronic lymphocytic leukemia (CLL) being much less prevalent The respective incidence rates are around one-fourth, one-third, and one-sixth of the one in the US. For these reasons, it is of interest to evaluate whether also their respective precursor conditions, *i.e.*, circulating t(14;18)-*IGH*/*BCL2* translocation, monoclonal gammopathy of unknown significance (MGUS), and monoclonal B-cell lymphocytosis (MBL) are also less common in Taiwan.
Aims: To know the epidemiology of the preursor conditions of lymphoid malignancies in Taiwan.
Methods: We screened 300 healthy Taiwanese individuals with the median age of 43 (range 23~73) for the presence of *IGH*/*BCL2* translocation in peripheral blood mononuclear cells by polymerase chain reaction. In another cohort of 302 healthy individuals with the median age of 53 (range 23-79), the screening for the appearance of MGUS by serum immunofixation and MBL by flow cytometry were done. The results were compared to the data from Western cohorts.
Results: In line with the epidemiological findings on the actual diseases, the prevalence rates of all three precursor conditions appear significantly lower in the Taiwanese population with 10.7% (32/300) of the cases positive for circulating *IGH*/*BCL-2* translocations, 1.3% (4/302) positive for MGUS, and 1.3% (4/302) positive for MBL, as compared with the frequencies of more than 50% for circulating *IGH*/*BCL-2* translocation, 3~5% for MGUS, and 3.5~7% for MBL in the Western populations. The differences, however, were similar to those observed for the incidence rates of FL, MM, and CLL, respectively, between Taiwan and the West. For MGUS and MBL, the numbers of positive cases were too small for analyses in terms of age. In the case of circulating *IGH*/*BCL2* translocations, the prevalence in the healthy population was higher in middle-aged to older people than in young adults (6.3% in the 3rd decade, 6.6% in the 4th decade, 13.8% in the 5th decade and 10.6% in the 6th decade), similar to the age-distribution pattern of FL.
Summary and Conclusions: The present study demonstrates lower rates for pre-neoplastic conditions in the Taiwanese population, *i.e.* of the presence of the circulating t(14;18)-*IGH*/*BCL2* translocations, MGUS, and MBL that is in line with the known lower frequency of their respective lymphoid neoplasms as compared to Western populations. These results confirm the role of the precursor conditions during the natural history of the actual diseases and the pre-neoplastic nature of these manifestations. Unraveling the molecular and genetic features of circulating *IGH*/*BCL2* translocations, MGUS, and MBL in healthy Taiwanese population will be key to understand the pathogenetic mechanisms responsible for the lower frequencies of FL, MM, and CLL as compared to the Western populations.

PB1716

PROGNOSTIC TESTING PATTERNS IN PATIENTS (PTS) WITH CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) TREATED IN US PRACTICES FROM THE CONNECT® CLL REGISTRY

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Background: Genetic aberrations detected by fluorescence *in situ* hybridization (FISH) and cytogenetic (CG) testing provide important prognostic information for CLL pts. The identification of genetic abnormalities has particular relevance in choosing immunochemo- or kinase inhibitor therapies, allogeneic stem cell transplantation, or clinical trials for CLL pts.
Aims: To analyze factors influencing decisions to perform FISH or CG testing in CLL pts.

Table 1.

MV analysis: Predictors of FISH/CG		
Time point/Practice setting	**Covariate**	**OR 95% CI**
LOT1 All sites	Academic center vs. community/government center	1.76 1.03–2.99
	White vs. other ethnicity	1.90 1.13–3.17
	Private insurance vs. other	1.44 1.08–1.92
LOT1 Community-Government sites	White vs. other ethnicity	2.40 1.26–4.58
	Age ≤ 75 vs. > 75	1.44 1.01–2.05
	RAI stage ≥ 2 vs. 1	1.52 1.07–2.14
LOT≥2 All sites	White vs other ethnicity	0.34 0.17–0.68
	Age ≤ 75 vs. > 75	1.45 1.01–2.07
LOT≥2 Community- Government sites	White vs other ethnicity	0.41 0.16–1.04
	Age ≤ 75 vs. > 75	1.65 1.05–2.61
	RAI stage ≥ 2 vs. 1	1.74 1.12–2.71

Methods: Connect® CLL is a large, prospective, longitudinal, multicenter, observational registry of 1494 CLL pts at 179 community (1311 pts), 17 academic (155 pts), and 3 government (28 pts) sites. Pts were enrolled within 2 months of initiating any line of CLL-directed therapy (LOT). Univariate (UV) and multivariate (MV) logistic regression analyses were conducted to identify characteristics associated with a decision to perform genetic testing at LOT 1 and at LOT ≥ 2.
Results: Baseline characteristics for the cohort were as follows: 63.8% of pts were male and the median age was 69 years (range 22–99). The median time from diagnosis to inclusion in the registry was 3.7 years (range 0–32) and 92.7% of pts had an ECOG PS score of ≤1. FISH or CG was performed at study enrollment in 861/1494 (58%) pts (36% CG, 49% FISH, 28% both). 65% of 889 pts were tested for FISH/CG prior to LOT1, 50% of 260 in LOT2, 45% of 345 in LOT≥3. Of 861 pts tested at enrollment, 29% had FISH/CG retested with a subsequent LOT. In UV analyses (14 predictors), FISH/CG were more often performed at academic sites (p 0.005), in pts age ≤75 (p 0.0002), at enrollment at LOT1 vs. LOT ≥2 (P<0.0001), in private insurance pts (p 0.002) and Rai stage ≥2 (P<0.0001). Table 1 describes independent predictors of performing genetic FISH/CG testing stratified by LOT (LOT1 vs. LOT≥2) and practice setting (all practice settings vs. community-government settings only).
Summary and Conclusions: Our results indicate that only a fraction of CLL pts are tested/re-tested for genetic alterations by FISH/CG. Given the significance of identifying del17p or complex CG in selecting each LOT, these results indicate a need for increased awareness of the importance of this testing in clinical practice.

PB1717

VALUE OF CIRCULATING GALECTIN-3 FOR PROGNOSIS OF CARDIOVASCULAR EVENTS IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA IN REMISSION

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Background: Perivascular leukocyte infiltrates and endothelial damage are vital in the development of chronic lymphocytic leukemia. As cell-to-cell interactions are critical in the processes of chronic lymphocytic leukemia, galectin-3 have become of interest as novel regulators of inflammation. Galectin-3 is produced by activated macrophages and it is predominantly expressed in subclinical atherosclerosis, unstable and stable coronary artery disease, heart failure. Therefore, galectin-3 is not only key player in inflammation and as well as in tumor progression by displaying intracellular and extracellular activities. However, the predictive role of galectin-3 in stable patients with chronic lymphocytic leukemia in remission is not understood.
Aims: Evaluate the prognostic value of circulating galectin-3 for cumulative survival in patients with chronic lymphocytic leukemia in remission.
Methods: One hundred fifty six out subjects with chronic lymphocytic leukemia in full or partial remission were enrolled in the study. Diagnosis and staging of chronic lymphocytic leukemia were defined by current clinical practice guidelines. All subjects gave their written informed consent to participation in the study. Observation period was up to 12 months. Blood samples for biomarkers measurements were collected. ELISA method for measurements of circulating level of galectin-3 was used. Hemodynamic evaluation was performed by transthoracic echocardiography. Echocardiography in B-mode was performed accordingly to Recommendation of American Society of Echocardiography on the scanner "MyLab 50" (Italy) using a transducer with a frequency of 2.5-3.5 MHz.
Results: Two hundred sixteen cumulative clinical events occurred in 51 patients (32,7%) within the follow-up, with their distribution being as follows: 7 deaths, 122 cardiac arrhythmias, 16 cardiac ischemic events, 3 strokes, 30 chronic heart failures and 38 hospital admissions for cardiovascular reasons. Medians of circulating levels of galectin-3 in free-events subject cohort and subjects cohort with cardiovascular events were 5,16 ng/mL (95% confidence interval [CI]=4,74-5,56 ng/mL) and 16,40 ng/mL (95% CI=14,80-18,01 ng/mL) (P<0.001). In multivariate logistic regression circulating VE-catherin independently predicted cumulative cardiovascular events (odds ratio [OR]=1,13; 95% CI=1,07–1,25; P=0.003) within 12 months of observation period. Results of the study showed that exaggerated circulating level of galectin-3 in patients with chronic lymphocytic leukemia in remission may consider biomarker with power predictive value for cardiovascular events whether for tumor progression did not. Probably it may be related with small size of the study or short-term period of the observation. However, association of galectin-3 with tumor progression was not found. Author think that it is needed more studies with higher statistical power to be recognized prognostic potential of Gal-3 in two directions: cardiovascular outcomes and tumor progression.
Summary and Conclusions: Among patients with chronic lymphocytic leukemia in remission increased circulating galectin-3 associates with increased cumulative cardiovascular events within 12 months.

PB1718

EFFICACY AND TOLERABILITY OF PENTOSTATIN CYCLOPHOSFAMIDE RITUXIMAB (PCR) REGIMEN IN ELDERLY, "SLOW-GO" CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) PATIENTS. A MONOCENTRIC EXPERIENCE

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Background: Chronic lymphocytic leukemia (CLL) is the most common adult leukemia and primarily affects the elderly. Treatment of CLL has evolved significantly in recent years. There is currently no standard of care for patients with CLL older than 70 years or patients with co-morbidities and an impaired physical condition ("Slow go"). These patients may be offered a mild chemotherapy regimen: chlorambucil or dose-reduced fludarabine or bendamustine. Dose-modified combination regimens such as Fludarabine, Cyclophosphamide, Rituximab (FCR)-Lite seem to deliver the FCR therapy with a lower toxicity, but this combination still has to be tested on a large scale, in less fit patients. Similarly, the use of pentostatin, cyclophospamide, rituximab (PCR) was investigated to achieve a reduced toxicity, with promising results, both in pretreated CLL patients than in frontline.
Aims: Starting in December 2010, we selected elderly patients with "slow-go" CLL to evaluate efficacy and safety of PCR regimen in this subset.
Methods: To date, we observed 16 cases of "slow-go" elderly CLL, most were high risk patients. They were treated with PCR regimen consisting of pentostatin (P:2mg/m^2), cyclophospamide (C:600 mg/m^2) and rituximab (R:375 mg/m^2) on day 1 (21-day cycles). Dose 1 of R was given on day 8 (Cycle 1) and was increased to 500 mg/m^2, from cycle 2. The dose of pentostatin was increased to 4 mg/m2, from cycle 3, in the absence of grade III-IV hematologic toxicity. This PCR regimen was given on a 21-day, 6-cycle schedule. Support from granulocyte colony-stimulating growth factor was provided, as required. Patients received prophylaxis with cotrimoxazole and lamivudine (as required).
Results: 13 patients (82%) were treated in frontline and 3 patients (18%) at relapse. The median age was 73 years and 88% were 70 years older. 69% (RAI stages 3-4) and 100% (Binet B-C). Cytogenetics showed Trisomy 12 (31%) and 13q14 deletion (19%). No other cytogenetic abnormalities were detected. IgV_H status resulted "unmutated" in 81% of cases. All patients had comorbidities and 87% had a Creatinìne Clearance (CrCl)<70 ml/min. The overall response rate (ORR) on the whole population was 88%, complete response (CR):25%, partial response (PR):63%. The ORR in frontline was 85%, CR(31%), PR(54%). All patients completed the planned six courses of PCR, without dose reductions or delayed. Toxicities were: grade III-IV neutropenia (18%) and anemia (6%); grade II thrombocytopenia (18%), grade II nausea (31%), grade II constipation (6%), grade II hypertransaminasemia (6%). We didn't observe severe or prolonged infections or other toxicities. There were no significant differences in the number of cycles administered, need for dose reductions, grade 3-4 toxicities in patients with CrCl<70 ml/min. At a median follow-up of 29 months (range: 6-44), the median progression free survival (mPFS) was 17.6 months (range: 3-42), 75% of patients are alive, 3 patients (25%) died for cardiovascular disease, respectively after 6, 12 and 12 months and 1 patient for severe pneumonia after 3 months from the end of therapy.
Summary and Conclusions: Therapeutic strategies for "slow-go" patients with CLL are still lacking, especially for elderly patients. In previous studies, PCR regimen demonstrated activity, both in frontline and at relapse. Moreover, PCR seems feasible and safe because loaded from toxicities less than FCR regimen. In our experience, PCR was well tolerated in elderly and "slow-go" CLL, also in patients with CrCl<70 ml/min. Then, PCR may be a treatment option in this subset of patients.

PB1719

IMMUNE THROMBOCYTOPENIA IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Chronic Lymphocytic leukemia (CLL) is sometimes associated with autoimmune cytopenia: autoimmune hemolytic anemia (5-25% of cases) and immune thrombocytopenia (1-8% of cases)]. The effect of immune thrombocytopenia (ITP) on the clinical outcome and survival of patients with CLL is controversial.
Aims: The aim of the study was to correlate of ITP-CLL evidence with biological features, phenotypic and cytogenetic abnormalities and disease outcome in patients with B-CLL.
Methods: We studied 175 patients with B-cell CLL. All patients were diagnosed according to the revised criteria of the National Cancer Institute-sponsored Working Group (NCI-WG) on Chronic Lymphocytic Leukemia (CLL) and were classified according to the Rai staging system. Twelve of them (6.9%) were with immune thrombocytopenia (IT). The diagnosis of immune thrombocytopenia was based on the presence on unexplained fall in platelet count to<100x10^9/L and on more than two indirect parameters: evidence of normal

bone marrow function (normal or increased megakaryocytes in bone marrow), no splenomegaly and no chemotherapy within the last month. The patients with thrombocytopenia due to bone marrow suppression (lymphocytes over 80% in bone marrow) were excluded from this analysis.
Results: Among clinical and biological variables, neither age, gender, tumor mass nor Rai stages at CLL diagnosis were significantly associated with ITP development. The ITP occurrence was significantly associated with ZAP-70 positivity (7/12; 58.3%). In contrast ZAP-70 was found in 44 of 119 B-CLL cases (27%, P=0.028). CD38 and P53 expressions was significantly higher (75% and 41.7%, respectively; P=0.038 and P=0.013, respectively) than in CLL without ITP (44% and 11.2%). Based on available FISH data, we found that among 12 cases with deletion of (11)(q22-23) region only one (9%) developed ITP. There was no statistical significance (p>0.05) between ITP development and cytogenetic deletion (13)(q14). The median overall survival of CLL-ITP patients was significantly shorter -68.6 months (95% CI: 49.5-87.6 months) than the patients without ITP -111 months (95% CI: 104.7-117.4 months; P=0.016) and overall survival dropped rapidly and was in stable rate after 12 months since the diagnosis. ITP cases had an increased risk of disease progression and mortality risk over 3 fold above the patients without ITP (P<0.001, P<0.05, respectively). CLL-ITP patients showed shorter median free of treatment period-2.08 months (95% CI 0-5.99), compared to CLL without ITP-45.10 (95%CI 36.50-53.70).
Summary and Conclusions: ITP-CLL is associated with a higher frequency of poor prognostic markers such CD38, ZAP-70 and P53 expressions and shortened free of treatment period and overall survival.

PB1720

IMPACT OF CLINICAL AND BIOLOGICAL VARIABLES ON TIME TO FIRST TREATMENT IN CHRONIC LYMPHOCYTIC LEUKEMIA
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Background: Chronic lymphocytic leukemia (CLL) is a heterogeneous disease with variable outcome. The identification of factors that could predict the clinical course of CLL is a crucial objective. Recently, prognostic models involving a set of clinical and biological risk factors have been proposed to evaluate the association with the time before initiating treatment and the prognosis for patients with CLL.
Aims: In our study we investigated time to first treatment (TFT), measured as the time elapsed between diagnosis and first treatment, and its relation with clinical and biological parameters and overall survival.
Methods: We retrospectively evaluated patients (pts) with de novo CLL treated at the University Hospital of Bari (Italy) between April 2006 and February 2013. At diagnosis pts were studied for clinical characteristics and biological prognostic factors: age, β-2 microglobulin, absolute lymphocyte count, sex, Rai stage, number of lymph node groups involved, pattern of bone marrow involvement, splenomegaly, mutational status of the immunoglobulin heavy chain gene variable region (IGVH), cytogenetic abnormalities detected by FISH. Median time to first treatment was calculated for all pts, divided into two groups according to Median time to first treatment. The first group included pts treated within 40 months from diagnosis; the second group pts treated after 40 months from diagnosis. Univariate analyses of each group were performed to evaluate the correlation between the clinical variables and time to treatment. In addition, overall survival was calculated and compared in the two groups.
Results: In total, 69 pts were included; 42 in the group of pts treated within 40 months from diagnosis, and 27 in the group treated after 40 months.
Statistically significant differences were seen between the 2 groups regarding the pattern of bone marrow involvement (diffuse *versus* other patterns of marrow involvement; P=0.05), number of lymph node groups involved, (one *versus* more than one; P=0.01); mutational status of the immunoglobulin heavy chain gene variable region (IGVH) (mutated *versus* unmutated; P =0.05), cytogenetic abnormalities by FISH (21 pts with 13q14 in the group of TFT >40 months and 2 in the group of TFT<40 months; P:0.01). No statistically significant difference was seen among the other clinical variables and TFT. Moreover, comparing the OS of the group with TFT >40 months with OS of the group with TFT<40 months, statistically significant difference was observed (P:0.01).
Summary and Conclusions: In our study the pattern of bone marrow involvement, the number of lymph node groups involved, the mutational status of the immunoglobulin heavy chain gene variable region and the cytogenetic abnormalities could help to recognize at diagnosis a subset of patients that may show rapid evolution to progressive disease. Multicentric studies and larger cohorts of patients are warranted to confirm these preliminary data.

PB1721

INCIDENCE OF SECOND CANCER IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKAEMIA: 19 YEARS EXPERIENCE FROM SINGLE CENTRE.
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Background: Patients with chronic lymphocytic leukaemia (CLL) have a higher incidence of second malignancies than the general population.
Aims: Our study aims to present the incidence in our population and predicting whether stage and previous chemotherapies could have a negative impact on the number of second cancer in patients of CLL at our Hospital.
Methods: We retrospective evaluated 178 patients of CLL registered at General Hospital of Limassol, in Cyprus over a period of 19 years (1995-2014). A second malignancy was defined as another malignancy at the time of diagnosis or during follow up.

Table 1. Results of study in parathensis there is the report of deaths related to second cancer (dead patients w/o cancer+dead patients with cancer).

		Early stage	Advanced stage
Patients	178	141	37
male	103	78	25
therapy		*22*	*10*
2nd cancer		*4*	*4*
death		*13 (11+2)*	*4 (3+1)*
w/o therapy		*56*	*15*
2nd cancer		*7*	*4*
death		*7 (3+4)*	*4 (2+2)*
Female	75	63	12
therapy		*13*	*4*
2nd cancer		*0*	*0*
death		*6 (6+0)*	*3 (3+0)*
w/o therapy		*50*	*8*
2nd cancer		*8*	*0*
death		*5 (3+2)*	*0*

Results: There were 178 patients, 75 females and 103 males. The average age, was 67 years (34-94 years). The common presenting features were lymphocytosis 86%, lymphadenopathy 82%, splenomegaly 20%, and hepatomegaly 7%. Analysing data for all the patients, at diagnosis, 79% were early stage, (Rai stage 0- 46,3%, Rai stage I- 14,7%, Rai stage II-18%) and 21% of cases were in advanced stage, (Rai stage III- 18,6%, Rai stage IV-2,4%). Twenty seven percent of patients received treatment at presentation or at disease progression, including chlorambucil, fludarabine, rituximab and bendamustine based regimens. During the follow up, we noticed 27 cancers, including two haematological malignancies along with cancer of breast, uterus, lung, prostate, colon, skin, pancreas renal and larynx. We also found two high grade transformation (Richter syndrome), two cases of prostate infiltration and one case of breast infiltration by chronic lymphocytic leukaemia (Table I). Median time between CLL and second cancer diagnosis was 5.01 years (0-16 years). According to correlation analysis, neither advanced stage nor previous chemotherapies influenced with statistical significance the incidence of second malignancy in patients with CLL in our Hospital. Moreover the second tumour did not predisposed shortness of patient's survival and chemotherapy has no impact in the time of cancer diagnosis.
Summary and Conclusions: The incidence of second cancer is 15,3% in our population. Advanced stage and previous chemotherapies have none impact in this incidence and did not influenced the survival of the patient, comparing with the survival of the non-cancer patients. Unfortunately, the majority of our patients has diagnosed before the facility for molecular and cytogenetic analysis became feasible in Cyprus, that didn't let us to include modern prognostic factors in our study.

PB1722

HYPOGAMMAGLOBULINEMIA AND INFECTIOUS EVENTS IN CHRONIC LYMPHOCYTIC LEUKEMIA
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Background: Infections are a major cause of morbidity and mortality in Chronic Lymphocytic Leukemia (CLL), related to humoral and cellular immune deregulations due to the disease itself and enhanced by immunosuppression associated to treatments. Hypogammaglobulinemia is the most common chronic immune defect in CLL, related to duration and stage of the disease, and correlates with the frequency and severity of infections observed in these patients.
Aims: Analyze and compare the frequency and type of infectious episodes requiring hospital admission in a population of patients diagnosed with CLL, with and without hypogammaglobulinemia.

Methods: Retrospective analyses of the clinical files of patients with CLL diagnosed between January 2004 and August 2014.
Results: Between January 2004 and August 2014, 151 patients were diagnosed with CLL. Twenty-five patients (16.6%) developed hypogammaglobulinemia, 6 at the time of diagnosis. The mean time from diagnosis of CLL to the development of hypogammaglobulinemia in the remaining patients was 38.2 months (min=4; max=97). Patients with a serum IGg<5g/L and recurrent or severe infections received IVIg replacement therapy. In the group with hypogammaglobulinemia (n=25), the median age at diagnosis was 71 years (min=50; max=88); 14 men (56%) and 11 women (44%). The initial staging (Rai) was 0 in 11 patients (44%), I in 8 patients (32%), III in 3 patients (12%) and IV in 3 patients (12%). The initial approach was watchful waiting in 16 patients (64%), with 9 patients (36%) requiring treatment. Sixteen patients (64%) required hospital admission related to infectious intercurrences, with 25.6 days as mean time of hospitalization (min=1; max=133). The total number of documented infections was 47 (mean of 3 episodes/patient; min=1; max=9): upper and lower respiratory tract infection in 14 patients (87.5%), urinary tract infection in 6 patients (37.5%); gastroenteritis, lower and upper limb cellulitis, oral candidiasis and febrile neutropenia in 2 patients each (12.5%). One patient developed cryptococcal meningitis and one patient developed septicemia. In the group without hypogammaglobulinemia (n=126), the median age at diagnosis was 70 years (min=37; max=93); 73 men (58%) and 53 women (42%). The initial staging was 0 in 90 patients (71.4%), I in 24 patients (19%), III in 6 patients (4.8%) and IV in 7 patients (5.6%). The initial approach was watchful waiting in 113 patients (89.7%) and treatment in 13 patients (10.3%). Twenty-two patients (17.4%) required hospital admission due to infectious events, with a mean time of 15.55 days (min=1; max=69) and a total number of 37 infections (mean of 1.7 episodes; min=1; max=5): upper and lower respiratory tract infection in 12 patients (54.5%), urinary tract infection in 6 patients (27.3%); gastroenteritis, septicemia and cellulitis in 3 patients each (13.6%); febrile neutropenia in 2 patients (9.1%). One patient developed a rickettsia infection.
Summary and Conclusions: The two groups were similar in terms of age and sex distribution. The proportion of patients staged III and IV was higher in the group with hypogammaglobulinemia. The percentage of patients with infectious events requiring hospital admission was significantly higher in the group with hypogammaglobulinemia (64% vs 17.4%). Fungal infections were only present in the group with hypogammaglobulinemia. A higher number of infectious events in this group required a longer time of hospital admission, reflecting their severity. Therefore, it is important to monitor immunoglobulin levels and to administer immunoglobulin replacement therapy in these patients.

PB1723

CUMULATIVE ILLNESS RATING SCALE (CIRS) – AN INDEPENDENT PROGNOSTIC PARAMETER FOR CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS' PRETREATMENT STRATIFICATION

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Background: Most of data about the clinical outcome of patients with chronic lymphocytic leukemia (CLL) come from patients participating in clinical trials. Such trials typically have strict eligibility criteria based on performance status and organ function. Little is known about the organ function of unselected patients (*i.e.*, regardless of whether they are eligible for trials). Adult comorbidity evaluation-27 (ACE-27), Cumulative Illness Rating Scale (CIRS) and hematopoietic cell transplantation comorbidity index (HCT-CI) are comorbidity indexes that take concurrent presence of nonmalignant diseases into account when explaining survival. They differ in both the number and categorization of comorbidities.
Aims: We investigated the prognostic significance of three comorbidity indexes in adition to standard prognostic tools in a sample of 74 patients with CLL.
Methods: We retrospectively evaluated the impact of ACE-27, CIRS and HCT-CI in a cohort of 74 adult patients with chronic lymphocytic leukemia (HLL) treated with Fludarabine-Cyclophosphamide combination. Also we tested impact of standard prognostic tool available in daily practice as age, gender, Rai/Binet clinical stage, LDH level, type of bone marrow infiltration and CD38 expression. Patient and survival data were acquired from inpatient hospital records. Cox hazard regression model was used to analyze overall survival according to standard prognostic factors and comorbidities classified by the ACE-27, CIRS and HCT-CI.
Results: Median follow up of our group was was 5 years (60 months) while median overall and progression free survival was 72 and 32.5 months, respectively. We found a significant association between CIRS and bone marrow infiltration pattern and overall survival (P=0.037; RR=2.0; 95%CI for RR 1.1-4.0 for CIRS>15 and P=0.014; RR=2.3; 95%CI for RR 1.2-4.5 for diffuse type of bone marrow infiltration). Other two comorbidity indexes – ACE-27 and HCT-CI and standard prognostic factors did not outperform the survival model, while in multivariate analysis CIRS>15 and diffuse type of marrow infiltration remained the most important predictors of overall survival (P=0,009 and P=0,004, respectively). We developed a new scoring system based on the CIRS score value and bone marrow infiltration pattern. Patients with CIRS≤15 and without diffuse type of marrow infiltration were of low risk (score 0), patients with CIRS>15 or diffuse bone marrow infiltration pattern corresponded with intermediate risk (score 1), while patients with both CIRS>15 and diffuse type of bone marrow infiltration represented high risk group (score 2). Median overall survival for patients with score 0, 1 and 2 was 96, 82 and 34 months, respectively, which makes this new score a good predictor of mortality among patients with chronic lymphocytic leukemia (P<0.001; RR=2.6; 95%CI for RR 1.5-4.5).

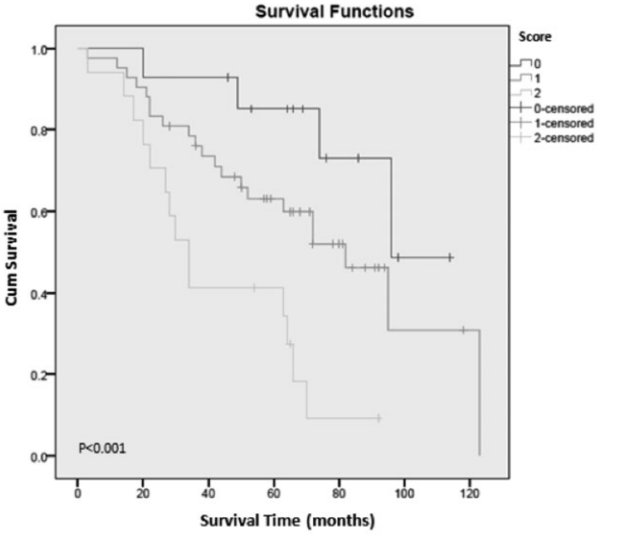

Figure 1.

Summary and Conclusions: In our group of adult CLL patients the overall survival is associated with the presence of comorbidities defined by the CIRS index. CIRS and type of bone marrow infiltration present a superior comorbidity risk-adjustment model for CLL survival prediction.

PB1724

SIDE EFFECTS AFTER RITUXIMAB INFUSION IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: The anti-CD20 monoclonal antibody rituximab is approved for the treatment of non-Hodgkin's lymphomas (NHL) and chronic lymphocytic leukemia (CLL). Infusional side effects are commonly seen during rituximab administration, mostly in the first cycle and in patients with high tumor load. Severe adverse events (SAE) may also occur, following which the drug needs to be definitively stopped in some cases. Very recently, Norin et al, have reported data of a Swedish national observational study focusing on SAE occurring in patients with CLL who underwent rituximab treatment (Leuk Res, 2015). These Authors evaluated 96 patients and found that 58% of them experienced at least one adverse drug reaction (ADR) during the first cycle. Only 5 patients (5%), however, reported grade ≥ 3 ADRs, with one grade 4 reactions. Only 2 patients had a leukocyte count >50.000/mL and no correlation between leukocyte count and ADR grade was found.
Aims: Starting in October, 2014, we are conducting a retrospective Italian multicenter study aiming to evaluate the incidence of side effects in patients treated in the last 5 years at our Institutions with rituximab for their hematologic malignancies or autoimmune disorders. We choose to focus on ADRs occurred in CLL patients aiming to evaluate the incidence of infusional side effects and, possibly, its correlation with clinico-biological features evaluated immediately before to start therapy.
Methods: To date, 130 patients have been evaluated: 60 with CLL, 34 with non-Hodgkin's diffuse large B-cell lymphomas, 33 with non-Hodgkin's indolent lymphomas, 1 with Hodgkin's lymphomas with lymphocyte predominance, and 2 with autoimmune disorders (1 immune thrombocytopenia and 1 acquired hemo-

philia A). Sixty patients with immunologically typical CLL were evaluated (40 were male and 20 female; mean age was 66,4 years, range 46-86; 1 patient with Rai stage 1; 29 patients stage 2; 17 stage 3; and 13 stage 4). Rituximab was given as first line treatment and variously combined with different drugs, the most used being fludarabine, cyclophosphamide, chlorambucil and bendamustine.
Results: Twenty-three patients (38%) experienced an adverse event during the first infusion of rituximab, while 37 patients (62%) did not. Overall, 13 patients (57%) had grade 1, 7 (30%) grade 2, 1 (4%) grade 3, and 2 (9%) grade 4 ADRs, according to National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE). No correlation was found with age, gender, concomitant presence of an autoimmune hemolytic anemia, spleen and lymph node involvement, platelet count, and type of associated-chemotherapy. Degree of bone marrow infiltration (p 0.03) and the concomitant infusion of rituximab plus chemotherapy with respect to a delayed administration (p 0.007) were significantly associated with the occurrence of ADRs. Quite surprisingly, patients with more elevated hemoglobin levels (p 0.02) and those with lower peripheral lymphocyte counts (p 0.03) displayed a greater risk to develop ADRs; these last patients also showed more severe ADRs. Most of side effects were easily managed. In the majority of cases rituximab was temporarily stopped, while steroids and anti-histaminic drugs were given. The monoclonal antibody was then safely re-started with a less rapid infusion. Only one patient definitively stopped rituximab treatment, due to grade 4 ADR (shock and dyspnea).
Summary and Conclusions: Overall, in our multicenter experience, side effects after rituximab were commonly seen in CLL patients during the first infusion. However, they were mostly limited to grade 1 and 2 (mild-moderate) and were easily managed. Rituximab was generally re-administered during the same course of the treatment. Only very few patients experienced severe infusion-related ADRs and rarely the monoclonal antibody had to be definitively stopped. Clinico-biological variables, including pharmacogenomic parameters, that could be useful in predicting the development of infusional side-effects are warranted in order to identify patients at higher risk for ADR and to develop appropriate preventing therapies.

PB1725

EPIDEMIOLOGY OF CHRONIC LYMPHOCYTIC LEUKEMIA ON REUNION ISLAND

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Background: Chronic Lymphocytic Leukemia (CLL) is the most common type of leukemia diagnosed in the Western world. The causes of its occurrence remain unclear: environmental factors are involved, such as farming related and chemical exposures. Family history of any hematological malignancy may also increase risk of CLL. Reunion Island is an 874.000 inhabitants tropical French overseas department located in the southwestern Indian Ocean. Because of its insularity and the significant interbreeding population, this territory lends itself to genetic peculiarities. The fraction of people of each ethnicity is not known exactly. A 2002 regional report estimated the distribution of different ethnic groups as 33% Persons of mixed race, 27% Caucasians, 20% Indians, 17% Africans and 3% Chinese.
Aims: In the absence of previous epidemiological study, we conducted a comprehensive review of epidemiological, prognostic and therapeutic characteristics of CLL Reunionese patients over a period of ten years, between 2004 and 2013.
Methods: Inclusion criteria were as follows: any French patient having a permanent residence on Reunion Island and matching the NCI/WG diagnosis criteria for the 2004-2008 period, or the IWCLL diagnosis criteria for the 2009-2013 period. Monoclonal B-cell lymphocytosis as well as small lymphocytic lymphomas were not included in the study. Variables analyzed were age at diagnosis of CLL, sex, prognostic markers (ZAP-70, lymphocyte doubling time, B2-microglobulin, and where available del (17p13)), stage at diagnosis and treatment intervention.
Results: A total of 134 cases of CLL were identified in this study. The age-standardized incidence rate was 1.23 per 100.000 with a male/female sex ratio of 1.98:1. Median age at diagnosis is 63 (range, 38-93 years). 37.3% of patients were under 60 years. A majority of CLL diagnosis (86.3%) were discovered incidentally after a blood cell count is performed for another reason. More patients were at early stage (Binet A) *versus* advanced (Binet B or C) at the time of CLL diagnosis (77% *versus* 23%). Median absolute lymphocyte count at diagnosis was 11.7 G/L. When treatment was indicated (30%), 2/3 patients (12%) received immunochemotherapy as first-line treatment. The median time to treatment was 21.9 months. The overall survival at 5 years is 85%.
Summary and Conclusions: All CLL diagnosis in Reunion Island are carried out *a priori* in one of the two Hospital Centers existing on the island, which are the only facilities able to perform blood lymphocytic immunophenotyping by flow cytometry. The French National Network of Registers FRANCIM analyses together CLL and small lymphocytic leukemia: the 2012 incidence rate on the whole French territory is estimated at 4.4 and 2.2, in men and women respectively, per 100,000. CLL incidence seems twice to three times lower on Reunion Island. Despite a comparison bias between national and Reunionese statistics, the difference appears significate. This Reunionese peculiarity might be explained either by genetic factors related to a highly mixed population, or by environmental factors. Another hypothesis which might explain the low CLL incidence would be an under-use of public and private medical facilities by the Reunionese, in particular elderly people. Besides, sharing between Binet classification stages is the same as the one reported in the literature. No significant differences were identified in overall survival compared with European CLL patients. The low CLL incidence observed on Reunion Island is probably due to multiple factors and requires further investigations. The determination of each patient's ethnic origin shall highlight a possible under-representation of one or several population categories.

PB1726

SECONDARY MALIGNANCIES AFTER FCR AND FC TREATMENT IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA. A SINGLE CENTER STUDY.

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Background: The combination of fludarabine, cyclophosphamide and rituximab (FCR) improved response rates and prolonged both overall survival (OS) and progression-free survival (PFS). On the basis of CLL8 trial FCR has become the standard treatment for fit patients with CLL.
Aims: In this report we presents of follow up results of FCR and FC therapy and adverse events as secondary malignancies.
Methods: 117 patients (50 previously untreated and 67 with relapsed and refractory) received six courses of FCR or FC. Median age was 56,5 (range 28-73) for FCR group and 57 (range 38-76) for FC group. 52 patients were in Binet stage B and 15 in stage C for FCR group, 38 patients were in Binet stage B and 12 in stage C for FC group. OR was 91% with CR rate 57% for FCR group, 86% and 50% for FC group.
Results: At follow up period 120 months median overall survival in FCR group was 61,3 month, in FC group – 44,6 months, in previously untreated FCR group median OS was 89 months and OS was not reached for previously untreated FC group, progression-free survival were 42,4 months for previously untreated FCR group and 24,4 month for previously untreated FC group (P=0,01). Infection complications III-IV grade were appeared in 15 patients: 4 (6%) for FCR group and 11 (22%) for FC group. 5 patients died due to infection: 2 patients in FCR group (2,9%) and 3 patients in FC group (6%). Secondary malignancies were occurred at 10 patients (8,5%): 6 patients in FCR group (8,9%) and 4 – in FC group (8%). Of these 10 patients, 5 patients (4,2%) had a solid tumor (lung, bladder, colon), 1patient (0,8%) had a melanoma, 4 patients (3,4%) had a Richter transformation.
Summary and Conclusions: FCR chemoimmunotherapy is highly effective in patients with CLL. The frequency of secondary malignancies was 8,5%.

PB1727

IBRUTINIB IN CHRONIC LYMPHOCYTIC LEUKAEMIA: A SINGLE CENTRE EXPERIENCE

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Background: Ibrutinib, a first-in-class Bruton's Tyrosine Kinase inhibitor, was available to National Health Service patients with relapsed/refractory Chronic Lymphocytic Leukaemia (CLL) through a Named Patient Supply (NPS) program from April to September 2014, funded by Janssen Pharmaceuticals. Approximately 600 patients with CLL were enrolled in the United Kingdom (UK). The James Paget University Hospital treated 23 patients with CLL through the NPS (including 10 patients from the Norfolk and Norwich University Hospital).
Aims: To evaluate the therapeutic response to ibrutinib in the clinical setting compared to clinical trial data.
Methods: Therapeutic response was assessed in October 2014 using clinical and laboratory data. The patient group was 65.2% male, with a median age of 74 years (range 45–89) and a median of 3 prior lines of therapy (range 1-6). Cytogenetic analysis was available for 20 (87%) patients. Five (25%) patients had 17p deletion, four (20%) had 13q deletion, three (15%) had trisomy 12p, two (10%) had 11q deletion, and six (30%) had no detectable abnormalities. Five patients were excluded from the analysis, including four who had received less than eight weeks of treatment and one who had withdrawn from the NPS early.
Results: Out of 18 patients, comprising a total of 242 drug-weeks, there was one (5.6%) Clinical Complete Response (CR), four (22.2%) Partial Responses (PR), nine (50%) Partial Responses with Lymphocytosis (PR+L), two (11.1%) with Stable Disease (SD), one (5.6%) with Disease Progression (DP) and histological transformation, and one (5.6%) death from sepsis. The Overall Response Rate (including PR+L patients) was 78%. Notably, both the patient

in Clinical CR and the patient with histological transformation had 17p deletion. Common side-effects included diarrhoea (16.7%), upper respiratory tract infections (11.1%), low platelet counts (<30x10^9/L in 5.6%), and lymphocytosis (>two-fold increase in 22.2%). None were treatment-limiting.
Summary and Conclusions: These encouraging early results must be interpreted cautiously, but they suggest that Ibrutinib has similar efficacy and side-effect profiles in the clinical setting as it does in clinical trials.

Chronic myeloid leukemia - Biology

PB1728

COMBINED EFFECTS OF PI3K INHIBITOR, COPANLISIB WITH ABL TYROSINE KINASE INHIBITORS IN PH-POSITIVE LEUKEMIA CELLS
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Background: ABL tyrosine kinase inhibitor (TKI), imatinib and second-generation ABL TKIs, nilotinib and dasatinib have demonstrated the potency against chronic myeloid leukemia (CML) patients. However, resistance to ABL TKI can develop in CML patients due to BCR-ABL point mutations. Moreover, ABL TKIs cannot eradicate leukemia stem cells (LSCs), thus, TKIs do not appear to lead to a cure the diseases. Therefore, new approach against BCR-ABL mutant cells and LSCs may improve the outcome of BCR-ABL-positive leukemia patients. Phosphoinositide 3-kinase (PI3K) pathway regulates cell metabolism, proliferation and survival. Furthermore, aberrant activation of PI3K signaling pathway has been shown to be important in initiation maintenance of human cancers. Copanlisib, also known as BAY80-6946 is a potent pan-class I PI3K inhibitor against PI3Kα and PI3Kδ with potential anti-neoplastic activity. Copanlisib is being investigated in a pivotal phase 2 clinical trial against hematological malignancies such as malignant lymphoma.
Aims: We hypothesized that targeting PI3K, in combination with ABL TKI, would result in enhanced therapeutic activity in Philadelphia chromosome (Ph)-positive leukemia cells including T315I mutation and ABL TKI resistant.
Methods: We investigated the combination therapy with a copanlisib and an ABL TKIs (imatinib, nilotinib and ponatinib) by using the BCR-ABL positive cell line, K562, murine Ba/F3 cell line which was transfected with T315I mutant, nilotinib resistant K562, ponatinib resistant Ba/F3 cells and primary samples.
Results: 72 h treatment of copanlisib exhibits cell growth inhibition against K562 cells in a dose dependent manner. The treatment of imatinib, nilotinib and ponatinib exhibits cell growth inhibition partially against K562 cells in the presence of feeder cell line, HS-5. We found that mRNA of PI3K subunit, p110δ is significantly increased after a co-culture with HS-5 in K562 and primary CD34 positive CML samples. We examined the intracellular signaling after treatment of copanlisib. High concentration of copanlisib reduced the phosphorylation of BCR-ABL, Crk-L and S6 ribosomal protein. Activity of poly (ADP-ribose) polymerase (PARP) was increased. Phosphorylation of BCR-ABL, Crk-L and S6 ribosomal protein was reduced after imatinib and copanlisib treatment. We investigated the copanlisib activity against T315I positive cells. Copanlisib potently induced cell growth inhibition of Ba/F3 T315I cells. Combined treatment of Ba/F3 T315I cells with ponatinib and copanlisib caused significantly more cytotoxicity than each drug alone. Phosphorylation of BCR-ABL and Crk-L was reduced and cleaved PARP was increased after ponatinib and copanlisib treatment. To assess the activity of copanlisib and ponatinib, we performed to test on CML tumor formation in mice. We injected nude mice subcutaneously with Ba/F3 T315I mutant cells. A dose of 20 mg/kg/day p.o of ponatinib and 6 mg/kg/three times per week i.p of copanlisib inhibited tumor growth and reduced tumor volume compared with control mice. We examined the intracellular signaling of tumor model. Phosphorylation of Crk-L and S6 ribosomal protein was reduced and cleaved PARP was increased. Treatments were well tolerated with no animal health concerns observed. We also found that the treatment of copanlisib exhibits cell growth inhibition against Ba/F3 ponatinib resistant cells, K562 nilotinib resistant cells and primary samples.
Summary and Conclusions: Our preclinical results indicated that administration of the PI3K inhibitor, copanlisib may be a powerful strategy against ABL TKI resistant cells and enhance cytotoxic effects of ABL TKI against those Ph-positive leukemia cells.

PB1729

DISTINCT ASSOCIATIONS BETWEEN UGT1A1 GENE PROMOTER POLYMORPHISM AND HYPERBILIRUBINEMIA IN CML PATIENTS TREATED WITH NILOTINIB AND RADOTINIB
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Background: Second-generation BCR-ABL tyrosine kinase inhibitors (2nd generation TKIs) including nilotinib (NIL) and radotinib (RAD) are approved for the treatment of newly diagnosed and other TKI failed chronic myeloid leukemia (CML). Uridine diphosphate glucuronosyltransferase 1A1 (UGT1A1) gene promoter polymorphism, distinct number of TA repeats in promoter, has been reported for an association with hyperbilirubinemia on nilotinib therapy (Singer *et al. Leukemia (2007) 21, 2311–2315*). However, the distribution of UGT1A1 $(TA)_7$ repeat polymorphism differs greatly between Caucasians and Japanese,

namely, the frequency of UGT1A1(TA)$_7$ repeat polymorphism is high in Caucasians, whereas it is low in Asians (Beutler *et al. Proc. Natl. Acad. Sci. USA 95 (1998)* 95, 8170–8174).
Aims: The aim of this study was to investigate the association between UGT1A1 gene promoter polymorphism and hyperbilirubinemia in Korean CML patients treated with NIL and RAD as a frontline therapy.
Methods: A total of 94 newly diagnosed chronic phase CML patients who treated with frontline NIL (n=42) and RAD (n=52) was screened for UGT1A1 promoter polymorphism genotype analysis. We used the High Pure PCR Template Preparation Kit (Roche, Germany) to prepare genomic DNA from whole blood and genotyped by direct sequencing of the 253- to 255-bp fragments produced by PCR.
Results: The (TA)$_6$/(TA)$_6$ homozygote and (TA)$_6$/(TA)$_7$ heterozygote were seen in all genotyped population and frequency of (TA)$_6$/(TA)$_6$ homozygote was 77.7% (73/94) in our patients. (TA)$_6$ /(TA)$_6$ homozygote predominated with 78.9% of the alleles in the RAD group and 76.2% in the NIL group. Relative risk for each genotype presented in Table 1, with hyperbilirubinemia defined as CTCAE grade 2 or greater observed at any post-baseline point. The relative risks for 6/6 genotype *versus* 6/7 genotype was 3.9 with 95% CIs of (0.7, 20.3) in the RAD group compared with 10.5 (2.0, 54.3) in the NIL group and NIL group showed significantly high association with UGT1A1 gene promoter polymorphism (P<0.05).

Table 1. Relative risk calculations prevalcence of hyperbilirubinemia.

	Radotinib				Nilotinib		
	Max grade≤1	Max grade≥2	Total		Max grade≤1	Max grade≥2	Total
(TA)6/(TA)6	19	22	41	(TA)6/(TA)6	28	4	32
	46.34%	53.66%			87.50%	12.50%	
(TA)6/(TA)7	2	9	11	(TA)6/(TA)7	4	6	10
	18.18%	81.82%			40.00%	60.00%	
Total	21	31	52	Total	32	10	42
Relative risk=3.9, 95% CI of (0.7, 20.3)				*Relative risk=10.5, 95% CI of (2.0, 54.3)*			

Abbreviation: CI, confidence interval.

Summary and Conclusions: This finding suggests that UGT1A1 gene promoter polymorphism may be an important determinant of hyperbilirubinemia in CML patients with NIL therapy, but not in RAD. However other mechanisms should be explored in patients who have significant hyperbilirubinemia with RAD therapy. Updated data with longer follow-up duration will be presented in the meeting

PB1730

IMPACT OF MULTIDRUG RESISTANCE GENE 1 (MDR1) C3435T POLYMORPHISM ON CHRONIC MYELOID LEUKEMIA RESPONSE TO TYROSINE KINASE INHIBITORS
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Background: Single nucleotide polymorphisms (SNPs) of multiple drug resistance (*MDR1*) gene are associated with altered P-glycoprotein (p-gp) activity and contribute to resistance to tyrosine kinase inhibitors (TKIs) in chronic myeloid leukemia (CML).
Aims: We aimed to demonstrate the association between MDR1 gene C3435T polymorphism and molecular response in newly diagnosed chronic phase (CP) CML patients to standard dose upfront imatinib and nilotinib therapy.
Methods: MDR1 C3435T was genotyped using polymerase chain reaction Restriction Fragment Length Polymorphisms (PCR-RFLP) at diagnosis. BCR-ABL1 transcripts level was measured by Real Time Quantitative polymerase chain reaction (RQ-PCR) at diagnosis then every 3 months.
Results: This study included 74 Philadelphia (Ph') positive CP-CML patients; 38 males and 36 females. Median age at diagnosis was 38 years (18-78). Median BCR-ABL1 level was 101%. Forty patients received imatinib (54%) while 34 received nilotinib (46%). Optimal response at 12 month was 35% in the imatinib arm *versus* 80% in the nilotinib arm (P=0.001). The frequency of MDR1 SNP C3435T was 46%, 32% and 22% for CC, TT and CT genotypes, respectively. Optimal response at month 12 differed significantly between imatinib and nilotinib among patients with MDR1 C3435TT genotype (11% *versus* 83%, respectively, P=0.002) while less significant difference was found between the two drugs in CC and CT genotypes (35% vs. 75% and 60% vs. 83%, respectively, P=0.042 & P=0.588).
Summary and Conclusions: MDR1 C3435TT may be used as an additional criterion for initiating nilotinib instead of imatinib as front line therapy for CP-CML patients. We demonstrated the usefulness of MDR1 SNP polymorphism in the identification of CML patients who may or may not respond optimally to imatinib.

PB1731

DIFFERENTIAL EXPRESSION OF ABCF2 IN NEWLY DIAGNOSED AND DASATINIB-TREATED CHRONIC MYELOID LEUKEMIA PATIENTS
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Background: *ABCF2* was previously identified by our group as genes differentially expressed in CML patients which are resistant to imatinib, in samples collected before and after dasatinib treatment (responsive and resistant) in a microarrays analysis study (SILVEIRA, RA *et al. Hematology*;19(1):31-41,2014). *ABCF2* gene is a member of the ATP binding cassette (ABC) transporter family and although its function is not clear, studies with colorectal and breast cancer showed an association of low expression of *ABCF2* and poor prognosis and non-response to palliative chemotherapy. The role of *ABCF2* in CML pathogenesis is unknown.
Aims: The aim of this study was to analyse the expression profile of *ABCF2* in CML patients without previous treatment, in CML patients treated with dasatinib (after imatinib failure) and in healthy donors.
Methods: Total RNA extraction of mononuclear cells from peripheral blood, transcription to cDNA, and qPCR were performed to analyze differential gene expression. *ACTB* and *GAPDH* were used as endogenous control. *geNorm* program was used to estimate the gene expression in arbitrary units (A.U.). Results were expressed as median and compared using non-parametric tests (*Mann Whitney* or *Kruskal-Wallis*). We evaluated 13 healthy donors (control group) and 39 CML patients treated with dasatinib in second line after imatinib failure: 25 responsive to dasatinib, all with complete cytogenetic response (CCyR), 15 of them with major molecular response (MMR) and 14 patients resistant to dasatinib. We also analyzed 9 samples collected at CML diagnosis from the group that was responsive to dasatinib. Only one patient of this group had no sample available after dasatinib treatment.
Results: *ABCF2* expression was down-regulated in the newly diagnosed CML samples and in patients treated with dasatinib compared to control group (0.15 [0.05 – 0.91] *versus* (*vs.*) 0.35 [0.04 – 3.07] *versus* (*vs.*) 2.5 [0.61 – 4.84] P< 0.0001). There was no difference of expression between patients at diagnosis and patients treated with dasatinib (all patients) (*P*=0.09). However, *ABCF2* expression was significantly lower in CML dasatinib-resistant patients when compared to dasatinib-responsive patients with MMR (0.35 [0.05 – 2.21] *versus* (*vs.*) 1.19 [0.14 – 3.07] P=0.02). The expression of *ABCF2* was down-regulated in 8 CML patients at diagnosis and its expression increased after treatment with dasatinib in patients that achieved MMR (0.22 [0.06 – 0.91] *versus* (*vs.*) 1.20 (0.14 – 2.73] P=0.049).

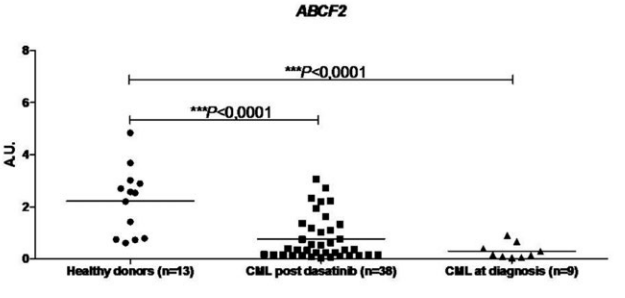

Figure 1.

Summary and Conclusions: *ABCF2* is down-regulated in newly diagnosed CML when compared to the healthy donors and to dasatinib treated patients. On the other hand, up-regulation of *ABCF2* was observed after treatment in patients which achieved MMR with dasatinib. *ABCF2* might be involved in mechanisms associated with the development of CML or resistance. Further studies are necessary to clarify the role of *ABCF2* in CML.

PB1732

DIDO 2 LOW EXPRESSION IN CML IS LINKED TO APOPTOSIS RESISTANCE
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Background: Chronic myeloid leukemia (CML) is a myeloproliferative neo-

plasm which patients are diagnosed by the *BCR-ABL1* oncogene presence. *BCR-ABL1* encodes Bcr-Abl, a tyrosine kinase protein responsible for CML pathogenesis and apoptosis impairment. CML patients are treated with tyrosine kinase inhibitors (TKI). However resistance to TKI have been described and can be associated with Bcr-Abl dependent and independent mechanisms. Therefore new therapeutic targets and CML pathogenesis, including the possible involvement of *DIDO*, should be investigated. *DIDO* is a gene that encodes transcription factors of apoptosis-inducing genes and has three different isoforms: *DIDO* 1, 2 and 3. In physiological conditions *DIDO* is present in small concentrations in cytoplasm, but during the process of apoptosis its protein expression rises and then it is translocated to the nucleus. Increased expression of *DIDO* is therefore associated with the induction of apoptosis process. In myeloproliferative diseases *DIDO* is defined as a tumor supressor gene. Expression changes of isoforms 2 and 3 were already described in the pathophysiology of myeloid neoplasms.
Aims: To evaluate the association between apoptosis resistance and Dido expression by the quantification of the expression of *DIDO* 1, 2 and 3 genes in CML patients in chronic and advanced phases and in healthy subjects.
Methods: 39 CML patients (median age = 45 years; 19 women and 20 men) and 16 controls (median age = 35 years; 10 women and 6 men) were studied. Peripheral blood mononuclear cells were isolated by Ficoll-Hypaque density gradient method and the mRNA was extracted by Trizol® method. The cDNA was synthesized by High Capacity cDNA Reverse Trasnscription® Kit in conventional PCR and gene expression analysis of isoforms 1, 2 and 3 of *DIDO* gene was performed by real-time PCR (Applied Biosystems 7500 Real Time PCR®). The results were expressed as relative units of expression. The gene expression comparison of *DIDO*1, 2 and 3 between health subjects and patients in different stages of the disease was assessed by Mann-Whitney statistical test.
Results: The *DIDO* 2 expression was lower in advanced stages (median=1.352 and P=0.0130) in comparison to controls (median=4.886 and P=0.0130). Comparison of isoforms 1 and 3 expression between CML patients and controls yielded no significant differences.
Summary and Conclusions: The results indicate that *DIDO* 2 low expression may be linked to apoptosis resistance in CML patients. In addition the results also suggest a different role for each of the three different gene isoforms in CML.

PB1733

DOWN-REGULATION OF MIR-203-B IN IMATINIB-RESISTANT CHRONIC MYELOID LEUKEMIA PATIENTS

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Background: Chronic myeloid leukemia (CML) is a myeloproliferative disorder characterized by the expression of the BCR-ABL oncoprotein, which is essential for the pathogenesis of the disease. Imatinib, an ATP-competitive selective inhibitor of BCR-ABL, has unprecedented efficacy for the treatment of CML. Nevertheless resistance to imatinib is observed in over 30% cases of CML. Several cellular and genetic mechanisms of imatinib resistance have been proposed, including overexpression of the *BCR-ABL* gene, the tyrosine kinase domain mutations, pharmacokinetic and pharmacodynamic factors.
Aims: The purpose of this study was to investigate the mechanisms of resistance to imatinib in CML patients.
Methods: We have analyzed mRNA level of *BCR-ABL* gene by quantitative real time RT-PCR in 114 patients and have studied BCR-ABL mutations in patients with high level of expression of fusion gene. Mutation status was studied by direct sequencing of *BCR-ABL* cDNA samples.
Results: *BCR-ABL* mutations were founded in 16 (32%) patients with resistance to imatinib treatment. The mutation spectrum included four missense mutation: M351T (7 cases), T315I (4), T315I+M351T (2), M244V (1) and H396R (1). Next we conducted a search for microRNAs specifically targeting 3'UTR of BCR-ABL, using the miRBASE program to scan human genome (http://microrna.sanger.ac.uk/) and analyzed microRNAs expression profile. The down-regulation of miR-203-b (25-fold), mir-323 (4-fold) and miR-196-b (2-fold) in the group of patients with resistance was discovered.
Summary and Conclusions: In conclusion, our data showed that mutations in the BCR-ABL kinase domain may cause, or contribute to, resistance to tyrosine kinase inhibitors in CML patients, but resistance mechanisms might also be regulated by microRNAs.

PB1734

A GENOME-WIDE ANALYSES OF DIFFERENTIALLY EXPRESSED GENES AND RELATED NETWORKS AFFECTED BY FISETIN AND HESPERETIN IN CHRONIC MYELOID LEUKEMIA CELLS

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Background: Diet is an important determinant of cancer risk based on various epidemiologic and case control studies. Therefore, there is a great interest to investigate bioactive food components for their cancer preventive and therapeutic potentials. Fisetin and hesperetin are members of the flavonoid polyphenols, found in several plant species. They have a wide range of pharmacological properties including antioxidant, anti-inflammatory and anticancer effects. Their chemopreventive/chemotherapeutic potentials were investigated in several cancer types such as colon and prostate cancers, however, the exact mechanisms of fisetin and hesperetin actions are not known in CML.
Aims: We aimed at determining the underlying molecular mechanisms of fisetin and hesperetin induced growth inhibitory effects in K562 CML cells based on changes in global gene expression patterns using genome-wide microarray analysis.
Methods: Expression profiling is accomplished using the Human HT-12v4 beadchip microarrays (Illumina, Inc.). Total RNA isolated from fisetin and hesperetin treated K562 CML cells was converted to biotin labeled cRNA, which were hybridized to beadchips. The BeadChips were analyzed using Illumina's Genome Studio in order to cluster genes. We obtained the list of differentially expressed genes based on p-value<0.05 and at least 2.0 fold change. Affected genetic networks were determined by using the Kyoto Encyclopedia of Genes and Genomes (KEGG) and Ingenuity Pathway Analysis (IPA).
Results: A total of 553 and 1734 genes were significantl regulated (P<0.05) in 50 μM and 100 μM fisetin treated K562 cells, respectively. Both 50 and 100 μM fisetin treatment resulted in the upregulation of common genes such as NFKBIA, NOXA, p21 and GADD45B. We also found each fisetin concentration altered expression of specific genes. For instance, 50 μM fisetin upregulated NFKBIZ and GADD45A while 100 μM fisetin treatment resulted in the upregulation of CDKN2D/ p19, TXNIP and SMAD5. Furthermore, MYC, MYB, C-KIT, TUBA1A and TUBAL3 were some of common downregulated genes after 50 and 100 μM fisetin treatment. 100 μM fisetin also caused downregulation of important genes such as FOXK1, FOXA2 and BCL-XL. Apoptosis modulation, TP53 network, TNF-α, KIT receptor and JAK/STAT signaling, adhesion networks, growth hormone receptor signaling and angiogenesis were modulated networks. On the other hand, a total of 1659 and 1201 genes were significantly changed (P<0.05) in 100 and 150 μM hesperetin treated K562 cells, respectively. DUSP1, CDKN1A, GADD45B and BIM were the examples of common upregulated genes in 100 and 150 μM hesperetin treatment. On the other hand, 100 μM hesperetin also upregulated some other genes such as p27, CASP4, DUSP5 whereas 150 μM hesperetin upregulated MT1A, DUSP3 and NFKBIA. STAT5A, TUBA1A, MYB, KIT, EPCAM and PCNA were the examples of common downregulated genes in 100 and 150 μM hesperetin treatment. 100 μM hesperetin treatment also resulted in the downregulation of EEF1G, POLR2B and STAT3. Furthermore, 150 μM hesperetin treatment downregulated genes such as MCM10, ABCC4 and POLE2. Translation initiation and elongation networks, replication and trancription networks, EGF, JAK/STAT and KIT receptor signaling pathways, growth hormon receptor signaling and PI3K pathway were altered networks.
Summary and Conclusions: Fisetin and hesperetin trigger apoptosis and growth suppression via affecting various significant targets in K562 cells, which could open the usage of new strategies together with fisetin to overcome difficulties in CML treatment.

PB1735

A STATE OF THE ART CUSTOM "DUPLEXED" REAL TIME PCR ASSAY FOR MINIMAL RESIDUAL DISEASE MONITORING OF CHRONIC MYELOID LEUKEMIA USING LOCKED NUCLEIC ACID PROBES.

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Background: In India treatment of Chronic Myeloid Leukemia (CML) is supported through an International Patient Assistance Program in which imatinib mesylate is provided to economically disadvantaged patients free of cost. As a result most of these patients opt for treatment and need regular molecular monitoring. However, commercial kits for BCR-ABL monitoring are cost prohibitive for our patients. It is also currently recommended to test for BCR-ABL copy numbers in triplicates to ensure making confident calls at low levels placing further economic strain in resource constrained settings especially if all quality control measures are to be followed. In that context it is required to develop cost effective BCRABL monitoring technologies. Globally, a majority of centres use the EAC protocol for CML, an assay that suffers from background noise. We addressed these problems by modifying the legacy real time PCR (qPCR) process. We cloned a duplex qPCR compatible plasmid that enables us to multiplex BCRABL and ABL reaction in a single well using FAM- black hole quencher1 (BHQ1) and HEX-BHQ1 probes modified with locked nucleic acids (LNA).
Aims: To develop a custom duplex PCR compatible plasmid and develop and standardize a LNA probe based assay for BCR-ABL and ABL.
Methods: A 1600bp amplicon from a newly diagnosed CML (E13A2) patient into a pJET1.2/blunt cloning vector. This was linearized and using Avogadro's number an estimate of copy numbers were arrived at and a standard curve was created. Accuracy of dilutions were confirmed by using Ipsogen's standards. Using Modified EAC protocol: BCR-ABL probe was truncated to 16

nucleic acids labelled with FAM and BHQ1 with three LNA modifications whereas the ABL probe was 19 nucleic acids (HEX and BHQ1) with 4 LNA modifications. Calibration to the WHO calibrator was done using a secondary standard (Asuragen Inc) to derive an international scale conversion factor. Assay precision was monitored using two levels of controls in the duplex assay (median NCN 0.1% and 0.05%). We compared the normalized copy number (NCN) derived from the duplex assay with the simplex assay in 87 samples of CML (NCN ranging from 0.002 to 108.5, median 0.14%).
Results: The plasmid was successfully cloned and confirmed by sanger sequencing. The duplex assay did not lead to loss of MMR in any patient also we did not face any issues with false positivity. We found a good correlation between the simplex and duplex assay (r^2=0.97). In all runs, slope was between -3.2 to -3.6, R^2>0.98. The international scale conversion factor was 0.79. Over 60 runs the BCRABL assay has a CV of 38.9% and 48.1% for high and low precision controls.
Summary and Conclusions: This assay has enabled us to maintain a high level of quality control (runs in triplicates, precision, no template as well as no reverse transcription controls) yet process 22 samples per run. As a result the cost of the assay is low. We would be happy to share this plasmid to other users from resource constrained setting such as ours. To the best of our knowledge, this is first paper to use LNA probes for CML minimal residual disease (MRD) monitoring. The use of a duplex PCR enables us to significantly decrease the cost of the assay ($5/patient) yet maintain a high standard of quality control.

PB1736

EXPRESSION LEVELS OF JAK/STAT SIGNALING GENES IN NEWLY DIAGNOSED, DRUG SENSITIVE AND RESISTANT CHRONIC MYELOID LEUKEMIA PATIENTS

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Background: Jak/STAT is a major intracellular signaling pathway that involved in immune response, cancer development (mostly leukemias) and hematopoiesis by stimulating cellular growth, proliferation and differentiation. This pathway relies on transmition of outside signals into the cells through cell membrane. The Jak/STAT mechanism composed of 2 main components including Janus kinase (Jak) and signal transducers and activators of transcription (STAT) proteins. Janus kinases, have four different types (Jak1, Jak2, Jak3 and Tyk2), found as an inactive form and associated with receptor protein on the cell membrane whereas there are seven types of STAT protein (STAT1, STAT2, STAT3, STAT4, STAT5A, STAT5B, STAT6) that are located in cytoplasm and activated by Jak kinases. The system is started with conformational change and trans-activation of Jak proteins by a signal from a number of cytokines. This activation is followed by STAT phosphorylation at tyrosine residue in transactivation domain of STAT protein. Phosphorylated STAT proteins become dimerized by interacting phosphotyrosine of one STAT and SH2 domain of another STAT, as a result, they either affect different pathways and cause signaling cascades or move into the nucleus and directy activate their target genes involved in proliferation, differentiation, cell growth, cell death or survival. Abnormal activation of Jak/STAT signaling pathway proteins are found in many types of hematological malignancies including AML, MDS, B-cell lymphomas and CML.
Aims: Despite high efficiency of tyrosine kinase inhibitors, the success of therapy is limited to multidrug resistance phenomenon. Recent developments in drug resistance mechanism in CML showed that many molecular interactions and signaling pathways are involved in mediating TKI resistance in patients. Jak/STAT signaling pathway is one of these pathways that have significant roles in various cellular mechanisms including leukemia initiation and drug resistance. The aim of this study is to examine the relationship between expression levels of Jak/STAT genes and clinical outcome of chronic myeloid leukemia (CML) patients who are newly diagnosed; treated with imatinib, nilotinib or dasatinib; responded positively to TKIs; imatinib, nilotinib or dasatinib resistant; or lost of their molecular response.
Methods: This study including 14 newly diagnosed CML patients, 5 patients who are CML diagnosed and treated with imatinib, 1 patient who positively responded to imatinib, 1 patient who lost molecular response, 1 imatinib resistant patient and 1 patient who has both imatinib and nilotinib resistance. Mononuclear cells were isolated from bone marrow samples of the patients and total RNAs were isolated, mRNAs were converted into cDNAs and expression levels of *JAK1-3, TYK2, STAT1-4, STAT5A-B* and *STAT6* genes were detected by Real-Time PCR.
Results: The results showed that expression levels of *Jak3, STAT1-4* and *STAT5A* genes had higher in TKI resistant patients, compared to other patient groups. Expression levels of *STAT5B, JAK1* and *JAK2* genes were higher in newly diagnosed and positively responded patients than in resistant patients while expression levels of *TYK2* was lower in imatinib-treated patients.
Summary and Conclusions: It was demonstrated for the first time that there is a relation between the clinical outcome of CML patients and expression levels of JAK-STAT genes that could make this signaling pathway a new target for more effective treatment of CML.

PB1737

PLASMA AND LEUKOCYTE METABOLOMICS AND METABOLISATION STUDY OF PATIENTS WITH CHRONIC MYELOID LEUKAEMIA

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Background: Metabolomics refers to the interpretation of information-rich data with the aim to understand metabolic processes and to find changes in metabolite levels as a potential biomarkers of the disease. Due to drug metabolizing enzymes several bioactive or toxic metabolites of tyrosine kinase inhibitors are produced. Therefore determination of drug metabolites may have clinical relevance and could be helpful for treatment adjustment.
Aims: The first aim of this work was to examine the influence of tyrosine kinase inhibitors and hydroxyurea on the leukocyte and plasma metabolite profiles from patients with CML compared with healthy controls. The second aim of this work was profiling of imatinib metabolites in plasma from patients with chronic myeloid leukaemia.
Methods: Leukocytes and plasma samples from healthy controls (n=10), patients before treatment (n=9), treated with hydroxyurea (n=5), imatinib (n=24), dasatinib (n=8) and nilotinib (n=10) were prepared from whole blood and metabolites were extracted by the chloroform/methanol/water mixture. Analysis of 400 metabolites was performed using high performance liquid chromatography (Ultimate 3000, Dionex) coupled with tandem mass spectrometry (QTRAP 5500, AB Sciex). Metabolites were quantified using MultiQuant 3.0 software and statistically evaluated in R programme language with statistics packages. For metabolisation study plasma samples were taken from patients on treatment by imatinib (24h after dose of 400 mg). Separation of imatinib metabolites was performed on Phenomenex Kinetex C18 column using UltiMate 3000 RS (Thermo Scientific) liquid chromatography. For detection in full scan mode and MS^2 fragmentation experiments Orbitrap Elite mass spectrometer (Thermo Scientific) based on exact mass measurement was used. Data were evaluated using MetWorks 1.3 SP3 and Mass Frontier 7.0 software.
Results: Total 170 metabolites were identified on the basis of its transition and retention time in all leukocyte and plasma samples. Significant changes in metabolic pathways were found. Both unsupervised and supervised statistic methods (PCA, PLS-DA, OPLS-DA, cluster analysis) separated all the studied groups. In plasma samples more than 90 potential metabolites in concentration range of 4 orders of magnitude were found. All metabolites were identified by exact m/z values and confirmed by MS2 and MS3 exact m/z fragmentation experiments. Changes in profiles of several metabolisations between patients were followed.
Summary and Conclusions: In this study metabolome of human leukocytes and plasma was defined and changes of metabolite levels were observed in all groups of patients compared to healthy controls. High Resolution Mass spectrometry using Orbitrap principle is able to detect and confirm high as well as low intensive metabolites and its fragments. Tyrosine kinase inhibitor – Imatinib offers complex metabolite profile. It has diagnostic potential for individualized treatment of patients with adverse effects or resistance on treatment. The work was supported by grant of IGA Ministry of Health, Czech Republic NT12218-4/2011.

PB1738

TO THE DIFFERENTIAL DIAGNOSIS OF ACUTE MYELOBLASTIC LEUKEMIA AND CHRONIC MYELOID LEUKEMIA STARTED WITH BLAST CRISIS

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Background: Despite the achievements of modern medicine sometimes there is a problem to differentiate chronic myeloid leukemia (CML) started with blast crisis from acute myeloblastic leukemia (AML), especially in the cases of Ph chromosome negative or BCR-ABL1- negative cases. Even in the presence of this marker one should have taken in mind that there are cases of Ph + AML as well.
Aims: The aim of our study was to precise the diagnose in patients with blast cells in peripheral blood and prominent hepato- and splenomegaly suspicious

on CML and reveal additional criteria for the diagnosis of CML started with blast crisis.
Methods: To solve this problem we have used peripheral blood and bone marrow cell culture method worked out by us. This method supports propagation of blast cells specific for each form and variant of leukemia and enables to reveal the malignant clone according to morphocytochemical investigation or phenotyping *in vitro* proliferating cells (Shvelidze, Saralidze *et al.* Atlas of Hematology, 2013. http://www.e-bookland.ge/Books/medical/ATLAS-of-HEMATOLOGY). We investigated peripheral blood and bone marrow cell cultures of 5 patients with CML presented with blast crisis at the time of diagnosis, 8 patients with CML at chronic stage, 2 patients with CML in accelerated phase and 7 patients with AML (3 cases of AML without maturation and 4 cases of AML with maturation).
Results: Our studies showed that in all cases of AML proliferation of blast cells is confirmed by abundance of blast cells in cultures during 2 weeks of cultivation and appearance of maturation part of them up to promyelocytes and myelocytes and rarely to the band neutrophils in late cultures. On contrary, in the cases of CML presented with blast crisis as well as in the cases of CML at chronic stage, rapid maturation of blast cells up to band neutrophils is obvious from the beginning of cultivation. Just from the second day of cultivation in cultures of patients with CML disappearance of blast cells and abundance of band and segmented granulocytes, mainly neutrophilic, but eosinophilic and basophilic also are observed (Figure 1). It must be noticed that from 2 patients with CML in accelerated phase in one case majority of cells in culture were matured granulocytes, that is characteristic for CML, while in another case culture material was presented by abundance of immature myeloid cells – myeloblasts, promyelocytes and myelocytes and a few band and segmented granulocytes that pointed to tumor progression. In the last case treatment was not effective at all. Figure 1. Abundance of band neutrophils with ring nuclei. 2-day- bone marrow culture of a patient with CML started with blast crisis. Rapid maturation of blast cells up to band neutrophils *in vitro* enabled to diagnose CML. May-Grünwald –Giemsa stain. X 1000.

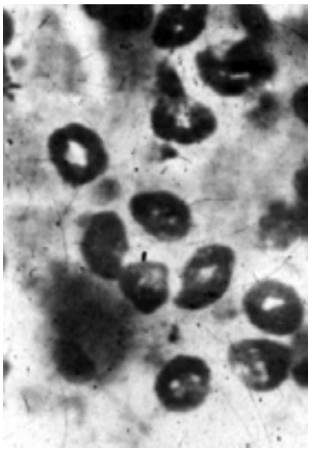

Figure 1.

Summary and Conclusions: Results of our study show that blood and bone marrow culture data of patients with CML can be successfully used as additional criteria for the differentiation of CML started with blast crisis from AML as far as in cultures of patients with AML abundant proliferation of blast cells is observed, whereas in the cases of CML started with blast crisis rapid maturation of blast cells up to band neutrophils from the beginning of cultivation is obvious. Abundance of immature cells in blood or bone marrow cultures of patients with CML points to tumor progression in spite of the clinical stage of disease and represents a bad prognostic marker. Cytochemical study or immunophenotyping of proliferated *in vitro* cells can help to determine the type of new proliferative clone.

PB1739

USEFULNESS OF THE TEMPUS™ BLOOD RNA STABILIZATION SYSTEM FOR OPTIMIZING RNA EXTRACTION METHOD FROM WHOLE BLOOD, MEASURING AND REPORTING ABL TRANSCRIPTS LEVELS IN CHRONIC MYELOID LEUKEMIA.

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Background: Chronic myeloid leukemia (CML) is a myeloproliferative disorder of blood stem cells. More than 95% of patients with detected translocation t(9;22) is characterized by the fusion between exons e13 or e14 of *BCR* gene, which are located in major breakpoint cluster region (*M-bcr*) and exon a2 of *ABL* gene. These fusions are described as b2a2 (e13a2) and b3a2 (e14a2). Because patients treated with tyrosine kinase inhibitors achieve lower levels of detectable disease, quantification of *BCR-ABL* transcripts with quantitative RT-PCR has become an essential tool in chronic myeloid leukemia monitoring. Major molecular response (MMR; *i.e.*, a ≥3-log reduction in *BCR-ABL* transcript levels) is used in current treatment guidelines to assess prognosis. Recent evidence suggests that deeper molecular responses (≥4-log reductions in *BCR-ABL* transcript levels), particularly when attained early during treatment, may have even better correlation with long-term outcomes, including survival and disease progression. Therefore, quantitative measurement of *BCR-ABL* transcripts in blood and bone marrow both aids in the initial diagnosis of CML is essential for routine post-therapy minimal residual disease monitoring. Establishing methods for secure long-term storage of RNA is critical to realize MRD monitoring. We describe the results of RNA stability in the same set for whole blood RNA collecting in Tempus Blood RNA tubes and tubes with EDTA.
Aims: All the steps of the preanalytical phase (blood collection, conservation and procedures of isolation RNA) are influencing on the number of copies of the *ABL* control gene in the analysis of *BCR/ABL* transcripts. Our aim was to show the differences in the stability of the RNA using EDTA tubes and tubes with a special stabilizing solution Tempus (Tempus™ VACUETTE® Blood RNA Tube) for the collection of peripheral blood and bone marrow in CML.
Methods: Various kits for stabilizing the RNA at the time of blood collection were developed: Ambion RNA later (Applied Biosystems) and the new Tempus Blood RNA kit (Applied Biosystems). The Tempus Blood RNA kit (VACUETTE® Tempus™ Blood RNA Tube) uses tubes containing a proprietary blend of reagents based on a patented RNA stabilization technology. In order to evaluate the Tempus Blood RNA kit, it was compared to unstabilized EDTA blood protocol. The RNAs extracted by the different methods were assessed for quantity control gene (*ABL*) and fusion gene transcripts *BCR-ABL* by QRT-PCR. After extraction, both RNA purity were measured by using both, the ratio A260/280, no significant changes in these ratios were observed in either system. The integrity of RNA after extraction was confirmed using the Agilent 2100 Bioanalyzer and was indicated by the RIN.

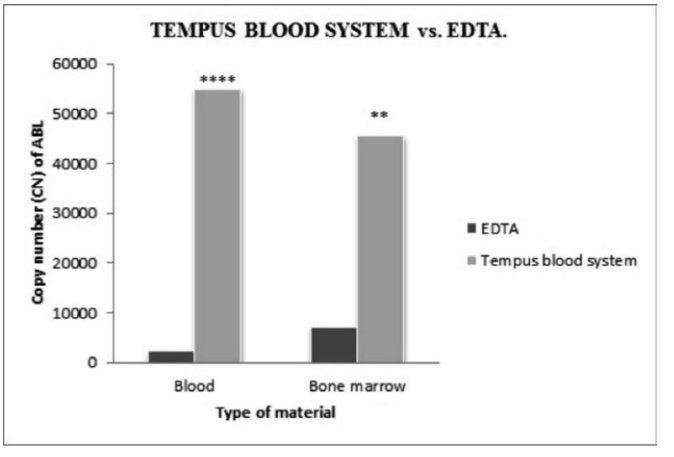

Figure 1.

Results: Our results strongly suggest the superiority of the stabilization system special tubes over the unstabilized EDTA blood. Tempus tubes are preventing the degradation of RNA at the time of the preanalytical phase during genetic analysis of CML patients. Using Tempus tubes we have achieved increased stability of RNA, which has a direct impact on increasing the number of copies of the *ABL* control gene (3-4 times) for the analysis of *BCR/ABL* and thus ultimately increases the sensitivity of QRT-PCR method. Tempus blood RNA systems have been shown to provide efficient stabilization of the blood RNA for several days at room temperature. Comparison of expression levels of *ABL control* gene in RNA (10 patients) extracted by EDTA or Tempus Blood RNA system. *ABL* expressions are expressed as copy number. P-value: in blood $P<0.0001$ and in bone marrow $P<0.0101$ (t-test).
Summary and Conclusions: In summary, molecular response measured by *BCR-ABL* QRT-PCR provides important prognostic information for the management of patients with CML treated with TKI therapies. The deeper molecular response (MR^4, $MR^{4.5}$, and MR^5) that is necessary to enroll a patient in a trial aiming at treatment-free remission (TFR).

PB1740

IMMUNE MONITORING OF THERAPEUTIC EFFICACY IN CHRONIC MYELOID LEUKEMIA

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Background: Different effects on the immune system are reported at the diag-

nosis and during therapy depending on Tyrosine Kinase Inhibitors (TKI) and interferon-alpha protocols. Some of these are dose-dependent effects. At present time, several clinical trials are establishing also the safety parameters for cessation of therapy in a selected group of Chronic Myeloid Leukemia (CML) patients. Although there is no consensus for laboratory evaluation of immune response for clinical trials, the Human Immunophenotyping Consortium (HIPC) aims at the standardization of protocols based on multicolor flow cytometry (Maecker *et al*. Nat Rev Immunol 2012).

Aims: The current study aimed at the evaluation of the immune status in treated CML patients to establish addtional efficacy parameters of different therapeutic strategies.

Methods: We used an extended 10-parameter panel for the analysis of T cells (Th1, Th2, Th17, Tregs), B cells (including plasmablasts), monocytes (classical and non-classical), dendritic cells (myeloid and plasmacytoid) and NK cells ($CD56^{bright}$ and $CD56^{dim}$) in CML immune monitoring. Additionally, activation and maturation profile, as well as memory status was investigated for T and B cells. Intracellular cytokine staining, phosphorylation studies, response to multiple stimuli and gene expression profiling of relevant sorted cells was also performed for evaluation of immune response in these patients. Deep analysis of specific subsets (NK, NKT and γδ T cells) and pathways (CD137, PD-1, CD96, CD226, TIGIT) were explored.

Results: Significant impact on NK cells, NKT and Th17 cells was observed in CML patients undergoing therapy with TKI. Maturation and activation status of different lymphocyte subsets was found affected by therapy. Optimal response was found associated to immune response robustness of CML patients.

Summary and Conclusions: In conclusion, CML successful treatment is highly influenced by the immune response and an immune-based monitoring could be an important tool in the near future.

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Chronic myeloid leukemia - Clinical

PB1741

EARLY BCR-ABL REDUCTION IS PREDICTIVE OF BETTER EVENT FREE SURVIVAL IN CHRONIC MYELOID LEUKEMIA NEWLY DIAGNOSED PATIENTS TREATED WITH ANY TKIS

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Background: Treatment of chronic myeloid leukemia (CML) aims at obtaining optimal response, as defined by international recommendations. As more tirosine kinase inhibitors (TKIs) are now available, much attention has been paid to the identification of early prognostic markers during treatment. A strong predictive value has been observed for early molecular response, with BCR-ABL ratio ≤10% at 3 months (mos), and BCR-ABL ratio ≤1% at 6 mos, being predictive for overall survival and event-free survival (EFS). Recently, the velocity of early BCR-ABL transcript elimination, namely the halving time, has shown to represent an additional prognostic index.

Aims: To evaluate the prognostic significance of the 3 mos time-point in our population.

Methods: We retrospectively analyzed the population of CML-CP pts treated in our Division, selecting pts who had a quantitative molecular evaluation at 3 mos. EFS was estimated with Kaplan-Meier method. Log-rank test was used to identify significant differences between curves. ROC analysis was used to calculate the optimal halving time thresholds for discriminating between outcomes. ABL was used as control gene.

Table 1. Responses and events according to BCR-ABL transcripts at 3 months

	BCR-ABL >10% at 3 months (n=12)	BCR-ABL ≤10% at 3 months (n=38)	p-value*
BCR-ABL ≤1% at 6 months	42.9% (3/7)	80% (24/30)	0.07
BCR-ABL ≤0.1% at 12 months	0% (0/9)	50% (15/30)	0.007
MR≥ 4, last follow-up	17% (2/12)	50% (19/38)	0.05
Total Events	75% (9/12)	13% (5/38)	<0.001

*P-values were computed using Fisher's test.

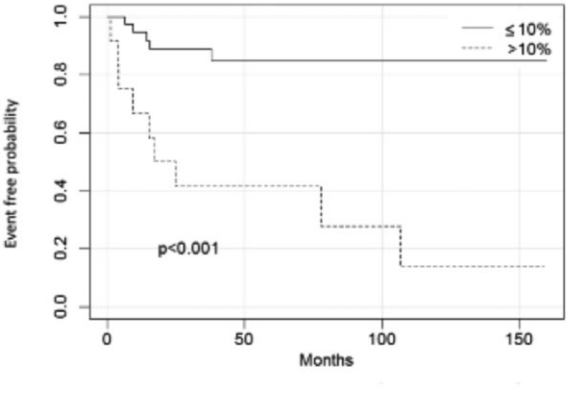

Figure 1. Event free survival by BCR-ABL levels at 3 months.

Results: 50 CML-CP pts, with a median follow-up of 57,5 mos (31-110.5) were analyzed. 36 pts (72%) were male, 14 (28%) female. Median age at diagnosis was 59 years. 33 pts were treated frontline with imatinib, 10 with nilotinib, 4 with dasatinib and 3 were enrolled in the GIMEMA rotational protocol nilo/ima. 76% of patients had a transcript ≤10% at 3 mos. 37 pts had the 6 mos evaluation and 39 pts had the 12 mos evaluation. 3/7 pts who had a transcript >10% at 3 mos had a transcript ≤1% at 6 mos (Table 1). None of the patients with a transcript >10% at 3 mos achieved an MR3 at 12 mos, compared to 50% in the other group (P=0.007). 14/50 pts had an event, defined as lack or loss of optimal response as per ELN recommendations, with a statistically significant difference among groups (94.6% in the group with 3 mos BCR-ABL >10% vs 66.7% in the group with BCR-ABL ≤10%, by 12 mos, P<0.001; 84.6% in the >10% group vs 41.7% in the ≤10% group, by the median time of follow-up, P<0.001) (Figure 1). No progressions occurred. 12 pts had to change drug because of an event (6 shifted to dasatinib, 6 to nilotinib), 3 for intolerance (1 shifted to dasatinib, 2 to nilotinib). Excluding these pts, in the group of pts >10% at 3 mos the probability of achieving a long-term MR4 was more than 60% lower compared to the other group [HR 0.36 95% CI (0.10;1.27)]. The halving time thresholds for discriminating between EFS was 25.5 days (specificity 84.8% and sensitivity of 83.3%; AUC 85.9%). None of the pts with a transcript >10% at 3 mos had a

halving time<25.5 days. Among pts with a transcript ≤10%, those with a halving time<25.5 days had an EFS of 93% vs 50% with a halving time >25.5 days (P =0.012). Those with a halving time<25.5 days had a reduction of risk of event of 92% (HR 0.08; 95% CI (0.02;035)). All pts were alive at the last follow-up.
Summary and Conclusions: Three different drugs, imatinib, dasatinib and nilotinib. are commercially available for frontline treatment of newly diagnosed CML. Clinicians are allowed to decide which of these therapies is most suitable for each pt, based on comorbidities, Sokal risk and age of the pt. Irrespective of the TKI used, the achievement of a BCR-ABL transcript ≤10% influences the outcome of the pts. Interestingly, we confirmed the importance of halving time in discriminating pts with the best outcome.

PB1742

GENERIC IMATINIB MESYLATE IS AS EFFECTIVE AS ORIGINAL GLIVEC IN THE MANAGEMENT OF CML

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Background: Unsustainable drug prices in chronic myeloid leukemia (CML) and cancer may be causing harm to patients. Advocating for lower drug prices is a necessity to save the lives of patients who cannot afford them (Experts in CML. Blood. 2013; 121(22):4439-4442). The patent date of imatinib mesylate in USA has just expired in January 2015. Patent expiration dates for imatinib may be different in different countries/regions. In Turkey, generic imatinib preparations are currently present. The only concern for generic imatinib is its efficacy over the original drug, Glivec or Gleevec.
Aims: The aim of this multi-center study is to assess the efficacy of generic imatinib over Glivec in terms of hematological, cytogenetic, and molecular responses in CML.
Methods: This work is designed as multicenter and retrospective study

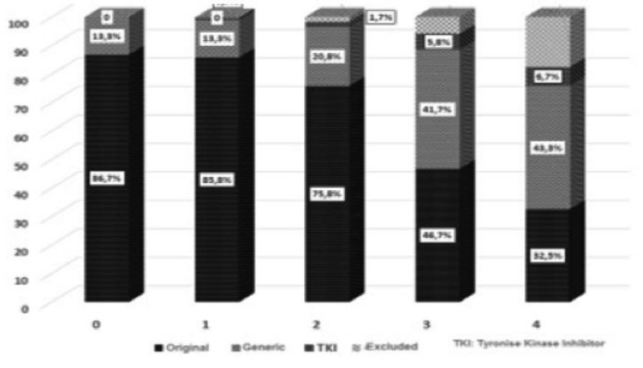

Figure 1. The ratio of drugs that were preferred by the clinicians for each evaluation.

Results: In this study, the retrospective data of 120 Turkish CML patients receiving imatinib from six different CML centers across Turkey were analyzed (68 females, 56.7%; 52 males, 43.3%). The mean age was 53 years (21-81). The most frequent ECOG performance status was "0" (65%). The distribution of genders between centers was similar. At the study onset, 86.7% of the patients (n=104) were using original molecules, and 13.3% of the patients (n=16) were using generic molecules. Original (86.7%), 16 generic imatinib mesylate. The patients were evaluated at 4 different time points for change of medication and efficacy. The mean period between each evaluation was 9 months. Initial evaluation showed that a patient who was using only original molecule, switched to second generation tyrosine kinase inhibitor (TKI) treatment. In this period, hematological response (HR) was observed in 99.2% of the patients, cytogenetic response (CR) was observed in 88.7% of the patients (47 of 53), and molecular response (MR) was observed in 75% of the patients. For each evaluation, the ratio of drugs that were preferred by the clinicians is shown in Figure 1. According to Figure 1, clinicians had a tendency to prefer generic molecules in each sequent visit, and this change rate was significant (P<0.001). The rate of using generic molecules was 13.3% in the initial evaluation, 20.8% in the second visit, 41.7% in the third visit, and 43.3% in the fourth visit. The rates of switching from original molecule to generic molecule, from original molecule to second generation TKI, and from generic molecule to second generation TKI are shown in Table 1. Accordingly, 11 patients, who were using original molecules during all cohort, switched to second generation TKI. On the other hand, only 1 patient, who was using generic molecules, switched to second generation TKI. Response to treatment is shown in Figure 2.

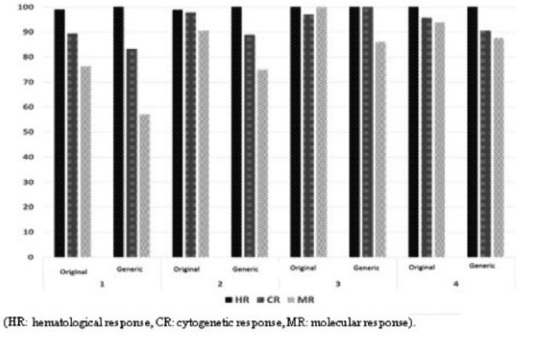

Figure 2. The ratio of CML treatment of the four evaluation points.

Table 1. The rates of switching from original molecule to generic molecule, from original molecule to second generation TKI, and from generic molecule to second generation TKI.

	Original → Generic		Original → Second generation TKI		Generic → Second generation TKI	
	n	%	n	%	n	%
First Visit	1/103	1,0	-	-	-	-
Second Visit	12/103	11,6	1/103	1,0	1/17	5,9
Third Visit	28/91	30,8	5/91	5,5	-	-
Fourth Visit	28/56	50,0	5/56	8,9	-	-

Summary and Conclusions: Therefore, we did not find any significant difference in HR, CR, and MR for original and generic drugs in each visit. Based on this data, generic imatinib mesylate is as effective as original Glivec in the management of CML.

PB1743

IMPACT OF TYROSINE KINASE INHIBITORS (TKIS) ON THE FERTILITY OF CHRONIC MYELOID LEUKEMIA PATIENTS (CML) IN A SINGLE CENTER IN ALGERIA

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Background: TKIs have dramatically modified the outcome of CML patients giving opportunity to 10% of patients that are in age to procreate to have a parental project.TKIs stopping leads to the relapse. We count the event of pregnancy and its evolution among patients with CML at Chronic Phase treated with TKIs in the two sexes at Blida in Algeria.
Aims: To evaluate in the two sexes fertility of patients treated by tyrosine kinase inhibitors.
Methods: Of 231 CML patients treated by TKIs, 29 pregnancies were noted among 19 women; we analyze the time of exposure to the TKIs before and at the time of the pregnancy and the hematologic, cytogenetic and molecular status before and after the pregnancy.
Results: Pregnancy of the spouse: - Husbands treated by Imatinib (IM): 10 cases, median age=34 years; IM (400mg/d); median time of exposure (MTE)=23 months; No interruption of IM before the conception; 3 early abortions; 12 pregnancies carried out in the term; 12 alive new-born without malformation. - Husbands treated by Dasatinib: 2 Cases (100mg/d; MTE before the conception (BTC)=12 months, not treatment stopping before the design; 02 pregnancies carried out in the term; 2 alive new-born, no malformations.
- Husbands treated by Nilotinib: 1 case (800 mg/d), MTE BTC=12 months, no stop of TKI before the conception; 1 pregnancy carried out in the term; 1 new-born living any malformation. Pregnancies not planned in 0 3 women treated by Imatinib: median age=31 years; median time of impregnation BTC : 19 months (4, 24, 39); Cytogenetic profile: CCyR=2 patients, MMR: 1 pt; no treatment stopping before the conception; exposure time to the IM of 6, 8 and 12 weeks; Evolution of the pregnancy without incidents; childbirth at 35 weeks=2 pts, 1 Caesarian (after acceleration phase); 03 alive new-born of weight (2400-3500 gr) without malformations; Evolution of the CML: loss of the hematologic remission at 20 weeks=3 patients of which one acceleration, relayed by interferon; No breast feeding; resumption of IM=3 pts, one blast phase (AML5) treated by Nilotinib, which died after 12 months; 2 pts with HR and CCyR after 12 months of recovery. Pregnancies planned in 5 women treated by Imatinib: 4 (G0P0), 01 (G2P1); median age=29 years; sokal high=2 pts: Intermediary=3

pts; MTE BTC=48 months (30-60) with IM 400 mg/d; cytogenetic profile=5 CCyR, 2 MMR 4.5 and 2 MMR 4; stop of IM BTC =5 pts, from 01 to 05 months; 2 early abortions 1 month from stopping; 5 pregnancies 3 to 5 months after stopping IM; Evolution of the pregnancy: without incidents, of which one treated by interferon; Childbirth: in the term = 4 pts; 5 alive new-born (twin) without malformations; 1 in progress; breast feeding: 3 pts (1 month); Evolution of CML=CHR maintained at 37 weeks=4 patients, a loss of the CHR; resumption of IM=4 Pts, MMR in one pt after 12 months of recovery OS=75 months; 3 still of evaluation, OS=61 months (46-83). Pregnancy in woman treated by Dasatinib: 30 years, sokal: Intermediary; G0P0; MTE BTC: 18 months, Cytogenetics: CCyR; no treatment stopping before the conception; pregnancy not planned; exposure time: 4 weeks; lost of follow up until the childbirth, new-born alive without malformations, Evolution of the CML: loss of HR; Resumption of Dasatinib, HR after 2 months, CCyR after 3 months, OS=65 months

Summary and Conclusions: Infertility is not an side effect of the TKIs; the major molecular response made it possible to carry out a pregnancy without relay, a non-response requires a relay and exposes to the risks

PB1744

SECOND REPORT OF ARAB LEUKEMIA NET (ALN) REGISTRY FOR CML IN THE MIDDLE EAST &NORTH AFRICA REGION(AFME).PART II RESULTS OF ADDITIONAL CHROMOSOMAL ABNORMALITIES(ACAS) IN EGYPT, A MULTICENTER RESULTS.

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Background: The first ALN report1 demonstrated that age-specific rates for CML in Egypt and Arab nations are lower by at least two decades compared to western populations1. Geographic and ethnic variations contribute to the variability of incidences among CML registries2. ACAs were reported in 5% of CML patients in chronic phase (CP). At diagnosis considered by ELN as a "warning" requiring careful patient monitoring, while ACAs emerging during treatment are considered by WHO classification as accelerated phase (AP) CML.ACAs influence response to Imatinib and outcome of TKI therapy. The occurrence of ACAs and additional mutations besides Ph chromosome(Ch) indicates that the leukemia has become at least partially BCR-ABL independent by secondary genetic acquisitions 2.Although the underlying mechanisms of resistance /disease progression are not fully understood for all ACAs, some are well identified as poor prognostic factors. such as the acquisition of additional Ph chromosome which might confer resistance via increasing the kinase activity of BCR-ABL3, the isochromosome 17q i(17q) which leads to inactivation of the tumor suppressor gene p53 and impedes the response to Imatinib via allowing the damaged cells to escape the DNA repair machinery of p53 and thus escape cell growth arrest or apoptosis, the extra 8 which is known to lead to c-Myc over expression that enhances the transformation of BCR-ABL positive cells or allows rapid growth of leukemic cells, and the extra 19 which might hinder Imatinib activity via hypermethylation / inactivation of neoplastic transformation silencing gene promoters such as ATG16L2 gene promoter4.

Aims: 1) To Release data of second ALN report concerning epidemiology of CML in Egypt, after enrolling 6 new centres,228 patients and adding ACAs to evaluate disease and clonal evolution. 2) Investigate the low mean age of CML in Egypt and the role of ACAs.

Methods: We analyzed data of 578 CML Egyptian patients (302 male and 276 female) followed-up for 5 years. Data were collected from 10 centers according to ELN2 and EUTOS recommendations2-3 by using a multicenter web based data registery portal, the ALN. (www.aln-afme.com). Chromosome banding analysis and FISH were performed according to the International System for Human Cytogenetic Nomenclature. we tested for ACAs defined as "major route" namely trisomy 8, duplication of Ph chromosome, i(17q), and trisomy 19.21.FISH was performed on at least 200 cells on bone marrow cells prepared according to cytogenetic techniques and by using DNA probes that hybridize at the BCR and ABL regions. RT-PCR, and real-time quantitative PCR were performed.

Results: Patients Median age was 43y, (40y for males, 41y for females), The age specific rates were highest for the age group of 30-35 years. Female patients presented with lower hemoglobin, higher platelet counts and smaller spleen size (P<0.0001). 98%of patients achieved CH respone,89% CYR,87% CYR,and 83% MMR. At diagnosis 87% patients were in CP-CML, 8.1% in AP and 4.9% in blastic phase. Sokal score: Low risk 57.8% Intermediate24.5% and High in 17.7%. EURO (Hasford) score (59% Low risk, 28.4% Intermediate risk and12.6% High risk). 42% of patients received first generation TKI, 54% second generation TKI, and 4% needed therapies plus TKI). Transplantation rate was 19%, PFS and OS were equal in female and male patients. ACAs were found in 62(11%) patients, they had lower cytogenetic and molecular response rates and longer response time to TKI and inferior outcome. ACAs were more frequent in younger, Imatinib resistant patients, and in blast phase. We identified loss of Y Ch in 18 patients (29%), trisomy 8 in 7 (11%), trisomy 19 in 12 (19%), i (17q) in 12(19%), other different single abnormalities in 8 patients (13%), complex karyotype with double ACAs in only 5 patient (8%).Four patient showed variant Ph Ch: t(9;22;22)(q34;q11;q11). Deletion of der(9) Ch in 17 cases (27%): (10 cases with loss of Y Ch, case with del(20)(q11q13), and 3 case with t(X;13)(q13;q32)). The cytogenetic and molecular response rates were uniformly lower in patients with ACAs, CCgR and MMR rates were significantly lower in patients with ACAs (68% vs 89% and 55% vs 86% respectively), responses were significantly slower in patients with ACAs, 54 patients presented with ACAs at diagnosis while 8 patients developed or acquired ACAs while on treatment.

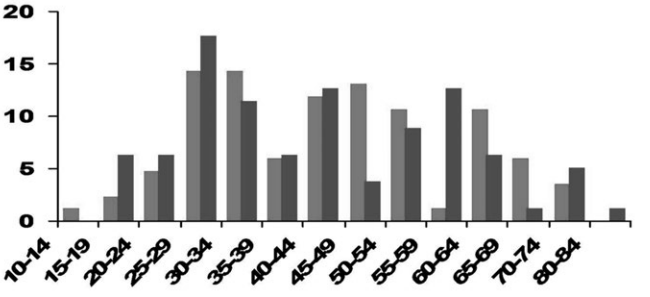

Figure 1.

Summary and Conclusions: The importance of ethnicity and gender differences in relation to disease incidence, and prognosis are major health policy focus2.To investigate the low mean age of CML in Egypt and evaluate role of ACAs on disease and clonal evolution, we analyzed CML data of 10 Medical Centers in Egypt. We identified 62(11%) cases with clonal ACAs. ACAs are more frequent in younger patients and adversely affected time and response rates to Imatinib treatment they were related to lower cytogenetic and molecular response rates and longer response time toTKI and inferior outcome and lower overall survival.

PB1745

EXTREME THROMBOCYTOSIS IN CHRONIC MYELOID LEUKEMIA IN THE ERA OF TYROSINE KINASE INHIBITORS (TKIS)

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Background: Thrombocytosis is a common feature in chronic myeloproliferative disorders. The incidence of thrombocytosis in CML is reported to be around 30 to 50%. Extreme thrombocytosis defined as a platelet (PLT) count >1.000x 10^9/l is uncommon in CML as well as isolated thrombocytosis.

Aims: Our aim is to study the clinical management and the therapeutic response of CML with extreme thrombocytosis.

Methods: From January 2001 to January 2015, 1314 patients affected by CML were followed in 15 different Italian hematological centers. We diagnosed 78 CML with extreme thrombocytosis at onset (5.9%); we conducted the study on 70 pts who have at least 6 months of follow-up and were in chronic phase; 2 pts in blast phase and in accelerated phase were excluded from the analysis.

Results: Our patients were 48 females and 22 males with a median age of 59 years (range 18-84). At diagnosis, median hemoglobin level was 11.6 g/dl, median WBC 32.7x 10^9/l and PLT count 1.466x 10^9/l. The Sokal score was high in 44 (62.9%), intermediate in 21 (30%) and low in 5 (7.1%). According to Hasford score 47.5% pts resulted high, 30.5% intermediate, 22% high; Eutos score available in 48/70 pts was high in 13 (27.1%) and low in 35 (72.9%). Pts' characteristics are summarized in Figure 1. PCR analysis showed the presence of p210 in all cases and the absence of JAK2 V617F mutation in 28 out of 30 pts (42.9% of all the pts) who performed the test. At diagnosis we registered 5 thrombotic/hemorrhagic episodes: 1 superficial thrombosis, 1 subarachnoid hemorrhage, 1 metrorrhagia, 1 epistaxis and 1 purpura. None of the two JAK2 positive patients showed thrombotic/hemorrhagic episodes. All but six pts received initial treatment with hydroxyurea and allopurinol. One patient underwent PLT apheresis. PLT count was generally unresponsive to initial treatment. Upfront treatment was imatinib in 53 pts, nilotinib in 11 pts and dasatinib in 6

pts. PLT count normalization was rapidly achieved after introduction of TKIs. Hematological response was reached at a median of 1 month, complete cytogenetic response after a median of 3 months (range 3-36) in 62/65 evaluable pts, and major molecular response in 53 out of 64 evaluable pts after a median of 9 months (range 3-101). Of the 53 pts who started imatinib 7 pts shifted to nilotinib for resistance (3 primary and 4 secondary), 3 to dasatinib for primary resistance, 1 to ponatinib for primary resistance and 2 pts are not on TKI at this time for intolerance. Four of these pts dead for causes independent from CML (1 pt for myocardial infarction, 1 pt for GVHD post-transplantation and 2 pts for second tumor at 3, 9, 38 and 147 months respectively) and 3 pts were lost during follow-up. Three patients of the 11 who started nilotinib stopped treatment with TKIs for resistance or intolerance, 1 shifted to dasatinib for secondary resistance. Sixty-three patients (90%) are alive, 58 of them on TKIs (33 imatinib, 14 nilotinib, 10 dasatinib, 1 ponatinib) at a median follow up of 65 months (range 7-168).

Table 1. Characteristics of patients and laboratory findings.

Number of patients	70
Male/female	22/48
Median age	59 years (18-84)
Median hemoglobin	11.6 g/dl (7.3-16.3)
Median white blood cell count	32.700 x 10^9/l (3.800-390.000)
Median platelets count	1.466 x 10^9/l (1.020-4.849)
Sokal score: low	5/70 (7.1%)
Sokal score: intermediate	21/70 (30%)
Sokal score: high	44/70 (62.9%)

Summary and Conclusions: The prevalence of extreme thrombocytosis in CML is 5.9% in a large multicenter observational study. Cytoreduction with hydroxyurea was not able to achieve normalization of PLT count, however pts with extreme thrombocytosis were easily and rapidly managed by TKIs. CML pts with extreme thrombocytosis resulted similar to the CML population considering the response to TKIs and low number of thrombotic/hemorrhagic complications. Patients on at least major molecular response are 48 out of 55 evaluable (87.3%) at a median follow-up of 65 months.

PB1746

ELEVATED BLOOD PRESSURE (BP) AND ADVERSE EVENTS OF HYPERTENSION (HTN) IN PHASE 1, 2, AND 3 TRIALS OF PONATINIB IN LEUKEMIA

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Background: Ponatinib is a potent, multitargeted tyrosine kinase inhibitor with proven efficacy in resistant Philadelphia chromosome–positive (Ph+) leukemia.
Aims: This analysis reports elevated BP and adverse events of HTN in phase 1, 2, and 3 trials of ponatinib in patients with leukemia.
Methods: Ponatinib safety and efficacy were evaluated in patients with relapsed/refractory hematologic malignancies in the ongoing phase 1 trial, in heavily pretreated chronic myeloid leukemia (CML)/Ph+ acute lymphoblastic leukemia (ALL) patients in the ongoing PACE (phase 2) trial, and in newly diagnosed chronic-phase CML patients in the terminated EPIC (phase 3) trial of ponatinib vs imatinib. All patients gave informed consent. Phase 1 and EPIC, but not PACE, excluded patients with uncontrolled HTN (defined as untreated systolic/diastolic >150/>100 mm Hg in phase 1 and >140/>90 mm Hg in EPIC). Elevated BP was defined by the single highest BP measurement (systolic/diastolic): grade (G) 1/pre-HTN 120–139/80–89 mm Hg, G2 140–159/90–99 mm Hg, G3 ≥160/≥100 mm Hg. HTN adverse events were reported by investigators.
Results: Elevated BP was frequent at trial entry (Table 1). G1/G2–3 rates were 44%/42% in phase 1, 37%/47% in PACE, and 51%/19% with ponatinib vs 52%/12% with imatinib in EPIC. Any increase in BP grade from baseline was also frequent, with rates of 74% in phase 1, 68% in PACE, and 68% with ponatinib vs 51% with imatinib in EPIC. In PACE, estimated systolic/diastolic BP increases over time were low, at 2.3/0.7 mm Hg per year. HTN adverse events were reported in 38%, 28%, 18%, and 2% of patients in phase 1, PACE, and the EPIC ponatinib and EPIC imatinib arms, respectively. Hypertensive crisis was reported in 2 patients in PACE and in 2 patients in the EPIC ponatinib arm. HTN adverse events did not lead to discontinuation or death. Few patients had dose modifications for HTN adverse events (0% in phase 1, 5% in PACE, and 3% with ponatinib vs 0% with imatinib in EPIC). A retrospective multivariate analysis of pooled patients showed that HTN adverse events were significantly associated with ponatinib dose intensity.

Table 1. Baseline BP and Any Increase in BP Gradea on Study.

	Normal BP[b] at Baseline				G1 BP at Baseline			G2 BP at Baseline		G3 BP at Baseline
	n	Increase to G1	Increase to G2	Increase to G3	n	Increase to G2	Increase to G3	n	Increase to G3	n
Phase 1, N=81	11	18%	45%	27%	36	47%	39%	27	70%	7
PACE, N=449	70	34%	31%	23%	167	53%	35%	157	62%	55
EPIC/ponatinib, n=154	46	57%	22%	7%	78	51%	14%	30	50%	0
EPIC/imatinib, n=152	54	65%	13%	2%	79	35%	5%	12	17%	6

[a]Based on single highest BP measurement; [b]<120/<80 mm Hg. Baseline BP was missing for 1 patient in the EPIC imatinib arm.

Summary and Conclusions: Increase in BP was frequently observed in ponatinib trials, including in patients on imatinib. Rates of HTN adverse events were relatively lower and seen primarily with ponatinib. While associated with ponatinib dose intensity, HTN adverse events rarely led to change in leukemia therapy. Given BP variability, investigator reporting of HTN adverse events may be a more reliable indicator of clinically meaningful HTN.

PB1747

PATIENTS WHO DO NOT ACHIEVE STABLE UNDETECTABLE BCR-ABL1 AFTER LONG-TERM IMATINIB OBTAIN DEEP MOLECULAR RESPONSES AFTER SWITCHING TO DASATINIB

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Background: Imatinib therapy has drastically changed the prognosis for patients with chronic myeloid leukemia (CML). Confirmed deep molecular response predicted significantly higher survival probabilities and no patient with confirmed deeper molecular response (*i.e.* $MR^{4.5}$) has experienced progression (Hehlmann R *et al. J Clin Oncol.* 2014). However, even after 8 years of imatinib administration, the cumulative incidence of ≥2 years of undetectable BCR-ABL1 (Stable $MR^{4.5}$) was 36.5% (Branford S *et al. Blood*. 2013). On the other hand, second generation tyrosine kinase inhibitor, nilotinib has been shown to be a more potent inhibitor of BCR-ABL than imatinib (Saglio G *et al. N Engl J.* 2010). Another study shows that patients with CML-CP with persistent minimal residual disease (MRD) on imatinib who switched to nilotinib experienced deeper confirmed molecular responses compared with those who remained on imatinib, and these responses were stable (Hughes P, *et al. Blood* 2014). Dasatinib, another second generation tyrosine kinase inhibitor also induces fast and deep cytogenetic and molecular responses that translate to better outcomes (Jabbour E *et al. Blood* 2014). However, it is not clear that dasatinib has a potential for achieving further molecular response after switching. Here, we conducted a multicenter, prospective 'CMR-CML' study and observed molecular response of CML-CP patients treated with dasatinib.
Aims: The primary endpoint of our study was complete molecular response (CMR) rate by 18 months after switching to dasatinib.
Methods: For the assessment of molecular response, a quantification of BCR-ABL transcripts by RQ-PCR analysis was applied. The analysis of BCR-ABL transcripts were performed at registration, 1, 3, 6, 9, 12, 15, and 18 months after switching to dasatinib. Measurement of BCR-ABL transcripts was performed as described previously (Yoshida C et.al. *Int J Clin Oncol.*2013). Undetectable level of BCR-ABL transcript (<50 copies/μgRNA), which is equivalent to 4.16-log reduction, was defined to be CMR in this study. Comparisons between the qualitative variables were carried out using the $\chi 2$ test. These sta-

tistical analyses were performed with the software package Stata version 11 (Stata Corp LP, College Station, TX, USA). For all analyses, the P values were 2-tailed, and a P value less than 0.05 was considered to be significant.

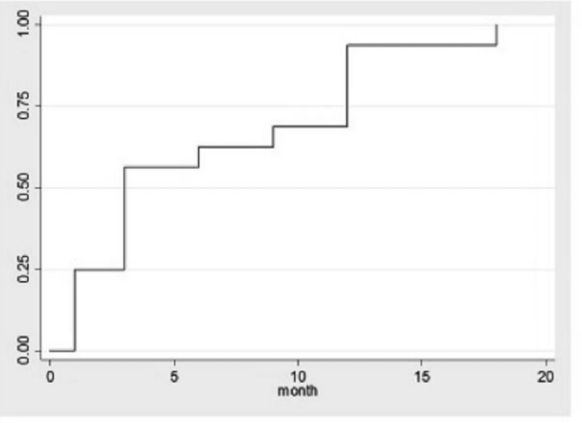

Figure 1.

Results: From August 6, 2011 to January 17, 2013, a total of 21 patients with CML-CP with persistent MRD after at least 2 years of imatinib therapy assigned in our study. Five patients were excluded because of several reasons. Finally, 16 patients were registered in this trial. The median age at registration was 50 years (range, 25-70 years), and 11 were men and 5 were women. All patients received a dosage of 100mg/day dasatinib once daily. All patients achieved CMR within 18 month. There were 4, 5, 1 patients obtained CMR by 1, 3, and 6 months respectively in 16 evaluable patients (Figure). Ten patients kept CMR at 16 month and 6 patients lost CMR (2 patients widowed this trial, 3 patients was suffered from recurrence). We investigated the possible associations between the achievement of early CMR and a number of baseline variables, including age, sex, lymphocyte count, International Scale (IS), and duration of imatinib therapy. There was higher cumulative incidence of CMR at 1 month when patients with a low IS at registration were compared with those with a high IS (0.013 vs 0.034 *P*=0.0201). Duration of imatinib therapy was significantly longer in patients who obtained CMR in 1 month (106±21 months vs 55±10 months *P*=0.0322). There was no difference in the frequency of female sex, age, lymphocyte count. Dasatinib therapy was well tolerable and toxicities were almost low grade. Non-hematological toxicities were liver dysfunction (grade 1 or 2, 43%), pleural effusion (grade 1 or 2, 37%), dyspnea (grade 1, 12%; grade 3, 6%), and gastrointestinal bleeding (grade 1, 12%). Hematological toxicities were anemia (grade 1or 2, 75%; grade 3, 12%), neutropenia (grade 1 or 2, 43%; grade 3, 6%), and thrombocytopenia (grade 1 or 2, 37%; grade 3, 12%).

Summary and Conclusions: This pilot study shows dasatinib has the ability to obtain further deep molecular response for the patients who do not achieve stable undetectable BCR-ABL1 after long-term imatinib therapy. Based on this study, randomized control study should be attempted in the future.

PB1748

UPDATED REPORT OF THE AUSTRIAN CML-REGISTRY

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Background: Tyrosin kinase inhibitors (TKI) treatment in CML has is standard of care, however, its treatment goals have become more stringent are directed by response kinetics. Since standardized BCR-ABL transcript monitoring has been established the ELN recommendations guide treatment according to the achieved molecular response. Clinical trials have demonstrated the predictive value of early molecular response with respect to consecutively achieved deep responses which in turn would allow for about half of the patient to remain in treatment free remission. In addition, the duration of sustained deep molecular response has recently been shown to be associated with a lower relapse rate after treatment discontinuation. Although second generation TKIs (2G-TKIs) are of superior efficacy in inducing deep molecular response the majority of patients in routine care are still receiving imatinib.

Aims: Assessing the current status of treatment and response thereto is a prerequisite to ascertain a high quality of disease management and to guide physicians to future treatment options for their patients. This registry aims to reflect the real life situation of CML treatment in Austria.

Methods: The Austrian CML registry is a database project under the patronage of the ASHO approved by an ethics committee. It is focused on diagnostic parameters, *e.g.*, conventional cytogenetics and/or FISH, BCR-ABL transcript levels, blood counts, general laboratory parameters, CML phase, concomitant diseases, CML specific treatment and including stem cell transplantation and adverse effects. Participating centres can contribute patient data after having obtained written informed consent.

Results: We summarize the CML registry data as of March 2015 and focus on treatment response. The CML registry cohort comprised a total of 434 patients with a median number of 4581 follow-up visits. At diagnosis 87.9%, 8.3% and 0.7% of evaluable patients were in early, late and secondary phase, respectively, whereas only 2.1%, 0.7% and 0.2% were in accelerated phase, myeloid and lymphatic blast crisis, respectively. First line treatment was imatinib, nilotinib or dasatinib in 301, 21 and 8 evaluable cases, respectively. The majority of patients had an optimal response according to ELN criteria at the last evaluable follow up (Figure1). We will present the results of the currently running final update on the registry cohort.

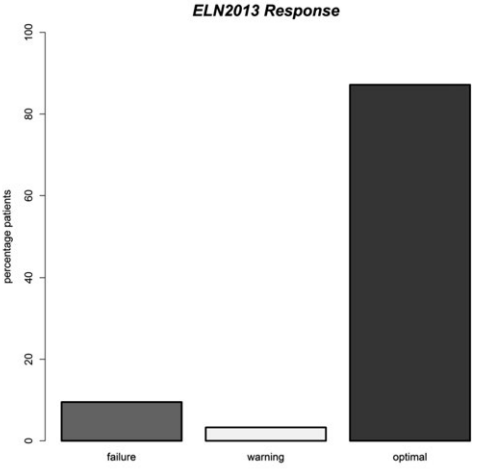

Figure 1.

Summary and Conclusions: The majority of patients fulfils the ELN 2013 criteria for optimal response. For years imatinib has been for years the mainstay of treatment and continues to be so. However, the use of 2G-TKIs has increased. The main focus of this registry for 2015 will be the percentage of patients reaching a sustained deep molecular response.

PB1749

UTILIZATION OF LABORATORY DATA TO QUANTIFY THE BCR-ABL1 TESTING LANDSCAPE IN USA

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Background: According to NCCN and ELN Guidelines, the monitoring of *BCR-ABL1* transcript levels is central to care of Chronic Myeloid Leukemia (CML) patients. Previous studies have shown that, in the US, a high proportion of CML patients do not receive the recommend frequency of BCR-ABL testing. Efforts have been made to standardize technical aspects of monitoring, but a large degree of variation remains in many other aspects of the BCR-ABL testing process, for example test requisition and result reporting. Therefore physicians are required to collect, assimilate and interpret a large amount of, often technical, information associated monitoring CML patients.

Aims: The aim of this study was to quantify the variation in the BCR-ABL testing

pathway, from test availability through to result reporting. An additional objective was to use a national database to analysis testing rates for CML patients.
Methods: Insurance claims data and independent information from clinical laboratories were used to map and evaluate the current US *BCR-ABL1* testing landscape. This study included analysis of test availability (including geographic reach), test request forms, turn around times (TAT), cost (as billed by the laboratory), methodology, sample reports and number of tests performed and billed.
Results: Between June 2013 and May 2014, *BCR-ABL1* testing was offered by 209 US clinical laboratories, of which, 137 laboratories conducted the testing in-house. The remaining 72 laboratories sent this testing to 26 laboratories, 22 of which were used by only a single referring laboratory. Geographic spread of the laboratories was not uniform, *e.g.* 16 laboratories in California whereas only one laboratory in each of 9 other states. 21% of laboratories stated they report on the International Scale (IS), with 77% not providing this critical information. Only 30% (6/20) of test request forms specified transcript types for which testing is available. TAT and cost varied from 2-14 working days and $187-$497 respectively. Only 22% (4/18) of laboratory reports included full test methodology details, graphical representation of historical results and clear interpretative comments in line with current NCCN guidelines. The testing frequency of CML patients was found to be<1 test per annum, much lower than current guidelines recommend.
Summary and Conclusions: Availability of quantitative *BCR-ABL1* testing appears to be adequate in the US, however, our data show that in those states with fewer laboratories offering BCR-ABL quantitation, a physician is less likely to have access to a laboratory offering testing on the International Scale. The heterogeneous landscape for *BCR-ABL1* testing is likely contributing to insufficiently-informed testing choices, testing frequency and, ultimately, treatment choices. The inadequate and inconsistent information available for physicians poses a barrier to supporting them in choice of testing laboratory and in determining actionable outcomes from patient test results. A more uniform and integrated approach is needed to better support physicians and to ensure optimal patient care.

PB1750

CORRELATION OF DASATINIB PHARMACOKINETICS WITH CLINICAL RESPONSE AND ADVERSE EVENTS IN NEWLY DIAGNOSED CHRONIC MYELOID LEUKEMIA CHRONIC PHASE

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Background: The second-generation tyrosine kinase inhibitor (TKI) dasatinib is used as the first-line therapy for newly diagnosed chronic myeloid leukemia (CML). However, some patients fail to respond or become intolerant to dasatinib. Several studies have shown a relationship between pharmacokinetics of dasatinib and molecular responses or significant adverse events.
Aims: To investigate pharmacokinetics of dasatinib and to identify an influence of transporter gene polymorphisms on dasatinib treatment in newly diagnosed CML patients.
Methods: Fourteen patients treated with dasatinib from whom were obtained a total of 118 pharmacokinetic profiles were enrolled in this study. Dasatinib plasma concentrations from samples obtained just prior to and 1, 2, and 4 h after oral dasatinib administration were analyzed by liquid chromatography-tandem mass spectrometry. Genotyping of 10 single nucleotide polymorphisms (SNPs) in genes involved in dasatinib pharmacokinetics (*ABCG2, ABCB1, CYP3A5*3, SLC22A1, ABCC2*) was performed by PCR-RFLP. The study protocol was approved by the Ethics Committee of Akita University Hospital, and all recipients gave written informed consent.

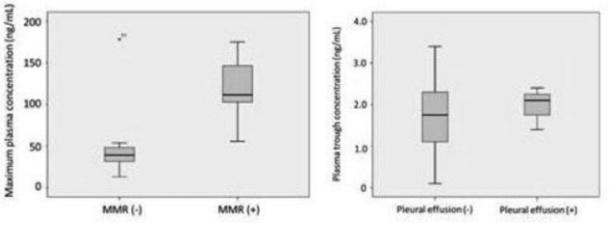

Figure 1.

Results: In this study, associations between dasatinib concentration, clinical response, adverse event, and 10 SNPs were investigated among newly diagnosed chronic phase CML patients. Seven patients achieved major molecular response (MMR) at 3 month and the other 7 patients did not. The plasma concentration at 2 h (C2h) or the maximum plasma concentration (Cmax) of dasatinib was significantly higher among patients with a MMR at 3 month than those without (*P*=0.051, *P*=0.035, Figure 1). Among 118 pharmacokinetics profiles, there were 8 adverse events of pleural effusion in 4 patients. Although there was not a significant difference in plasma trough concentration of dasatinib (C0h) between groups with pleural effusion and without (*P*=0.350), it was observed in patients with more than 1.4 ng/mL of C0h which could be translated into 2.8 nM (Figure 2). 5 patients have ABCG2 421C/C and 9 patients have ABCG2 421C/A. There were significant differences of plasma concentration at 2 h (C2h) after dasatinib administration between two groups (*P*=0.019). However, the other SNPs were not associated with dasatinib pharmacokinetics in this study.
Summary and Conclusions: Higher dasatinib C2h/max was associated with the likelihood of achieving a MMR at 3 month in our small CML patient cohort. Additionally, pleural effusion was not observed in patients with lower dasatinib trough concentration. Although a prospective study with a larger patient population is necessary to validate these findings, in addition to *BCR-ABL1* mutation analysis, our data indicate that clinical dasatinib blood-level testing may improve the efficacy and the safety of dasatinib therapy among newly diagnosed CML patients.

PB1751

«FLAG» REGIMEN IS EFFECTIVE FOR CHRONIC PHASE INDUCTION FOR CHRONIC MYELOID LEUKEMIA BLAST CRISIS.

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Background: Only hematopoetic stem cell transplantation (HSCT) should be curative in chronic myeloid leukemia blast crisis (CML BC). It seems that few patients obtain stable second chronic phase (CP) on monotherapy with tyrosin kinase inhibitors (TKI)and then might be switched to HSCT. We suggest that more patients may benefit with high doses of cytostatics in combination with TKIs.
Aims: To evaluate whether high doses of cytostatics in combination with TKIs may allow to obtain more stable response and switch more patients to HSCT.
Methods: We studied the results of different therapeutic approaches for CML BC in 19 patients (9 females and 10 males) with median age 43 years at the moment of the BC onset (range 21-63). CML was initiated in BC in 4 pts, whereas other pts develop BC on TKIs therapy. Median time from CML diagnosis was 44,5 mo (range from 1,7 to 139 mo). Type of BC was myeloid in 9, lymphoid in 7 and nondifferenciated in 3 pts. Pts were treated with FLAG based regimen+TKI (n=8), low dose chemo+TKIs or mono TKI (n=11). All pts obtained monoTKIs before FLAG regimen. The therapy was intensified due to lack or loose of second CP. 8 pts had SCT. Type of SCT was 4 unrelated alloSCT, 2 related alloSCT, 2 haploidentical SCT. 5 pts after FLAG and 3 pts after low dose chemo or monoTKIs undergo SCT.
Results: Second CP was obtained in 7/ 8(87.5%) patients after FLAG and only in 3/11 (27%) pts after low dose chemotherapy induction (P=0,02). Moreover 3 and 2 pts after FLAG induction have CCyR and MMR, accordingly. No pts on other therapies reach CCyR. 3 patients were undergone HSCT after 1 of FLAG course. Other patients received one or more FLAG consolidation. Consolidation did not lead to stable CP. All patients had rapid increase of blast count. There are no differences in duration of response in patients with low and high dose cytostatic therapy. The median time to HSCT from BC onset was 12 mo (range from 5,6 to 94 mo). The probability of survival is higher for transplanted patients vs not transplanted patients (25% vs 15%, p 0,014). 50% patients are alive after HSCT with median overall survival time after HSCT 14,5 mo (range from 4,4 to 38 mo). Median time to HSCT in alive and dead pts was 6,6 and 12,8 mo accordingly. There were no differences in overall survival after HSCT between treatment groups.
Summary and Conclusions: HSCT should be the goal of treatment strategy in BC CML. FLAG regimen in combination with TKIs is effective for CP induction and allow us to switch more pts to HSCT in compare with less aggressive strategies. Intensive chemo+TKIs may be able pts to reach CCyR or even MMR, but the responses are unstable and whether pts should obtained several courses of chemo for response deepening is questionable. It seems that pts should undergo HSCT as soon as possible after CP induction.

PB1752

FLOW CYTOMETRY-BASED ASSAY FOR DETECTION OF BCR-ABL FUSION PROTEIN IN BLOOD CELLS FROM CML PATIENTS

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Background: Chronic myeloid leukemia (CML) is currently diagnosed using RT-PCR and/or FISH to reveal the presence of the fusion mRNA transcripts for BCR-ABL, or of the characteristic Philadelphia chromosome. RT-PCR is also used to monitor the effects of treatment by sensitively measuring transcripts representing minimal residual disease (MRD). It has not been possibly to use flow cytometry to identify the neoplastic cells but such a method would be helpful in the workflow of a hematopathology lab.

Aims: We have now developed a method that successfully detects and enumerates cells harboring the fusion protein BCR-ABL by flow cytometry in CML patients.
Methods: The method uses the *in situ* proximity ligation assay (PLA) (Söderberg et al 2006, Leuchowius et al 2009), where two antibodies target the BCR and the ABL part, respectively, of the fusion protein. The antibodies are equipped with DNA oligonucleotides that – when brought in proximity – guide the formation of a circular DNA molecule as a template for localized DNA amplification through rolling circle amplification (RCA). Each RCA-product is then labeled with around 1,500 fluorophore-coupled DNA oligonucleotides, allowing cells to be detected by flow cytometry.
Results: The method has proven very sensitive, able to detect very low number of cells in patient samples, and it allows identification of patients in relapse at a very early state. We have analyzed 25 CML patient samples using PLA-based flow cytometry, and the results were compared to the routine RT-PCR analysis. More than 50% of the analyzed samples were positive for both methods, ranging from 75 IS% down to 0.03 IS% for the RT-PCR. Five of the samples were positive only in RT-PCR, while three were positive only in the PLA method.
Summary and Conclusions: Since the method is developed for readout with flow cytometry all the advantages with multicolor fluorescence analysis can be achieved simultaneously. In addation, the method allows for standard cytological markers to further characterize the malignant cells.

PB1753

Abstract withdrawn

PB1754

LOW HEMOGLOBIN LEVELS AT DIAGNOSIS ARE ASSOCIATED WITH WORSE TREATMENT RESPONSES AND EVENT FREE SURVIVAL IN CHRONIC MYELOID LEUKEMIA PATIENTS IN CHRONIC PHASE RECEIVING TYROSINE KINASE INHIBITORS

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Background: SPECIAL;Despite progress in prognosis of patients with chronic myeloid leukemia in the era of tyrosine kinase inhibitors (TKI), values of current available three prognostic scoring systems, namely Sokal, Hasford and EUTOS scores, for predicting survival outcomes and treatment response remained inconclusive. Current Sokal and Hasford score developed in pre-TKI era and they both included many baseline clinical variables but without baseline hemoglobin levels, a seldomly discussed variable. The value of baseline hemoglobin levels for predicting treatment responses and survival outcomes might be worthy to be reevaluated.
Aims: This study aims to exam the role of hemoglobin level at diagnosis in association with survival outcomes and treatment responses in chronic myeloid leukemia patients in chronic phase (CML-CP) receiving frontline TKIs and to validate the three scoring systems.

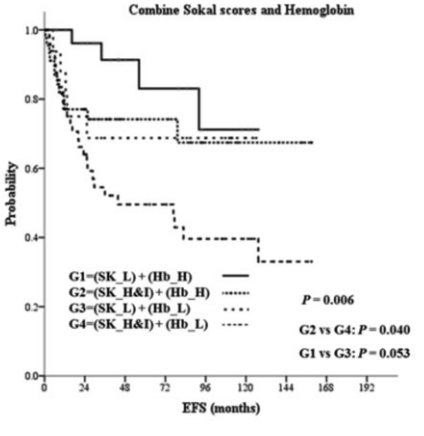

Figure 1.

Methods: SPECIAL;A total 172 consecutive patients with CML-CP patients were screened retrospectively from January 2001 to April 2014. Patients who previously recieved interferon-alpha or any chemotherapeutic agents or in combination with TKIs treatment, patients who were lost follow-up within 3 months of diagnosis, and patisnts who had no adequate data to calculate baseline risk socres were excluded. 143 patients were enrolled to further analysis after screening. The cut-offs of baseline hemoglobin level were defined as 12.3 mg/dl for male and 9.0 mg/dl for female sperately in consideration for gender difference in hemoglobin levels by adopting the receiver oprator characteristic (ROC) curve method.
Results: With a median follow-up of 54.0 months, 5-year overall survival (OS), progression-free survival (PFS), and event-free survival (EFS) were 87.5%, 85.9% and 66.8%. We found Sokal and Hasford, but not EUTOS score were predictive for difference in EFS and PFS; however, none of the three scoring systems were predictive for difference in OS. Lower baseline hemoglobin levels was an independent adverse factors for EFS (hazard ration 2.06; 95% confidence interval 1.12-3.78, P=0.019) and associated with worse treatment responses: the proportion of patients with lower *versus* higher hemoglobin levels achieving optimal responses were 52.2% vs 71.1% for 3-month early molecular response (P=0.021), 20.0% vs 51.4% for 6-month complete cytogenetic respone (P<0.001), and 18.0% vs 45.1% for 12-month major molecular response (P<0.001). Furthermore, combining hemogobin level and Sokal score further distinguised a population at risk for worse EFS and treatment responses.
Summary and Conclusions: The results highlights the need for revisiting tradition CML scoring systems in the TKI era and suggests baseline hemoglobin levels at diagnosis might be considered for new scoring systems.

PB1755

NILOTINIB RESULTS IN IMPROVED RATES OF MOLECULAR RESPONSE IN TURKISH NEWLY DIAGNOSED CML-CP PATIENTS: A 24-MONTH UPDATE

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Background: Nilotinib, a more potent and selective BCR-ABL-1 inhibitor than imatinib is approved by FDA and EMA for the treatment of newly-diagnosed Ph+ CML patients in the chronic phase (CML-CP).
Aims: The aim of the present study was to evaluate the efficacy and safety of nilotinib in a Turkish population of newly-diagnosed Ph+ CML-CP patients.
Methods: The study was a multicenter, open-label, single-arm phase II clinical trial. All patients were to be treated with nilotinib (AMN107, Tasigna®) 300 mg BID for 24 months.

Table 1. Baseline characteristics and molecular responses of the study patients.

Characteristics (n=96)	
Age, year (range)	46 (19-78)
Gender (Female/Male)	39 (40.6%)/57 (59.4%)
Palpable spleen size (baseline), cm	0.0 (0-17)
Leukocyte count, K/mm³	26.5 (1.7-289.4)
Platelet count(baseline), K/mm³	343.0 (75-1268)
Molecular response	
Cumulative MMR rate by 24th month (n=96)	77 (80.2)
Time to MMR (days) (n=77)	260.0±157.5
BCR-ABL level Month 3 (n=89)*	
<%10	77 (86.5)
≥%10	12 (13.5)

Data are expressed as median (minimum-maximum) or number (%) or mean±standard deviation, where appropriate.
MMR (Major molecular rate): BCR-ABL/control gene ratio of ≤%0.1 measured by RQ-PCR as %
* The number of patients who were still on treatment at 3rd month.

Results: As of April 30, 2014, of the 96 patients out of a total 112 enrolled that had 24 month follow-up, 77 completed active treatment period. General characteristics of the patients at baseline and molecular response by 24 months are presented in Table 1. Cumulative major molecular response rates at 3rd, 6th, 9th, 12th, 15th, 18th, 21st and 24th months were 23.4%, 47.9%, 56.4%, 61.7%, 71.9%, 79.2%, 82.2%, and 80.2%, respectively. For the assessment of deeper molecular responses (defined as transcript levels of BCR–ABL on the International Scale [BCR–ABLIS] ≤0.0032%]), complete molecular response with a 4·5 log reduction required at least 32000 control genes. According to this evaluation, within 24 months follow-up 19 patients achieved MR4.5 (19.7%) by 12 months whereas 45 patients achieved increased MR4.5 (46.8%) ratio by 24 months in out of 96 patients. Out of 96 patients, 39 patients had temporally or permanently discontinued treatment. Permanent discontinuation rate due to adverse event was 9.6% (9 patients) which was the most common reason among all patients (19.8%>19 patients) who permanently discontinued during 24 months. Thrombocytopenia was the most frequent (10.4%) AE, followed by hyperbilirubinemia (8.3%) and increased lipase level (7.3%). Despite published peripheral arterial occlusive disease (PAOD) reports related to nilotinib usage, there were no PAOD events reported at our cohort.
Summary and Conclusions: By 24 months, in Turkish patients with newly diagnosed CML, the cumulative MMR rate was 80.2%. Only one progression occurred, during the first year of therapy. Additionally results showing that 46.8% of these patients achieving MR4.5 by 2 years suggest that high efficacy was achieved with nilotinib, an approved first-line therapy for newly diagnosed chronic phase of CML (CP-CML) and can be an option for future candidate CML treatment regimen. Treatment free remission approach for the patients with sustainable responses during long term follow-up. This study contribute to well established nilotinib profile for chronic phase CML patients which is a licensed alternative for the treatment of newly diagnosed Ph+ CML-CP in Turkey.

PB1756

INCIDENCE OF SECOND NEOPLASMS IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA ANALYSED IN THE FRAME OF INTERNATIONAL RESEARCH PROJECT EUTOS POPULATION BASED STUDY IN RUSSIAN FEDERATION

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Background: Success of tyrosine kinase inhibitors (TKIs) in chronic myeloid leukemia (CML) gives hope for a durable disease free survival. Therefore long-term effects of TKI treatment should be studied. A significant event for CML patients (pts) is the development of other tumors, second neoplasms (SN) which can be defined as prior malignancies (pM) if detected before CML diagnosis and second malignancies (sM) which occur after the CML diagnosis during TKI treatment.
Aims: To evaluate the incidence and variants of SN in CML pts treated in clinical practice in Russian Federation (RUS).
Methods: We included 199 CML pts from 7 centers of 6 administrative districts of RUS into RUS part of EUTOS PBS-study. The inclusion criteria were: Ph'/bcr-abl-positive CML diagnosed in 1.10.2009-31.12.2012 verified by cytogenetic/molecular data, age of pts ≥ 18 years (y). Median (Me) age was 50(18- 82)y, sex ratio M/F(%) was 100/99 (50/50)pts. At the time of diagnosis phases of CML were the following: chronic phase (CP) in 187(94%)pts, accelerated phase in 11 (5,5%) pts, blast crisis in 1(0,5%) pts. Me time from diagnosis to imatinib (IM) therapy was 0,6 (0,5-15) months (mo). Pretreatment before IM was the following: hydroxyurea in 99 (50%) pts; IFN-α in 2 (1%) pts. Me follow-up since the CML treatment start was 35.2 (0,5-85)mo (Me for n=192; 7pts were without TKI therapy).
Results: Total rate of sN was 7% (14 of 199 pts). Two of those cases were sM revealed at 22mo (Encephaloma) and 23mo (adenocarcinoma of the colon) after diagnosis of CML, respectively. These 2pts died due to progression of sM after 29 and 32 mo of CML diagnosis respectively. A pM before CML diagnosis was in 6% (12 of 199 pts). Their Me age was 69(33-73)yy, sex ratio (M/F) 4/8pts. The Me time from diagnosis of a pM to CML diagnosis was 61 (28-429) mo. The pMs were: breast cancer (4/12 of cases), cancer of uterus and ovaries (2/12 of cases), rectal cancer, tumor of the upper third of the shoulder, kidney cancer, prostate cancer, basal cell carcinoma of the eyelid, GIST. Me time of IM therapy was 36 (7- 49)mo. Most of the pts among 12 cases had good results of TKI therapy: 7pts achieved major (4/7) and complete molecular (3/7) response (Me 13 (extr.6–30) mo). Three out of 12 pts with pM in anamnesis died (2 pts not achieved even hematologic response). Pts with sM achieved complete and partial cytogenetic response to 12mo of IM therapy. Mortality in the whole group of 199pts was 15,6% (31cases) and 5 of them (2,5%pts) or 35% (5 of 14 pts) died due to SN progression: 3pts with sM+ 2pts with pM, all 5 were in CP CML.
Summary and Conclusions: Identified cases of SN and CML combination underline a need for understanding the relationship between CML, other malignancies and their therapy. Assosiation of sM annd CML needs deeper analysis during longer observation time.

PB1757

OUTCOME OF FRONTLINE TREATMENT WITH GENERIC IMATINIB ACCORDING TO ELN 2009 BASED NATIONAL GUIDELINES. EXPERIENCE FROM University CLINIC OF HEMATOLOGY, CLINICAL CENTER OF SERBIA, BELGRADE

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Background: Serbia was the first country in Europe introduced generic imatinib (ANZOVIP®, Actavis). Lot of concerns and many issues were arisen by general and medical public, especially by patients knowing reports about treatment failures. All patients in Serbia were switched to the same drug together with newly diagnosed patients. To realize real efficiency of the drug, we made evaluation of newly diagnosed patients only.
Aims: Retrospective analysis of response to generic imatinib in frontline treatment.
Methods: As the largest institution, Clinic of Hematology CCS, we analyzed cohort of 49 patients with CML in chronic phase, frontline treated by generic imatinib from August 2012 to March 2014. All patients were treated according to ELN 2009 based National guidelines with regular cytogenetic follow up and molecular monitoring after 12 months (after CCgR). In all patients the follow up of treatment was at least 6 month.
Results: Our 49 patients, had median age of 47yrs ranging from 24 to 74yrs. There were 31 male and 18 female patients. In all patients CHR was achieved within 3 months of treatment. In 17 pts after their approval, we have performed early cytogenetic evaluation at 3 mths: 3/17 pts was without mitoses, 10/17 pts (58%) had Ph<35%, and 4 were non responders (Ph from 50-100%). According to our National guidelines evaluation at 6 and 12 months were performed. At 6 months, 23/49 pts achieved CCgR (46.9%), 38/49 (77.5%) was in majorCgR (ELN 2009 optimal), and 9/49 pts had insufficient response (no major CgR). At 12 mths similar evaluation revealed 26/49 pts achieving CCgR (53%), and ELN 2009 suboptimal response was noted in 7/49 pts (14.3%). Unfortunately we have also noted that some patients lost their previously achieved response after one year of treatment. Also 3 patients developed sudden blast phase. Taken together failure >12 mths was in 5+3 pts (16%). Besides 7 pts (14.3%) never achieved optimal response by 400mg imatinib up to one year of treatment. Seven patients (14.3%) were treated by imatinib escalation (800mg), and 4 achieved CCgR. 9 pts were treated with second line nilotinib (failure by ELN 2009) and 5 of them achieved secondary CCgR. From other four patients without optimal response to nilotinib, 3 of them were sent to BMT. Molecular evaluation was performed at M12 and M18 from start of the treatment. Due to technical reasons it was performed in more than half of the patients, 30 of 49 (61%), and optimal molecular response at M12 of M18 was achieved in 25 of 30 analyzed patients (at least MR3) but in 5 patients MR3 was not achieved even they are in continuous CCgR up to at M18.
Summary and Conclusions: Our pilot data are based on small number of patients from single center, but provided data that generic imatinib used in Serbia (ANZOVIP) is not much inferior to branded imatinib. Larger series of patients from whole Serbia (*e.g.* 90-100) will provide more accurate data about efficiency of generic imatinib in the first line treatment.

PB1758

THIRD-LINE TREATMENT WITH 2ND GENERATION TYROSINE KINASE INHIBITORS (TKIS) IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA WHO FAILED TWO PRIOR TKIS: A SINGLE CENTER EXPERIENCE OF 21 PATIENTS

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Background: Although most of the chronic phase chroic myeloid leukemia (CML-CP) patients do well under imatinib (IM), some quit IM due to resistance and/or intolerance. 2nd generation tyrosine kinase inhibitors (2GTKIs) (dasatinib

(DAS) and nilotinib (NIL)) can be used in patients who have intolerance and resistance to IM, and approximately 50% of patients failing to respond to previous treatments may respond to 2GTKIs. Limited data is available, displaying the outcome of CML patients who failed two lines of TKI, and received 2GTKIs as a 3rd-line treatment option.
Aims: The aim of this study is to report our single center experience on CML patients who received 2GTKIs as a 3rd-line treatment option.
Methods: Two hundred and nine CML-CP patients who were diagnosed between 1999-2013 and received IM were evaluated. Patients' demographics, Sokal risk scores, treatment outcomes and durations, and the follow-up periods were noted from the patients' files retrospectively. Patient monitorization was performed according to European LeukemiaNet (ELN) recommendations, and in case of resistance, mutational analysis was performed. The cumulative incidence of major molecular response (MMR) and complete cytogenetic response (CCyR) rates during the follow-up periods were calculated. The choice of 2GTKIs depended on mutational status and comorbidities of the patient, and the disease phase at the time of the switch (*i.e.* in patients with blastic crisis (BC), DAS was the treatment choice). Also in Turkey DAS was in the market before NIL became available, so at that period of time, DAS was the only option for 2nd-line TKI treatment.

Table 1.

Table 1. Baseline characteristics and treatment outcomes of patients received 3rd-line 2GTKIs. (F, female; M, male, HU, hydroxyurea; IFN, interferon; Ara-C, cytarabine; IM, imatinib mesylate; MMR, major molecular response; CCyR, complete cytogenetic response; PCyR, partial cytogenetic response; CHR, complete hematologic response; DAS, dasatinib; NIL, nilotinib) *Patient had splenectomy prior to the diagnosis of CML so Sokal score could not be calculated. ϕPatient died after stem cell transplantation.

Patient #	Age, years/ Gender	Sokal Risk Score	Treatment prior to IM	Reason for stopping IM	Duration of IM treatment (months)	2nd-line treatment	Reason for stopping 2nd-line treatment	Duration of 2nd-line treatment (months)	3rd-line treatment	Duration of 3rd-line treatment (months)	Reason for stopping 3rd-line treatment	Best response under 3rd-line treatment	Follow-up duration (months)	Outcome
1	20/M	Low	None	Resistance	22	NIL	Resistance	2	DAS	26	Continued	MMR	48	Alive
2	57/M	Low	INF+ARA-C	Resistance	83	DAS	Intolerance	25	NIL	12	Intolerance	PCyR	177	Dead
3	32/M	Low	HU	Resistance	12	NIL	Resistance	31	DAS	6	Continued	CHR	30	Alive
4	33/M	Low	HU	Resistance	26	DAS	Resistance	28	NIL	3	Intolerance	CHR	145	Alive
5	55/M	High	None	Resistance	23	DAS	Intolerance	52	NIL	4	Continued	CHR	79	Alive
6	57/M	Intermediate	HU/IFN+ARA-C	Resistance	68	DAS	Intolerance	20	NIL	34	Continued	MMR	131	Alive
7	47/F	Low	HU+IFN	Resistance	89	DAS	Intolerance	36	NIL	13	Continued	MMR	208	Alive
8	29/F	/*	HU	Resistance	31	DAS	Resistance	32	NIL	4	Intolerance	CHR	112	Deadϕ
9	51/M	Intermediate	HU	Resistance	69	DAS	Intolerance	19	NIL	33	Continued	MMR	161	Alive
10	39/F	Intermediate	HU	Resistance	49	DAS	Resistance	56	NIL	23	Continued	MMR	172	Alive
11	48/F	Low	HU+IFN	Resistance	80	DAS	Intolerance + Resistance	24	NIL	12	Progression	None	144	Dead
12	46/F	Low	INF+ARA-C	Resistance	42	DAS	Resistance	11	NIL	2	Intolerance	CHR	144	Alive
13	65/F	Intermediate	None	Intolerance	2	DAS	Intolerance	24	NIL	22	Continued	MMR	48	Alive
14	39/M	Intermediate	HU	Resistance	18	NIL	Intolerance	1	DAS	14	Resistance+ Intolerance	CHR	80	Alive
15	67/F	High	None	Resistance	23	DAS	Intolerance	12	NIL	42	Continued	MMR	77	Alive
16	66/F	Low	None	Intolerance	2	DAS	Intolerance	42	NIL	1	Intolerance	MMR	45	Alive
17	25/M	Low	None	Resistance	32	NIL	Intolerance	5	DAS	10	Continued	MMR	67	Alive
18	52/M	Low	HU+IFN	Resistance	60	DAS	Resistance	24	NIL	1	Progression	NA	100	Dead
19	36/M	Low	None	Resistance	20	NIL	Resistance	19	DAS	7	Continued	CCyR	46	Alive
20	50/M	Low	None	Resistance	63	DAS	Resistance	12	NIL	22	Continued	MMR	97	Alive
21	53/M	High	HU	Resistance	123	NIL	Intolerance	5	DAS	1	Continued	CHR	129	Alive

Results: One hundred and twenty-two patients were male (58%), and the median age was 44 years (range, 18-92 years). The rates of low, intermediate and high Sokal risk scores were 44%, 39% and 17%, respectively. One hundred and seventy-eight patients (85%) were in early CP (ECP), whereas 31 (15%) were in late CP (LCP). One hundred and forty-four (68.9%) of the patients had only received IM as a TKI treatment, of which 129 (61.7%) were still on IM after a median follow-up of 86 months (range, 13-142 months) at the time of the analysis. The cumulative MMR and CCyR rates under IM were 73.6% and 79.9%, respectively. Sixty-five patients (31.1%) received 2GTKIs, of which 45 had DAS and twenty received NIL as 2nd-line treatment. One patient having an allogeneic hematopoietic stem cell transplantation (AHSCT) after IM failure received DAS for the post-transplant relapse. Among patients receiving DAS, BC was the reason for starting treatment in 5, and nine patients receiving 2nd-line DAS, and one patient receiving 2nd-line NIL died due to BC. Two patients underwent AHSCT after receiving 2nd-line DAS+chemotherapy, of which one died due to disease progression and the other one remained in remission. The cumulative MMR rates under 2nd-line DAS and NIL were 40% and 55%, respectively. Twenty-one patients (15 NIL and 6 DAS) received 2GTKIs as a 3rd-line treatment, and at the time of the analysis, 13 patients were still on treatment, five and 3 patients had to stop therapy due to intolerance and resistance, respectively. During the follow-up, four patients died (3 due to disease progression, and one after AHSCT) (Table 1). Eight patients had 3rd-line treatment for less than 6 months (median 2.5 months), and the remaining 13 had a median follow-up of 22 months under 3rd-line 2GTKIs. Ten patients achieved MMR after a median of 22.5 months (Table 1). Among patients who were refractory to 2nd-line treatment (n=10), only three had gained MMR and one achieved CCyR, whereas 7 of the remaining eleven had achieved and/or maintained MMR under 3rd-line 2GTKI treatment where switching from the 2nd-line treatment was due to intolerance.
Summary and Conclusions: 2GTKIs can be a reasonable choice for the 3rd-line treatment even in patients who are resistant to prior 2 lines of TKI treatment, especially when 3GTKIs are not available and AHSCT is not an option.

PB1759

PDGFRB SUSTAINS THE "WITHDRAWAL SYNDROME" AFTER IMATINIB DISCONTINUATION

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Background: The Swedish group reported that 30% of patients who stopped imatinib presented by the first 2 months musculoskeletal pains, localized to shoulders and hips or extremities, like a rheumatic polymyalgia (Richter J, JCO 32: 25 -2014). Some of these cases have been described as sensitive to paracetamol, whereas other patients need long-term treatment with corticosteroids.
Aims: The aim of this study was the strict molecular and clinical follow-up of patients who stopped imatinib for intolerance in order to do a precocious diagnosis and start an appropriate treatment for this "withdrawal syndrome".
Methods: Seven patients discontinued imatinib in 2014 at the Hematology Unit of the Pisa University; median age was 70 years (range 54-75); all patients received imatinib for more than 3 years, with a median time from diagnosis of 10 years. Initial Sokal risk score was low in 4 patients, intermediate in 2, and high in one patient; EUTOS score was low in all cases, except for one. All patients were in deep molecular response (at least MR4.5) from a minimum of 24 months at the moment of discontinuation. Treatment was interrupted because of non-hematological toxicities (PAOD, bleeding, alopecia, eyes, liver, skin toxicity). BCR-ABL1/ABL1 IS ratio was monitored by quantitative PCR every month. Reactive C protein, interleukin-2, interleukin-10, TNFa, PDGFb, VEGF, and interferong were measured in the plasma by ELISA.
Results: Two of our 7 patients developed the "withdrawal syndrome" after 2 and 3 weeks from the imatinib discontinuation, respectively. They presented muscolo-skeletal pains, localized to shoulders, arms and hip; these symptoms were graded as 2. The C-reactive protein serum level was normal in one case and only slightly increased in the other one; lactate dehydrogenase and protein electhrophoresis were normal in both cases. None of the 7 patients lost the deep molecular response during the follow-up (median 6 months, range 3-11 months). Interleukin-2, interleukin-10, TNFa, IFNg, and VEGF were in the normal range; interestingly, in both patients presenting the "withdrawal syndrome" PDGFRb resulted increased (2.5 fold higher than the highest value in the normal range). Both these patients did not respond to paracetamol 1g trice/day, thus corticosteroids were given (16 mg methyl-prednisolone per day), with tapering within 8 weeks up to 4 mg. Both patients showed a clear improvement within days; after 8 weeks steroid was discontinued, but symptoms, even if milder, reappeared. Both patients still receive 4 mg/day. PDGFb in this phase was still high, even if significantly reduced in respect of the onset of the syndrome.
Summary and Conclusions: Our study confirms, even if in a limited experience, the feasibility of the TKI discontinuation, but also the presence of the "withdrawal syndrome". It is well known that imatinib inhibits PDGFRb; PDGFRb has been also reported to contribute to synovitis by re-directing synovial fibroblasts toward a more aggressive phenotype (Rosengren S, 2010), and increased expression of PDGFb was also detected in the inflamed synovia, supporting its relevant role in the inflammatory processes (Reuterdhal C, 1991).
Consequently, we could hypothesize that the discontinuation of imatinib, stopping the PDGFRb inhibition, could activate the PDGF-mediated arthalgias.

PB1760

CROSSING OF BLOOD-PLACENTAL BARRIER BY TYROSINE KINASE INHIBITORS USED FOR TREATMENT OF CHRONIC MYELOID LEUKEMIA: THEORETICAL AND PRACTICAL ASPECTS

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Background: The issue of safe usage of tyrosine kinase inhibitors (TKIs) in chronic myeloid leukemia (CML) patients on late stages of pregnancy is unclear. TKI ability to cross the blood-placental barrier (BPB) in humans and association with the adverse effects is rarely evaluated in practice due to rareness of cases and ethical reasons. The obtained data are scarce. We hypothesized whether BPB crossing can be predicted by a model based on molecular physicochemical properties of available TKIs.
Aims: To compare the theoretical calculations of BPB crossing of TKIs used for CML treatment with the data obtained in practice and association with clinical outcomes.
Methods: The relative rate of TKIs penetration through BPB was evaluated by so called clearance index (CI) in comparison with antipyrine as free passive diffusion reference with CI=1. The CI of imatinib, nilotinib, dasatinib and bosutinib. was estimated using Quantitative Structure-Activity Relations (QSAR) approach (Hewitt et al, Environ Res, 2007) according to the equation (picture 1). Concentration of imatinib in available cases was simultaneously measured

at labour in blood plasma of mother, cord blood and homogenized placenta by HPLC-mass spectrometry technique. Information of therapy and pregnancy outcomes was taken from Russian CML pregnancy registry and local database of Uzbekistan.

Results: Calculated CI values for imatinib, nilotinib, dasatinib, bosutinib were 0,36, 0,33, 0,44 and 0,67 correspondingly showing moderate but not negligible rate of BPB transfer compared to antipyrine. In 3 of 17 women with CML who received TKI (15 imatinib, 2 nilotinib) since 2nd-3rd trimester (due to lack of response) imatinib concentration was measured. The fetal:maternal ratio ranged from 0,12 to 0,25 and imatinib concentration in placenta was higher than in maternal blood (Table 1). All 17 newborns had no birth abnormalities, low weight<2500 g was in 6 cases. In 5 of 6 cases infants were born preterm at week 35-37, their further development was normal.

Table 1. Concentration of imatinib measured at labour and fetal:maternal ratio.

ratio of case	Imatinib concentration, ng/ml maternal blood plasma	placenta	cord blood	fetal:maternal
1	896,5	ND	109,5	0,12
2	897,5	1095	226	0,25
3	520,6	702,10	115,5	0,22

$$CI=-0,00246TPSA+0,244\sum C_2H_5+0,139\sum Hal+0,569$$

TPSA -topological polar surface area of a TKI molecule
$\sum C_2H_5$ – number of ethyl groups
$\sum Hal$ – number of halogen atoms

Equation for prediction of clearance index of tyrosine kinase inhibitors (TKI)

Figure 1.

Summary and Conclusions: A simple QSAR model based on diffusion-like properties of TKIs used for CML treatment showed the moderate rate of BPB transfer compared to antipyrine. Data obtained at practice showed reduced level of imatinib in cord blood plasma and higher level in placenta compared to maternal blood. Factors which were not accounted in the theoretical model (active transportation mechanisms, extensive metabolic clearance at the fetal side) might specifically prevent TKIs from crossing BPB. Cases of preterm delivery with low weight of children in mothers who got TKIs during pregnancy need further evaluation.

PB1761

ACHIEVING EARLY OPTIMAL RESPONSE IS ASSOCIATED WITH BETTER HEALTH-RELATED QUALITY OF LIFE IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA TREATED WITH IMATINIB OR NILOTINIB AS FRONTLINE THERAPY

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Background: Tyrosine kinase inhibitors (TKIs) as the first-line therapy have improved the outcome of patients with chronic myeloid leukemia in chronic phase (CML-CP). Health-related quality of life (HRQoL) is becoming increasingly recognized as an important component of the management of CML.

Aims: To prospectively assess the HRQoL in newly-diagnosed patients with CML-CP treated with imatinib or nilotinib and to identify the factors associated with the HRQoL outcomes.

Methods: From June 2011 to July 2011, 59 newly-diagnosed patients with CML-CP from Peking University People's Hospital, who were enrolled in the ENESTchina trial, were randomly assigned to receive imatinib 400 mg daily (n=27) or nilotinib 300 mg BID (n=32). Response to first-line TKI therapy was assessed according to European LeukemiaNet 2013 recommendations for the management of CML. HRQoL was measured with the Short Form 36 Health Survey (SF-36) at baseline and every 3 months during the 3 years follow-up. Fisher Exact tests or chi-square tests were used to compare subjects treated with imatinib or nilotinib. The Kaplan-Meier method was used to assess statistical significance in the time-to-event analyses. The longitudinal change in HRQoL was further analyzed in a mixed model approach to linear regression for repeated measurements.

Results: Among the 59 patients (35 man and 22 women), the median age was 37 years (range, 18-74 years). Patients in the two treatment arms were comparable in terms of demographics (*e.g.* gender, age, education and house register), clinical characteristics (*e.g.* Sokal risk score and interval from diagnosis to treatment) and HRQoL scores at baseline. With a median follow-up of 37 months (range, 11-38 months), patients in the nilotinib arm had significant higher cumulative incidences of complete cytogenetic response (P=0.0077) and major molecular response (P=0.0248) compared with those in the imatinib arm. Higher proportions of optimal responses at 3 months (81.5% vs. 68.8%, P=0.263), 6 months (77.8% vs. 62.5%, P=0.204) and 12 months (51.9% vs. 37.5%, P=0.269) were also observed in the nilotinib arm, but without statistical significance. Patients in the two arms had similar rates of event-free survival (84.4% vs. 85.2%, P=0.958), progression-free survival (88. 9% vs. 87.5%, P=0.893) and overall survival (100% vs. 90.6%, P=0.199 at 3 years. Regarding HRQoL, although there was no difference on the SF-36 physical and mental assessments between patients in the two arms during the follow-up, role-physical functioning subscale was improved in patients treated with imatinib (P=0.0421) over time, furthermore, social functioning (P=0.0293) and mental component summary (P=0.0043) were improved in those treated with nilotinib. In total population, patient characteristics at baseline (including gender, age, education, house register and Sokal risk score), TKI used (imatinib vs. nilotinib) and early responses to TKI therapy (optimal response vs. non-optimal response) at 3, 6 and 12 months was analyzed in order to identify the independent factors affecting the HRQoL. Multivariable analyses by mixed model showed that patients with university graduate or above was associated with better vitality (P=0.0468), achieving optimal response at 3 months improved physical functioning dramatically (P<0.0001), and achieving optimal response at 6 months (P=0.0432) or 12 months (P=0.0483) improved social functioning remarkably.

Summary and Conclusions: Imatinib or nilotinib as the first-line therapy improved the HRQoL of CML-CP patients significantly, especially for those achieving optimal response at 3 months, 6 months or 12 months.

PB1762

"REAL-LIFE" FRONTLINE DASATINIB TREATMENT IN UNSELECTED ELDERLY PATIENTS WITH CHRONIC MYELOID LEUKEMIA

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Background: Dasatinib has been recently licensed for first line treatment of patients with chronic myeloid leukemia (CML). However, very few data are available as to toxicity and efficacy of dasatinib in unselected elderly CML patients.

Aims: To address this issue, we revised a "real-life" cohort of 54 CML patients in chronic phase aged >65 years treated with frontline dasatinib in 24 Italian Centers from 6/2012 to 12/2014 focusing on toxicity and efficacy data.

Methods: The main clinical features of the patients at diagnosis were as follows: M/F 27/27, median age 75.4 years [interquartile range (IQR) 70.6 – 79.5), median Hb 12.3 g/dl (IQR 11.1 – 13.6), median WBC 51.5x 10^9/l (IQR 28.1 – 100.0), median PLTS 430x 10^9/l (IQR 233 – 770). According to Sokal risk classification, 2 patients (3.7%) were low risk, 32 (59.3%) intermediate risk, 15 (27.8%) high risk while 5 (9.2%) were not classificable. 30/54 patients (55.5%) had ≥ 2 comorbidities requiring concomitant therapies: according to ECOG scale, performance status at baseline was 0 – 1 in 46 patients (85.1%) and 2 in 8 patients (14.9%).

Results: Median interval from diagnosis to dasatinib start was 23 days (IQR 14 – 35). Dasatinib starting dose was 140 mg/day in 1 patient (1.8%), 100 mg/day in 40 patients (74.1%) and<100 mg/day in 13 patients (24.1%), respectively. After a median period of treatment of 13.7 months (IQR 6.4 – 20,6) all patients were evaluable for toxicity; on the whole, grade 3 – 4 hematological and extra-hematological toxicities were reported in 5 (9.2%) and 9 (16.6%) patients, respectively. Overall, 9 patients (16.6%) permanently discontinued dasatinib due to toxicity (2 patients in the first 3-month period of treatment and 7 beyond that period). Pleural effusions of all WHO grades occurred in 10 patients (18.5%): in 4 of them the pleural effusion occurred during the first 3-month period of treatment. As to treatment efficacy, 5 patients were considered too early to be evaluated (<3 months of treatment) and 49 were evaluable for cumulative response; on the whole, 45/49 patients (91.8%) achieved complete cytogenetic response (CCyR) and 33/49 (67.3%) also a major molecular

response (MMolR). Response to treatment at different time-points is shown on Table 1. After a median period of observation from dasatinib initiation of 15,5 months (IQR 9.2 – 24.7), only 1 patient died from unrelated cause (senectus) while in MMolR: cumulative event-free survival (EFS) at 12 months was 82.4% (95%CI 71.3 – 93.5).

Table 1.

	3rd month	6th month	12th month
Not evaluable:	12	15	16
Too early	5	6	16
Not performed	7	9	0
Evaluable	42	39	38
Discontinuation	2 (4.7%)	4 (10.2%)	7 (18.4%)
Less than CCyR	6 (14,3%)	2 (5.1%)	2 (5.2%)
CCyR only	21 (50.0%)	10 (25.7%)	9 (23.6%)
MMolR	13 (31.0%)	23 (59.0%)	20 (52.6%)

Summary and Conclusions: Present data shows that dasatinib could have a major role in the treatment of unselected patients aged >65 years; indeed, dasatinib seems very effective and has a favourable safety profile also in elderly subjects with comorbidities.

PB1763

QUALITY OF LIVE IN PATIENTS WITH CML: QUESTIONNAIRE BASED STUDY

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Background: With imatinib therapy, the survival of patients with CML has improved dramatically. In recent years, second generation TKI (2G TKI) led to more increased responses. Despite the demonstrated superiority of efficacy and toxicities as previous treatments, several adverse events of TKIs remain. Because CML treatment is lifelong, it is important for patients to maintain adherent to treatment. Correlations between poor adherence to TKI treatment and adverse events and quality of life (QoL) have been demonstrated among CML patients in clinical settings. The FACT-leu is a validated tool that measures leukemia-specific and more general QoL concerns. However, there are few studies about QoL of CML patients in Korea.
Aims: Therefore, we evaluated the QoL of CML patients treated with TKIs.
Methods: The CML working party of the Korean society of Hematology was produced by the questionnaire included the questions of FACT-leu. We distributed the questionnaire over the online or offline. QoL of patients with CML was analyzed using these questionnaires.
Results: A total of 384 patients responded to the survey between March 2014 and June 2014. Patients with high compliance of TKI (taking 90% or more drugs) were 88.28% and lower compliance (taking 70% or less drugs) were 1.82%. In queries about poor adherence to TKI therapy, most common cause was forgetfulness (18.49%). FACT-Leu scale was higher in patients with 2G TKIs than imatinib (122.9±22.65 vs 116.0±25.00 (P=0.005)). Female, older patients showed meaningful worse QoL in FACT-leu scale.
Summary and Conclusions: In 2G TKI era, QoL of CML patients seemed that is better than before. Additional prospective studies are warranted using adequate measuring tool of QoL.

PB1764

SAFETY AND USEFULNESS OF REDUCING THE DOSE OF IMATINIB TO 300 MG IN CHRONIC MYELOID LEUKEMIA (CML) PATIENTS WITH SUSTAINED DEEP MOLECULAR RESPONSE.

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Background: Tyrosine kinase inhibitors have dramatically changed the treatment of CML and nowadays most of the patients are able to achive sustained deep molecular responses in this setting. This scenario has led to an increasing interest on therapy discontinuation studies but this strategy is not recommended outside clinical trials
Aims: We have reduced the dose of Imatinib (IM) from the standard 400 to 300 mg/day, in some patients with deep and stable deep responses in order to improve tolerability and costs of the treatment. In this analysis we review whether this strategy was safe and clinically useful.
Methods: Patients with chronic phase CML that decreased IM 400 to 300 mg/day at least during 3 months and who had molecular responses analyzed before and after the change were retrospectively selected from our database. BCR-ABL ratio was measured by automated means (GeneXpert, Cepheid, CA, USA) according to the International-Scale. Hematological parameters such as hemoglobin level and mean corpuscular volume were also analyzed before and after the IM dose change. Data were analyzed using SPSS v11.0.

Table 1. Patient's responseti IM 300MG/DAY (N=13).

RESPONSE BEFORE DOSE REDUCTION	MONTHS IN UNDETECTABLE DEEP RESPONSE BEFORE DOSE REDUCTION	CAUSE FOR DOSE REDUCTION	IMPROVED TOXICITY	WORST RESPONSE AFTER DOSE REDUCTION	RESPONSE AT LAST FOLLOW UP	MONTHS WITH IM 300MG
≥RM4.5 with UD[a]	14	≥RM4.5 with UD	NA[c]	≥RM4.5 with UD	≥RM4.5 with UD	12
≥RM4.5 with UD	44	≥RM4.5 with UD	NA	≥RM4.5 with UD	≥RM4.5 with UD	4
≥RM4.5 with UD	12	≥RM4.5 with UD	NA	≥RM4.5 with UD	≥RM4.5 with UD	11
≥RM4.5 with UD	33	≥RM4.5 with UD	NA	≥RM4.5 with UD	≥RM4.5 with UD	5
≥RM4.5 with UD	26	≥RM4.5 with UD	NA	RM4with DD[b]	RM4and UD	19
≥RM4.5 with UD	17	≥RM4.5 with UD	NA	≥RM4.5 with UD	≥RM4.5 with UD	9
≥RM4.5 with UD	25	≥RM4.5 with UD	NA	≥RM4.5 with UD	RMC4.5	12
RM4 and UD[a]	15	Pancytopenia	YES	MMR	RM4and UD	50
RM4 and UD	0	Pancytopenia	YES	MMR	≥RM4.5 with UD	34
CCR[a]	0	Heart failure	YES	MMR	RM4with DD	24
MMR[a]	0	Neutropenia	YES	≥RM4.5 with UD	≥RM4.5 with UD	117
≥RM4.5 with UD	11	Neutropenia	YES	≥RM4.5 with UD	RM4and UD	30
RM4.5 with DD[a]	0	Limb Edema	YES	≥RM4.5 with UD	Exitus[a]	7

[a]≥RM4.5 with UD : At least RM4.5 and undetectable disease. [a]RM4 and UD: RM4 and undetectable disease. [a]CCR: Complete cytogenetic response. [a]MMR: Major molecular response. [a]RM4.5 with DD: RM4.5 with detectable disease. [a]RM4 with DD: RM4 with detectable disease. [c]NA=Not applicable. [a] Exitus= not CML related.

Results: A total of 13 patients (7 males, 6 females) with chronic-phase CML diagnosed between 1998 and 2012 were included in the present study. Median age at diagnosis was 67 years (range 23-78). According to Sokal score, 3 were high risk, 4 intermediate and 6 low; according to EURO score, 1 was high risk, 6 intermediate and 6 low; according to EUTOS score, all were low risk. First line treatment was IM 400 mg in 10 (76.9%), IM 600 mg in 1 (7.1%) and Interferon-alpha in 2 (16%). The reason for dose reduction was toxicity in 6 patients (46%): neutropenia (2), pancytopenia (2), limb edema (1) and heart failure (1). In all of them the adverse event improved after lowering the dose. All patients that reduced the dose due to toxicity were at least in CCR. In the other 7 patients (54%) the dose was reduced because of sustained deep response; RM4.5 or better and undetectable disease (≥RM4.5 with UD). Median time in ≥RM4.5 with UD, prior to dose reduction was 24.4 months (range 12-33; Table 1). Median time of treatment with 300 mg/day was 12 months (range 4-117). Comparing molecular response before dose reduction and at last follow up, 3 patients improved and 10 maintained it; if we compared molecular response before the dose reduction and the worst response during follow up, just 3 patients worsened (2 from RM4 and UD to MMR, and 1 from RM4.5 and UD to RM4 with detectable disease), but none of this changes were clinically relevant. The median hemoglobin increase was 1.2 g/dl (range 0.4-2.3; P=0,054). The median decrease in mean corpuscular volume was 3.7 fL (range 0.4-11.5; P=0,006) (Wilcoxon Test). With this univariate analysis we cannot rule out that other concurrent causes could contribute to this hematological change. Additionally, there was an economical benefit derived from the dose reduction, since 334 accumulated months of treatment with IM 300 mg/day, produced savings of 357.046 Euros (savings for 13 patients would be of 166.764 Euros per year). Out of the 13 patients, 12 continue with 300 mg, and one patient died due to causes not related to CML
Summary and Conclusions: In our experience IM dose reduction due to medical decision or toxicity in patients with sustained deep response has been safe, clinically useful and economically beneficial. This could be a useful strategy in some patients with deep responses until treatment discontinuation could be recommended in common clinical practice.

PB1765

INFLUENCE OF CYP3A5 AND SLCO1 ON IMATINIB RESPONSE AMONG EGYPTIAN PATIENTS WITH CHRONIC PHASE CHRONIC MYELOID LEUKEMIA.

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Background: Imatinib (IM) was approved as a molecular target drug that selec-

tively inhibits Bcr-Abl tyrosine kinase which causes Philadelphia-positive chronic myeloid leukemia (CML) and so far been the first-choice treatment in CML with excellent results. However, only a proportion of patients achieve major molecular response. Hence, the need to find whether there are some factors that affect the response to treatment is essential. Although IM pharmacokinetics is influenced by several enzymes and transporters, little is known about the role of pharmacogenetic variation in IM metabolism.
Aims: This study aimed to investigate the frequencies of mutational status of CYP3A5 and SLCO1 in CML patients undergoing imatinib treatment and to determine whether these two genes could predict the response to imatinib therapy in CML patients.
Methods: We investigated the mutational status of SLCO1 and CYP3A5 by Polymerase Chain Reaction followed by restricted fragment length polymorphism in 62 Philadelphia positive newly diagnosed Egyptian CML patients in chronic phase. All patients received imatinib therapy and were followed for at least one and half years. The response to imatinib therapy was evaluated by recording the hematological response, cytogenetic response at 6 month, and molecular response at 12 month of imatinib treatment according to the European Leukemia Net criteria.
Results: 11 patients were excluded from the study as they showed treatment failure to imatinib at 6 month of treatment so they were shifted to 2nd line TKIs. Six (54.5%) out of these 11 patients were mutant for CYP3A5 while five were wild, however four (36.3%) out of them were mutant for SLCO1 and seven patients were wild type. The remaining 51 patients who continued in the study showed that the mutant CYP3A5 was more common with low hemoglobin level (P 0.03) and low platelets count at diagnosis (P 0.001) however, there was no relation between SLCO1 mutational state and patients characteristics. Also there was no relation between mutational state of either CYP3A5 or SLCO1 with the sokal score of the patients at diagnosis. Patients with mutant CYP3A5 showed suboptimal cytogenetic response to imatinib at 6 month *versus* patients with wild type (P 0.07). At 12 month of imatinib treatment, 74.1% of patients with mutant CYP3A5 had no MMR *versus* 66.7% of patients with wild type had MMR (P 0.004). However there was no significant relation between mutational state of SLCO1 with neither cytogenetic response at 6 month nor molecular response at 12 month of imatinib treatment.
Summary and Conclusions: CYP3A5 mutant gene was associated with poor imatinib efficacy while the SLCO1 was not associated with the response to imatinib treatment in Egyptian patients with CML in chronic phase.

PB1766

IS GENERIC MESILATE OF IMATINIB LESS INFERIOR THAN GLIVEC? ANALISYS IN BRAZILIAN COHORT
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Background: The improvement of the treatment for LMC patients after the introduction of tyrosine kinase inhibitor (TKI) Imatinib (Glivec®, Novartis) speaks for itself. After the developed of Glivec, others TKIs became available on the market. These new drugs, however, come to market with prices to astronomical levels. The introduction of therapies with generic drugs is extremely relevant. In Brazil, mesilate of imatinib (IM) become available in a generic formulation (gIM) by January 2014 with a productive development partnership signed Farmanguinhos/FIOCRUZ, Vital Brazil Institute and Cristália Pharmaceutical Chemicals.
Aims: The objective of this study was analyzed our Brazilian CML patients cohort and evaluate the molecular response of these patients before and after the exchange of the drug (Glivec vs. gIM) and monitoring a small group of CML patients considering only gIM as first line therapy.
Methods: We analyzed and compared two different groups. The first one has 171 patients who start with Glivec as first line therapy and followed them between 2001 and 2014. All these patients switched to gIM in 2014. The second group has 11 patients who started the treatment with gIM in 2014. For those, the follow-up was 12 months.
Results: The median time follow-up for patients whom start Glivec was 5.4 years (1.1 – 13.8), while on the other group was 0.4 (0.3 – 1) years. We observed a slight predominance of female on the Glivec first line therapy cohort. Most of the patients (96.8%) were diagnosed in chronic phase in both group; 96.8% and 92.3% respectively. Considering the group who start with Glivec as front line therapy before switch to generic, 9.9% (17/171) of the patients lose MR during the treatment. One of them presented a L452I mutation. In spite of that, they still switch to gIM. Among 17 patients, 5 (29.4%) gain MR after switch to generic IM. At this time, these 17 patients are excluded from subsequent analyzes. After switch to gIM, 5.8% of the patients (9/154) lost MMR response or more. Three of them acquire a mutation, 1 patient at 7 months and the other 2 patients, 3 months after change the treatment: L273M, L387M and F359V. L387M mutation was already described as sensitive to IM. F359V-mutated cell lines demonstrated decreased sensitivity to imatinib compared with CML cell lines wild type. One patient present E453A mutation before change the drug. The median time to lose response was 7 months. Only 3.3% (5/154) of the patients gain MR after change to generic IM. We had 11 patients in our cohort who star the treatment with generic IM. Taking account the 3 months cutoff, 72.7% (8/11) of the patients presented levels ≤10% BCR-ABL1 transcripts and 5 of these 8 patients (62.5%; 5/8) achieved MMR at 12 months. None of them discontinuous the treatment.
Summary and Conclusions: In our patient cohort of "good responders", taking into account that all of the patients who had to switch to second generation TKIs were excluded from this study, the generics were at least non inferior to the original drug in terms of maintenance of response state, efficacy and tolerability when used in the upfront setting, as well as when used subsequently. Prospective studies are needed to address the effectiveness of generic IM in CML patients.

PB1767

A POSSIBLE CONNECTION BETWEEN CIRCULATING 25-HYDROXY VITAMIN D AND MOLECULAR RESPONSE IN CHRONIC MYELOID LEUKEMIA
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Background: Vitamin D deficiency is a common problem all over the world. Growing evidence suggest that there is connection vitamin D and cardio-metabolic disease and cancer. Low-circulating 25-hydroxy vitamin D (25-OH-D) has been associated with various malignancies, although it remains unclear whether vitamin D deficiency exerts as a causal effect on the development of malignancies.
Aims: In this study we aim to evaluate whether there is link between circulating 25-OH-D levels and molecular response in chronic myeloid leukemia (CML)
Methods: Subjects : A total of 61 patients with CML (31 women, 30 men) were recruited in this cross-sectional study from hematology clinics of Katip Celebi University Ataturk Training and Research Hospital between June 2014 and September 2014.The study was approved by the human research ethics committee of the hospital and informed consent was obtained from all participants. Vitamin D status as defined; severe deficiency ≤10 ng/mL; deficiency 10-20 ng/mL; insufficiency 20-30 ng/mL; sufficiency ≥30 ng/mL (1). Treatment responses were evaluated according to the ELN recommendations. Statistical: All analyses were performed using the Statistical Package for the Social Sciences Software, version 18.0 (SPSS Inc., Chicago, USA). In view of the subjects number for each groups, patients were divided into two groups according to their vitamin D levels (group 1 ≤10 ng/mL, group 2 >10 ng/mL) and two groups were compared with clinic and laboratory feature using Mann-Whitney U test. Among the groups difference in gender distribution was analyzed byx2 analysis. To evaluate vitamin D levels on molecular response, we used binary logistic regression analysis. In the model, molecular response was defined as a dependent variable and subjects were categorized to their vitamin D levels; ≤10 ng/mL an >10 ng/mL and also which were added the analysis as a categorical variable. To clarify effect of potential confounders; sokal score, gender and type of treatment, we added these variables in the model as a consecutively. All reported confidence interval (CI) values are calculated at the 95% level. A two-sided p value of<0.05 was considered statistically significant.
Results: According to the vitamin D levels; 37 (60.7%) patients had ≤10 ng/mL, 19 (31.1%) patients had 10-20 ng/mL and 5 (8.2%) patients had 20-30 ng/mL. In our study population no subjects had sufficient vitamin D levels. In unadjusted logistic regression models, increased vitamin D levels were significantly associated with molecular response. After adjusting potential confounders, this association was slightly diminished but it is still remained
Summary and Conclusions: In present study, we demonstrated for the first time that lower circulating 25-OH-D levels were independently associated with molecular unresponsive in subjects with chronic myeloid leukemia after adjusting for potential confounders.There are some limitations to the current study. The size of study population was relatively small and the cross-sectional design of the study cannot prove causality. These findings are initial and provoking, but this association should be verified in another larger population using a prospective-cohort design. Taken together, our results indicate for the first time that decreased circulating 25-OH-D levels were associated with molecular unresponsive in patients with CML. 25-OH-D may contribute to molecular response in the patients

PB1768

ANALYSIS OF MINIMAL RESIDUAL DISEASE AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT) FOR CHRONIC MYELOID LEUKEMIA (CML) BY REAL-TIME QPCR FOR BCR-ABL GENE AND ITS CORRELATION WITH RELAPSE
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Background: hronic myeloid leukemia (CML) is a clonal bone marrow disease characterized by the presence of the Philadelphia chromosome, resulting from translocation between chromosomes 9 and 22 producing the BCR-ABL hybrid gene., which encodes tyrosine kinase proteins and regulate cell growth. The development of targeted therapies for the treatment of this disease, such as imatinib mesylate, changed the landscape treatment of CML. Hematopoietic stem cells transplantation (HSCT) still remains the only curative modality of therapy. After HSCT, relapse can be observed even very late. The amount of leukemic cells in relapse (molecular, cytogenetic or hematologic) and relapse phase are the main prognostic factors found, and the response to the interventions (infusion of donor lymphocytes or imatinib mesylate) are best when relapse is identified early. The use of real-time PCR for the detection of BCR-ABL gene for monitoring of recurrence in patients after HSCT shows a good alternative of tracking disease, however cut-offs which would define relapse are not yet established in the literature. Besides, there have been recent changes in the PCR methodology including the padronization of an international scale, used as monitorization for TKI therapy. Few studies evaluated this method for monitoring patients after HSCT.
Aims: The main objective of this study is assessing the significance of the results of real-time PCR for quantification of BCR-ABL gene in patients previously diagnosed with CML who underwent HSCT. The intention is to analyze if the results of this test are reliable predictors of the risk of disease recurrence and what is the range of values that is considered at increased risk for relapse, which would allow early intervention.
Methods: We retrieved databases and records of 57 patients diagnosed with CML who were treated with HSCT from 2003 in the HC-UFPR. Type of relapse (hematology or cytogenetic) and correlation with BCR-ABL values obtained by real-time PCR, is the primary endpoint.
Results: We identified 57 patients which received HSCT from 2003-2014. All them had been molecularly monitored by Real Time Q-PCR and have more than one value measured. 21 patients had positive Q-PCR results. From those, 17 had persistent positive results, and 12 of them ultimately relapsed. Four patients had intermitent low values of BCR-ABL and so far no one of them relapsed. No patient with persistent negative brc-abl relapsed. IS ratio of patients who relapsed were significantly higher than those who didn't relapse, which had IS showing 2-3 log reduction. Patients with intermitent low values of BCR-ABL had IS showing 3 log reduction altenating with negative results.
Summary and Conclusions: Although there were changes in BRC-ABL techniques, real time Q PCR can be used for monitorization of relapse after transplant. Persistent positive results highly correlated with relapse within the present cohort of patients. However, low levels aternated with negative results did not resulted in a higher rate of relapse. Patients with persistent negative measuses did not relapse at all.

PB1769

MONITORING CLINICAL CARDIOLOGY WITH TRANSTHORACIC ECHOCARDIOGRAPHY AND ELECTROCARDIOGRAPHY IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA TREATED WITH TYROSINE KINASE INHIBITORS SECOND GENERATION

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Background: Long term prognosis of patients with chronic myeloid leukemia (CML) has significantly improved during the "age of tyrosine kinase inhibitors", though the occurrence of adverse events on medium and long term in patients with chronic comorbidities, of which cardiopulmonary and metabolic diseases must be routinely evaluated, has become a clinical concern albeit the cardiac safety profile has been prospectively investigated. During the phase II and III clinical trials of both first and second-line therapy, we reported our experience in cardiologic monitoring of patients with CML treated with second-generation TKI (nilotinib and dasatinib) on second-line therapy.
Aims: Identify clinical, electrocardiographic and transthoracic echocardiogram alterations in CML patients treated with nilotinib or dasatinib.
Methods: Clinical evaluation, electrocardiograms and transthoracic echocardiogram were performed to all CML patients on treatment with nilotinib or dasatinib during the period between October 2010 and January 2015, at the beginning of treatment and every 6 and 12 months after that according to the occurrence or not of abnormalities detected at baseline. The exclusion criteria were; clinically significant cardiac disease (congestive heart failure), left ventricular ejection fraction (LVEF) < 45%, past myocardial infarction or unstable angina (during the last 12 months), resting bradycardia (< 50 beats/min), left bundle branch block, ventricular bypass, acquired or congenital long QT syndrome (including family history), QTcF interval >450 msec, patients taking medications that could extend the QT interval and those who could not discontinue their treatment before initiating nilotinib or dasatinib. The doses used of tyrosine kinase inhibitors were 400 mg every 12 hours for nilotinib and 100 mg every 24 hours for dasatinib per os.

Results: Baseline characteristics and electrocardiogram and transthoracic echocardiogram abnormalities are shown on table 1, along with the identified changes in the monitoring during treatment of the clinical, electrocardiogram and transthoracic abnormalities recorded.

Table 1.

Baseline characteristics		
	Nilotinib 83 (100%)	Dasatinib 51 (100%)
Male, n	54 (55%)	29(57%)
Age, years median (range)	47.9 (17-79)	42
Older than 60 years	24 (29%)	7 (13.7%)
Congestive heart failure	2 (2.4%)	1 (1.9%)
Ischemic cardiac disease	1 (1.2%)	0 (0%)
Cardiopulmonary comorbidities	2 (5.40%)	2 (7.14%)
Systemic arterial hypertension	9 (11%)	3 (6%)
Type 2 diabetes mellitus	5 (6%)	4 (7.8%)
Hypercholesterolemia	0	0
Hypertriglyceridemia	0	1 (2%)
QTc higher than 480 ms	0 (0%)	0 (0%)
QTc higher than 500 ms	0 (0%)	0 (0%)
LV diastolic dysfunction	26(31.32%)	17 (33.33%)
LVEF higher than 55%	83 (100%)	48 (94.11%)
Pericarditis without effusion	10 (12%)	3 (6%)
PAH	3 (3.6%)	5 (10%)
PHA slight/mild/severe, n	2(2.4%)/1(1.2%)/0	3(6%)/1(2%)/1(2%)
Mitral minimum insufficiencies	4(5%)	1(2%)
Follow up monitoring		
	Nilotinib 56 (100%)	Dasatinib 46 (100%)
# follow up transthoracic echocardiogram	130 (1-8)	77 (2-6)
# follow up electrocardiograms	185 (1-6)	105 (2-7)
Congestive heart failure	2 (2.4%)	1 (1.9%)
Ischemic cardiac disease	1 (1.2%)	0 (0%)
Cardiopulmonary comorbidities	2 (5.40%)	2 (7.14%)
Systemic arterial hypertension	4 (7%)	3 (6%)
Type 2 diabetes mellitus	6 (11%)	3 (6%)
Hypercholesterolemia	7 (12.5%)	0
Hypertriglyceridemia	14 (25%)	1 (2%)
QTc higher than 480 ms	0 (0%)	0 (0%)
QTc higher than 500 ms	0 (0%)	0 (0%)
LV diastolic dysfunction	27 (48%)	7 (15%)
LVEF decrease FEVI (≥10%)/FEVI normal	4 (7%)	8 (17%)
PAH	6 (10.71%)	8 (16%)
PAH slight/mild/severe	2(3.5%)/3(5%)/1(1.8%)	4(8%)/2(4%)/2(4%)

Summary and Conclusions: The cardiac adverse events in patients with CML undergoing treatment with second-generation TKI are not common and may be related to factors independent from treatment like age, cardiopulmonary and metabolic comorbidities and pharmacological interactions. Treatment was discontinued on those patients with recurrent PAH on dasatinib dose adjustment, though there was a temporary suspension in one nilotinib patient due a severe PAH, extended QT syndrome was not observed in any of the patients, however 8 patients undergoing treatment with dasatinib (16%) showed PAH (2 severe, 2 mild, and 4 slight), 5 were temporary suspensions and 2 resulted in definitive suspensions due severe PAH; In our experience the results do not differ significantly from the international publications. To our knowledge is the first report to adverse events in México for PAH secondary to second-generation TKIs.

Hematopoiesis, stem cells and microenvironment

PB1770

EVALUATION OF PLURIPOTENT MARKERS IN CELLS OBTAINED FROM DIFFERENT HUMAN HEMATOPOETIC SOURCES

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Background: Clinical use of hematopoetic stem cells (HSC) is a developing field for treatment of most hemoglobinopaties, hereditary blood diseases and cancers. Even though use of HSC's are practical they are not as effective as pluripotent stem cells (PSC). In last years the thought of possible presence of PSCs in other adult tissues has become an attractive research area for search and isolate of PSCs from adult tissues.
Aims: In our study we refined cell populations using different isolation methods. Cells obtained from three different human hematopoetic sources and analysed for expression of pluripotent markers like NANOG, OCT3/4 and SSEA4.
Methods: Human peripheral blood (PB), apheresis material (AM) and cord blood (CB) were used as hematopoietic sourses. Two isolation method performed to all materials. 1) Total mononuclear cells obtained by eryhtrocyte lysis method. 2) MNC layer and erythrocyte layer were isolated with ficoll density gradient method. The presence of pluripotent markers such as NANOG, OCT3/4 and SSEA4 and their celullar location were detected with immunofluorescence staining in all three layers. Also western blotting method used for determinig NANOG and OCT3/4 protein expression.
Results: The results obtained from western blotting and immunofluorescence methods showed expression of NANOG, OCT3/4 and SSEA4 proteins in PB, AM and CB. And also immunofluorescence images demonstrated cytoplasmic and nuclear presence of these proteins. OCT3/4 protein of immunofluorescence determinations showed cytoplasmic locations in all layers. Futher investigations need to be done for enlighten this protein function in hematopoetic cells because OCT3/4 has different isoforms. NANOG protein showed cytoplasmic location in PB and AM, both cytoplasmic and nucleic location in CB. Western blotting results indicated that NANOG protein create a ladder pattern mostly cause of post-translational modifications. NANOG ladder pattern also changed between layers. These findings showed different layers had different post-translational modifications so different type of NANOG protein.
Summary and Conclusions: As a result, these findings showed that it is necessary to investigate the function of PSC markers in differentiated adult cells. Another conclusion is, among the lysis and ficoll density gradient method, lysis method has the highest cell recovery amount.Consequently, this study provided us new informations and viewpoints about expression of PSC markers in adult tissues.

PB1771

ALTERATIONS IN THE BONE MARROW MICROENVIRONMENT CAN ELICIT DEFECTIVE HEMATOPOIESIS: COMPARISON OF APLASTIC ANEMIA, CHRONIC MYELOID LEUKEMIA AND NORMAL BONE MARROW.

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Background: Normal hematopoiesis involves complex interactions between hematopoietic cells and the bone marrow (BM) microenvironment. Examples of extremes at both ends of dysregulated hematopoiesis are BM failure in aplastic anemia (AA) and myeloproliferative disease such as chronic myeloid leukemia (CML). The specific causes of and mechanisms involved in dysregulated hematopoiesis are unknown.
Aims: To better understand the interactions between hematopoiesis and the BM microenvironment, we investigated changes in the hematopoietic stem cell (HSC) compartment and the BM microenvironment of patients with AA (n=10), CML in chronic phase (n=10) and normal marrow (n=10).
Methods: BM biopsy specimens were analyzed by semiquantitative immunohistochemistry (mean positive cell number/high power field) for osteopontin, osteonectin, osteocalcin, nestin, stromal-derived factor-1 (SDF-1) expression, and to identify endothelial cells, lymphocytes (CD3, CD4, CD8, CD20, CD56), macrophage (CD169), and HSCs (CD34, CD117).
Results: The lowest numbers of HSCs were in AA specimens, and the highest numbers were in CML specimens. The highest numbers of T and B lymphocytes were found in normal control specimens (P<0.01). Natural killer (NK) cells were occasionally observed in the AA specimens, but were absent from the CML and control specimens (P<0.01). Macrophages were absent from the CML specimens, whereas the number of macrophages (P<0.01) was highest in the AA specimens. There were significant differences between the stromal cell components of AA specimens and CML specimens. Numbers of cells positive for osteopontin (P<0.01), SDF-1 (P<0.01) and nestin (P<0.01) expression were higher in the AA than in the CML specimens. No nestin+ cells were observed in CML specimens. Numbers of cells positive for osteocalcin (P<0.01) and osteonectin (P=0.015) expression were higher in CML than in AA specimens. The highest numbers of endothelial cells were in CML specimens (P<0.01).

Table 1.

TABLE 1. Semiquantitative immunohistochemical analysis of bone marrow cells from patients with aplastic anemia, chronic myeloid leukemia and control

Antigen	AA	CML	Control	*P* value*
Hematopoietic stem cells				
CD34	1.36 ± 1.22	14.31 ± 15.44	9.49 ± 3.02	0.013
CD117	2.84 ± 2.06	34.00 ± 21.83	11.17 ± 4.25	<0.01
Lymphocytes and macrophages				
CD3	18.67 ± 12.88	26.28 ± 10.44	70.83 ± 30.70	<0.01
CD4	1.56 ± 1.25	0.74 ± 1.14	9.87 ± 3.65	<0.01
CD8	12.41 ± 9.79	16.96 ± 6.27	41.17 ± 14.11	<0.01
CD20	1.01 ± 1.07	0.00 ± 0.00	7.37 ± 4.98	<0.01
CD56	0.11 ± 0.12	0.00 ± 0.00	0.00 ± 0.00	<0.01
CD169	1.53 ± 0.96	0.00 ± 0.00	1.05 ± 1.07	<0.01
Stromal cell components				
Osteopontin	5.79 ± 2.08	0.10 ± 0.32	4.45 ± 1.11	<0.01
Osteocalcin	113.40 ± 88.54	261.70 ± 323.93	145.20 ± 149.28	0.275
Osteonectin	0.40 ± 0.17	2.13 ± 0.73	2.60 ± 1.10	<0.01
SDF-1	2.96 ± 2.76	0.43 ± 0.49	1.94 ± 0.47	<0.01
Nestin	8.04 ± 8.41	0.00 ± 0.00	3.62 ± 1.21	<0.01
Endothelial cells	39.20 ± 21.46	186.90 ± 140.95	18.00 ± 7.64	<0.01

Values are expressed as mean ± standard deviation.

*Analysis of variance (ANOVA) was used to test for differences between the 3 types of bone marrow specimens.

AA, aplastic anemia; CML, chronic myeloid leukemia; SDF-1, stromal-derived factor-1

Summary and Conclusions: The BM of AA patients demonstrated increased numbers of macrophages and NK cells, which could result in cytotoxic and/or immune-mediated marrow damage; or, alternatively, be a consequence of HSC injury. The BM of CML patients demonstrated decreased numbers of SDF-1-expressing and absence of nestin-expressing cells, which may result in reduced homing of HSCs and proliferation of malignant hematopoietic cells. There were significant differences between AA and CML specimens in the numbers of cells expressing osteoblast-derived extracellular proteins such as osteopontin, osteocalcin and osteonectin. Our findings suggest that changes in the components of the BM microenvironment might be related to defective hematopoiesis that may lead to BM failure and/or myeloproliferative disease.

PB1772

ENDOPLASMIC RETICULUM STRESS RESPONSE IN G6PC3 DEFICIENT WHITE BLOOD CELLS

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Background: Severe Congenital Neutropenia type 4 (SCN4) is a rare autosomal recessive disease due to mutations in the glucose-6-phosphatase beta (G6PC3) gene. G6PC3 is a typical metabolic enzyme in the endoplasmic reticulum (ER) suggested hydrolyzing glucose-6-phopshate (G6P) in glucose and phosphate. This enzyme catalyzes the final step of glycogenolysis in non gluconeogenic tissues like neutrophils. In the lumen of ER glucose-6-phosphate can be metabolized by hexose-6-phosphate dehydrogenase (H6PD) as well, which is responsible for ER redox homeostasis. The first diagnosed patient in our country has a non-sense mutation in the first exon at position W73Term causing a lack of G6PC3. Beyond severe inborn neutropenia and consequent inflammatory episodes the phenotype comprises other anomalies including congenital heart defects, urogenital anomalies prominent superficial veins, facial dysmorphism, growth and developmental delay.
Aims: The question arises as to what kind of disturbances can cause a defect of a metabolic enzyme leading to developmental malignancies-beyond affecting neutrophils? Since data are already presented in G6PC3 KO mice, we were interested in the differences or similarities between human and rodent WBC-s.
Methods: Whole blood from healthy and patient with SCN4 was collected and purified obtaining total white blood cell (WBC) fraction. Enzymatic and Western-blot measurements were made on these protein samples.

Results: Neutrophils from G6PC3-deficient WBC-s proved the lack of G6PC3 on Western blot, and also on the enzymatic level. Surprisingly we could not identify any metabolic aberrations in G6PC3-deficient WBC-s. G6PC3 deficient cells showed increased level of Grp78 and phosphorylated eIF2-α compared to the control one. Other ER stress enzymes are still under investigation. Interestingly H6PD was also decreased in the mutant cells vs. control cells.
Summary and Conclusions: Lack of G6P reduction or hydrolysis suggests that endoplasmic reticulum stress can be responsible for increased apoptosis in G6PC3-deficient neutrophils. However, the involvement of the PERK-eIF2α-ATF4 signaling pathway in SCN4 is still unclear, these alterations may at least in part be responsible for the phenotype of G6PC3 deficiency. Our results suggest that trying to maintain the ER redox environment with small molecules, like ascorbic acid, may help in neutrophil granulocyte surviving. We continue our investigations towards this direction. This work was supported by the Hungarian Scientific Research Fund OTKA 101226

PB1773

EXPANSION OF UMBILICAL CORD BLOOD HEMATOPOIETIC STEM CELLS ON BIOCOMPATIBLE NANOFIBER SCAFFOLDS USING CO-CULTRE SYSTEM WITH BONE MARROW DRIVED MESENCHYMAL STEM CELLS

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Background: Hematopoietic stem cell transplantation (HSCT) is a therapeutic approach in treatment of hematological malignancies and inability of the bone marrow. Umbilical cord blood (UCB) known as an alternative for hematopoietic progenitor/stem cells (HPSC) for in allogeneic transplantation. The advantage of this source of HPSC is the low aptness of GVHD due to low number of T lymphocytes. The main hindrance in application of HPSC derived from umbilical cord blood is the low number of stem cells in the cord blood. So, ex vivo expansion of HSCs is the useful approach to overcome this restriction.
Aims: The aim of using this systems is to produce appropriate amount of hematopoietic stem cells, which have the ability of transplantation and long term hematopoiesis.

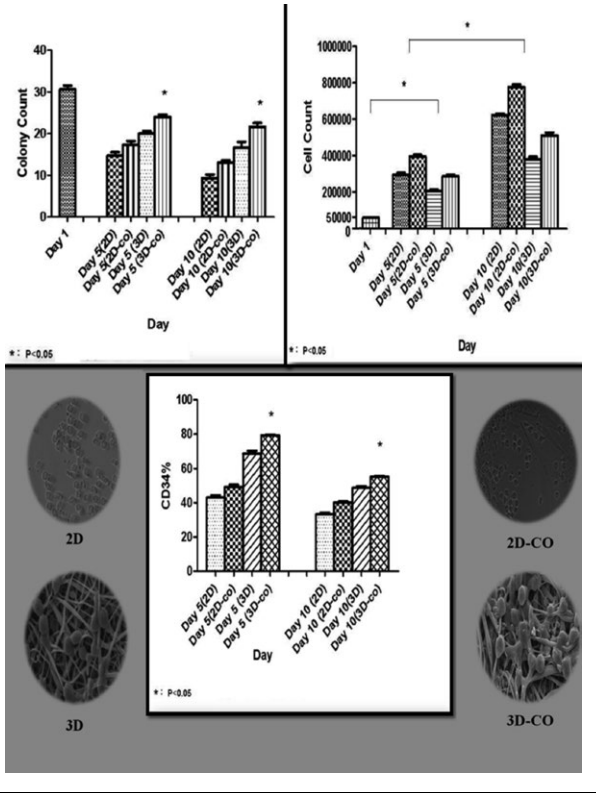

Figure 1.

Methods: The routine system of hematopoietic stem cells expansion is using expansion cytokine cocktail including, SCF, TPO and FLT3L in 2- Dimensional (2D) microenvironment. In this microenvironment cells only affected by cytokines and there is not any influence of cell – cell interactions. Synthetic biomaterials such as nanofibers based on Polyethersulfone(PES) is used to produce synthetic niches. These 3D structures leads to activation of adhesion, proliferation and differentiation of CD34+cells with highly similarity to bone marrow microenvironment. Considering the important role of mesenchymal cell lineages in the bone marrow hematopoietic stem cells fate through the production of secretory factors andcell-cell interactions, in this study we used mesenchymal stem cells seeded on the bottom surface of the nanofiber scaffolds to provide their supportive role in hematopoietic stem cell proliferation during the three-dimensional culture system.Accordingly, we set up four culture systems consist of two-dimensional (2D), three-dimensional (3D),two dimensional + co-cultured by mesenchymal stem cells (2D-CO) and three-dimensional + co-culture by mesenchymal stem cells(3D-CO). We sorted CD34+ cell by MACS technology and the cells were cultured in StemSpan serum-free medium containing SCF+TPO+Flt3L.
Results: The results of this study showed that coculture of HPSC with MSC in three dimensional scaffold could able to expand of CD34 cells with minimal differentiation in comparison to other systems.
Summary and Conclusions: Establishing a cell culture system with maximum similarity to the bone marrow microenvironment lead to improve culture conditions and proliferation of hematopoietic stem cells. this include three-dimensional (3D) nanofiber scaffold system and use of chemical molecules existing in matrix of bone marrow.also given important role of mesenchymal stem cells in fate of hematopoietic stem cells,using this cell resulted improved proliferation of hematopoietic stem cells.it is recommended to additional studies for more investigate the required characteristics of stem cells for bone marrow transplantation and evaluate enhanced abilities of this system.One of the challenges in this system is the harvesting hematopoietic stem cells amidst of of fiber scaffolds and mesenchymal stem cells.To overcome this problem, use of biodegradable fibers in future studies is recommended.

PB1774

Abstract withdrawn

PB1775

EFFECTS OF STORAGE IN PARAMETERS OF FULL BLOOD COUNT (FBC)

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Background: It is common knowledge that the FBC has to be analyzed as soon as possible to get an accurate result. However due to the heavy workload and the increase demand for FBC, a delay before analysis is seen many times. Therefore it is vital to know the storage effects that are likely to affect the FBC.
Aims: FBC is a laboratory test which is routinely done as one of the first steps in the diagnostic workup. There is often a post collection interval before analysis of results where external factors such as storage temperature, and the duration of sample storage can affect the results. This study analyses the storage effects of components of FBC at different temperatures over time.
Methods: A descriptive cross sectional study was conducted in the laboratory of CSTH. K_2EDTA blood samples were randomly taken from 102 apparently healthy individuals aged between 20-70 years. Baseline measurements were analyzed within 30 min of collection through a fully automated haematology analyzer Sysmex SX 500i. Samples were divided into 3 portions and each of them was kept at 4±2^{o}C, 23±2^{o}C and 31±2^{o}C up to 48hrs. All were repeatedly analyzed after 6hrs, 24hrs, and 48hrs. Statistical analysis was conducted using SPSS 17.O.
Results: Among parameters of the FBC, red cell count and haemoglobin were stable at 4^{0} C, 23±2^{o}C and 31±2^{o}C throughout the study (48 hrs).White blood cell count (WBC) was increased at 31±2^{o}C and 23±2^{o}C. Machine refused to read the differential count of some samples after 24hr of storage. Neutrophil and Basophil counts were significantly increased with time throughout the study in all temperatures. Storage caused a significant decline in platelet count and an increment in MPV at all temperatures after 6hr. (P<0.05). At 4^{0}C MCV and RDW were decreased whereas it was significantly increased at RT and 23±2^{o}C.
Summary and Conclusions: In order to prevent variability in FBC parameters, it is preferred to analyze the blood sample as soon as possible. For an accurate platelet count, samples should be analyzed within 6hr. Haemoglobin remains stable at any temperature even at 48 hr. Neutrophil count keeps increasing with storage time at different temperatures. Differential counts were rejected by the machine in most cases after 1day of storage.

Hodgkin lymphoma - Clinical

PB1776

CLINICAL OUTCOME OF VERY REFRACTORY HODGKIN'S LYMPHOMA

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Background: Although Hodgkin's lymphoma (HL) is largely curable with first-line therapy, approximately one-third of patients will not have a complete response to frontline treatment or will subsequently relapse. Only in 50% of these patients will effective salvage be achieved with conventional therapies. The prognosis is particularly poor for those patients who are refractory or relapse following an autologous stem cell transplant (ASCT). Reduced-intensity conditioning (RIC) is increasingly used as a potentially curative option.
Aims: We performed a retrospective analysis of 25 adult patients (15 women/10 men) with HL, who relapsed within 12 months or had progressive disease after ASCT, in order to analyze clinical outcome.
Methods: Disease status at ASCT was complete remission in 7 patients (28%), sensitive disease in 14 (56%), and refractory disease in 4 (16%). Median time from ASCT to relapse was 7 months (range 0.5-11); 12 patients (48%) had stage 3-4 at relapse/progression. B symptoms were present in 40% of the patients, bulky disease in 20% and extranodal involvement in 56%. Treatments following relapse/progression were the standard BEACOPP regimen in 10 patients (40%), 2nd ASCT in 5 (20%), brentuximab vedotin without further therapy in 2 (8%) and RIC in 8 (32%); the therapeutic regimens used as a "bridge" to RIC were brentuximab vedotin in 6 (24%) and bendamustine in 2 (8%).
Results: Overall response rate was 64%, including 36% complete remission and 28% partial response. Progression occurred in 24% and 12% had stable disease. No difference in response rate was found among the treatment regimens. RIC was administered in 8 patients (4 in complete and 4 in partial remission). Estimated overall survival at 40 months was 72% for the whole population, 88% for patients who underwent RIC and 58% for those who did not. Estimated progression-free survival at 40 months was 50%; 62% for the RIC group and 42% for the non RIC group. After a median follow-up of 36 months (range 3-84), 18 of the 25 patients are alive (72%) and 11 (44%) still in complete remission (45% of them underwent RIC).
Summary and Conclusions: Patients with HL who fail ASCT have a very poor prognosis, and the goal of treatment should be to ultimately refer them to RIC. Even if several questions are still open, this approach should be proposed for these poor prognosis patients. Brentuximab vedotin is useful in order to reduce the disease burden before RIC.

PB1777

THERAPEUTIC RESULTS OF TUNISIAN NATIONAL HODGKIN LYMPHOMA PROTOCOL :MDH2008 UNICENTRIC EXPERIENCE ABOUT 71 PATIENTS

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Background: Hodgkin lymphoma(HL) is a curable hemopathy. Several groups have established management protocols for this disease. The Tunisian group has adopted a third version of a national consensus: MDH2008
Aims: assessment of therapeutic response of hogkin lymphoma treated according the national protocol HL 2008.
Methods: Between Mai 2009 and decembre 2013,71 patients were enrolled in HL-2008 protocol (a third version of prospective trial) at the haematology department of Hedi Chaker hospital (Sfax). HL-2008 defined 5groups : G1 : favorable early stage, G2 : unfavorable early stage, G3 : advanced stage and localised stage with mediastinal bulk(MTI>0.35), G4 : early stage in elderly patients, G5 : advanced stage in elderly group. We recommanded 2 cycles ABVD+radiotherapy (IFRT) for G1, 4 cycles of ABVD + IFRT for G2, 8cycles of BEACOPP (4 escalated+ 4 baseline) for G3. Elderly groups recieved 6 and 8 cycles of ABVD for G4 and G5 resectively. Early assessment of response was recommanded after 2 cycles in each group and an escalation to BEACOPP-R took place if response was less than 75%. The date of point is in january 2015. Our study determine therapeutic response, the overall survival (OS), event free survival (EFS), relapse free survival (RFS) and factors forecast which influenced these survivals.
Results: Median age was 33 years (range16-82) with sex ratio=0.87. 62% of patients were treated in G3, 21% in G2, 7% in G5, 6% in G1 and 4% in G4. 86% of patients were in complete response after the first line therapy. 7% of patients were refractory. OS, EFS, RFS were respectively 91%, 82% and 92%. 5 relapse were noted. There were grade 3-4 hematological toxicities only in patients treated with BEACOPP. 3 toxic deaths were noted 1 with escalated BEACOPP and two with ABVD treating elderly(G5). In univariante study, chemosensivity (2cure) and therapeutic response at the end of treatment were 2 predictive prognostic factors for OS (P=0.01, P<0.001), EFS (P<0.001, P<0.001). Bulky mediastinal disease was a significant adverse prognostic factor for the EFS and the RFS (P=0.03, P=0.009).
Summary and Conclusions: Comparatively to the second version, intensive chemotherapy for unfavorable HL(advanced stage and bulky medaistinal disease with stage II), RFS and OS are better.

PB1778

SECOND NEOPLASMS IN LONG SURVIVORS OF HODGKIN'S LYMPHOMA: A RETROSPECTIVE ANALYSIS IN A SINGLE CENTER

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Background: Prolonged survival in patients with Hodgkin's Lymphoma (HL) seems to be associated with an increased risk of developing secondary malignancies.
Aims: Ours objectives were to describe and analyze incidence of secondary malignancies, to detect predictors and their overall survival.
Methods: Single-center retrospective analysis of patients diagnosed and treated for LH in the period 1975 to 2015, at the General Hospital of Segovia (Spain). Statistical analysis was performed using SPSS[a] (v.15).
Results: 101 patients were analyzed. Median age was 33 years [10-88]. 59.4% were male. 60% had an advanced stage at diagnosis (IIB-IV). The most common histology was nodular sclerosis (n=48). 82.2% patients had a favorable prognosis (Hasenclever ≤2). 87.1% patients were treated with chemotherapy (schedule ABVD the most used). 62.5% of patients received radiotherapy (RT) as first-line therapy. 10 patients only received RT as single therapy. The median follow-up was 88 months [0-422]. 16 second malignancies were detected. Median onset was 10 years [2-26]. Differences in median onset between hematologic malignancies and non-hematologic (4 vs16 years) were observed, but the difference was non stadistical significantly, perhaps due to small sample size. Comparing patients with secondary neoplasia against the global serie, only the stage (P=0.013) and number of deaths showed significant differences (P=0.005). Cumulative incidence (CI) was 35% at 300 months (CI in second malignancies is shown in Figure 1). The median overall survival stimated was 310 months in global serie (patients with second neoplasms: 300 months). Nowadays, 71 patients (70.3%) still alive, but only 6 affected by second neoplasia are alive.

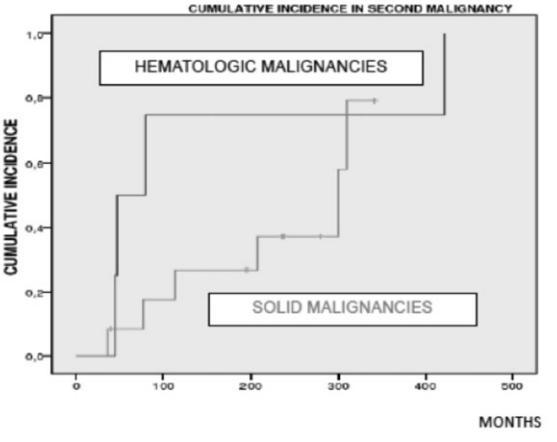

Figure 1.

Summary and Conclusions: HL is considered curable in a high percentage of patients, but carries a incidence of second malignancies in long survivors with an increased risk at 25 years of diagnosis, as happened in our series of a single center with median onset of 16 years. A previous publication (Kg, AK. Blood, 2014) describe an increased risk of hematologic neoplasia in patients who had received chemotherapy with alkylating agents type MOPP plus extensive radiotherapy later or with the combination chemotherapy (ABVD-MOPP). We found similar data in our serie featuring 2 cases who were treated with MOPP and mantle type RT, and other 2 with ABVD-MOPP. Another publication showed higher risk associated with BEACOPP schedule, results against to our findings: None of the 11 patients who received BEACOPP (standard or scalated) developed second neoplasia. In any case, the absence of these neoplasms along the track (last hematologic neoplasia in 1996), may be due to the change in therapeutic schemes and extent of RT (field involved vs extend field). Patients

who developed non-hematological malignancies, 8 were treated with RT (all type Mantle). Therapeutic schemes were different, but in those patients who did not receive RT, all except one received ABVD scheme, no differences in both arms. Appearance of secondary neoplasms in field radiation is to be expected, as shown in our serie; however, there is also an increased risk in patients treated with alkylating agents.
In our retrospective study of single center, stage at diagnosis proved to be a predictor factor of development of second neoplasia related with higher mortality. Therefore, our data resemble previously publications as increase in the CI throughout evolution. Secondary malignancies may be lower with actual therapeutic schemes although longer follow-up would be necessary.

PB1779

ENDOCRINOPATHIES AND SECOND MALIGNANCIES IN LYMPHOMA SURVIVORS: A SINGLE CENTRE EXPERIENCE

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Background: Improvements in the treatment of both Hodgkin's and non Hodgkin's Lymphomas (HL and NHL) have resulted in an increasing number of long term survivors. But this patient's population is at high risk of developing serious late therapy related complications that can negatively affect their lives or lead them to an early death. The most common secondary diseases in the people cured from lymphomas are cardiovascular diseases, second malignancies (mainly solid tumours) and endocrinopathies that in the majority of the cases are thyroid dysfunctions.
Aims: In our institution the HL and aggressive NHL long term survivors are followed up in a dedicated clinic since September 2014. Here we report preliminary data on second malignancies and thyroid dysfunctions.
Methods: We have collected retrospective data on thyropathies and second tumours in 216 lymphoma survivors.
Results: We have analyzed data regarding 216 patients coming in our clinic from 15 September 2014 to 16 February 2015, 119 were affected by HL and 97 by NHL. One hundred fifteen are females, 101 males; median age at observation is 53 (range 22-89). All of them are in complete response for lymphoma for at least 5 years from the completions of curative therapy. Thirty patients (28 females and 2 males) had thyropathies (13.8%),namely hypothyroidism interesting 25 of them and multinodular goiter in 5 cases. Twenty six had a previous HL but only 4 developed endocrinopaties after a NHL. According to lymphoma therapies, 26 (24 HL and 2 NHL) of them had neck and/or mediastinal radiotherapy. They are the 26.5% of all receiving irradiation in the chest and neck; the other 4 did not had radiotherapy. Twenty four patients (11%) experienced a second cancer, 3 of them had 2 neoplasms, so we documented 27 second tumours. They were: 8 breast, 7 skin basocellular, 3 colon and sigma, 3 thyroid, 2 prostatic, 1 lung, 1 bladder carcinoma, 1 testis and 1 cutaneous appendages cancer. Regarding the previous therapies all but 2 the females with breast cancer had undergone to mediastinal radiotherapy; 5 out of 7 with cutaneous cancer developed the lesion in the sites of previous irradiation; 1 out of 3 with thyroid cancer had mantel radiotherapy, the one with lung cancer had MOPP chemotherapy and mantle radiotherapy; no one of the intestinal cancers had abdominal radiotherapy, the one with urinary cancer had abdominal radiotherapy and MOPP/ABVD regimen. Median age of breast cancer in our setting is 50.5 (range 38-70). The median time between diagnosis of lymphoma and diagnosis of second malignancy was 21 years (range 5-41).
Summary and Conclusions: Our data confirm the incidence of thyroid diseases in lymphoma survivors particularly in people with prior HL. In our Department we described also numerous cases of second neoplasms in the lymphoma survivors population. That outline the importance of a plan for early diagnosis of cancers in this setting of patients.

Indolent Non-Hodgkin lymphoma – Clinical

PB1780

CLINICAL AND OUTCOME IN PATIENTS WITH ALK POSITIVE AND NEGATIVE ANAPLASTIC LARGE-CELL LYMPHOMA

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Background: Anaplastic large-cell lymphoma (ALCL) accounts for approximately 3% of non-Hodgkin´s lymphomas (NHL). It is a peripheral T-cell lymphoma characterized by expression of CD30. Currently the WHO recognized two kinds of ALCL based on anaplastic lymphoma kinase (ALK) expression. In previous studies, ALK positive ALCL was associated with younger patients and a better prognosis. The International Peripheral T-Cell Lymphoma Project Study reported a 5 year overall survival of 70% and 49% in ALK positive and ALK negative ALCL respectively.
Aims: To study the clinical characteristics and outcomes of patients with systemic ALK-positive and negative ALCL in our population.
Methods: We retrospectively analyzed 39 consecutive cases of ALCL referred and treated at the Instituto Nacional de Cancerologia, México
Results: A total of 1951 charts of NHL patients from 2008 to 2013 were reviewed. ALCL was confirmed in 49 (2.5%). Ten patients had primary cutaneous anaplastic lymphoma, thus 39 patients were analyzed.
Twelve patients were ALK-positive (33%) and 27 (77%) Alk-negative. Median aged was 26 (17-48) vs 47 years (22-76) respectively (P=0.40). Clinical characteristics are shown in table 1. There were not significance differences between groups. Seventeen patients achieved a CR (44%), 3 (8%) partial responses, 5(13%) had refractory /relapsed disease and 14 patients (36%) could not be evaluated for response, 12 died before treatment completion. The median follow up was 39 months with an overall survival (OS) of 47%, median EFS was not reached. The multivariate analysis for OS in all group identified B2-microglobulin ≥2.7 mg/dL as the only prognosis factor (P=0.01). No difference in overall response, overall survival or event-free survival was found in relation with ALK status.

Table 1.

	ALCL ALK negative n(%)	ALCL ALK positive n(%)	p
Range, y			
<40	7 (29)	8 (67)	0.71
≥40	17 (71)	4 (33)	
Performance status 0-1	12 (52)	7 (70)	0.45
Ann Arbor stage III-IV	19 (83)	9 (75)	0.67
B symptoms	19 (83)	11 (92)	0.64
Bulky disease	9 (39)	6 (50)	0.53
Extranodal disease	11 (48)	5 (42)	0.72
Albumin ≤3.5 mg/dL	15 (65)	8 (89)	0.38
DHL elevated ≥213 UI/L	13 (56)	6 (67)	0.70
Hemoglobin ≤10 g/dL	4 (17)	3 (33)	0.37
Lymphocyte ≤1200 cel/mm3	15 (65)	7 (78)	0.68
2microglobulin ≥2.7 mg/dL	11 (50)	7 (78)	0.23
IPI score			0.56
Low/ Low-intermediate	9 (39)	5 (50)	
High-intermediate/ High	14 (61)	5 (50)	
Treatment			
CHOP	13 (72)	6 (54)	0.039
CHOEP	-	4 (36)	
Other	5 (28)	1 (9)	
Radiotherapy	6 (25)	7 (58)	0.050
Overall response (CR+PR)	10 (71.4)	8 (88.9)	0.61
Relapsed	7 (29)	2 (17)	0.68
3 years OS	49.5%	70%	0.38
10 months EFS	50%	80%	0.15

Summary and Conclusions: Interestingly, we found no difference in outcome between Alk positive and negative patients. There are controversial reports about the prognostic significance of ALK status in ALCL. Gascoyne and cols. reported a 5 years OS of 93% vs 37%. However a GELA study did not find such a difference, while an elevated B2-microglobulin was a poor prognostic factor (OS 84% vs 22%). We also found B2-microglobulin to be a significant prognosis factor.

PB1781

BENDAMUSTINE PLUS RITUXIMAB TREATMENT IN FIRST LINE/ RELAPSED HCL: A SINGLE CENTRE EXPERIENCE

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Background: Hairy Cell Leukemia (HCL) is a low grade Non Hodgkin lym-

phoma that presents with cytopenia and splenomegaly. Diagnosis is confirmed by the presence of lymphocytes CD103, CD25, CD11c positive on bone marrow. Treatment is based of purine analogs with which patients reach an hematological response. However relapse still occurs suggesting the presence of residual disease. Recently it has been discovered the presence of B-RAF V600 mutation in HCL, and experience with B-RAF inhibitor had shown major responses. Bendamustine experience in hematologic neoplasms lead to clinical and long lasting response, especially in low grade lymphomas. The drugs acts as an alkylating agent but it shares some characteristic with purine analogs.
Aims: We previously observed its promising clinical activity with MRD eradication as well, here we report updated results.
Methods: We treated a total of 9 patients (2011-2014) with bendamustine (60-70 mg/ m^2 days 1,2) in association with rituximab (375 mg/m^2 day 3). Cycles were repeated every 28 days up to 6 cycles. Before and after treatment patients had an abdomen ultrasound and bone marrow evaluation. Complete response was considered absence of marrow involvement and splenomegaly post treatment. MRD was evaluated by cytometry or IgH rearrangement with Pcr techniques. Patients' characteristics are summarized in Table 1.
Results: Treatment was well tolerated in all patients, with neutropenia (grade 2) and skin toxicity (grade 2). One patient had to stop treatment due to pulmonary embolism. We did not observe any serious infective complication. Overall response rate (ORR) was 100% with 7/9 complete response (CR) and 2/9 partial response (all in patient with relapsed disease). After a median follow up 25,7 months patients are all alive without any relapse. In evaluable patients MRD eradication has been achieved in 5/9 cases Patients treated at diagnosis obtained 100% CR and were MRD negative.

Table 1.

Cases	Age	Sex	Previous Treatment	Status pre tp	N of tp	Toxicity/Grade	Response	MRD
Pt 1	46	M	Cladribrine-rituximab	1ST Relapse	4	-	CR	NEG 1
Pt 2	58	M	-	Diagnosis	4	-	CR	NEG 1
Pt 3	63	M	Cladribrine	1ST Relapse	4	-	CR	NEG 2
Pt 4	73	M	IFN-a, cladribine	2nd relapse	4	-	PR	POS
Pt 5	67	M	-	Diagnosis	5	Hematologic/II	CR	NEG 1
Pt 6	78	M	-	Diagnosis	6	Hematologic/II	CR	NEG 1
Pt 7	75	M	-	Diagnosis	6	-	CR	NEG 1
Pt 8	74	M	Splenectomy	1st relapse	6	SKIN,EP	PR	POS
Pt 9	54	M	Cladribine	1st relapse	6	-	CR	NA

1 Flow cytometry
2 IgH rearrangement with Pcr techniques

Summary and Conclusions: Here we confirmed our first report on bendamustine in HCL with a longer follow up and additional cases. Results are encouraging and this approach is well tolerated also in elderly patients. Data of bendamustine in HCL as first line therapy are not yet described in literature and in our experience results are at least comparable to those with purine analogs. First data on MRD are interesting as well. Future will bring B-RAF inhibitors which are not still available and with whom resistance has already been described, but in the in the meanwhile bendamustine could be suggested as a treatment option for HCL patients.

PB1782

EXPERIENCE WITH VEMURAFENIB IN THE TREATMENT OF HAIRY CELL LEUKEMIA

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Background: Targeted inhibitory therapy with first line BRAF-inhibitors has been used successfully in HCL patients refractory to or relapsing following previous lines of therapy, including cladribine and rituximab. Careful selection of eligible patients is mandatory since vemurafenib might cause secondary skin malignancies, photosensitivity, QTc-prolongation, liver enzyme elevations and arthralgia. Nevertheless, vemurafenib treatment has the advantage of perfect oral bioavailability, possibility of outpatient treatment, and the lack of hematological toxicity.
Aims: Treatment of relapsed/refractory hairy cell leukemia patients is a challenging task. We report on our experience gained with vemurafenib, a treatment option targeting the disease-defining genetic event, the BRAF V600E mutation.
Methods: Since 2012 January, six out of our 75 hairy cell leukemia patients were found to be refractory to at least two or three lines of therapy or relapsed repeatedly following previous treatments and thus were candidates for vemurafenib treatment. The National Institute for Quality and Organizational Development in Healthcare and Medicines permitted the off label use of vemurafenib in each case. All patients were male, median age was 67 yrs (40-83). One patient was refractory to four previous lines of therapy, the other patients relapsed, on average three times (2-6), and were ineligible for cladribine treatment (4/6 patients) or had cladribine-intolerance (1/6). Before starting vemurafenib, every patient underwent a dermato-oncological and ophtalmological screening. Treatment was planned for 56 days with 240 mg BID regime. Three-four months after treatment splenic ultrasound and bone marrow biopsy were performed to assess the response.
Results: Treatment lasted for 56 days, starting dosage was 240 mg BID. Three of six patients had no complaints during therapy. In two cases asymptomatic and reversible indirect hyperbilirubinaemy was detected without any other clinical signs of hemolysis of hepatic transaminase elevation. Dosage modification (240 mg once a day) was indicated in a patient with reversible grade 1 toxicoderma. In one case treatment was interrupted arbitrarily by the patient after 14 days because of grade 3 arthralgia. In another patient vemurafenib was withhold for 10 days because of community acquired pneumonia. Treatment responses were as follows: partial remission in 4/6, minor response 1/6, and still not evaluable in a recently treated patient A 83 year old patient received vemurafenib treatment for only 10 days when he had to be hospitalised due to progressive cardiac decompensation anaemia, renal insufficiency and hyperuricaemy. The patient died a few days later due to cardiac cause, an autopsy report was not available. Interestingly, in an 80-ys-old patient vemurafenib treatment resulted in gradual and stable normalisation of blood counts while the degree of bone marrow infiltration decreased from the initial 90% only to 70% at three months after having finished therapy. With a median follow up time of 12 (1-22) months until now we observed no overt relapse in our HCL patients treated with vemurafenib.
Summary and Conclusions: Vemurafenib offers a feasible outpatient treatment option for relapsed/refractory patient without hematologic toxicity. Questions to be answered in prospective studies are the optimal dosage and duration of vemurafenib, retreatment and the possibility to combine treatment with a MEK inhibitor.

PB1783

PREVALENCE OF HEPATITIS B REACTIVATION IN PATIENTS WITH INDOLENT NON HODGKIN LYMPHOMA CD20+ DURING MAINTENANCE THERAPY WITH RITUXIMAB

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Background: Anti CD20 antibody (Rituximab) based chemotherapy regimens increase the HBV reactivation risk although sporadic HBV reactivation cases are reported in patients on maintenance with Rituximab single therapy. We evaluated how many reactivation occurred among patients Hepatitis B core antigen positive (HBcAb+) and Hepatitis B surface antigen negative (HBsAg-) who received maintenance therapy with Rituximab.
Aims: The aim of this study is to assess the prevalence of HBV reactivation among patients HBcAb+/HBsAg- during maintenance therapy with Rituximab.
Methods: Here we report our experience about 98 patients with indolent non Hodgkin Lymphoma CD20+ who received maintenance therapy with Rituximab (schedule: 375 mg/mq every 2 months for 2 years) from January 2007 to January 2015. Patients received different chemotherapy regimens during induction: 45% (44/98) with R-CHOP, 34% (33/98) with R-FN, 13% (13/98) R-Bendamustine, 3% (3/98) with R-Fludarabine, 3% (3/98) with R-Leukeran and 2% (2/98) with Rituximab monotherapy. We performed blood tests for HBV (HBsAg, HBsAb, HBeAg, HBeAb, HBcAb) in all patients before starting maintenance therapy and liver function tests before each administration of Rituximab. None of these patients received prophylactic therapy with antiviral drugs during induction and maintenance therapy.
Results: 32% of the patients (32/98) were HBcAb positive. 58% of the patient (57/98) completed therapy with Rituximab and 30% of them (17/57) were HBcAb positive; one of these patients occurred HBV reactivation. 42% of the patients (41/98) are still in maintenance therapy and 36% of them (15/41) were HBcAb positive with risk of HBV reactivation too.
Summary and Conclusions: In patients HBcAB+/HBsAg- treated with Rituximab in single therapy is indicated the prophylaxis with lamivudine. In our single centre experience HBcAB+/HBsAg- patients didn't received therapy with antiviral drugs during maintenance therapy with Rituximab; one of our patient occurred HBV reactivation. In terms of cost-benefit, we reported an advantage in the monitoring approach that was used in our patients in respect to universal prophylaxis with a total savings of about € 3.400,00 for each patient. More study are necessary to estabilish the clinical utility of prophylactic therapy with lamivudine during the maintenance therapy with Rituximab.

PB1784

ARE THE FOLLICULAR LYMPHOMAS CURABLE DISEASES? RETROSPECTIVE STUDY ON 146 PATIENTS WITH AT LEAST 10 YEARS OF OBSERVATION

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Background: Follicular lymphomas are usually defined as incurable diseases with a natural history characterized by several relapses. This study was launched to evaluate how many patients, after a long observation period, don not relapse or do not experience a new chemotherapy treatment.
Aims: We aim to identify which clinical characteristics or therapeutical approaches are associated with this cohort of favourable patients.
Methods: All patients with histologically confirmed diagnosis of follicular lymphomas grade I-II or IIIa were selected from our data base starting from January 2000 untill December 2004 in such a way to have at least 10 years of observation for alive patients. We divided patients in two cohorts, cohort 1 with patients relapsed or progressed and cohort 2 with patients never relapsed or progressed.
Results: From January 2000 to December 2004, 146 patients were diagnosed and treated in our Institution. Thirteen patients were excluded from the analysis, 8 because of lost to the follow-up and 5 did not obtained at least a partial remission. Finally 133 patients were selected for the study. The median age at diagnosis was 61 years (range 30-87). Stage I-II in 47 patients, III-IV in 86. Bone marrow biopsy was positive in 87 patients, FLIPI 0-1 in 35, FLIPI 2 in 43, FLIPI 3 in 40 and FLIPI 4 in 15 patients. According to treatment 96 patients were treated with antracycline containing regimens, 24 with fludarabine containing regimens and 13 were observed or treated with radiotherapy. Rituximab was used in 92 patients, as sequential treatment in 70 or chemotherapy combined in 22; 41 patients did not use rituximab. Patients relapsed or progressed represent cohort 1 (85 patients) and those without relapse or progression represent cohort 2 (48 patients). The statistically significant differences between the two cohorts were: elderly patients (P 0.05), symptomatic patients (P 0.05), FLIPI and FLIPI2 high score (P 0.005), lack of complete remission (P 0.0000) all observed in cohort 1. The overall survival with a median period of observation of 115 months (range 2-185) was 71%, considering the two groups the overall survival in cohort 1 was 62% with a median of 142 months and it was 94% in cohort 2 with median not reached. In univariate analysis normal value of beta2 microglobulin (P 0.05) and the use of rituximab (P 0.01) were associated with a better overall survival; in multivariate analysis treatment with rituximab manteined a statistically significance.
Summary and Conclusions: In conclusion this retrospective monocentric study confirms that about one third of follicular lymphoma patients could be considered cured particularly if rituximab was used in the treatment. At the present time all patients with follicular lymphoma are treated with combined immuno-chemotherapy, moreover after induction therapy patients are started on manteinance. We can therefore hope for the future in an improvement of survival results.

PB1785

A RETROSPECTIVE ANALYSIS OF SPLENIC MARGINAL ZONE LYMPHOMA: PROGNOSTIC FACTORS, ROLE OF WATCH AND WAIT, AND THERAPEUTIC APPROACHES IN THE RITUXIMAB ERA

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Background: Splenic marginal zone lymphoma (SMZL) is an indolent lymphoma recognized as a distinct entity in the WHO classification. Arcaini's scoring system (Blood, 2006)-based on Hgb<12 g/dL, albumin<3.5 g/dL, elevated LDH-is useful to stratify patients prognostically. Given the lack of standard criteria guiding treatment initiation, the watch and wait (W&W) approach in asymptomatic patients is recommended. In symptomatic patients it has been suggested that, apart from splenectomy, rituximab +/- chemotherapy is the best option.
Aims: The aims of our study were to identify risk factors at diagnosis, to assess the progression rate within a W&W strategy, and to analyze the outcome of different therapies in the post-rituximab era.
Methods: : We retrospectively examined the clinical files of 83 patients with SMZL managed at our center in Rome between 2000 and 2013. Patients were stratified according to Arcaini's scoring system. Asymptomatic patients were managed with a W&W policy. Splenectomy was performed in 21 patients with a symptomatic spleen enlargement and limited bone marrow or nodal involvement. Patients not eligible for a splenectomy or with a more generalized disease were treated with chemotherapy alone (12) and, after its introduction, with rituximab + chemotherapy (R-chemo) (8).
Results: The median age at diagnosis was 66 years. The male/female ratio was 1.1. HCV antibodies were positive in 3.7% of cases. The 10-year overall survival was 93% (CI: 84.7-100%). Notably, no patient died of disease progression. The 5- and 10-year progression-free survival (PFS) were 77% and 62%, respectively. In univariate analysis, negative predictors of a worse PFS were splenomegaly (>18 cm) and bone marrow infiltration (>30%). Patients with a low Arcaini score had a better 5-year PFS (87%) than those with an intermediate (76%) or high (44%) risk score (P value=0.01). Fifty asymptomatic patients underwent a W&W program. The median PFS of this population was 45 months; at 10 years, 17% of patients are still on W&W (Figure 1). Sixty-five patients, either at diagnosis or after a W&W period, were treated; those treated with splenectomy or R-chemo first line had similar results, while those treated only with chemo had an inferior outcome. However, when analyzing separately patients with a score<1, splenectomy alone resulted in a highly significant PFS advantage compared to the other treatment approaches (Figure 2).

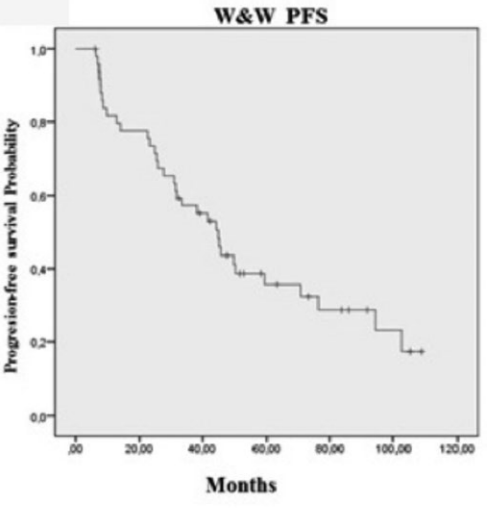

Figure 1.

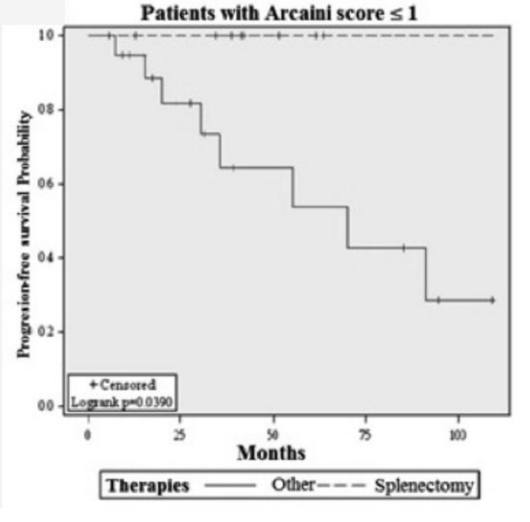

Figure 2.

Summary and Conclusions: This real-life single center study offers an insight into the natural history of indolent SMZL, confirming its very good prognosis. We found a negative prognostic impact of a marked splenomegaly and marrow infiltration. At variance from previous reports, we did not observe a high prevalence of HCV, especially as this is a Center/South-Italian case series. Moreover, we confirm that Arcaini's staging system, that defines 3 separate risk groups, is a powerful prognostic stratificator. The W&W approach allows a median PFS of 45 months, longer than that reported in follicular lymphoma patients. Finally, our data confirm the inferiority of chemotherapy alone against splenectomy and R-chemo. The subgroup of low risk patients treated only with splenectomy fared very well. For such patients, splenectomy could remain the best first-line approach even in the rituximab era. Prospective studies are needed to confirm these results.

PB1786

SINGLE-AGENT IBRUTINIB DEMONSTRATES LONG-TERM ACTIVITY AND SAFETY IN PATIENTS WITH RELAPSED/REFRACTORY WALDENSTRÖM'S MACROGLOBULINEMIA

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Background: Bruton's tyrosine kinase (BTK) is an essential component of the B-cell receptor pathway and functions via the activation of downstream signals mediating B-cell growth, adhesion, and survival. Ibrutinib, a first-in-class, once-daily, oral, covalent inhibitor of BTK, has emerged as an attractive treatment option for patients with Waldenström's macroglobulinemia (WM). Recently, ibrutinib was approved by the FDA for the treatment of WM, representing the first FDA-approved agent in WM. The first-in-human trial of ibrutinib was an open-label, phase 1 study in patients with relapsed/refractory (R/R) B-cell malignancies including WM (Advani, *J Clin Oncol*. 2013) and demonstrated encouraging activity of ibrutinib (75% overall response rate) in WM, thus initiating further studies.
Aims: To report long-term activity and safety outcomes of ibrutinib in 4 patients with R/R WM who enrolled in this phase 1 study and then continued into an extension study.
Methods: All patients provided written informed consent before enrollment. Four patients with R/R, histologically confirmed WM and adequate hematologic, renal, and hepatic function received oral ibrutinib between 560 mg/day and 12.5 mg/kg/day until progressive disease or unacceptable toxicity. Patients were required to have IgM levels ≥1000 mg/dL with bone marrow infiltration. After 6 months of therapy, patients with objective response or stable disease were rolled over from the parent study (PCYC-04753) into the extension study (PCYC-1103-CA) at a fixed dose of ibrutinib 560 mg daily. Adverse events (AEs) were assessed by NCI CTCAE v3.0. In the 1103 study, only data on AEs grade ≥3, serious AEs (SAEs), and AEs leading to dose modification or discontinuation were captured. Disease response assessments included laboratory assessments (serum IgM) and radiographic imaging when applicable. Best clinical response was assessed per the 3rd International Workshop of WM (IWWM).
Results: Three of 4 WM patients achieved durable partial responses accompanied by ≥50% reductions from baseline in IgM levels (~80% to 90%), which reached a plateau after 1 year of therapy. Responses are ongoing after 4 years of therapy with no evidence of progression in other clinical features attributed to WM. Additional clinical improvements included sustained increases or stabilization in hemoglobin levels (without the use of erythropoietic growth factors or transfusion); reduction in lymphadenopathy (present in 3 of 4 patients at baseline); and improvement in hematocrit over time. Grade 3/4 AEs included neutropenia (1 patient) and thrombocytopenia (1 patient), and SAEs included febrile neutropenia (2 patients), pneumonia and pneumonitis (1 patient), and atrial fibrillation (1 patient). In addition, the same patient experienced a second episode of grade 2 atrial fibrillation (not an SAE), leading to dose modification to ibrutinib 420 mg daily. All grade 3/4 AEs and SAEs were resolved without sequelae and were assessed by the investigator as unrelated to ibrutinib.
Summary and Conclusions: This case series is the first to demonstrate extended activity and tolerability of ibrutinib in R/R WM, accompanied by early and rapid decline in IgM levels and increase in hemoglobin levels. Single-agent ibrutinib induced profound and durable responses, with a favorable safety profile, in this difficult-to-treat patient population—consistent with findings of a phase 2 trial (Treon, IWWM, 2014).

PB1787

BENDAMUSTINE IN ELDERLY PATIENTS WITH INDOLENT NON-HODGKIN'S LYMPHOMA: A REPORTED EXPERIENCE IN ROUTINE PRACTICE ON 76 PATIENTS

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Background: The combination of bendamustine and rituximab (RB) has been shown to be an appropriate option for first line treatment or treatment for relapsed / refractory patients (pts) with indolent non-Hodgkin's lymphoma (NHL) and Chronic Lymphocytic Leukemia (CLL).
Aims: The objective of this retrospective study was to compare the toxicities of RB between pts<65y and ≥ 65y among pts with indolent NHL or CLL in relapse.
Methods: Analysis was performed retrospectively on pts treated with RB between January 2009 and December 2013 in relapse. The treatment consisted of rituximab (375 mg/m^2 D1) and bendamustine (90 mg/m^2 D1-2) every 28 days. Fisher and Wilcoxon tests were used, and the probability of adverse event (AE) during cure was compared according to the age in generalized linear mixed models.
Results: Among the 76 pts included, 41 pts (54%) were<65y (median age= 57y, range 33-64), and 35 pts (46%) ≥65y (median age= 74y, range 65-80). In the cohort of pts<65y, 33 pts presented with indolent NHL (24 FL, 6 MZL, 2 SLL, 1 tricholeucocyte), 7 pts CLL, 1 MCL. In pts ≥65y, 26 pts presented with indolent NHL (15 FL, 7 MZL, 3 SLL, 1 Waldenström), 8 pts CLL and 1 MCL. The PS was ≥2 in 18% of pts<65y vs 32% of pts ≥65y, with high LDH in 29% vs 53% of pts. The median number of prior treatments was 4 in<65y and 6 for ≥65y. Fifty-seven (75%) pts received at least 4 cycles of RB and 34 (45%) pts at least 4 cycles, corresponding to 344 cycles overall (179 (52%) in pts<65y and 165 (48%) in pts ≥ 65y). Eighty-six percent of pts had at least one AE during their treatment, wich was G3/4 in 47% of the pts. The occurrence of G1/4 AE per cycle was 51% in the whole cohort without significant difference between age groups (53% and 49%, respectively in pts<65y and pts ≥65y, P=0.65). Among them, AE was noticed grade (G) 3/4 in 50 cycles corresponding to 28 (16%) cycles in pts<65y and 22 (13%) in pts ≥65y (P=0.64). Hematotoxicity was present in 29 pts (70%)<65y and 27 pts (77%) ≥65y. Probability of hematotoxicity per cycle was 20% for<65y and 18% for ≥ 65y (P=0.70). Thrombocytopenia and neutropenia were the most frequent G3/4 hematotoxicity, occurring in 12% and 17% of the pts<65y and pts ≥65y respectively. Nausea affected 34% of pts<65y vs 43% pts ≥65y. Twenty-seven percent of pts<65y and 31% pts ≥65y developed infections. Pulmonary toxicities G1/4 with bronchitis were rare (3% per cycle) but significantly more frequent in pts ≥65y, 5% per cycle compared to pts<65y, 1% per cycle (P<0.036). A dose adjustment was required for 10 pts (24%)<65y related to thrombocytopenia in 7 pts; the same happened to 12 pts (34%) ≥65y, because of thrombocytopenia, deep neutropenia, cutaneous rash G3, maculopapular rash, severe sepsis or digestive disorder. Treatment was discontinued in 13 (32%) pts<65y (4 pancytopenia, 2 thrombocytopenia, neutropenia G4, 1 severe sepsis and 1 pneumonitis) and in 11 (31%) pts ≥65y (4 fever infections (bronchitis), 2 febrile aplasia, 1 cutaneous rash G3, 1 pancytopenia, 1 thrombocytopenia, 1 mucositis G3 and 1 gastrointestinal intolerance) (P=1).
Summary and Conclusions: The toxicities of RB combination and their probabilities do not differ significantly with the age, except for respiratory disorders. Bronchitis G1/2 appears to occur more frequently in pts ≥ 65y. This has to be noticed in the perspective of association of BR with new agents such tyrosine Kinase inhibitors that may increase this risk of pulmonary toxicity in elderly patients.

PB1788

INCIDENCE OF SECONDARY PRIMARY NEOPLASM IN A COHORT OF PATIENTS WITH FOLLICULAR LYMPHOMA. A SINGLE CENTER REPORT

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Background: Follicular lymphoma (FL) account approximately one third of all non-Hodgkin lymphomas, it is know that primary affect males and white population; also is established that any patient diagnosed for a primary cancer have an increased risk to be diagnosed for a second primary neoplasm (SPN), respect to follicular lymphoma there are few reports about a 25% increased risk for all non-Hodgkin lymphomas not related with histologic subtype. Although is recognized that chemotherapy, radiotherapy and radioimmunotherapy (RIT) increased this risk; there are recent reports about the use of radioimmunotherapy with 90Y-Ibritumomab-Tiuxetan (90Y-IT) that show an increased risk for SPN. RIT is available in our center since 2005 and we have been accumulated a long experience. Considering this we analyze our data and compare the incidence of SPN in all patients with FL treated in our institution with different schedules including or not 90Y-IT.
Aims: To analyze the incidence of second primary neoplasm in FL patients diagnosed and treated in our center, searching for relationship with RIT
Methods: A chart review was carried using the registry of diagnosed patients, from the Department of Hematology, all consecutive patients diagnosed of Follicular lymphoma of any grade according to WHO since 2001 were included, a review of clinical records: demographic and clinical data, incidence of previous cancer and therapies, SPN (basocellular skin and *in situ* cervix carcinoma were excluded), number of chemotherapies, relapses, therapy with 90Y-IT, actual status and cause of dead were recorded.
Results: A total of 251 FL patients were registered, Male/female ratio: 107/144, mean age 59.9 years (15-86), Stage I: 7.2%, II: 11.0%, III: 24.1%, IV: 57.8%, FLIPI: Low-risk: 62.15%, Intermedium: 13.9%, High-risk: 10.3%. A 27.8% of patients receiving two or more chemotherapy schedules, 10,5% had underwent an auto-SCT. 100 patients (39.8%) received 90Y-IT (55 as a 2nd or 3rd line of therapy and 45 as a consolidation therapy). The mean follow-up for all patients is 108 months (median 49 m). Respect to incidence of neoplasms we found that 38 (15.1%) patients have a registry of at least 2 primary cancers, in 16 (42.1%) of them FL diagnosis were the second primary neoplasm. In the 22 patients who developed a SPN after FL diagnosis, 11 had been received two or more lines of therapies and 5 of them including RIT.
According to relationship with 90Y-IT, in 3 SPN were diagnosed before the use of RIT and in one at the same time of RIT, and in 8 patients the diagnosis of SPN occur after RIT at a mean time of 32.3 months. For all patients the SPN were recorded at a mean time of 24.5 months after FL diagnosis.
Summary and Conclusions: Even this work is a single institution reports, summarize relevant information outside clinical trials about the incidence of second primary neoplasm in one cohort of follicular lymphoma patients treated (100) or not (151) with 90Y-IT. In our experience 90Y-IT not increase significantly the risk of SPN. A more exhaustive analysis will be presented in case of acceptance.

PB1789

COMBINATION OF FLUDARABINE, MITOXANTRONE, DEXAMETHASONE AND RITUXIMAB (R-FND) IN THE TREATMENT OF INDOLENT NON-HODGKIN B CELL LYMPHOMA-RETROSPECTIVE STUDY OF 60 CHINESE PATIENTS

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Background: Indolent non-Hodgkin B cell lymphomas (NHL) are a group of lymphoid malignancies that typically follows a protracted clinical course with frequent relapses. Although it is generally considered incurable, effective treatment regimens exist. The combination of fludarabine, mitoxantrone and dexamethasone (FND) resulted in high complete remission (CR) rate in the pre-rituximab era. Data are scarce on whether the addition of rituximab (R-FND) would result in a better CR rate or longer remission. Moreover, this combination has never been properly evaluated in Chinese patients, a population that reportedly has a much lower prevalence of indolent NHL.
Aims: To determine the efficacy of RFND regimen in indolent lymphomas in Chinese patients
Methods: Consecutive patients who are diagnosed with indolent NHL (follicular lymphoma, marginal zone lymphoma, lymphoplasmacytic lymphoma and low grade B cell lymphoproliferative disease (B-LPD) not otherwise classified) were recruited into this retrospective study from January 2012 to December 2014. The R-FND regimen (Rituximab 375mg/m^2 on D1, Fludarabine 40mg/m^2 daily D1-3, mitoxantrone 10mg/m^2 on D1 and dexamethasone 20mg daily D1-5) was used as frontline treatment in our institution. Baseline demographic data were collected. Response rate and toxicity of the regimen was evaluated. Overall survival and progression free survival was analyzed using Kaplan-Meier method.
Results: Sixty patients (29 men, 31 women) (follicular, N=22; marginal zone lymphoma, N=27; lymphoplasmacytic lymphoma, N=4; B-LPD not otherwise classified, N=7) at a median age of 61 years were treated, with R-FND given as frontline therapy in 50 patients and salvage therapy in 10 patients. Stage III/IV disease occurred in the majority of the cases (N=42, 70%). The median follow up time was 23 months. Complete remission (CR) was achieved in 49 out of 59 evaluated patients (83.1%), and partial remission (PR) in 8 patients (13.6%), giving an overall response rate (ORR) of 96.7%. The response was also durable, with a progression free survival at 2 years of 81.3%. The overall survival at 2 years was 93.4%. Haematological toxicities were the most common adverse effects, with grade III/IV anaemia, neutropenia and thrombocytopenia being 10%, 88% and 17% respectively. Clinically significant cytomegalovirus (CMV) reactivation occurred in 8 patients (13%) with 2 of them developing CMV retinitis.
Summary and Conclusions: R-FND is a highly effective regimen with high CR rate and long duration of remission. However, due care should be taken with respect to haematological toxicity.

PB1790

A MULTICENTER, RETROSPECTIVE ANALYSIS OF LOW-GRADE PRIMARY FOLLICULAR LYMPHOMA OF THE GASTROINTESTINAL TRACT: TREATMENT AND OUTCOME

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Background: Primary gastrointestinal (GI) follicular lymphoma (FL) has been regarded as a relatively rare malignant disease, accounting for 1-3.6% of primary non-Hodgkin lymphomas of the GI tract. The primary FL of the GI tract occurs most often in middle-aged adults with a 2:1 female predominance. The most frequent primary occurrence location is duodenum. The low-grade primary FL of the GI tract which is especially located at duodenum characterized as an indolent. Although the various therapeutic strategies have been developed for FL, there is no definite guideline for the treatment of low-grade primary FL of the GI tract.
Aims: In this study, we analyzed our experience of low-grade primary FL of the GI tract according to the treatments.
Methods: A total of 12 patients who diagnosed as a low-grade primary FL of the GI tract between June 2005 and June 2014 from 5 institutions in Korea were retrospectively analyzed. Low-grade primary FL of the GI tract was defined as Lugano stage I or II and WHO histologic grade 1 or 2. Endoscopy and immunohistochemical pathologic work-up were performed at diagnosis. The patients were classified according to the treatment strategies such as watch & wait group (n=4) and treatment group (n=8). Treatments included various chemotherapies (CTx) and radiotherapy (RTx).
Results: Median age was 44 years (range, 33-66) and a female predominance was observed (male:female=4:8). All patients had an Eastern Cooperative Oncology Group (ECOG) performance score of 0 or 1. The most common reason for initial evaluations of primary FL of the GI tract was health screening (n=10, 83.3%). Only two patients received initial evaluations due to GI symptoms. The small intestine, especially the 2nd or 3rd portion of duodenum was the most common primary site of involvement (n=8, 66.6%). There were similar clinical characteristics between the two groups. Exceptionally, white blood cell (WBC) count (P=0.024) at diagnosis was higher in the treatment group. The primary site of involvement was not different between the two groups. However, the only primary site of the watch & wait group was duodenum (n=4, 100%). In the watch & wait group, the maintenance of stable disease status was observed in all patients. However, one patient in the watch & wait group eventually received additional RTx after the initial observational period of 19.4 months according to the decision of the attending physician. In the treatment group, two patients received CTx such as CVP (cyclophosphamide, vincristine and prednisone, n=1) or CHOP (cyclophosphamide, doxorubicin, vincristine and prednisone, n=1) and six patients received RTx (median 3600 cGy, range, 2520-4140). These eight patients in the treatment group achieved complete remission (CR) after treatment, but one patient with low-grade primary FL of the rectum who received 6th cycles of CVP CTx relapsed at 30.5 months after achieving 1st CR. She received 4 cycles of weekly rituximab monotherapy and re-achieved CR. After a median follow-up of 33.1 months (range, 3.6 to 107.7 months), all 12 patients were alive.

Table 1. Clinical characteristics of primary follicular lymphoma of the GI tract.

Characteristics	Watch & Wait group	Treatment group	All patients	*P. value*
No. of patients, n (%)	4 (33.3%)	8 (66.7%)	12 (100%)	
Age, median (range), years	48 (46-54)	42 (33-66)	44 (33-66)	*0.471*
Gender, Male/Female, n (%)	1 (25.0%)/3 (75.0%)	3 (27.5%)/5 (62.5%)	4 (33.3%)/8 (66.7%)	*0.594*
WBC x 10^9/L, median (range)	5.61 (5.2-6.93)	6.90 (5.82-9.33)	6.80 (5.20-9.33)	*0.024*
Primary location of involvement				*0.460*
Duodenum, n (%)	4 (100%)	4 (50.0%)	8 (66.6%)	
Rectum, n (%)	0 (0%)	3 (27.5%)	3 (25.0%)	
Ampulla of vater, n (%)	0 (0%)	1 (12.5%)	1 (8.4%)	
Ann Arbor stage, IEA/IIEA, n (%)	4 (100%)/0 (0%)	7 (87.5%)/1 (12.5%)	11 (91.7%)/1 (8.3%)	*0.667*
Lugano stage, I/II, n (%)	4 (100%)/0 (0%)	7 (87.5%)/1 (12.5%)	11 (91.7%)/1 (8.3%)	*0.667*
1st line treatment				*0.002*
Watch & Wait, n (%)	4 (100%)	0 (0%)	4 (33.3%)	
Chemotherapy, n (%)	0 (0%)	2 (25.0%)	2 (16.7%)	
Radiotherapy, n (%)	0 (0%)	6 (75.0%)	6 (50.0%)	
Final follow-up status, CR/SD, n (%)	1 (25.0%)/3 (75.0%)	8 (100%)/0 (0%)	9 (75.0%)/3 (25.0%)	
Overall survival, median (range), months	13.5 (3.6-21.3)	46.6 (16.0-107.6)	33.1 (3.6-107.7)	

Summary and Conclusions: In this study, we confirmed that the watch & wait strategy was safe in low-grade primary FL of duodenum. In addition, radiation alone also was an appropriate strategy for the low-grade primary FL of the GI tract. However, close monitoring would be necessary for the patients with non-duodenal site of the low-grade primary FL. Further studies with more patients would be needed to confirm appropriate treatment strategies for the low-grade primary FL of the GI tract.

PB1791

RITUXIMAB INDUCES HYPOGAMMAGLOBULINEMIA IN PATIENTS WITH NON HODGKIN LYMPHOMA

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Background: Rituximab (R) is a monoclonal antibody that binds the CD20 antigen on all peripheral B cells. Its favorable toxicity profile and effectiveness have led to its wide use in induction and maintenance regimens for Non Hodgkin Lymphoma (NHL).
Aims: This retrospective single center study aimed to evaluate the hypogammaglobulinemia (hypoIg) associated with R use.
Methods: We performed serial quantitative serum immunoglobulin (SIg) concentration at the baseline, after chemotherapy, during and after R maintenance therapy.
IgG, IgA and IgM deficit were respectively defined by level below 700 mg/dL, 70 mg/dL and 40 mg/dL. Symptomatic patients were defined as having 2 or more non-neutropenic infections in a 6-month period after or during R.
Results: 123 patients with indolent NHL and SIgG studies were analyzed, 47,1% were relapsed or refractory. The median age of patients was 60 years (range: 28-80). The histologies included follicular lymphoma (FL) (n=77), small lymphocytic lymphoma (SLL) (n=14), marginal zone lymphoma (ML) (n=20), mantle cell lymphoma (MCL) (n=12). Patients received a median of 13 doses of R (range: 6-27). The median follow-up of surviving patients was 4,4 years. Before treatment with R, 11/123 (8,9%) had low SIgG levels (5 FL, 1 MCL, 4 SLL, 1ML) and 3/11 (27,2%) required, during R maintenance treatment, Intravenous Immunoglobulin (IVIG) administration. After R-chemotherapy, IgG deficiency appeared in 29/123 (28,4%), 2/29 needed IVIG. After or during R maintenance 25/123 (20,3%) showed IgG deficiency after a median of 9 R cumulative doses; the deficit occurred in the 80% (20/25) within the fourth R maintenance dose and in no one after the sixth R administration. In this category, 10/25 (40%) were symptomatic and 4/25 (16%) required IVIG. All 10 patients who needed IVIG showed at least two Ig isotypes deficiency.
Summary and Conclusions: We observed that R administration was associated with a high risk of hypoIg. In addition, we found that the number of R doses

correlated to the development of symptomatic hypoIg. Finally we observed that the risk of hypoIg increased in patients who received maintenance R. The decision to introduce therapy with IVIG in non-neutropenic patients was related to repeated episodes of infection. HypoIg often is underestimated also for the presence of confounding symptoms. Our study suggests that the baseline and periodic Ig monitoring should be considered in these patients subset.

PB1792

LONG TERM OUTCOME OF PATIENTS WITH GASTRIC MARGINAL ZONE LYMPHOMA RECEIVING FLUDARABINE, MITOXANTRONE AND RITUXIMAB AS FIRST-LINE TREATMENT

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Background: Gastric mucosa-associated lymphoid tissue (MALT) B-cell lymphoma represents the most frequent localization of extranodal marginal-zone lymphoma (MZL), characterized by a well established association with *Helicobacter pylori* (HP) infection. The first-line treatment for HP positive patients is HP eradication therapy; however, there is no consensus on the standard management for patients with HP negative or persistent disease after HP eradication. Current approaches include radiation therapy, chemotherapy and immunotherapy with rituximab, either alone or in combination. Despite the activity of many alkylating agents and nucleoside analogues against MALT lymphoma, evidence about the superiority of a specific regimen toward others is still lacking.
Aims: in this study we have investigated long-term efficacy and safety of fludarabine and mitoxantrone in association with rituximab (R-FM) as first-line treatment for gastric MALT lymphoma with HP negative or persistent disease after HP eradication.
Methods: A cohort of 13 patients (M/F: 5/8; median age 65 yrs) diagnosed with gastric MALT lymphoma by gastroscopy biopsies and treated between August 2005 and March 2012 was retrospectively analyzed. All patients completed staging with whole body CT scan and bone marrow (BM) biopsy. Five patients had stage I disease, 7 stage II and 1 stage IV, HP was positive in 9/13 patients. Induction treatment consisted of fludarabine (25mg/m^2 i.v. on days 2 to 4), mitoxantrone (10mg/m^2 i.v. on day 2) and rituximab (375 mg/m^2 i.v. on day 1), administered for up to 6 cycles every 28 days. Final response assessment including esophagogastroduodenoscopy with random biopsies and CT scan was done 4-6 weeks after completion of therapy according to the 2007 Revised Response Criteria. During follow-up period CT scan was performed together with esophagogastroduodenoscopy every 6 months for the first 2 years (annually for stage I patients). Thereafter, endoscopy was performed annually until the 5th year, after this period the patients continued only clinical follow-up.
Results: All patients (13/13, 100%) achieved a complete remission (CR), a median of 4 cycles (range 3-6) of R-FM were given and all were evaluable for response and toxicity. Treatment-related toxicities were mainly hematologic, with grade 3-4 neutropenia observed in 11/13 patients (84.6%), grade 2 thrombocytopenia and anemia in 1 and 2 patients, respectively. All but one cases required secondary neutropenia prophylaxis with filgrastim, 1 patient had grade 3 febrile neutropenia. The same patient was hospitalized while on treatment because of non-neutropenic fever with detection of Candida Albicans by stool culture; 2 months later experienced CMV reactivation, too. Two patients developed prolonged pancytopenia, with slow recovery after about 12 months. Grade II nausea was documented in 4/13 patients, no other adverse events occurred. After a median follow-up of 60 months (range 24-110) all patients were alive, 1/13 had disease relapse after 8 months and received total gastrectomy; estimated 9-year progression-free survival and overall survival were 92.4% (Figure 1) and 100%, respectively.

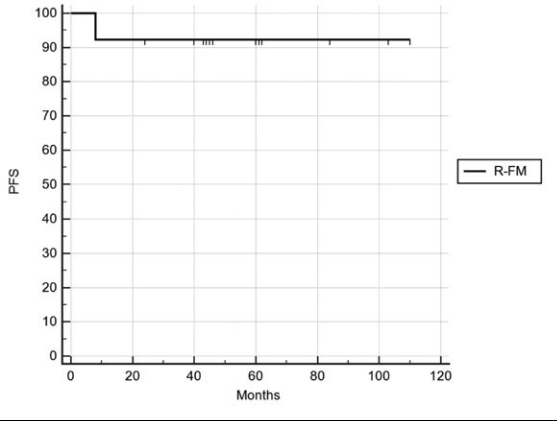

Figure 1.

Summary and Conclusions: This small series of homogeneously treated patients suggests R-FM regimen has a high long-term efficacy as frontline treatment for gastric MALT lymphoma. However, the high incidence of grade 3-4 hematological toxicity makes this treatment less safe compared to the previously published results of other regimens such as rituximab in combination with chlorambucil or bendamustine. It could remain a suitable option for patients with advanced-stage disease, while exluding stage I.

PB1793

THE ROLE OF FLOW CYTOMETRY IN THE DIAGNOSIS AND FOLLOW UP OF GASTRIC LNH

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Background: The diagnosis of lymphoma is mainly based on morphology, but the diagnostic accuracy is greatly increased by the use of ancillary techniques like immunophenotyping, citogenetic and molecular tests. This multiparametric approach is recommended by WHO classification.
Aims: To evaluate the role of flow cytometry in the study of gastric biopsies with suspect of NHL. We have assessed the validity of integration of histological and cytometric assays to optimize diagnosis of lymphoproliferative diseases and to lower the number of inconclusive cases at histological exam. The aim of flow cytometry was to define lineage and clonality of lymphoid proliferation, thus giving a support to final assessment.
Methods: 27 patients with primary gastric lymphoma underwent, from January 2007 to June 2010, to double gastric biopsy at diagnosis or during follow up. Diagnosis was MALT in 15/27 (56%), DLBCL in 8/27 (30%) and other histological type in 4/27 (14%). Every patient underwent complete staging and the stage was I/E in 12/27 and II/E in 15/27. Therapy was antibiotic eradication of HP in 5/27 and chemo-immunotherapy in accordance with guidelines. Samples for histology were fixed with formalin and stained with HE, then tested by antibodies for CD3, CD5, CD10, CD20, CD21, CD23, CD30, CD38, CD43, CD45RO, CD79a, CiclinaD1, BCL-2, BCL-6, Ki67, EMA, κ e λ. Samples for flow cytometry were put in saline, sent to laboratory within thirty minutes and processed with standard methods. A cytometric pattern was defined as pathological if there was an evident atypical assempbly of B or T lymphoid antigens along with clonal restriction for Ig light chains or TCRVβ repertoire.
Results: We have carried out 36 biopsies in a population of 27 patients and we obtained a final diagnosis in all cases. Diagnosis was: NHL in 9/36 (8 MALT and 1 follicular lymphoma), plasmacytoma in 1/36, and benign conditions in the remaining 26/36. In all cases there was strict concordance between the two methods, but in one single cases positivity of flow cytometry led to histological revision and, finally, to full concordance.
Summary and Conclusions: This study demonstrated that flow cytometry is an easy and reliable diagnostic tool for gastric lymphoma: assumed that its goal is to assess lineage and clonality, it is fast, sensitive and specific. It is synergistic to histology, especially in difficult cases like biopsy performed during a revaluation after chemotherapy or antibiotic therapy, or reactive conditions where lymphoid infiltrates can mimic lymphoma. Histological diagnosis certainly remains the gold standard for lymphoma diagnosis but flow cytometric typing can usefully support histology to reach a correct diagnosis in 100% of cases.

PB1794

IMPROVED DETECTION OF CHLAMYDOPHILA PSITTACI DNA IN OCULAR ADNEXAL MALT LYMPHOMA WITH PCR OPTIMIZATION

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Background: The pathogenic association between *Chlamydophila psittaci* (*Cp*) infection and ocular adnexal MALT lymphoma (OAML) has been suggested, but large geographic variation (0-87%) has been reported in the prevalence of Cp infection in patients with OAML. Although its cause has not been elucidated fully, this geographic discrepancy may be in part due to the methodological biases or suboptimal PCR.
Aims: Therefore, we examined the detection rates for *Cp* DNA in patients with OAML according to the different PCR condition.
Methods: Six OAML patients diagnosed between April 8, 2014 and August 11, 2014 were included in this study. DNA was extracted from paraffin-embedded tissues and touchdown enzyme time release (TETR)-PCR was performed to identify *Cp* DNA as previously reported (Madico *et al.*, J Clin Microbiol 2000;38:1085-93). Then, we performed TETR-PCR with modified annealing temperature (beginning at 67C vs 62C in original protocol) using same extracted DNA samples.
Results: Four (67%) patients were male and median age was 50 years (range, 41-77 years). The primary site of OAML was conjunctiva in 4 (67%) patients

and orbital soft tissue in 2 (33%) patients. At presentation, each 3 (50%) patients were diagnosed as stage I and IV, respectively. In initial TETR-PCR according to the original protocol, *Cp* DNA was detected in none of 6 patients. When TETR-PCR was performed with modification of annealing temperature, Cp DNA was identified in 4 (67%) patients. Direct sequencing could be performed in 3 of 4 positive cases, and specificity of amplified fragments was confirmed.
Summary and Conclusions: PCR optimization with modification of annealing temperature could improve the detection of *Cp* DNA in patients with OAML. Future prospective validation in a larger sample size is necessary.

PB1795

SYMPTOMATIC BONE INVOLVEMENT IN WALDENSTROMS MACROGLOBULINAEMIA

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Background: Bone involvement by Waldenstroms macroglobulinaemia (WM) is very uncommon. The presence of bony lesions may point towards possible high-grade transformation, or the diagnosis of a different IgM paraprotein-producing disorder (*e.g.* IgM myeloma).
Aims: We describe symptomatic skeletal involvement in 4 cases of previously diagnosed WM patients and evaluate the diagnostic value of bone biopsy to allow tailored management decisions to be made.
Methods: Four patients with WM were evaluated when they developed progressive bony pain, using MRI, PET-CT and bone biopsy. An image-guided biopsy of affected bone was performed in each case and the results were correlated with their bone marrow histology and other clinical and laboratory data. Patient characteristics are shown below:

Table 1.

Patient ID	Age/ Sex	IPSSWM	ECOG	Isotype	Genetics	Time from diagnosis	Prior therapy
A	53/M	1	0	IgMk	N/A	New diagnosis	None
B	60/M	2	1	IgMk	N/A	2 years	DRC x 6 to VGPR
C	57/F	2	1	IgMk	L265P Glu343Valfs*2	1 year	DRC x 6 to MR
D	44/F	2	0	IgGK	L265P	3 years	DRC x 6 to PR

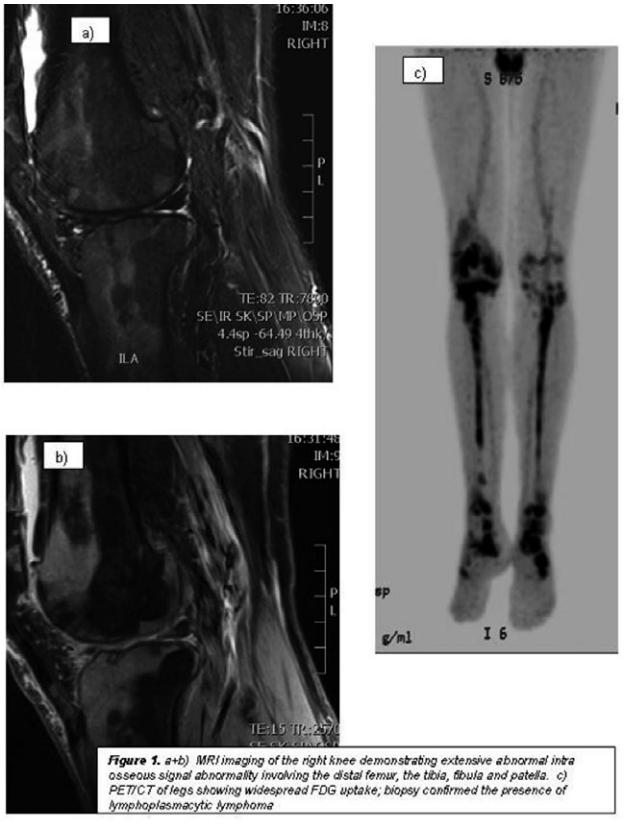

Figure 1. *a+b) MRI imaging of the right knee demonstrating extensive abnormal intra osseous signal abnormality involving the distal femur, the tibia, fibula and patella. c) PET/CT of legs showing widespread FDG uptake; biopsy confirmed the presence of lymphoplasmacytic lymphoma*

Figure 1.

Results: In all cases, patients were investigated due to unrelenting bone or joint pains, including shoulder & upper arm (24 months); knee (6 months); ankle and lower limb (6 months); knees (8 months) with limitation of movement and/or joint swelling. The bone disease was evident at the outset, at 12, 18 and 38 months from diagnosis respectively; 1 patient had newly diagnosed WM needing treatment, 2 had evidence of progressive disease 6 and 14 months post last treatment and the 4th patient had achieved a minor response to therapy at the time of bone disease. MRI scans demonstrated abnormal intraosseous signal characteristics in the affected bones in all cases which enhanced with contrast and showed low signal intensity on the T1 weighted sequences and areas of high signal intensity on the T2 weighted sequences. PET-CT scans were performed in 3 cases and showed patchy diffuse uptake correspomding with MRI signal abnormality in 2 cases, but no focal tracer uptake in the third case. There was no evidence of osteolysis on the CT component. Biopsy of affected bones demonstrated a heavy infiltrate (70-90%) of lymphoplasmacytic lymphoma with identical characteristics to the iliac crest biopsy. In all cases, the bones were diffusely infiltrated by CD20+ cells, with a scattering of CD138+ cells. There was no evidence of transformation to high grade disease in any case. Blood work showed a median Hb of 120 g/L, median platelets 270x 10^9/L, median M-protein of 17 g/L (range 5-22 g/L), raised alkaline phosphatase and LDH in 1 patient, and normocalcaemia in all. All 4 went on to receive chemoimmunotherapy (patient A also had radiotherapy to the humerus) which improved the bone symptoms in 2, but too early to assess in 2, and are alive with a median follow up is 7 months (range 1-20).
Summary and Conclusions: In our cohort of 4 patients who developed symptomatic bone disease there was appendicular skeletal infiltration by lymphoplasmacytic lymphoma; the possibility of skeletal disease in WM should be considered patients with bone or joint pain and the significance of bone disease in WM deserves further characterisation. Image-guided bone biopsy helps to confirm the diagnosis and rule out transformation to high grade lymphoma or an alternative diagnosis of IgM myeloma.

PB1796

BORID REGIMEN IN PATIENTS WITH MANTLE CELL LYMPHOMA

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Background: Mantle cell lymphoma (MCL) is seen in predominantly elderly and male patients, and responsive to chemotherapy but usually remission periods are short. There is no standart treatment regimen but addition of rituximab to treatment protocols increased response rates. Bortezomib has been shown to have synergistic effect with rituximab and alternative combination regimens [eg. BORID (bortezomib-rituximab-dexamethasone)] have been implemented.
Aims: To evaluate the effect of BORID regimen to prognosis and survival in MCL.
Methods: We investigated 15 patients diagnosed MCL between 2001-2014 in our clinic and treated with BORID regimen during follow up because of relapsed disease. Demographic data collected from patient files retrospectively.

Table 1.

Table 1: Patient characteristics and treatment regimens.

Patient	Age/Sex	1st	Rs	PFS	2nd	Rs	PFS	3th	Rs	PFS	4th	Rs	PFS	5th	Rs	PFS	6th	Rs	PFS	OS
1	63/M	R-CHOP (8)	PR	7	ICE (3)	N	1	BORID (2)	N	-	-	-	-	-	-	-	-	-	-	17
2	53/F	R-CHOP (2)	PR	16	BORID (6)	PR	21	ARA-C+R	-	-	-	-	-	-	-	-	-	-	-	80
3	70/M	R-CHOP (8)	CR	11	BORID (3)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	34
4	55/M	R-CHOP (6)	CR	26	RT	CR	14	BORID (1)	-	-	-	-	-	-	-	-	-	-	-	65
5	60/M	R-CHOP (8)	CR	12	DHAP (4)	N	1	uln (2)	N	-	R-ICE (1)	N	-	IV MTX+ARA-C	N	-	BORID (3)	N	-	42
6	59/F	R-CHOP (8)	N	1	BORID (1)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10
7	66/M	CHOP (8)	PR	7	R-DHAP	PR	9	Cpc	N	-	BORID (3)	PR	3	R+B (6)	N	-	-	-	-	42
8	69/M	Chlr	PR	23	CVP (4)	N	1	R-CHOP (4)	PR	4	R main.	PR	17	BORID (5)	PR	-	R+B	-	-	51
9	67/F	CHOP (8)	CR	-	ASCT	CR	17	BORID (1)	-	-	-	-	-	-	-	-	-	-	-	42
10	73/M	CDOP (8)	PR	10	BORID (5)	N	1	Chlr	N	-	ARA-C (6)	PR	8	Len	PR	6	-	-	-	86
11	48/M	Nordic	CR	-	ASCT	CR	5	R-DHAP (2)	CR	-	ASCT	CR	6	BORID (2)	N	-	R+B+ARA-C (2) Ib (1)	N	-	25
12	66/M	ARA-C+R (2)	N	1	BORID (3)	N	-	-	-	-	-	-	-	-	-	-	-	-	-	7
13	54/M	CHOP (3)	N	1	DHAP (4)	PR	5	ASCT+R	CR	65	BORID (2)	N	-	R-HyperCVAD (4)	CR	-	R main.	CR	57	152
14	71/M	CVP (1), R-CHOP (2)	N	1	Nordic	N	2	BORID (2)	N	-	-	-	-	-	-	-	-	-	-	12
15	46/F	R-CHOP (8)	PR	4	IT MTX+ARA-C+RT	CR	6	R-ICE (2)+ASCT	CR	26	CHOP (2)	N	-	BORID (6)	N	2	-	-	-	56

Rs: Response to treatment; PFS: Progression free survival; PR: Partial remission; CR: Complete remission; N: Non-responsive;

R: Rituximab; Len: Lenalidomide; Cyc: Cyclophosphamide; Chlr: Chlorambucil; B: Bendamustin; ARA-C: Cytosine arabinoside; ASCT: Autologous Stem Cell Transplantation; OS: Overall survival, Ib: Ibrutinib; RT: Radiotheraphy; R main: Rituximab maintenance

Results: 73% of patients were male, and the median age was 65 years (range, 46-73 years). All of the patients were in advanced stage (III-IV) at diagnosis and according to MIPI score all patients were high risk. Mean hemoglobin level was 10.7 g/dL (range, 6.2-14.8 g/dL). Mean leukocyte and lymphocyte counts were 8.900x 10^9/L (range, 2.79-561 x 10^9/L) and 2.2 x 10^9/L (0.7-522 x 10^9/L), respectively. LDH was normal in seven (47%) patients and high in 8. Ki-67 index was examined in 9 patients, and it was >%50 in 3. Median number of treatments prior to BORID was 2 (range, 1-5) and median BORID cycle was 3 (range, 1-6). Treatment regimens are listed in Table 1. BORID was discontinued after first cycle in 3 patients (20%) because of side effects; 1 patient had tumor lysis syndrome, 1 had elevated liver enzymes and 1 grade 4 polyneuropathy. Response rates for BORID were as follows: 1 patient was non-responsive and lost the follow-up after two cycles, 1 patient was partial responsive after 6 cycles and she received 12 courses of BORID totally, after all she had complete response. 1 patient lost the follow up after 3 cycles, 1 had tumour lysis syndrome and died after first course of BORID, 1 patient had progression and CNS involvement after 3 cycles, 1 patient had hepatotoxicity and lost the follow-up after first course, 2 patients had partial remission, 6 patients were non-responsive and one of them died because of pneumosepsis after second course of BORID, 1 patient had grade 4 polyneuropathy after first cycle but she remained progression free for 23 months. 4 patients received SCT (3 autologous, 1 autologous and allogeneic), and median survival was 41 months (range, 7-152 months).

Summary and Conclusions: Cure in MCL is not possible with current treatment strategies. Combination of bortezomib and rituximab may be appropriate treatment regimen in relapsed MCL especially for patients who are not candidate for SCT or with comorbidities, but to evaluate the efficacy and toxicity of BORID in relapsed MCL prospective studies are needed.

Infectious diseases, supportive care

PB1797

THE MANAGEMENT OF INVASIVE FUNGAL INFECTION WITH POSACONAZOLE ORAL SUSPENSION MAINTENANCE TREATMENT FOLLOWING INTRAVENOUS ANTIFUNGAL REGIMENS IN ROUTINE UK CLINICAL PRACTICE (MAINTAIN)

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Background: There are limited data on the use of posaconazole oral suspension (POS) as a maintenance treatment for invasive fungal infection (IFI) following initial treatment with an intravenous (IV) antifungal agent/s.
Aims: To describe a population of patients who received POS following previous IV antifungal treatment for possible, probable, or proven IFI and describe the IV antifungal agents prescribed in those patients, prior to POS, the rationale for initiating POS maintenance therapy, and the dose and duration of POS received.
Methods: A retrospective analysis of 25 consecutive eligible adult patients who received oral POS following IV antifungal treatment was conducted in three hematology centers in the UK. Study data were obtained from hospital records, including patient case notes and hospital administrative and clinical databases. Data is presented as descriptive statistics.
Results: *Description of patient population:* The patient population reported in this analysis comprises 25 adults with a median age of 54 years (interquartile range [IQR]: 43-61 years), of whom 15 were male and 22 were defined as White British. The most common primary diagnosis was acute myeloid leukaemia (n=13). 7 patients had a previous IFI prior to the case under review; 7 had documented long-term immunosuppression, and 17 patients were stem cell transplant recipients. *Antifungal treatment:* 12 out of 25 patients received prophylaxis prior to this episode of IFI. All patients were given IV antifungal treatment prior to the initiation of oral POS maintenance treatment. The two most commonly prescribed IV antifungal treatments prior to POS initiation were either caspofungin or liposomal amphotericin B. The most common reasons for treatment initiation were; persistent temperature and suspicious CT result. For the majority of the patients who were subsequently prescribed POS the center attributed rationale for doing so, was to “facilitate hospital discharge” (n=16). All 25 patients received POS at the normal recommended dose; of the 19 patients with a recorded stop date, the median duration of POS therapy was 27 days (IQR: 15-87.5 days). Table 1 shows the mean number of diagnostic and monitoring tests of those patients that had the specified test.
Summary and Conclusions: This analysis suggests that in a UK patient population diagnosed with hematological malignancy and receiving treatment for an IFI, POS is frequently used following iv therapy, as a step-down treatment. Physicians most frequently stated that this treatment decision was to allow the early discharge of patients from hospital. The sample size although relatively small, has similar demographics to already published data. Use of POS, by allowing the discontinuation of iv therapy, may reduce the burden on hospital resources and may deliver considerable cost savings to the NHS by decreasing length of stay. Furthermore, other benefits may include allowing patients to receive treatment at home, in their preferred environment, where appropriate and also reduce the risk of nosocomial infections.

PB1798

IMMUNOLOGICAL MEMORY OF HEPATITIS B VACCINE IN CHILDREN WITH CANCER

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Background: Children with cancer are at risk of severe infections especially who have not completed their primary immunizations. Immune competence decreases not only due to chemotherapy-induced neutropenia, but also due to the reduction of serum antibody titers gained from previous immunizations. Hepatitis B infection in cancer patients occurs as a result of recurrent blood transfusions and also due to suppressed immune system. The national immunization program plays an important role in decreasing subsequent hepatitis B infection in children with cancer, however, the efficacy of the immunization strategies employed is questionable.
Aims: We aimed at evaluating the acquired immunity from the routine hepatitis B vaccination in cancer patients after recovery of their immune system.
Methods: case control study was conducted on 22 patients with cancer(13 males,9 females, mean age 7.2±3.14 years) who were registered in and followed up at pediatric oncology unit of Zagazig university hospital and who completed their standard chemotherapy at least 6 months prior to the study. Twenty two age and sex matched healthy children were enrolled as a control group. Data abstraction form was designed to capture the appropriate information

from the individual medical records including full clinical, transfusion and laboratory data.. HBsAb and HBcAb concentrations were determined in the patients and healthy subjects serum by ELISA. HBs AB titer >10 mIU/mL was considered as baseline protective titer for preventing HBV infection.
Results: The frequency of non immune subjects in children with cancer was significantly higher than those in healthy children (P-value=0.001),where anti-HBs antibody titer was more than 10 mIU/mL in 54.5% of patients and less than 10 mIU/mL in 45.5% of patients. While in healthy controls, 90.9% had antibody titer more than10 mIU/mL and 9.1% had antibody titer less than 10mIU/mL. No significant relation was found between loss of immunity against HBV and age of patient, type of cancer and duration of chemotherapy.
Summary and Conclusions: Children with cancer who received chemotherapy are at increased risk for HBV infection. HBV revaccination should be considered at least six month post-intensive chemotherapy.larger studies still needed to confirm our finding.

PB1799

THE EVALUATION OF FEBRILE NEUTROPENIA EPISODES IN CHILDHOOD MALIGNANCY; SINGLE CENTER EXPERIENCE

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Background: Febrile neutropenia (FN) is an oncologic emergency that may cause serious complications or death. Rapidly diagnosis and empirical antimicrobial therapy can decrease morbidity and mortality in FN. Agents for empirical therapy have choosen by clinicians must be bacterisidal, broad-spectrum and least toxic. Guidelines and current studies have anticipated about high risk factors and empirical therapy in FN.
Aims: To evaluation of febrile neutropenia episodes (FNEs) in our clinic is aimed in current study.
Methods: We retrospectively analyzed data of 131 FNEs of 48 patients who were admitted to the Pediatric Hematology and Oncology Department of Dr. Behçet Uz Children's Hospital, Izmir, Turkey between January 2012 and March2014.
Results: The median age at diagnosis of FNEs was 7.4years (3 months-17 years). Thirty-two of the 48 patients were male and 16 were female. The diagnosis were leukemia at 31, lymphoma at 2 and solid tumor at 15 of patients. Seventy-four per cent of 131 FNEs were determined in patients with leukemia. Focus of clinical infection were determined in 78% of episodes. The distribution of infection's focus were shown at figure 1. Thirty of FNEs (23%) were documented microbiologically and 60% of them were gram negative bacteria, 30% of them were gram positive bacteria and 10% of them were fungus. Empirical antibiotherapy was applied with monotherapy in 28% of episodes as monotherapy with piperacillin-tazobactam. Piperacillin-tazobactam was also used in 47% of FNEs as duotherapy with aminoglycosides. Other empirical antibitherapy choose were 16% meropenem and 9% cefoperasone-sulbactam. Empirical antifungal was applied in 10% of episodes. Modification of therapy was made as adding antibiotics in 37% (adding aminoglycoside or glycopeptide) or changing antibiotics in 22% (changing to meropeneme) of FNEs. Fourty-eight per cent of FNEs had got response with monotherapy or duotherapy like controlled fever with clinical stabilization. Fever control was provided in 72% of episodes in first 72 hours and also in 83% of episodes in 5 days. Two of 48 patients (4%) were died during neutropenic fever due to pneumonia.

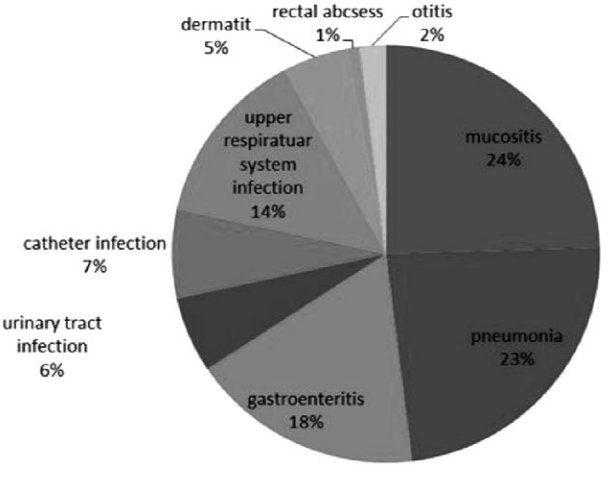

Figure 1. The distribution of infection's focus.

Summary and Conclusions: This study presents clinical findings of patients with FN in childhood malignancy at single center. FNEs were the most in leukemia than the other malignancy. In current study, we identified that we modificated to therapy in FNEs and this value is higher then expected. Even so, fever control rate of us was successfull in FNEs for initial 5 days. This study will contribute to rapidly and efficacious empirical therapy can decrease morbidity and mortality in FNEs.

PB1800

AFTER ADMISSION TO INTENSIVE CARE UNIT NON-TRANSPLANT PATIENTS WITH HAEMATOLOGIC MALIGNANCIES HAVE BETTER SURVIVAL OUTCOME THAN RECIPIENTS OF HAEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Patients with haematological malignancies admitted to Intensive Care Units (ICU) have a high mortality rate and poor survival. Identifying predictors of mortality and survival may be useful for clinical decision making and can help to improve survival. The differences in mortality rate and survival outcomes between recipients of haemoipietic stem cell transplant (HSCT) and non-transplant haematologic patients have been poorly studied.
Aims: to compare survival rate after intensive care management between recipients of haematopoietic stem cell transplant and non-transplant patients with haematologic malignancies.
Methods: prospective observational study of all consecutive patients with haematologic malignancies admitted to the ICU. Patients have been undergone an HSCT or not. Period: December 2012 through April 2014. Variables that were analyzed included: demographics, haematological disease, stage of the haematological disease, main reason for admission into the ICU, critical score evaluation (APACHE II y SOFA), organ failure, organic support therapy, death rate in the ICU and in hospital during the following 28 days after admission. Comparisons of quantitative and qualitative variables between independent groups were analyzed by Student t-test, or Mann-Whitney U test for non-normally distributed variables, and chi-square test, Fisher's exact test in 2x2 tables, depending on sample size.
Results: 63 consecutive patients were included and their data analyzed. Twenty seven have undergone an HSCT and 36 did not. HSCT recipients tended to be younger [median age 51 (34-55) vs. 57 (43-66); P=0,01] and they were admitted to the ICU in a worst clinical condition [SOFA: 10 (8-14) vs. 8 (4-11); P=0,01] and APACHE II [26 (19-29) vs. 21 (16-28); P=0,3]. The most frequent cause of admission to the ICU was respiratory failure (77,8%), without differences between both groups (P=0,5). However, recipients of HSCT needed more frequently invasive mechanical ventilation (IMV; P=0,007) and suffered significantly more liver failure (77,8% vs. 30, 6%; P<0,001) and renal failure (85,2% vs. 44,4%, P<0,001). Haemodynamic failure was also more frequent in the HSCT recipients than in non-transplant patients (88, 9% vs. 63, 9%; P=0, 02). Hospital mortality rate 28 days after ICU admission of patients with HSCT was higher (92,6%) than for non-HSCT patients (44,4%; P<0,001). There were no significant differences between both groups in the rest of analyzed variables
Summary and Conclusions: recipients of an haemopoietic stem cell transplant have a significantly higher mortality rate and worst survival than non-transplant haematologic patients after intensive care admission. The causes for such an outcome could be the worst clinical condition at ICU admission, more need for invasive mechanical ventilation and higher rate of organ failure in HSCT recipients. Knowing the reasons for these differences merit further investigation and may help to improve survival of both settings of patients

PB1801

INVASIVE PULMONARY ASPERGILLOSIS IN ACUTE LEUKEMIA:ABOUT 57 CASES

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Background: Invasive pulmonary aspergillosis (IPA) is a serious fungal infection and it's associated with high mortality.
Aims: In this study, we evaluate the incidence and treatment outcome of IPA in acute leukemia (AL).
Methods: Our retrospective study included patients with *novo* acute leukemia (ALL or AML) under which occurring IPA during chemotherapy following in department of Hematology CHU Hédi CHaker Sfax between January 2009 and December 2013. The treatment of acute leukemia is based in chemotherapy and /or corticosteroids, and it is done in conventional rooms.Invasive pulmonary aspergillosis was diagnosed based on the revised definitions of invasive fungal

disease from the EORTC/MSG clinical, microbiological and radiological criteria. Accordingly, the patients were diagnosed as having "proven," "probable," or "possible" IPA.For this, a Clinical data including clinical manifestations, diagnostic results, and treatment was collected for every patient.
Results: We analyzed 247 patients with acute leukemia. IPA was diagnosed in 57 patients.the incidence of IPA was 23% during 5 years. The average age was 29 years old (3-53 years old). All our patients had at least 3 criteria according to EORTC/MSG (neutropenia<500 / mm3 and prolonged, fever despite broad-spectrum antibiotics and immunosuppressive agents in the previous 30 days). The aspergillus antigenemia was positive in 26 patients (45%).The broncho-alveolar lavage (LBA) was done in 22 cases including (38%). For this, patients with aspergillosis were diagnosed with probable IPA in 45%, possible IPA in 46.5% and proven one in 5 cases (8.5%). All patients were treated with amphotericin B and relay by voriconazole. The Evolution was favorable in 28 cases (49%) with early deaths in 10 patients (18%). Twenty patients (35%) are alive with an average decline about 1 year.
Summary and Conclusions: The prevalence of the IPA in our series is higher than the literature (15%) because patients are treated in conventional rooms. The prevalence of proven IPA in our study is very low because of the difficulty of histological documentation (technic problem and deep thrombocytopenia). Wherever; the precocity of diagnosis by TDM imagery and the treatment with voriconazole have improved the mortality rate in our series.

PB1802

DIAGNOSTIC UTILITY OF ADDING SERUM GALACTOMANNAN TEST TO STANDARD TECHNIQUES OF DIAGNOSING INVASIVE FUNGAL INFECTION IN PATIENTS OF ACUTE LEUKEMIA WITH NEUTROPENIA
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Background: Invasive fungal infection (IFI) (esp. Invasive Aspergillosis) is very common in patients of acute leukemia undergoing induction chemotherapy. Case fatality rates are approximately 50-60% in various studies. It is usually diagnosed as probable case based on CT, as per EORTC/MSG criteria. Serial Galactomannan (GM) monitoring can be helpful in diagnosing IFI early.
Aims: To study the diagnostic & prognostic significance of weekly Serum Galactomannan level monitoring and the development of invasive fungal infection based on radiological imaging.
Methods: Prospective single centre cohort study which included 100 patients of acute leukemia with neutropenia. Weekly GM levels were done with CT chest done as and when clinically indicated. Results of GM levels were compared with CT positivity at any time point during the phase of neutropenia. GM levels were also assessed to predict the survival.
Results: Patients of acute leukemia with neutropenia (mean age 24 8 years, 62% males) were followed for a period of 1 month of which 37% patients expired with median day of expiry 40 days. ALL and AML patients were 34 and 66%, respectively with fever being most common (92%) presenting symptom. 49% patients had evidence of fungal pneumonia on the basis of CT chest with median day of positivity was 12 days (IQR 3-16 days). 74% patients had GM positive value at any time point out of which 20% had persistent GM index positive. Highest value of GM index was 0.701 at day 14 correlating with nadir ANC of 30/cumm at day 14. GM levels were positive in 43%, 63%, 69% and 54% patients at day 0, 7, 14 and 21, respectively. The median GM levels at day 7 (0.8 vs 0.4; P=0.001), 14 (0.87 vs 0.49; P=0.00) and 21 (0.92 vs 0.46; P=0.00) were higher in CT positive group as compared to CT negative group. There was a significant negative correlation between ANC and GM levels (P=0.007). High GM levels at day 7 (p value=0.018) and day 14 (p value<0.001) were predictor of development of invasive fungal infection on 1st or 2nd CT. Low GM levels at day 14 (1 vs 0.63; P<0.001) and post induction CR status (98% vs 8%; P<0.001) were significantly associated with better survival.
Summary and Conclusions: High GM levels at day 7 were predictor of development of invasive fungal infection on CT. High GM levels negatively correlates with the degree of neutropenia. Low GM levels at day 14 and post induction CR is associated with better survival.

PB1803

INFECTIOUS COMPLICATIONS AFTER AUTOLOGOUS HEMATOPOIETIC TRANSPLANTATION:EARLY AND LATE EVENTS
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Background: Infectious complications occur in most patients receiving high dose therapy (HDT) and autologous stem cell transplantation (ASCT) both during neutropenia,related to the conditioning regimen and later on due to gradual immunological reconstitution of lymphoid and immune effector cells. Fatal infections are rare, but they represent a mayor cause of morbidity and treatment related mortality.
Aims: We retrospectively analyzed the incidence and type of infections (early:day (d) 0-100, late:from d 100) after ASCT for haematological malignancies at our hospital from January 2002 to February 2015.
Methods: We studied 103 ASCT performed to 98 patients: 62 males and 41 females;median age:55 years (23-69); the diagnosis were: 42.7% Multiple Myeloma, Non Hodgkin Lymphoma(follicular 10.67%,Large cell B diffuse 10.67%,T 5.8%,anaplastic 0.97%,plasmablastic 0.97%),Hodgkin Lymphoma 19.4%,amyloidosis 1.9%, acute myeloid leukaemia 5.8%, acute lymphoblastic leukaemia 0.97%. Conditioning regimens were:BEAM 19.4%, escalated CBV 28.1%,melfalan 41.7%,BUMEL 2.9%,BEA 5.8%,other 1.9%.
All patients received antibiotic and antiviral prophylaxis during conditioning and for at least 6 months.Vaccinations were administered as stated in EBMT guidelines.(one dose of polysaccharide pneumococcal vaccine at 7m).
Results: Early infections: 94.2% patients have febrile neutropenia; fever of unknown origin represented 44.3% of episodes, the documented infections were as follows:see table 1. We have a fatal sepsis caused by multirresistant pseudomonas aeruginosa.Mortality due to infection:1.03%. Late infections: 75 episodes were recorded in 43 patients. Mortality due to infection was 7.14%. The incidence of Herpes zoster reactivation was11.2% at 6m(3-48) postransplantation; 54.5% were receiving corticosteroids and 36.2% had relapsed. Urinary tract infections 12.2% at 57.5m(10-117). Bacterial Pneumonia:20.4% at 43m(4-78);50% received corticosteroids and 65% had relapsed. Influenzae pneumonia 2%at 27.5 m(14-41).Invasive fungal infections(aspergillus) pneumonia 3.1%: 2 probable,1 proven at 31.5m(4-59).Oropharyngeal candidiasis 3.1% at 45.5m (10-86). Pneumococcal infection: 8.2% at (45.5m (15-76):(5 invasive pneumococcal disease:1 arthritis, 3 bacteriemic pneumonia,1meningitis), 3 pneumonia; 2 patients have multiple episodes;mortality rate was 25%;50%of patients had progressed.and 62.5% received corticosteroids. Catheter related infections: 5.1% at 17m(4-46). Gastroenteritis 3.1%(1 campylobacter, 2 clostridium difficile) at 13m (7-23). 1(1.02%)episode of bacteriaemia by Listeria monocytogenes without focal infection at 8m. 1(1.02%)Visceral Leishmaniasis at 33m. Cryptosporidium infection 3.1% (1 systemic, 2 diarrhea) at 40.5m(8-73).

Table 1.

Microbiologically documented infection 37.1%)	Clinically documented infections (13.4%)
2 Herpes zoster	6 pneumonia
3 oral herpes simplex	1 maxilar sinusitis
3 UTI (urinary tract infection)Ecoli	
2 low respiratory tract: 1 Hemophilus influenzae ,1 Stenotrophomona maltophilla	1 otitis
3 oral candidiasis: 1 C.albicans,1 C.tropicalis	
1 Campylobacter gastroenteritis	5 enterocolitis
18 bacteriemia (50% gram positive)	
1 celulitis EColi	
2 candidemia (1 C.parapsilosis,1 C.tropicalis	
1 vulvar abcess Enterococo faecium	

Summary and Conclusions: Our incidence of infectious complications during neutropenia period is similar to that previously described in literature. The incidence of fungal infections is low. 43.8% of our patients suffered late infections and the mortality was 16.2%.Herpes zoster is an important cause of morbidity late after ASCT. Pneumococcal infections stand out due to its continuous occurrence late after transplantation and its high mortality; perhaps the use of the 13-valent pneumococcal conjugated vaccine could help to prevent it. Constant surveillance and prompt therapy must be held for infections, even late after autologous transplantation.

PB1804

RITUXIMAB-ASSOCIATED HEPATITIS B VIRUS (HBV) REACTIVATION IN LYMPHOPROLIFERATIVE DISEASES: CASE REPORTS
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Background: Reactivation of hepatitis B virus (HBV) after rituximab-based chemotherapy regimens in patients with B chronic lymphoproliferative disorders is well-recognized as a potentially serious complication in HBV immune patients. It has been observed in patients positive for hepatitis B surface antigen (HBsAg) and in HBsAg negative patients who had HBV infection in the past.

The risk of HBV reactivation differs according to both the patient's HBV infection status prior to systemic chemotherapy and the degree of immunosuppression due to chemotherapy. The overall incidence of reactivation with use of rituximab is unclear, but ranges from 2% to 35% of patients.
Aims: To discuss the relationship between Rituximab-based therapy and B hepatitis reactivation, and to estabilish the management of hepatitis associated with the use of Rituximab, prevention and treatment.
Methods: We conducted a prospective study on nine patients with lymphoproliferive diseases, whom treated with Rituximab, in our institution from 2013 to 2014. Data were collected and analysed from medical records. We were focused on risk factors for HBV reactivation, clinical characteristics, biological data and management. Lymphoproliferative histologies were diffuse large B-cell lymphoma (n = 3), follicular lymphoma(n=1) CLL (n=2), mantle-cell lymphoma (n=1), and marginal zone lymphoma (n=2). All patients received Rituximab-containig chemotherapy: FCR (Fludarabine, Cyclophosphamide, Rituximab), and R-CHOP (Rituximab-Cyclophosphamide, Doxorubicine, Vincristine and Prednisone). Four patients were HBV positve; one of them was treated by pegylated Interferon and was completely recovered before starting Rituximab.
Results: The median age at diagnosis was 56 years (rang 37-72), and seven patients were male. HBV screening data were available for all patients at baseline. 4 patients were HBsAg positive, five of them were HBsAg negative. The time from last rituximab to reactivation was 3 months (range 0–12), HBV reactivation occured usually at the completion of chemotherapy (upper than 3 months after the last dose of Rituximab), for 3 patients in the periods between cycles (median occurrence was 17 days). Clinical manifestations of hepatitis B reactivation were variable and ranged from asymptomatic to acute hepatitis (2 patients). Serum transaminase (ALT, AST) levels were elevated, high total bilirubin and hepatitis serology tests revealed reappearance of HBsAg and hepatitis B e antigen (HBeAg), loss of anti-HBsAb, and positivity for hepatitis B virus (HBV) DNA (3 patients were HBV DNA negative). Lamivudine and entecavir (nucleoside analogues) were the most antiviral used for the treatment of HBV reactivation. Six patients were treated : 05 of them received Lamuvidine schudeled at 100 mg daily. Entecavir was prescribed for one patient who received previously Interferon. The remaining 3 (33% patients) were HBsAg negative and anti-HBsAb negative and anti-HBcAb positive, did not undego treatment and they were closely monitored for HBsAg, anti-HBsAb, HBV-DNA, and transaminase levels. The hepatitis flare was controlled (improvement of liver function tests, reduction of HBV DNA levels) and treatment was well tolerated. At the last follow-up, two patients died because of fulminant hepatitis and severe peumonia
Summary and Conclusions: Rituximab-based therapy may cause serious HBV-related complications and even death, by increasing the risk of HBV reactivation both in inactive carriers and those with resolved HBV. Baseline HBV serology is recommended for all patients receiving immuno-chemotherapy regimen, and HBsAg positive patients should receive anti-HBV prophylaxis to decrease virus reactivation and death rates.

PB1805

DIAGNOSTIC VALUE OF PLASMA LEVELS OF PRESEPSIN (SOLUBLE CD14-SUBTYPE) FOR FEBRILE NEUTROPENIA IN PATIENTS WITH HEMATOLOGIC MALIGNANCIES

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Background: Previous studies reported that procalcitonin (PCT), C-reactive protein (CRP) and cytokines such as interleukin (IL)-6 and IL-8 may be useful as biomarkers of bacterial infections in the management of febrile neutropenia (FN). CD14, one of the surface markers in monocytes/macrophages, is a lipopolysaccharide-binding protein complex receptor, and its fragment "presepsin (soluble CD14-subtype)" has recently been reported as a novel diagnostic biomarker of infection, especially sepsis (Endo S, *et al*. 2012). However, the superiority of this test in FN, compared with the other markers, is unknown.
Aims: The purpose of the study was to examine whether presepsin was a superior diagnostic marker for bacterial infections in comparison with PCT, CRP, IL-6 and IL-8.
Methods: We prospectively evaluated the utility of these biomarkers in patients with hematological disorders who developed FN during chemotherapy between Nov 2013 and Feb 2014. FN was defined as an axillary temperature ≥ 37.5 □ recorded once with a neutrophil count<500/μl or<1,000/μl with an expected decline to<500/μl. Patients were closely and frequently monitored to assess precisely differences in kinetics among these markers. Thus, blood samples for these measurements were collected simultaneously at the following time points: once before chemotherapy as a control, 3 times per week until onset of FN after chemotherapy, at onset of FN, at 8, 16, 24, 48 and 72 hours after the onset of FN, and every 48 hours after that until resolution of fever, in accordance with the protocol. Plasma presepsin was determined by the PATHFAST® Presepsin kit (LSI Medience Corporation, Japan). Serum levels of CRP, PCT and cytokines were measured by nephelometry, electrochemiluminescence immunoassay and the Bio-Plex Pro Cytokine Assay® system (Bio-Rad Laboratories, CA), respectively.
Results: A total of four patients aged 29–54 years were enrolled and four FN episodes were evaluable. The underlying diseases were acute leukemia in three patients and one case of non-Hodgkin's lymphoma. Concerning the cause of FN, Patient no.1 (P1), P2, P3 and P4 were finally diagnosed as having a fever of unknown origin, suspected drug fever, clinically documented local infection and Staphylococcus aureus bacteremia, respectively. The number of times each of these patients was sampled was 18, 17, 18 and 15 respectively. A result was considered to be positive if it met the following criteria: 314 pg/mL for presepsin, 0.30 mg/dl for CRP, 0.05 ng/mL for PCT, and the levels of the baseline controls sampled before chemotherapy for both IL-6 and IL-8, respectively. The presepsin peak levels from the onset of FN to 24 hours in P1, P2, P3 and P4 were 330, 148, 348 and 460 pg/mL, respectively. The corresponding levels of CRP, PCT, IL-6 and IL-8 in P1, P2, P3 and P4 were 1.8, 6.0, 11.9 and 4.9 mg/dl; 0.04, 0.34, 0.36 and 0.22 ng/mL; 14 (baseline, 27), 107 (23), 69 (25) and 24 (8.4) pg/mL; and 52 (62), 787 (58), 56 (150) and 26 (13) pg/mL, respectively. In P2, where no infection was suspected, presepsin was the only marker that did not increase. This suggests that presepsin might be useful to differentiate infectious from non-infectious causes of FN.
Summary and Conclusions: Our results suggest that presepsin may be more useful than CRP, PCT, IL-6 and IL-8 in the management of FN, especially when attempting to rule out the presence of serious bacterial infections.

PB1806

EFFECTIVENESS OF SUPPORTIVE THERAPY WITH LOW-DOSE LENOGRASTIM FOR B-CELL LYMPHOMAS

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Background: To date, over 30 kinds of malignant lymphomas have been classified by the WHO classification. Although R-CHOP chemotherapy is currently considered the standard therapy for B cell lymphoma, the beneficial effects of G-CSF, namely, decreases in the number of neutrophils, the prevention of febrile neutropenia, and maintenance of the treatment interval, have also been reported. We performed R-CHOP therapy in our hospital between 2009 and 2014 and assessed its curative effects based on the number of neutrocytes when combined with three G-CSF agents (filgrastim, nartograstim, and lenograstim), which were used as supportive therapy in our hospital.
Aims: We would like to confirm whether the usage of G-CSFs which are filgrastim, nartograstim, and lenograstim is considered a safe supportive therapy for R-CHOP chemotherapy.
Methods: White blood cell counts were measured before administering G-CSF to a total of 177 patients scheduled for the next cycle of R-CHOP chemotherapy in our hospital between February 2009 and February 2014. The effectiveness and side effects of the 3 drugs were examined through analysis of variance.
Results: The median age of patients who underwent a total of 177 cycles of R-CHOP chemotherapy was 61 years old. The Filgrastim group was significantly older than the Nartograstim and Lenograstim groups (Filgrastim: 75±5 years old ($P=1.64\times10^6$), Nartograstim: 61±17 years old, Lenograstim: 58±12 years old). The proportion of females was high in the nartograstim group (P=0.004). No significant difference was observed the incidence of infiltration to the bone marrow between the Nartograstim and Lenograstim groups. LDH levels were significantly lower (LDH: 200±24mg/dL, P=0.036) in the Filgrastim group immediately prior to chemotherapy. Furthermore, white blood cell counts before the next course of chemotherapy were significantly lower in the Filgrastim group (WBC: 4352±1412/μL, P=0.006), whereas no significant difference were observed between the Nartograstim and Lenograstim groups.
Summary and Conclusions: It was difficult to directly compare filgrastim with the other 2 drugs due to the large number of elderly patients in the study hospital. On the other hand, no significant differences were observed in effectiveness between nartograstim and lenograstim and no influence was noted on the white blood cell count immediately prior to the next course of chemotherapy. We herein confirmed that no significant differences existed between the Nartograstim and Lenograstim groups; therefore, the usage of these agents is considered a safe supportive therapy for R-CHOP chemotherapy.

PB1807

INVASIVE FUNGAL INFECTION IN ACUTE LEUKEMIA PATIENTS-A SINGLE CENTER EXPERIENCE

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Background: Invasive fungal infection (IFI) is one of the most severe complications that apppear in patients with acute leukemia. The outcome of the acute leukemia is significantly influenced by the presence of severe fungal infection during induction, consolidation or salvage therapy.
Aims: To evaluate the outcome of patients with proven or probable IFI at diagnosis or during therapy. We analyzed the risk factors for invasive fungal infection in our study group.

Methods: We evaluated 91 patients diagnosed with acute leukemia during a 2 year period between March 2012 and October 2014. Of the 91 patients included, 63% was diagnose with acute myeloid leukemia. Median age at diagnosis was 52 years old. The number of episodes of febrile neutropenia in all patients and the appearance of probable or confirmed invasive fungal infections in the course of these episodes was analyzed.
Results: In our cohort the incidence of IFI during 90 episodes of febrile neutropenia analyzed was 18,6%. More than 50% of invasive fungal infection appeared after induction chemotherapy and the median duration of neutropenia grade IV OMS at this patients was 22 days. Infection with Aspergillus spp. was the most frequent diagnosed fungal infection (14 patients). Invasive candidiasis was diagnosed in only 3 patients. The most common location was the lung. 2 patients were diagnosed with probable cerebral aspergillosis. Despite the good response of azole based therapy in majority of patients, 5 patients required second line of antifungal therapy and death appear in 4 patients with invasive fungal infection. In our study group the only risk factor which statistic significantly influenced IFI risk was duration of neutropenia more than 21 days (P=0,006).
Summary and Conclusions: IFI is an important cause of morbidity and mortality in patients diagnosed with acute leukemia. The high percentage of patients with invasive fungal infection after induction chemotherapy (9,9% in our study) with significantly delays in subsequent consolidation courses is a important issue. Correct assessment of the risk of infection in these patient populations associated with appropriate prophylactic measures can significantly reduce the risk of these forms of infection.

PB1808

INFECTION HISTORY MAY BE USED TO PREDICT OF BENEFIT OF IVIG THERAPY IN HYPOGAMMAGLOBULINAEMIC HAEMATOLOGY PATIENTS

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Background: Intravenous immunoglobulin (IVIg) has been shown to reduce the risk of infections in unselected patients with hypogammaglobulinemia secondary to hematological malignancies. It is not known whether a history of infections can predict benefit from IVIg and can therefore by used to select patients for therapy
Aims: To determine the risk of serious infections when selecting hematological patients for IVIg based on a history of infections and IgG levels.
Methods: A retrospective review of the rate of hospitalisation for infections before and during IVIg therapy in hematological malignancy patients selected for therapy on the basis of serious or recurrent infections was conducted. Results were compared with hypogammaglobulinemic haematology patients not selected for therapy. Characteristics of the groups were compared with Chi-squared tests and event rates expressed as rate ratios with estimated variance to determine 95% confidence limits.
Results: A total of 35 treated patients were treated with IVIg for 1194 months and had 776 months of observations prior to starting treatment. By comparison 57 patients with low IgG levels were identified with hypogammaglobulinemia (IgG<7g/L) who did not require IVIg, with the total observational period being 2587 months. The untreated group had fewer cases with severe hypogammaglobulinemia (51% v 77%, P<0.01) but were otherwise similarly matched for diagnosis, age and sex. The treated group showed a rate of hospitalisation for bacterial infection of 0.42 per patient year prior to treatment, reducing to 0.14 (relative risk 3.1 (1.6-5.9) for infection prior to treatment). The risk of infection prior to treatment also compared favourably to the 0.08 hospitalisation per patient year in those never treated (relative risk 5.5 (3.0-10)). An IgG level of<4g/L did not predict rates of infection compared with an IgG level >4g/L in either the untreated group or the treated group prior to the commencement of IVIg.
Summary and Conclusions: In this retrospective study, selecting hypogammaglobulinemic hematology patients to have IVIg given or withheld on the basis of a history of infections appears safe and effective.

PB1809

IMPACT OF POSACONAZOLE PROPHYLAXIS ON INVASIVE FUNGAL INFECTION, ASPERGILLUS GALACTOMANNAN AND PERSISTENT FEBRILE NEUTROPENIA DURING AML INDUCTION THERAPY: A SINGLE-CENTER, REAL-LIFE EXPERIENCE

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Background: Posaconazole prophylaxis has been shown to prevent invasive fungal infection(IFI) in acute myeloid leukaemia (AML) patients during induction therapy. The role of serum aspergillus galactomannan(GM) in diagnosis of invasive fungal infection and the optimal management of persistent febrile neutropenia during posaconazole prophylaxis is less clear.
Aims: The aims of this study are to determine in patients having AML induction chemotherapy with posaconazole prophylaxis, the incidence of IFI, the role of GM in diagnosis and premptive antifungal treatment and the management of persistent febrile neutropenia for more than 96 hours despite broad-spectrum antibiotics.
Methods: We collected prospective data on 22 consecutive patients undergoing AML induction chemotherapy with posaconazole prophylaxis between August 2013 and November 2014. Induction chemotherapy comprised duanorubicin 45-90 mg/m^2 for 3 days and cytarabine 100 mg/m^2 for 7 days. Oral posaconazole was started on day 1 of chemotherapy. Patients not in complete remission received salvage reinduction chemotherapy. Serum posaconazole levels were not done. GM was performed once or twice weekly.Patients who had persistent febrile neutropenia for more than 96 hours despite broad spectrum antibiotics were investigated with computed tomography. Respiratory consult for broncho-alveolar lavage was discretionary. Empirical antifungal therapy was commenced if the patient was clinically unstable or had radiological findings of probable IFI.
Results: IFI(aspergillus flavus sinusitis) was diagnosed in 1 out of 22 patients (4.55%) during the first cycle of induction chemotherapy. Probable invasive pulmonary aspergillosis was diagnosed in one patient during salvage chemotherapy. Both infections were fatal. Both patients had radiological findings but negative serum GM levels and diarrhoea at the time of diagnosis. One patient had a single positive serum GM (GM index of 0.9) two weeks prior to the diagnosis of IFI but was deemed to be false-positive as he was afebrile and asymptomatic. Serum GM was done on 179 occasions but was positive only once (0.56%) which was deemed false-positive. Persistent febrile neutropenia for more than 96 hours despite broad spectrum antibiotics were seen in 17 patients (77.27%). Computed tomography showed radiological findings suggestive of chest infection in 9 patients (40.9%) and sinusitis in 3 patients (13.63%). Positive blood cultures were seen in 10 patients with febrile neutropenia (45.45%). 4 patients(18.19%) had antifungal treatment. BAL was performed in one patient and did not reveal any specific microbiology.
Summary and Conclusions: Posaconazole is effective antifungal prophylaxis in AML patients undergoing induction chemotherapy. Diarrhoea may impair posaconazole absorption and may have lead to invasive fungal infection in the two patients but posaconazole levels were not done. Therapeutic drug monitoring may be useful as IFI are uncommon with adequate levels. Serum GM is not useful in the diagnosis and initiation of preemptive antifungal therapy with posaconazole prophylaxis. Persistent febrile neutropenia should not be automatically an indication for empirical antifungal therapy in all patients. Individualised decision making is needed in this context. Assessment of serum posaconazole levels, computed tomography, broncho-alveolar lavage with BAL galactomannan and the clinical status of the patient may be the best approach to initiate antifungal therapy with posaconazole prophylaxis in persistent febrile neutropenia of unknown origin. Empirical antifungal therapy is an alternative option but may increase treatment cost and drug resistance. Invasive fungal infection in the setting of posaconazole prophylaxis is difficult to treat and often fatal.

PB1810

THE EPIDEMIOLOGY OF INVASIVE FUNGAL INFECTIONS INHEMATOLOGIC ADULT PATIENTS IN WROCLAW University HOSPITAL, POLAND

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Background: The invasive fungal infections (IFIs) are associated with a very high mortality in hematologic patients mainly because of asymptomatic course and delayed diagnosis.
Aims: The aim of the study was to analyze the clinical feature and outcome of invasive fungal infections in adult patients diagnosed and treated in Wroclaw Medical University.
Methods: The retrospective analysis of patients with hematologic malignancies and patients undergone hematopoietic stem cell transplant (HSCT), who were hospitalized in our hospital between January 2011 and December 2014, was performed. Proven, probable and possible IFIs were diagnosed according to the definitions of EORTC/IFICG. Galactomannan and mannan antigens were detected by ELISA whereas culture of pathogenic fungi was based on EUCAST recommendations.
Results: In analyzed period, invasive fungal infection was diagnosed in 55 patients, of which 34 (62%) was proven, 19(34,5%) probable, and 2(3,5%) possible. Aspergillosis was diagnosed in 50/55 patients, candidosis in 3/55 patients, mucormysosis in 1/55, and cryptococcosis in one patient (1/55). The major pathogens isolated form clinical specimens were: *Aspergillus fumigatus* (21/55,38.2%), *Aspergillus flavus* (2/55, 3.6%), *Aspergillus niger* (1/55, 1.8%), *Absidia* (1/55, 1.8%), *Mucor* (1/55, 1.8%), *Candida glabrata* (3/55, 5.5%). In two patients two different *Aspergillus spp*. (*A. fumigatus* and *A. flavus*) were identified from lower respiratory tract. The infection was mostly localized in lungs (48/55,87.2%), and central nervous system (4/55, 7.3%). Disseminated infection was noted in two patients (3/55, 5.5%). IFIs were most common in patients with acute myeloid leukemia (27/55, 49%) and patients undergone HSCT (8/55, 14.5%). The major risk factors in this patients were prolonged neutropenia and degree of immunosupression. A total of 39 patients (71%) died.

Summary and Conclusions: The most common pathogen of invasive fungal infection in the cohort of hematologic patients was *Aspergillus fumigatus*. Constant monitoring of the epidemiologic trends and insights in timing of fungal infections in this patients may help to improve the effective prevention and treatment strategies.

PB1811

A COMPARISON OF IMMATURE GRANULOCYTE (IG) PARAMETER BETWEEN A HEMATOLOGY ANALYZER AND MANUAL MICROSCOPY

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Background: Left shift in granulopoiesis, has been seen in cancer or infection. An accurate marker has been IG (immature granulocyte which includes promyelocytes, myelocytes and metamyelocytes), blasts and bands are excluded from the measurement. In the peripheral blood smear, the appearance of immature granulocytes (IG) is a common finding in infection, inflammation, hematologic malignancy disorders and other factors that stimulate the bone marrow such as Growth-Stimulating Factors (G-CSF). Also, increased granulation in neutrophils indicates serious infection or patients undergoing chemotherapy.
Aims: To compare the manual IG with the IG provided from an automated hematology analyzer.
Methods: A total of 143 unselected whole-blood samples were collected in tubes with K3-EDTA and were analysed in approximately 3 h of collection. Patients who had their blood counts monitored on a daily basis were excluded. The automated IG% results (a percent of the total white blood count) provided from the hematological analyzer (Sysmex-XE 5000) were divided into three groups IG<1 (61 samples), IG 1-10 (61 samples), IG >10 (21 samples) and compared to manual IG counts. The first two groups were similar in regard to sex (39 males-22 females) and age (8-86 years avg=59,4 years). In the third group there were 14 males and 7 females, the age was 18 to 88 years (avg=58,4 years). The peripheral blood smears, from flagged reported samples (Imm Gran), were stained with May-Grünwald- Giemsa by Sysmex SP-100 slide system. The immature granulocytes and granularity of the neutrophils, were generated by two expertised physicians. Mean value comparison using paired t-tests was used in order to examine the statistical significance among the groups. The significance level was defined as $P<0.05$. All statistical analysis was performed using SPSS 20 and Mc Excel 2010.
Results: In the first group with the automated IG<1, the manual IG count was zero, so there was an agreement between visual microscopy and SYSMEX XE-5000. In the second group there was also an agreement in comparison to the manual IG. The difference of the mean values of IG1-10 between the manual IG and the automated IG is not statistically significant (p-value >0,05) showing that there is no difference between the two methods. In the third group there was a difference. The manual IG percentage revealed a higher number of immature granulocytes than the automated IG. The difference between the microscopic and manual results increased with increasing levels of IG. In the third group, the number of specimen was very low so, in order to acquire reliable results, a larger number of samples should be generated.
Summary and Conclusions: Immature granulocytes (IG) are a predictive useful parameter for the presence of infection, sepsis, inflammation, treatment with oncolytic drugs or bone marrow activation. For blood samples whose IG was >1%, a microscopic examination should be operated. In addition, taking into consideration IG% as a marker, a serious number of spurious blood film reviews might be reduced and minimized. Also, the implication to shorter turn-around times would enhance the diagnostic effectiveness.

PB1812

VORICONAZOLE AND CASPOFUNGIN COMBINATION THERAPY IS EFFECTIVE AND SAFE FOR PROBABLE AND POSSIBLE INVASIVE PULMONARY FUNGAL INFECTION IN PEDIATRIC ACUTE LEUKEMIA

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Background: Aspergillus species have emerged as an important cause of life-threatening infections in immunocompromised patients. Despite timely diagnosis and appropriate antifungal therapy, clinical outcome might be disappointing, necessitating treatment with a combination of antifungal agent.
Aims: in this study, we report the efficacy of voriconazole and caspofungin in pediatric acute leukemia.
Methods: We identified 30 pediatric acute leukemia patients who received the combination of CAS and VRC for invasive fungal infection (IFI) from April, 2009 to May, 2013 in pediatric department of Yeungnam university hospital. Medical records of patients were reviewed and analyzed retrospectively. We analyzed data that included the following: Demographic characteristics, underlying disease and disease state, radiological findings and outcome in patients with IFI.
Results: Of the 30 consecutive patients who receive CAS and VRC during study period, 8 patients were not evaluable for the following reasons: pneumonia not attributed to fungus (n=3), non-infectious pulmonary infiltration (n=5). The study group comprised 11 boys and 11 girls, with mean age was 7 years (range, 0.8-13.3) years. Underlying diseases were acute lymphoblastic leukemia (ALL) in 11 of these patients, acute myeloid leukemia (AML) in 11 of patients. No patient had received hematopoietic stem cell transplantation nor immunosuppressive treatment at the onset of IFD. IFD were classified as probable in 9 (41%) and possible in 13 (59%) patients, respectively. All but 1 patients had received previous empirical antifungal monotherapy with conventional amphotericin B, liposomal amphotericin B and fluconazole (range 1-21 days) before start of VRC and CAS combination therapy. One patient had started *de novo* combination antifungal therapy. The median duration of VRC and CAS combination therapy was 45 (range, 5 – 99 days) days. Loading and maintenance dose of CAS were 63.1±7.7 mg/m^2 and 47.2±6.0 mg/m^2 respectively. Those of VRC were 5.9±0.5 mg/kg and 3.6±0.60 mg/kg respectively. The survival rate of 100 days after initiation of combination therapy is 90.9%. Twenty patient (90.9%) had response to combination therapy. (19 complete response and 1 partial response). Age, sex, underlying disease, disease state, treatment phase, oxygen and intravenous immunoglobulin therapy did not affect overall response and overall survival by univariate analysis. VRC and CAS combination therapy was well tolerated except for 2 patents. Two had mild increased in liver enzyme.

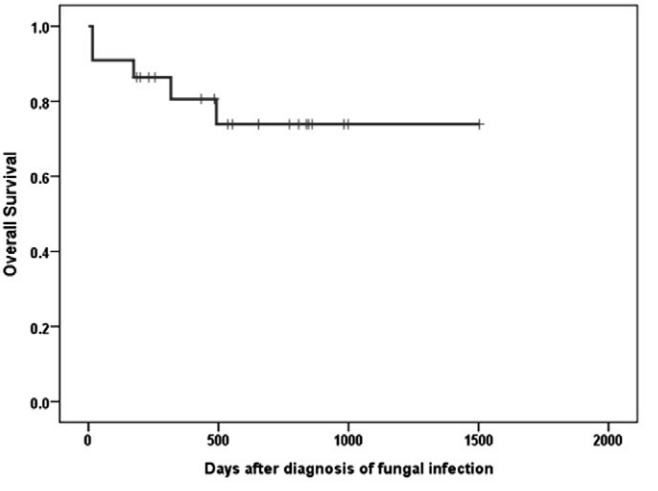

Figure 1.

Summary and Conclusions: Voriconazole and caspofungin combination therapy is effective and safe treatment for serious invasive pulmonary fungal infections in pediatric acute leukemia

PB1813

POSACONAZOLE AS PRIMARY PROPHYLAXIS REDUCES INVASIVE FUNGAL INFECTIONS IN AML PATIENTS: A SINGLE CENTRE MATCHED PAIRED ANALYSIS

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Background: Prevention and prompt treatment of invasive fungal infections (IFI) in acute myeloid leukemia (AML) patients can improve overall survival by reducing infection-related mortality and allowing to receive full planned chemotherapy in a timely manner.
Aims: Since January 2013, AML patients undergoing intensive chemotherapy in our institute and potentially eligible for bone marrow transplantation received posaconazole (PSZ) as IFI prophylaxis. The aim of the present study was to evaluate tolerability and efficacy of PSZ and to optimize our clinical practice.
Methods: From January 2013 to October 2014, 35 patients treated for newly diagnosed AML in our institute received PSZ for antifungal prophylaxis.
Non promyelocytic AML patients received a fludarabine, cytarabine and idarubicin containing regimen (FLAI) as first line treatment. M3 AML patients were treated according to GIMEMA AIDA 2000 protocol.
PSZ was given at the standard dose of 200 mg for 3 times/day, concurrently with a fat snack or with at least 100 ml of an acidic drink. Because of unpredictable absorption rates PSZ serum levels (TDM) were assessed routinely according to a validated high-performance liquid chromato-graphic (HPLC) method as described. To detect factors affecting PSZ exposure we analyzed each period of hospitalization as a single independent event.
We retrospectively compared through a matched-paired analysis 22 non M3 AML patients treated with PSZ as IFI prophilaxys to a control historical series of 22 patients who had received fluconazole (FLC) or itraconazole (ITZ). Of 22 patients in PSZ cohort, 18 received subsequent induction cycle 2 and 6 received

consolidation therapy, therefore accounting for a total of 46 episodes of prolonged neutropenia. In FLC/ITZ arm 22 patients received cycle 2 and 6 received consolidation therapy, for a total of 50 episodes of prolonged neutropenia. Matching variables were sex, age, response to induction. Patients median age, sex and days of severe neutropenia were not significantly different between the two cohorts. The incidence of IFI and the days of empirical or targeted intravenous antifungal therapy were analyzed.
Results: PSZ showed a good tolerability profile with no serious adverse events clearly related to prophylaxis occurring. A median number of 3 TDM for each period of hospitalization was performed (range 2-6). The achievement of a plasmatic PSZ concentration >0,7 mcg/mL is considered optimal for prophylaxis efficacy; in 39/65 (60%) episodes of hospitalization and treatment, with at least two TDM, the threshold PSZ serum concentration was reached, with stable plasmatic levels. Median PSZ plasmatic value at first assessment was 0.73 mcg/mL (range 0.1-3.9). The strongest negative factors affecting PSZ absorption are the temporary discontinuation of prophylaxis and the concomitant assumption of proton pump inhibitors. No proven or probable IFI were observed in the PSZ cohort. Data coming out from the matched paired analysis demonstrated a significant reduction in IFI incidence in our institution: 0% in PSZ cohort Vs 12% (6 proven/probable IFI episodes over 50 severe neutropenic periods) in FLC/ITZ cohort (P<0.05). Mean days of targeted/empirical intravenous antifungal therapy for single patient in PSZ cohort was 0 days Vs 2.2 days in FLC/ITZ cohort (P<0.05).
Summary and Conclusions: Our clinical experience confirms the benefit and potential cost-effectiveness of primary prophylaxis with PSZ in AML patients receiving intensive treatment, as no patients in PSZ cohort experienced IFI nor received empirical intravenous antifungal therapy.

PB1814

IMPACT OF CHEMOTHERAPY REGIMENS WITH ESCALATED ANTHRACYCLINES ON INCIDENCE OF FEBRILE NEUTROPENIA IN PATIENTS WITH ACUTE MYELOID LEUKEMIA
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Background: Modern chemotherapy schemes of acute myeloid leukemia include remission of induction regimens with escalated doses of anthracyclines (escalated "7+3" regimen). Changes in incidence of febrile neutropenia are not clear in patients receiving remission induction regimen "7+3" with escalated anthracycline dose (90mg/m^2) comparing to standart "7+3" chemotherapy regimen(45mg/ m^2).
Aims: The aim of the study was to assess the influence of "7+3" chemotherapy regimen with escalated anthracyclines on incidence of febrile neutropenia in patients with acute myeloid leukemia comparing to standard "7+3" chemotherapy regimen.
Methods: 56 hospitalization episodes of patients with acute myeloid leukemia on chemotherapy from March 2013 to January 2014 were reviewed. Incidence of febrile neutropenia (fulfilled criteria of Freifeld *et al.*, 2011) was compared in the groups receiving standard "7+3" regimen and "7+3" with escalated anthracyclines. Groups were comparable by age and gender characteristics and adjusted for other possible confounders.
Results: No statistically significant difference in incidence of neutropenia in patients receiving the escalated "7+3" regimen comparing to the standard one was found (71% to 70% accordingly, P=1, exact Fisher's criterion).
Summary and Conclusions: Use of "7+3" chemotherapy regimen with escalated anthracycline dose in treatment of acute myeloid leukemia does not increase the risk for development of febrile neutropenia in patients.

PB1815

PREDICTORS OF OUTCOME AND SEVERITY IN ADULT FILIPINO PATIENTS WITH FEBRILE NEUTROPENIA
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Background: Febrile neutropenia carries a significant mortality rate of 5-21.5%, warranting early recognition and institution of appropriate therapy. In the Philippines, there is a lack of studies on prognostic factors that predict poor outcome in patients with febrile neutropenia. It is important to identify these factors to recognize patients who will benefit from early aggressive therapy and closer monitoring.
Aims: The study aimed to describe the clinical, laboratory, and microbiologic profile of adult Filipino patients with febrile neutropenia, and to determine specific parameters that are potentially associated with severe outcomes, complications, and mortality.
Methods: This is a retrospective study of adult febrile neutropenia patients, regardless of cause, admitted at the Philippine General Hospital from January 2010-October 2014. Patients were described in terms of clinical, laboratory, and microbiologic presentation, and stratified according to the presence or absence of severe outcomes. Prognostic factors were then identified using univariate and multivariate logistic regression analysis.
Results: A total of 115 febrile episodes in 102 patients were identified. There was no difference in median age or gender ratio between the complicated and non-complicated groups; leukemia (48.7%) was the most common primary underlying disease in both groups. Most patients (50.43%) had infections of the respiratory tract, with Gram negative organisms predominating in the complicated group. The factors that significantly predicted poor outcome in the univariate analysis were non-treatment/relapse of the underlying disease (OR 2.28; 95% CI, 1.04-4.98; P=0.040), prolonged fever >7 days prior to admission (OR 3.24; 95% CI, 1.16-9.01; P=0.024), non-recovery from neutropenia (OR 2.17; 95% CI, 1.01-4.68; P=0.048), and severe thrombocytopenia<50,000/uL (OR 3.45; 95% CI, 1.52-7.84; P=0.003.) Meanwhile, completeness of antibiotic therapy significantly predicted a better outcome (OR 0.26; 95% CI 0.12-0.57; P=0.001.) Using the factors that reached significance in the univariate analysis, subsequent multivariate analysis yielded prolonged fever (OR 2.43; 95% CI, 0.77-7.74), isolation of a pathogen on cultures (OR 2.69; 95% CI, 1.04-6.98), and nadir absolute neutrophil count (ANC)<100 during admission (OR 1.96; 95% CI, 0.75-5.12) as significant predictors of poor outcome. The factors that significantly correlated with better outcome were granulocyte colony-stimulating factor (G-CSF) use (OR 0.31; 95% CI, 0.11-0.85) and completeness of antibiotic therapy (OR 0.26; 95% CI, 0.10-
Summary and Conclusions: Adult febrile neutropenia patients with prolonged fever >7 days prior to admission, known pathogen on cultures, and nadir ANC<100 during admission were at significant risk of developing worse outcomes, whereas those with G-CSF use and complete antibiotic therapy were significantly associated with better outcomes. These prognostic variables might be useful in identifying patients that need more intensive treatment and closer monitoring.

PB1816

CHEMOTHERAPY-INDUCED NEUTROPENIA AMONG PEDIATRIC CANCER PATIENTS IN EGYPT: RISKS AND CONSEQUENCES
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Background: Chemotherapy –induced neutropenia (CIN) is the major dose-limiting toxicity of systemic chemotherapy, and is associated with substantial morbidity, mortality and costs.
Aims: The aim of the current work was to identify risk factors that may predispose pediatric cancer patients, treated with myelo-suppressive chemotherapy, to CIN and associated sequels.
Methods: 113 neutropenia episodes were analyzed, risk factors for CIN were classified into; patient-specific, disease-specific and regimen specific while consequences associated with CIN were divided into infectious and dose modifying sequels. Both risks and consequences were analyzed to target high risk patients with appropriate preventive strategies.
Results: 28% of our patients presented with single neutropenia attack while 72% of them experienced recurrent attacks during their treatment cycles. Mean absolute neutrophil count (ANC) was 225.5±128.5 (10^9/L), ranged from 10-497(10^9/L) started at 14.2±16.3 days (ranged 2-100) after the onset of chemotherapy and resolved within 11.2±7.3 days either with (45.1%) or without (54.9%) granulocyte colony stimulating factor (G-CSF).No significant association could be found between any patient character or disease stage and the risk for CIN. However, certain malignancies (ALL, Neuroblastoma and Burkitt's lymphoma) and certain regimens (induction blocks for ALL, AML) had the worst myelotoxic effect with severe and prolonged neutropenia episodes. G-CSF significantly shortened the neutropenia episodes and enhanced bone marrow recovery. Febrile neutropenia was the leading complications among our cases (73.5%), associated with several documented infections particularly mucositis (54.9%), respiratory (45.1%), GIT (38.9%) and skin (23.9%) infections. 6% of our cases died of infection –related complications. Neutropenia was responsible for treatment discontinue (13.3%), dose delay (13.3%), and dose reduction (5.3%) of our patients. The mean cost for each episode in our service was 9386.5±6688.9 Egyptian pounds (L.E) which represented a significant burden on health care providers.
Summary and Conclusions: Although this study is preliminary survey with relatively small number of patients, our findings are relevant to clinical care of pediatric cancer patients in our region. Special attention to CIN prevention should be directed to hematologic malignancy cases especially at early cycles. Severe and prolonged neutropenia are life-threatening events that need aggressive management.

PB1817

MENTAL DISORDERS OBSERVED DURING THE APPLICATION OF VORICONAZOLE
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Background: Among the side effects of the drugs from the group of antifungal agents voriconazole are the mental disorders by different psychopathological registers.
Aims: The aim was to study the typology of mental disorders and therapeutic approaches in the event of psychosis in patients with hematological diseases complicated by fungal infection, during the application of voriconazole.
Methods: There were studied 15 patients with various hematological diseases (AML, ALL, MM, AA) by the clinical method in the period from 2006 to 2014, in which observed the development of mental disorders during therapy with voriconazole in connection with invasive fungal infections (aspergillosis, candidiasis).
Results: Of the 15 patients, 11 demonstrated hallucinatory syndrome. Development hallucinosis in most cases within 1-2 days underwent almost stereotypical dynamics-the formation of psychopathological disorders began with demonstrations insomniac disorders (violation of falling asleep, frequent awakenings), joined by vivid nightmares with disturbing content, accrued unexplained anxiety, persistent daytime (4 patients of the 11 alarm combined with hypothymia (depressed mood). Then, against the clear consciousness and stored criticism, manifest verbal perceptual deception by type of acoasma (hiss, scratches) or elementary functional hallucinations (addressed to the patient isolated words or phrases arising on a background of real sounds of medical equipment or personnel speech), which gradually became more complicated. With closed eyes formed visual hallucinatory disorders that were in the nature of sensuous vividness, and were represented by a set of bright, moving images of people and (rarely) animals of normal size or reduced. It is noted high involvement of the patient in what is happening-"people" were talking among themselves and with the patient, "commented" his actions and thoughts. There was not noted the increasing of anxiety on the background of experienced by patients deceptions of perception. In 4 patients, hallucinatory disorders are not formed and mental disorders limited by insomniac, anxiety disorders and hypothymia and had more short-term character than in the previous group of patients. Described disorders in patients persisted for 6±2 days on average. Formation of described psychopathological disorders occurred on the background of significant somatogenic asthenia caused by severe hematologic malignancies and its complications (including infectious origin) and intoxication by chemotherapeutic agents. In the history of the patients showed no manifest psychopathological disorders. Treatment of mental disorders in the studied patients was phased. The aim of the first phase was psychosis reduction, the second – the support treatment to prevent relapse of psychotic disorders. Preference was given to intravenous administration of psychotropic drugs (in most cases – through a central venous catheter) that correspond to the guidelines for the management of patients with haematological diseases. In hallucinatory disorders the main therapeutic intervention focused on the first stage; the treatment have a short duration and include the use of butyrophenone antipsychotics (haloperidol 10 mg / day). In necessary of the rapid sedation or relief the anxiety/insomniac disorders diazepam was administered in therapeutic scheme (20 mg/day).
Summary and Conclusions: During the application of voriconazole the mental disorders may occur (most often formed hallucinatory syndrome) that require psychopharmacological correction.

Myelodysplastic syndromes - Biology

PB1818

IN VITRO EVALUATION OF A BACTERIAL BIOLUMINESCENT REPORTER ASSAY FOR ASSESSMENT OF RESPONSE TO DECITABINE

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Background: Decitabine (DAC) is a cytidine analogue and hypomethylating agent used in the management of patients with myelodysplastic syndrome (MDS) and increasingly in acute myeloid leukemia (AML). Cellular uptake via the hENT1 transporter and subsequent phosphorylation by deoxycytidine kinase (dCK) produces the active metabolite decitabine triphosphate (DAC-TP). DAC-TP is incorporated into DNA, where it covalently binds to and inactivates DNA methyltransferases (DNMTs), leading to hypomethylation and potential re-expression of silenced tumour suppressor genes. DAC is also known to have a direct cytotoxic effect on leukaemic cells. DAC has been compared extensively with the related compound azacytidine (AZA) clinically. Only 10-20% of intracellular AZA is converted to DAC-TP leading to hypomethylation, compared with 100% DAC-TP from DAC. Trials have shown AZA to be clinically favourable, indicating that a lower intracellular concentration of DAC-TP could be beneficial. Current opinion is that DAC may be useful following appearance of resistance to AZA. Therefore a tool that could assist in predicting individual patient benefit from treatment with DAC is needed, particularly considering associated costs to healthcare services for this drug (~$80,000 pa per patient).
Aims: This study aimed to assess the feasibility of detecting DAC and DAC-TP using a previously validated biosensor sensitive to the cytidine analogue cytosine arabinoside (ara-C) (Alloush *et al., Clin Chem.* 2010;56(12):1862-70). Biosensor HA-1 is a *cdd*-deficient, bioluminescent bacterium with inducible expression of human dCK that is specific for toxic analogues of cytidine. Biosensor HA-1 has been formulated into an assay capable of predicting response to treatment within 8-hours and is currently undergoing clinical evaluation for ara-C.
Methods: Direct application of DAC to biosensor HA-1 using a clinically relevant dosing range (0.1-10 mM) equivalent to 15-150 mg/m^2 resulted in a dose-dependent increase in light output, with maximum 2.6-fold increase over control. A concurrent decrease in bacterial growth was observed as previously noted with ara-C, confirming intercalation of bacterial DNA with DAC-TP. Direct comparison of DAC with ara-C demonstrated an equivalent response by biosensor HA-1 for both time to peak (5.25 hours) and scale of increase of light output
Results: Subsequently leukaemic cell lines, HL-60 and K562 were exposed to DAC (0-10 μM) to assess whether DAC uptake and metabolism to DAC-TP during the initial 1-hour post-dosing would predict cytotoxicity at 72 hours. Assessment of cytotoxicity of DAC performed following daily dosing of DAC (0.1 – 10 mM) on HL-60 and K562 cells over a 72-hour period indicated that HL-60 cells were more susceptible to DAC-induced cytotoxicity than K562 cells. Concurrent assessment of accumulation of parent DAC and DAC-TP using biosensor HA-1 indicated that K562 cells produced more DAC-TP than HL-60 cells in the initial 1-hour post-dosing with DAC (≥1 mM), implying that the cytotoxicity observed may be more related to accumulation of parent DAC than metabolite. Previous *in vitro* studies have observed that the IC_{50} concentration of DAC is associated with maximal hypomethylation, indicating that DAC-TP could be responsible for the suppression of proliferation observed
Summary and Conclusions: Studies are on-going in our laboratory to directly assess DAC-TP using biosensor HA-1 and compare with hypomethylation in cell lines and patient blasts to define the intracellular drug kinetics of DAC, and further dissect the role of parent drug and metabolite in DAC-mediated toxicity.

PB1819

LOW RPS14 EXPRESSION IN THE NON 5Q- MYELODYSPLASTIC SYNDROMES

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Background: The *RPS14* gene, located on chromosome 5 and involved in the ribosomal protein synthesis, has been reported as a causal factor in the 5q-syndrome, where its up-regulation during treatment with lenalidomide has been associated with best responses. *RPS14* expression in non-5q-MDS was reported in 53%>71% of cases. Interestingly, in low and intermediate-1 IPSS subgroups, patients with lower *RPS14* expression had longer OS.

Aims: The aim of this study was to assess the *RPS14* expression in a larger series of non-5q- MDS patients.
Methods: A total of 112 patients, 45% females and 55% males, with a median age of 71-year (range 19-89), were enrolled in 5 different Italian institutions from March 2010 to October 2014. Nine patients were affected by CMMoL; the prognosis of the remaining 103 cases was determined according to the IPSS in low (36%), intermediate-1 (31%), intermediate-2 (21%), and high risk (12%). About 40% of cases were affected by RCMD, 24% by RAEB-2, and 13% by RA. Twelve bone marrow samples from healthy donors have been used as normal references.
Results: In healthy donors, the mean *RPS14* expression was 0.94±0.26; in the whole MDS series, it was 0.57±0.42. In comparison with healthy controls, the 52% of MDS cases showed lower *RPS14* expression levels: 79% in the RA, 56% in the RAEB1, 44% in the RAEB-2, and 41% in the RCMD subgroups. When patients were stratified according to the IPSS, in the half of the low, intermediate-1 and intermediate-2 cases *RPS14* was under-expressed, opposite to one third of the high risk and 13% of the CMMoL patients only. No relationships with age or sex were observed. To evaluate if the haploinsufficiency would be responsible of the low expression of *RPS14* gene, we performed the copy number assay on 32 MDS, 15 healthy donors, and 3 patients with 5q- syndrome: in 91% of cases the copy number assay excluded the haploinsufficiency.
Summary and Conclusions: Our study showed in a large series of patients that a lower *RPS14* expression interests the half of the non-5q- cases, especially those affected by RA and at low and intermediate IPSS risk. Other authors previously reported that low expression of *RPS14* was not due to promoter hypermethylation. Here we demonstrated that also the haploinsufficience is not the cause of the *RPS14* low expression. Moreover, our findings suggest a possible role for lenalidomide in non-5q- MDS, especially in low risk patients.

PB1820

MOLECULAR MAPPING OF THE PDGFRB/SPTBN1 GENE FUSION TRANSCRIPT AS A RESULT OF THE T(2;5)(P16.2;Q33.1) IN AN IMATINIB MESYLATE-RESPONSIVE MDS

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Background: PDGFRB is a member of the tyrosine kinase family and PDGFRB gene rearrangements, already reported in myeloproliferative neoplasms (MPN), lead to its constitutive activation, ultimately affecting cell proliferation and migration. Patients with PDGFRB rearrangements respond to imatinib mesylate therapy. The t(2;5)(p16.2;q33.1) (PDGFRB/SPTBN1) has been reported only in one case of atypical MPN. SPTBN1 is a cytoskeletal protein with a role in mitotic spindle assembly.
Aims: The aim of the study was to fully characterize the partner fusion gene products formed as a result of the t(2;5)(p16.2;q33.1), encountered for the first time in a MDS patient.
Methods: A 78-years-old man presented for investigation of macrocytic anemia in 2012. Bone marrow smear showed mild dysplastic features in the granulocytic and megakaryocytic series. Bone marrow biopsy showed 60% cellularity and 4% CD34+ cells. The findings were consistent with MDS diagnosis. One year later, a new biopsy showed 23% CD34+ cells and dyserythropoiesis. The patient was administrated imatinib mesylate and since July 2014 has achieved hematological remission. Following karyotypic analysis on bone marrow cells, interphase and metaphase FISH studies with specific BAC probes (UCSC Genome Bioinformatics, Source Bioscience) were performed in order to refine the chromosome breakpoints and detect the partner genes. RP11-100O5/RP11-368O19 BAC probes encompassing the PDGFRB gene at 5q33.1 and RP11-423N19/RP11-1022E1 BAC probes flanking the SPTBN1 gene at 2p16.2, were fluorescently labeled by nick-translation and used as dual-color break-apart probes. For the molecular characterization of the partner genes, 5' rapid amplification of cDNA ends, followed by single step 5'RACE PCR was performed using a 5'Gene Racer primer (Life Technologies) and a reverse primer for PDGFRB exon 15. Second step PCR was carried out with a 5'Gene Racer nested primer and a reverse nested primer for PDGFRB exon 15. Following cloning, sequence analysis of the PCR products was conducted to fully characterize the fusion transcripts.
Results: At diagnosis the karyotype was: 46,XY,t(2;5)(p16;q33)[20]/46,XY[5]. iFISH detected PDGFRB and SPTBN1 gene rearrangements in 56% of the cells. Based on those results, the patient was administrated imatinib mesylate and has favorably responded thus far. Metaphase FISH revealed translocation of 5' PDGFRB at 2p16.2 and fusion with the 3' SPTBN1 onto derivative chromosome 2. Accordingly, the 5' of SPTBN1 was translocated at 5q33.1 and fused with the 3' PDGFRB onto derivative 5. For the molecular characterization of the fusion gene transcripts, cloning and sequencing of the 5'RACE PCR products, showed that SPTBN1 exons 1-3 were fused to PDGFRB exon 12 onto derivative chromosome 5.
Summary and Conclusions: Cytogenetics identified the PDGFRB/SPTBN1 fusion gene generated from the rare translocation t(2;5)(p16.2;q33.1). Thorough molecular mapping analyses revealed that the genomic breakpoints were located at exon 3 of SPTBN1 gene and exon 12 of PDGFRB gene, leading to a PDGFRB/SPTBN1 fusion transcript comprising exons 1-3 of SPTBN1 gene and exon 12 of PDGFRB gene onto derivative chromosome 5. Importantly, the involvement of tyrosine kinase gene PDGFRB in t(2;5), encountered for the first time in a MDS case, prompted the administration of a successfully tailored-based signal transduction therapy (imatinib mesylate) to the patient.

PB1821

EVALUATION OF P53 PROTEIN EXPRESSION IN MYELODYSPLASTIC PATIENTS: PROGNOSTIC IMPACT IN A SINGLE CENTRE EXPERIENCE

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Background: AML follwing MDS has a very poor outcome, so accurate prediction is essential, expecially in patients who could potentially be cured by stem cell transplantation. A strong association between p53 protein expression, the TP53 mutation and an adverse outcome has been reported in various hematologic malignancies including MDS. Strong nuclear staining of the p53 protein by immunhistochemistry (IHC) has been used as a surrogate marker for TP53 gene mutation in hematologic and other malignancies; a strong correlation of p53 nuclear expression with TP53 mutation has also been demonstrated.
Aims: To assess the role of p53 expression in MDS patients as a new prognostic tool, as well as its feasibility in routine clinical practice, we analyzed the prevalence of p53 expression in a cohort of 62 consecutive bone marrow (BM) biopsies from patients with MDS at diagnosis, and correlated our findings to other validated prognostic markers and clinical outcome
Methods: The DO-7 antibody (DakoCytomation, Denmark), which labels both wild and mutant-type p53 proteins, was used to detect p53 expression (a colorectal carcinoma positive control was included on each slide). Diagnosis according to the 2008 WHO Classification was: isolated del (5q) in 4 cases, RA in12, RARS in 5, RCMD in 8, RAEB-1 in 15, RAEB-2 in 18.
Results: Among patients with strong p53 immunostaining in ≥1% of BM cells, we found significant correlations with BM blasts (70% RAEB-1/2, vs 32% in negative cases). IPSS-R cytogenetic risk was int/poor/very poor in 36% of positive and 0% in negative IHC cases. IPSS score was int-2/high in 54% of patients with strong positivity vs 20% in negative cases. BM fibrosis was present in 70% of positive vs. 30% of negative IHC cases. Transfusion dependency was observed in 50% of patients with p53 strong expression vs. 20% of negative cases. Moreover, we found that strong p53nuclear expression was associated with a significantly worse outcome (87% disease progression plus leukemic evolution vs. 20% in negative cases) and shorter median OS (18 months vs. 35 in negative cases).
Summary and Conclusions: Our data indicate that IHC p53 protein expression, evaluated in bone marrow biopsies by a widely available method, is a highly predictive marker and thus a helpful tool in risk assessment and the decision making process in MDS.

PB1822

MYELODYSPLASTIC SYNDROMES: AN INTEGRATED WORKUP FOR A CORRECT DIAGNOSIS

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Background: The diagnosis of MDS is determined by bone marrow's histology and is integrated by conventional cytogenetic (CC) analyses that, although, are unable to show chromosomal abnormalities in about 30% of cases. Array Comparative Genomic Hybridization (aCGH) detects copy number aberrations in half of MDS diploid by CC. Recently, *TET2, ASXL1, EZH2, CBL, IDH1/IDH2, DNMT3A,* and *UTX* mutations have been described in MDS.
Aims: Therefore, we evaluated the prognostic and predictive implications of FISH, aCGH and mutation assays when added to CC.
Methods: Fifty patients, 15 females and 35 males, with a median age of 72-year (range 39-88) were enrolled in a single institution (Hematology Unit, University of Pisa, Italy), between March 2013 and October 2014. Patients' prognosis was determined according to IPSS in low (42%), intermediate-1 (33%), intermediate-2 (17%) and high risk (8%). Samples were evaluated by CC including, conventional karyotyping and FISH for chromosome 5, 7, PDGFRA, and

PDGFRB. Moreover, aCGH was performed using SurePrint G3 Human CGH Microarray, 8x60K (Agilent), and mutations of TET2, TP53, ASXL1, and EZH2 were determined using qBiomarker somatic mutation PCR array (Qiagen).
Results: After FISH analysis, additional copy number losses of chromosome 3 and 5 were observed in 1 and 2 cases, respectively. According to aCGH, 20 patients (40%) showed copy number aberrations (Table). Seventeen patients had a mutation: 12 in TP53, 4 in ASXL1 and 1 in TET2 sequence.

Table 1.

Table: Chromosomal abnormalities detected by aGCH

PATIENTS'ID	CHROMOSOMAL ABNORMALITIES
2	arr[hg19] 13q12.3q14.3(31092502-53311418)x1 (22,2 Mb)
3	mosaic trisomy 14 (50%); arr[hg19] 20q11.21q13.2(30914548-51319788)x1 (20,4Mb)
8	monosomy 7 (70%);arr[hg19]12 p13.2p11.23(12006149-26807222)x1 (14.7 Mb) (**ETV6**)
9	arr[hg19] 13 q14.2q14.3(48459403-52345261)x1~2(mosaic 30%)(3.8 Mb)
10	monosomy 7 (40%)
14	arr[hg19] 3q21.2-q21.3 (125672997-128686163)x1 (3Mb) (**GATA2**)
16	arr[hg19] 3p26.1(4134024-4505821)x1 (371kb)
17	arr[hg19] 1q25.1 (173884057-174002363)x3 (118kb)
19	arr[hg19] 16q23.2(79627703-79630630)x1 (2,9kb) (**MAF**)
23	mosaic trisomy 8 (55%)
24	mosaic loss Y (45%)
25	arr[hg19] 5p14.1(26847820-27350339)x3 (502.2kb)
28	mosaic trisomy 8 (55%)
30	arr[hg19] 14q12q21.3(250475054769463 9)x2~3 (mosaic 55%)(22.6 Mb)
32	arr[hg19] 20 p12.1(14875511-15199538)x1 (324kb)
34	arr[hg19] 11q14.1q22.3(83083530-129302517)x1 (46,22Mb)
37	arr[hg19] 5q14.3q33.2(87462725-155217488)x1 (67,7Mb)
38	arr[hg19] 17p13.2(3519758-3543664)x1 (23 Kb) (**CTNS**)
39	loss Y
40	arr[hg19] 5q31.1q33.3(135.602.022-156.376.769)x1 (20,7Mb)
41	arr[hg19] 5 q14.3q33(87686665-154433763)x1~2(mosaic 35%)(66.75 Mb)
42	multiple alterations
43	multiple alterations (**MAF**)
44	arr[hg19] 16q13(56672571-56673791)x1 (1.2 Kb) (**MT1A**)

Summary and Conclusions: 1) The study showed the reduction of CC failures (only 16%); 2) Because of FISH results, a patient received azacitidine and another one lenalidomide; 3) Three MDS patients with copy number aberrations detected exclusively by aCGH progressed in acute leukemia being lethal: one patient had copy number loss of chromosome 3, one had copy number losses of chromosomes 8 and Y and the last one had copy number loss of *ETV6* locus. 4) One patient with *TET2* mutation did not respond to the azacitidine. Three out of 4 patients with *ASXL1* mutation had a good response to epoietin. TP53 mutations were observed in 12 cases of which 2 developed acute leukemia, 2 were resistant, and 2 sensitive to epoetin. In conclusion, our results support the relevance of an integrated work-up for MDS and provide a preliminary estimation of copy number aberration and mutation frequencies. The prognostic role of aCGH and somatic mutations needs to be determined in properly sized series.

PB1823

CD38 EXPRESSION ON CD34+ BONE MARROW CELLS (BM) AS A TOOL IN LOW GRADE MYELODYSPLASTIC SYNDROMES (MDS)

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Background: The diagnosis of MDS can be difficult, especially in the absence of markers of clonality. Flow cytometry (FCM) has tried to be a useful tool but the methods described so far are poorly standardized and time-consuming.
Aims: Recently, a study has shown that a reduced expression of CD38 in the CD34 + BM cells below a threshold value diagnosed low-grade MDS with high sensitivity and specificity, (Goardon et al, Haematologica, 2009, 94:. 1160-1163). Our aim was to test the effectiveness of this method.
Methods: We analyzed 140 BM samples sent to our laboratory between 2012-2015 with suspected MDS. The samples were processed within 24 hours of aspiration and were labeled with an antibody panel that included CD45-FITC, CD34-PE-Cy5 CD38 PE, CD10 PE-Cy7, CD117-APC and CD19-APCCy7. A sufficient number of nucleated cells was acquired on a FACSCanto (Becston Dickinson) flow cytometer and CD38 expression was analyzed in CD34 + myeloid and lymphoid blasts. The expression of CD38 was quantified as a ratio as the MFI of CD38 on CD34 + cells divided by the MFI of isotype control staining. The result was considered pathological below the threshold value described in the study (CD38 IMFR<110)
Results: Of the total of 140 patients, 71 patients were diagnosed with MDS using current WHO criteria (2008): 39 RCDM, 7 RCUD, 2 AR, 10 CMML, 3 RAEB-1, 7 RAEB-2, 1 RARS,1 5q-, 1 MDS / MPD. In them, the ratio CD38 IMFR gave altered in 46 (64.5%). (Media 95.2, range 10.3-314.1). 69 patients did not meet criteria for SMD, of which 61 (88.4% patients) had a normal ratio (mean 145.9, range: 63.8-808.8).
In our case, the test gave a sensitivity of 64.5% and a specificity of 88.4% in the diagnosis of MDS.
Summary and Conclusions: Our results confirm that CD38 expression is reduced in CD34 + cells from MDS patients and may be helpful in the diagnosis of low-grade MDS.

PB1824

COMPARISON BETWEEN CYTOMORPHOLOGY AND CYTOGENETIC ANALYSIS FOR THE DIAGNOSIS OF MYELODYSPLATIC SYNDROME IN COHORT OF 374 PATIENTS

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Background: Cytogenetic analysis is an essential step to establish the prognosis in patients with myelodysplastic syndrome (MDS). In our center, the investigation of cytopenia includes a systematic cytogenetic analysis.
Aims: The aim of this work was to compare the efficiency in first intention of bone marrow morphologic analysis to karyotype analysis to reach the diagnosis of myelodysplastic syndrome.
Methods: A monocentric cohort of patients investigated for one or more cytopenia(s) was analysed. Patients diagnosed with MDS with blasts excess, acute leukaemia, bone marrow localization of lymphoma or solid tumor were excluded. Blood, bone marrow smears morphology and karyotype were compared according to the final diagnosis (MDS, immune, metabolic, inflammatory, congenital cytopenia).
Results: Between 2010 and 2013, samples of 374 patients were analysed. Median age of the whole cohort was 66.8 y.o (1-91). 72% of patients had one cytopenia (mostly anaemia in 50%), 20% were bicytopenic and 8% were pancytopenic. Analysis of bone marrow smears revealed no dysplasia, a uni-or multi-lineage dysplasia or an abnormality of cellularity in respectively 53%, 42% and 5% of cases. Cytogenetic disclosed a normal karyotype in 84% and at least one abnormality in 16% of patients. Loss of Y chromosom, recurring abnormalities and del 5q were the most frequent karyotype abnormalities respectively 7%, 3,7%, 3%. The diagnostic of MDS was retained in 105 patients (29%) whereas 269 patients (71%) were classified as having a differential diagnosis of cytopenia (immune, metabolic, inflammatory, congenital). MDS subtypes were refractory anemia (49%), refractory cytopenias with multilineage dysplasia (20%), refractory cytopenia with unilineage dysplasia (17%), refractory neutropenia (1%), refractory thrombopenia (3%), del 5q syndrome (4%) and MDS-unclassified (6%). In group of MDS patients compared to non-MDS patients, blood test revealed a deeper anaemia (10.2 vs 11 g/dL, P< 0.0001), a higher mean corpuscular volume (94 vs 89 fL, P<0.0001), a higher red-cell distribution width (15.15 vs 13.8, P<0.0001). Then, bone marrow cytomorphology showed more often at least one lineage dysplasia 86.7% vs 30% without dysplasia (P<0.0001) and at least one karyotype abnormality in 32.6% vs 10% without abnormality (P<0.0001). Finally, table 1 summarizes sensitivity, specificity, positive predictive (PPV) and negative value (PNV) of morphological and cytogenetic analyses for the diagnosis of MDS

Table 1.

Table 1. Sensitivity, specificity, PPV, PNV of cytomorphology and cytogenetic analysis to confirm or exclude diagnosis of MDS

	Sensitivity %	Specificity %	PPV %	PNV %
DIAGNOSIS OF MDS				
BONE MARROW MORPHOLOGY				
Erythroid dysplasia (>20%)	59	79.5	52.3	83.3
CYTOGENETIC ANALYSIS				
Loss of Y chromosom	8.5	92.5	31	72
Other karyotype abnormalities	9.5	98.5	71.5	73.5
Defavorable karytoype	8.5	100	100	73.7
DIAGNOSIS of NON-MDS				
BONE MARROW MORPHOLOGY				
No dysplasia	86.6	68.7	52	92.9
CYTOGENETIC ANALYSIS				
No cytogenetic abnormality	32.4	90.3	56.6	77.39

Summary and Conclusions: For the diagnosis of myelodysplastic syndrome, cytomorphology of bone marrow is more sensitive but less specific than cytogenetics. Our results suggest that in case of cytopenia without blast excess or malignant infiltration, blood and bone marrow smears microscopic examination could be performed frontline and cytogenetic examination could be delayed at the time of morphology confirmation after 1 to 3 months.

Myelodysplastic syndromes – Clinical

PB1825

MYELODYSPLASTIC SYNDROMES IN PATIENTS AFFECTED BY AUTOIMMUNE DISEASES

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Background: Background: Several immunological abnormalities have been reported among patients affected by myelodysplastic syndromes (MDS). On the other hand, a relatively limited number of studies have explored the occurrence of MDS during the course of systemic autoimmune diseases (AD)
Aims: Aim of the study: to estimate characteristics and frequency of MDS among patients with systemic autoimmune diseases (AD).
Methods: Methods: A retrospective systematic search through the electronic health records of patients admitted at the Rheumatology University Medical Center from 2009 and 2014 who were concomitantly evaluated by the Division of Hematology was performed to select those patients with systemic AD and MDS. To refine the search the ICD-9-CM diagnosis code for MDS was utilized. Medical charts of eligible patients were retrieved and data were collected with regard to demographics, type of AD, AD duration, prior treatments, bone marrow biopsy, and cytogenetic profile. Categorical variables were compared using chi square test and Fisher's test; continuous variables were compared using Student's t-test. A 2-tailed value of $P<0.05$ was taken to indicate statistical significance.
Results: Results: Out of the medical records of 3800 patients, we identified 23 patients with AD and MDS. Patients' median age at the diagnosis of MDS was 65 years, with a 1.09:1 female to male ratio. Rheumatoid arthritis and seronegative arthritis were the most frequent underlying AD condition (7/23, 30%) followed by large and small vessel vasculitis (7/23, 30%), Systemic Lupus Erythematosus (3/23, 13%), Sjogren's syndrome and myositis (2/23, 8%). Moreover, one patient was affected by Systemic Sclerosis and one by Behçet's syndrome. Anemia (21/23, 91%) was the most common hematologic presenting abnormality, followed by thrombocytopenia (9/23, 39%) and neutropenia (8/23, 35%). Three patients out of 23 presented with a trilineage cytopenia (13%). In the majority of the patients the diagnosis of MDS was subsequent to that of AD, with a median period between the two diagnosis of 4 years. Prior to MDS diagnosis, about one third of the patients received cytotoxic drugs, among which MTX was the most commonly prescribed (5/23, 22%) followed by azathioprine and cyclophosphamide (2/23, 8%). Regarding MDS, the most common diagnosis was refractory anemia with excess of blasts (RAEB I and II) (4/16, 25%) followed by sideroblastic anemia (2/16, 12%) and refractory anemia (2/16, 12%). A progression to leukemia was documented in 2 patients.
Summary and Conclusions: Conclusions: Our study is limited by its retrospective design. However, our results documented that the frequency of MDS in AD is not negligible and might be suspected especially in older patients presenting with "unexplained" cytopenias.

PB1826

HYPERFERRITINEMIA IS ASSOCIATED WITH IMPAIRED SURVIVAL IN PATIENTS DIAGNOSED WITH MYELODYSPLASTIC SYNDROMES RESULTS FROM THE FIRST POLISH MDS REGISTRY

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Background: Myelodysplastic syndromes (MDS) are a heterogeneous group of clonal stem cell disorders characterized by ineffective hematopoiesis, cytopenias and risk of progression to acute myeloid leukemia (AML).
Aims: The influence of serum ferritin concentration on survival and acute myeloid leukemia transformation in MDS patients remains controversial. The data for Central European population is scarce and so far there is no description for Poland. Aim of this study was to perform an retrospective analysis of relationship of ferritin concentration with red blood cell transfusion dependency, survival and acute myelogeneous leukemia transformation.
Methods: We retrospectively evaluated data of the 819 MDS patients (58% male; median age 70 years), included in the MDS Registry of the Polish Adult Leukemia Group (PALG). Analysis was performed for 190 patients diagnosed with MDS maximal 6 months before inclusion to registry in order to avoid selection bias (shorter survival of higher risk MDS patients).
Results: Patients with hyperferritinemia higher than 1000 ng/l *versus* patients with ferritin concentration lower than 1000 ng/l had median survival of 320 days vs 568 days respectively (p log-rank=0.014). It was shown that the following factors significantly worsened survival: ferritin concentration higher than 1000 ng/l (P=0.0023; HR=2.94), RBC-transfusions dependence (P=0.0033; HR 2.671), platelet transfusions dependence (P=0.0033; HR 2.671), hemoglobin concentration lower than 10 g/dl (P=0.0036; HR 2.97), platelet count lower than 10 G/l (P=0.0081 HR=5.04), acute leukemia transformation (P=0.0081; HR 1.968).
Summary and Conclusions: Taking into account relatively low number of patients in previous studies exploring the topic of hyperferritinemia in MDS patients, the study provides important insights.

PB1827

HIGH P53 PROTEIN EXPRESSION IS ASSOCIATED WITH WT1 MRNA LEVEL AND DISEASE STAGE PROGRESSION IN PATIENTS WITH MYELODYSPLASTIC SYNDROME.

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Background: Myelodysplastic syndrome (MDS) is a heterogeneous disease. Several clinical findings were reported as prognostic markers. Identification of p53-positive cells by immunohistochemistry in bone marrow (BM) was demonstrated to correlate with presence of TP53 mutations, indicative of poor prognosis. Wilms' tumor gene (WT1) mRNA expression was associated with the WHO classifications and risk evaluation of MDS. But there was no report about correlation between p53 expression and WT1 mRNA level.
Aims: We assessed BM biopsy specimens for p53 expression by immunohistochemistry in association with other clinical parameters including WT1 mRNA expression in adult MDS patients.
Methods: We performed a retrospective study involving 29 BM biopsy samples from 24 patients with MDS between 2013 and 2014. The percentage of p53 staining positive cells was assessed based on a total manual count of 1000 BM hematopoietic cells. Clinical parameters were corrected from patient medical charts.
Results: The study included 17 males and 7 females. The median age at procedure was 71 years (range, 28-84). Five patients received sequential BM biopsies when clinical findings changed. According to WHO 2008 classification of MDS, 14 specimens were RCMD, 3 were RCMD-RS, 6 were RAEB-1 and 6 were RAEB-2. The result of cytogenetic analysis of 26 samples, showed complex karyotypes with more than 3 abnormalities found in 7 samples including 4 of complex karyotype with -5/5q-, isolated del5q was not found in this cohort and 11 were normal karyotype. Nine patients (11 specimens) were treated with azacitidine after first biopsy. With the median post-procedure follow up of 5.7 months (1-23.3 months), leukemic transformation and death occurred in one patient (two specimens) and two patients (three specimens), respectively. Positive rate of p53 expression in BM was as follows;<1%, 5 specimens; 1-3%, 8; 3-5%, 4; 5-10%, 4; >10%, 8. In 5 patients with sequential biopsies, p53 expression level of first sample was lower than it of second sample except in one patient who received azacitidine treatment after first biopsy. WT1 mRNA

expression level in peripheral blood (PB) was as follows;<100 copies, 11; 101-1000copies, 8; >1000 copies, 6; 4 patients were not tested for WT1 mRNA level at the time of BM biopsy. Higher level of p53 expression was significantly associated with greater WT1 mRNA expression level in PB (r=0.40, P=0.05), complex cytogenetic abnormality (r=0.64, P<0.001), increasing with WHO2008 classification (r=0.68, P<0.001), IPSS (r=0.50, P=0.01), and IPSS-R (r=0.53, P=0.006). But it was not associated with percentage of CD34 positive cells in BM. In spite of short duration of follow-up time, high level of p53 expression was associated with overall survival (r=0.54, P=0.003).
Summary and Conclusions: High p53 protein expression in BM is associated with WT1 mRNA expression level and disease stage progression. It can be a predictor of survival in adult MDS.

PB1828

BONE MARROW OSTEOPONTIN AS A PROGNOSTIC FACTOR IN MYELODYSPLASTIC SYNDROME

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Background: Osteopontin, a glycophosphoprotein encoded by a single gene on chromosome 4q13, is expressed and secreted in numerous human cancers and plays a role in tumor progression and metastasis. Also, high osteopontin level is associated with shortened survival in hematologic malignancies. Bone marrow (BM) osteopontin levels are currently not reported, and relationship between BM osteopontin and the clinical course of disease has not been demonstrated in myelodysplastic syndrome (MDS).
Aims: The objectives of present study were to investigate 1) the prognostic value of BM osteopontin, 2) whether osteopontin level in response to chemotherapy can be used to predict therapeutic response in patients with newly diagnosed MDS.
Methods: From July 2012 to February 2014, 24 patients [12 with refractory cytopenia with multilineage dysplasia (RCMD) and 12 with refractory anemia with excess blasts (RAEB)] were enrolled. The RAEB patients were treated with hypomethylating agent, while RCMD patients received supportive care. The patients with RAEB were divided into three separate groups: in marrow complete remission (mCR), in progression of disease (PD), and in stable disease (SD) after chemotherapy. The levels of BM osteopontin were analyzed at the time of diagnosis from RCMD and RAEB patients, and after 3 cycles of hypomethylation chemotherapy from RAEB patients. The BM samples obtained at the time of diagnosis in RCMD patients, and at the time of diagnosis and after chemotherapy in RAEB patients.
Results: Osteopontin levels were significantly higher in the RAEB group compared to the RCMD group (120.62±20.42 vs. 62.62±7.40 ng/mL, respectively; P<0.001). Osteopontin levels did not change significantly with chemotherapy response (Figure 1).We analyzed the association between osteopontin levels at diagnosis and survival in RCMD and RAEB patients. RCMD patients were divided into two subgroups with high (≥ 63.1 ng/mL) and low (< 63.1 ng/mL) osteopontin levels at diagnosis. The osteopontin level had no significnat impact on survival among thess subgroups (P=0.157 for each variable; Figure 2). RAEB patients also were divided into two subgroups with high (≥ 115.3 ng/mL) and low (< 115.3 ng/mL) osteopontin levels. The low osteopontin level had a trend toward survival advantage (P=0.087 for each variable; Figure 3).

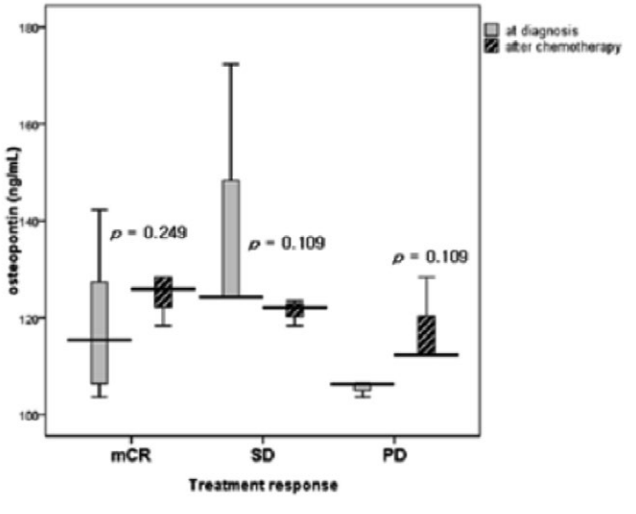

Figure 1. BM osteopontin level in response to chemotherapy in RAEB group.

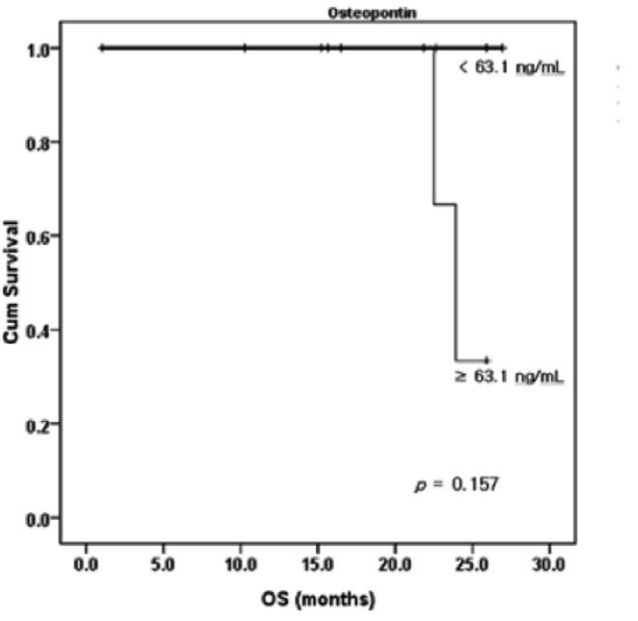

Figure 1. BM osteopontin level and survival in RCMD group. The patients were divided into two subgroups with high (≥63.1 ng/mL) and low (<63.1 ng/mL) osteopontin levels at diagnosis.

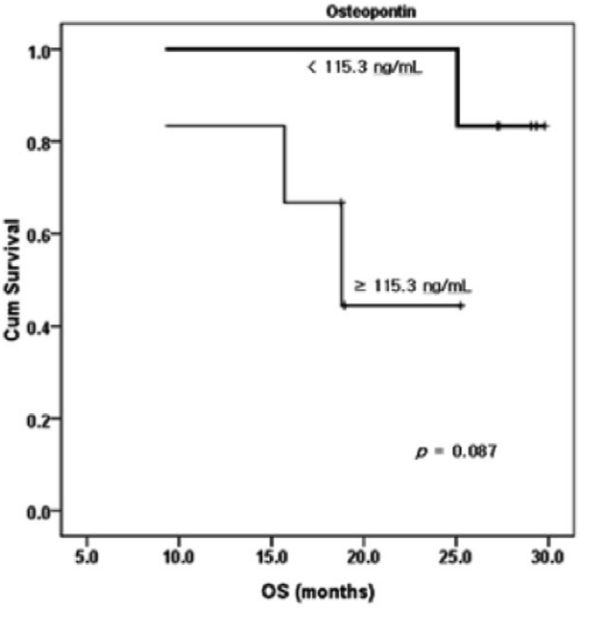

Figure 1. BM osteopontin level and survival in RAEB group. The patients were divided into two subgroups with high (≥115.3 ng/mL) and low (<115.3 ng/mL) osteopontin levels.

Summary and Conclusions: The present study demonstrated that RAEB patients have higher levels of marrow osteopontin than RCMD patients, and that these patients tend to show a shorter survival. In RAEB patients, the low osteopontin level had a trend toward survival advantage. Thus, these result may contribute to the understanding of the role of marrow osteopontin as the prognostic factor in MDS patients.

PB1829

HIPERFERRITINEMIA IN MYELODYSPLASTIC SYNDROME (MDS) PATIENTS - RETROSPECTIVE STUDY FROM A SINGLE CENTRE EXPERIENCE

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Background: Most myelodysplastic (MDS) patients have anemia and many of them require red blood cells (RBC) transfusions leading to iron overload. Hema-

tological improvement (HI) during iron chelation therapy (ICT) was first pointed out more than twenty years ago. This phenomenon seems to be more frequent after introduction of Deferasirox. The most simple test assessing iron overload is serum ferritin concentration.
Aims: Assessment of hyperferritinemia incidence in MDS patients at the moment of MDS diagnosis, and correlation between ferritin level and AML transformation in patients diagnosed with MDS.
Methods: The retrospective data collection from a single center experience (Department of Hematology County Hospital, Timisoara, Romania) between December 2003 and December 2012 included 121 patients (68 men and 53 women) with MDS. All the patients had complete blood count and serum ferritin level, and complete follow-up data.
Results: Ferritin level above 1000 ng/mL was found in 38 patients (31%) (Group 1) and ferritin level ≤1000 ng/mL in 83 patients (69%) (Group 2). Most patients with significant hiperferritinemia, were RBC transfusion dependent (81% of patients). Among patients with ferritin level ≤1000 ng/mL, 38% were RBC transfusion dependent. Serum hemoglobin concentration was lower in Group 1 patients in comparison with Group 2 patients (7,5 g/dL vs 9,4 g/dL, P<0,001). The most frequent MDS subtype in Group 1, were patients with refractory anemia (RA) (30%), compared with patients with ferritin ≤1000 ng/mL-15% (P<0,04). According to IPSS score, there were no differences between studied groups. Median follow up was 12 months. There was an improved overall survival (OS) in RBC transfusion independent patients compared to RBC transfusion dependent patients, but mean OS was not significantly statistically different in studied groups. No correlation was found between ferritin level and time to AML transformation.
Summary and Conclusions: Hiperferritinemia >1000 ng/mL does not influence survival and time to AML transformation in MDS patients. The most frequent MDS subtype in patients with ferritin level>1000 ng/mL was MDS RA. Among patients with ferritin level >1000 ng/mL, 81% were RBC dependent.

PB1830

EPIDEMIOLOGICAL, CLINICAL, THERAPEUTIC AND PROGNOSTIC FEATURES OF MYELODYSPLASTIC SYNDROM IN FARHAT HACHED HOSPITAL SOUSSE TUNISIA.

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Background: The myelodysplastic syndrom (MDS) is a frequent entity in hematology. The clinical manifestations are characterized mainly by hemorrhagie, anemia or infection. An early diagnosis is essential in order to initiate an appropriate management to improve the prognosis of this affection.
Aims: The aim of our study is to report the epidemiological, clinical and therapeutic features of patients with MDS.
Methods: This retrospective analysis included 114 patients with MDS who were treated and followed between 2005 and 2013.
Results: The sex ratio was 1.The median age was71 years with a range from 11 to 99 years. Three patients had a family history of hematological diseases, and two had a personal history of neoplasia treated with chemotherapy and radiotherapy. The circumstances of discovery were an anemic syndrome, hemorrhagic syndrome and hemorrhagic syndrome in 68.7%, 5.2% and 2.6% of cases, respectively. The blood count showed anemia in 89.5% with VGM>100fl in 48.7% of cases, thrombocytopenia in 61.4% of cases, and leukopenia in 24.3% of cases. The myelogram showed dyserythropoiesis, dysgranulopoiesis, dysmegacaryopoiesis in 44.3%, 65,2% and 44.3% of cases, respectively. The karyotype was normal in 75.4% and abnormal in the remaining cases. According to the latest WHO classification, refractory anemia, sideroblastic anemia, refractory anemia with excess blasts, refractory anemia with excess blasts type 2, 5q- syndrome, refractory cytopenia with multi line and unclassifiable in 26.4%, 7%, 22.4%, 20%, 1.7%, 2.6%, and 20%, respectively. According to the IPSS, patients were classified very low risk, low risk, intermediate risk, high risk and very high risk in 10%, 36%, 30%, 17% and 7%, respectively. The management was symptomatic with red blood cell transfusions in 66% of cases, platelet transfusion in 56% of cases. The MDS was stable in 51% of cases and transformed into acute myeloid leukemia (AML) in 12, 3% of cases and only 5.26% of patients received chemotherapy. Only one patient aged 30years was underwent allograft of hematopoietic stem cell transplantation and two patients received revlemid. The overall survival at 2 years was 59% and 48% at 5 years.
Summary and Conclusions: Specific treatment for the kind of MDS should be started early in order to improve the management and prognosis of these patients.

PB1831

A MULTICENTRIC STUDY TO EVALUATE THE EFFICACY OF EPOIETIN ZETA (BIOSIMILAR OF EPO ALPHA) IN LOW RISK MYELODISPLASTIC PATIENTS

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Background: Erytropoietin (EPO) is a valuable option in the treatment of low risk myelodisplastic syndrome (MDS). Since the expiration of EPO alpha patent, epoietin biosimilars are becoming an increasingly important therapeutic option as anti-anemic drugs. Clinical efficacy and safety of biosimilar EPOs have been demonstrated in renal failure and chemotherapy anemias, but not in the treatment of myelodisplasic syndrome (MDS) patients.
Aims: Our study was designed to evaluate safety and efficacy of erythropoietin zeta (Retacrit, Hospira), a biosimilar of EPO alpha, to treat anemia of low risk MDS, such as low and Intermediate-1 (Int-1) patients according to International Prognostic Scoring System (IPSS).
Methods: We collected data from 32 MDS patients (14 males, 18 females), aged 58-84 (median 76.5), treated with EPO zeta in Sicily. According to WHO classification, 15 out of 32 subjects were diagnosed as refractory anemias (RA), 13/32 as refractory cytopenias with multilineage dysplasia (RCMD) and 4/32 as refractory anemias with excess blasts-1 (RAEB-1, bone marrow blasts<10%). Considering cytogenetic subgroups, karyotype was favourable in 28 out of 32 patients while was intermediate in the remaining 4. According to IPSS risk stratification, 17/32 patients were classified in the low risk group and 15/32 in the Int-1. Four patients were transfusion dependent before anti-anemia treatment with EPO. To start EPO treatment: 1) Emoglobin (Hb) had to be below 10 g/dl; 2) Serum iron, folate and B12 vitamin had to be in the in the normal range or corrected before therapy onset; 3) the maximum allowed transfusional need was of 3 units of packed red blood cells (PRBC) per month in the 90 preceding days; 4) serum EPO levels had to be below 500 UI/mL. Patients were treated with EPO zeta, 40.000 U s.c./week. After 8 weeks, whether there was no or suboptimal response (raise in Hb of less than 1.5 g/dl and/or no transfusion independence), EPO dose was raised to 80.000 U s.c./week. Treatment was continued for 24 weeks.
Results: Patients were considered as responders either when Hb levels raised of at least 1.5 g/dl from basal levels (and this data had to be confirmed in two consecutive evaluations) or when there was at least a 50% reduction of the trasfusional needs as evaluated before starting EPO. Responders were 23 out of 32 patients (71.9%); for 9/23 responders (39%) EPO dose had to be raised to 80.000 U/week to obtain the best results. No side effects greater than G1 according to WHO were reported in our cohort of patients during the 24 weeks treatment period.
Summary and Conclusions: In our series, efficacy of EPO zeta in low risk MDS patients was consistent with that reported in the literature using the originator EPO alpha in similar patient populations. Larger prospective randomized studies comparing originator and biosimilar EPOs will provide a definitive answer. However, biosimilar EPO zeta seems to be a good option to treat anemia in low risk MDS, thus saving resources considered its favourable pharmaco-economic profile.

PB1832

MANAGEMENT OF MYELODYPLASTIC SYNDROMES:EXPERIENCE OF MILITARIAN HOSPITAL OF RABAT MOROCCO

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Background: Myelodysplastic syndromes (MDS) are a disorder of hematopoietic stem cell that affects essentially old population.
Aims: In this study we aim to show characteristics of management of MDS in our department and give a small example about Moroccan way to treat them with a few resources.
Methods: This is a retrospective study including all patients managed in our training for MDS since 2006 to February 2015.
Results: A total of 78 patients were included. The median age was 65.94 years; the rate of men was more likely higher than women with a sex ratio [M/F] of 1.7. At diagnosis, the international prognosis scoring system (IPSS) was calculated for only 85, 89% (67 patients), 41.79% (28 patients) had low risque, 35.82%(24 patients) had intermediate 1 IPSS,14.92% (10 patients) intermediate 2,just five patients had a high risque with range of 7%.Reviesed IPSS(R-IPSS) was also calculated for only 61 patients (78%),9.8% had a very low R-IPSS,44.26% had low risque,29.5% had intermediate risque,10 patients had high (9%) and very high (6,55%) risque. Concerning management, we decided to wait and see for 41% of patients, 34.6% needed best supportive care, Erythropoietin was prescribed for 28% of our patients, 21.7% received Azacithidine, three of our patients had Hydroxyurea,one of them had Thalidomide, and only two of them have been selected to have an allo stem cell l transplantation. Overall Follow up is about 42 months. The overall survival rate was 77, 78% at 2 years, and 44, 44% at 5 years.
Summary and Conclusions: Despite our few resources our results are the same of most publications concerning survivals in MDS.

PB1833

THE MDS/MPN PATIENTS TREATED WITH 5-AZACITIDINE: A CLINICAL REAL LIFE EXPERIENCE

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Background: The myelodysplastic/myeloproliferative disorders (MDS/MPN), according to the World Health Organization classification, include cases that have clinical, laboratory, and morphologic features intermediate between myelodysplastic syndrome (MDS) and myeloproliferative neoplasm (MPN). This is an infrequent entity and there is few data in the literature regarding treatment.
Aims: To evaluate the efficacy of 5-azacitidine (5-AZA) treatment in patients (pts) affected by MDS/MPN disorders.
Methods: A retrospective analysis of the MDS/MPN pts, into a large cohort of MDS or AML with blast<30% subjects, treated with 5-AZA in 10 centers in Lombardia region was performed. Response to therapy was considered evaluable if pts had reached, at the time of observation, at least 6 cycles of 5-AZA. The Kaplan Meier method was applied to evaluate the survival.
Results: From a population of 187 pts, data about 9 cases of MDS/MPN were carried out. Of these 4 were female and 5 male. Median age was 65 (range 23-81). Median bone marrow blasts was 5% (range 2-15); in two cases blasts were over 10%. In 3 pts Red Blood Cells transfusion dependence was present. In the other 6 cases median hemoglobin level was 10.2 g/dl (range 9-15). Only 1 patient had Platelet count (PLT) <10.000/mm^3, in 3 case PLT was <50.000/mm^3 and in 2 cases PLT was >300.000/mm^3. In 3 pts the Neutrophil count was ≥ 10.000/mm^3, in 1 case was<1500/mm^3. The median number of 5-AZA courses administered was 6 (range 5-20). A response to the treatment (including the cases of "stable disease" maintenance) was achieved in 5 cases on 7 evaluable. The median survival was 10 months (Figure 1).

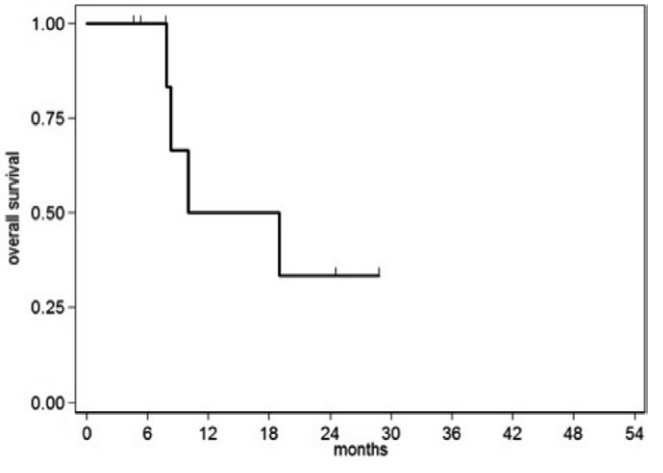

Figure 1.

Summary and Conclusions: This abstract is intended to be only a descriptive report of a disease entity rarely considered. The small sample size does not allow to draw any conclusion about the observed population. Nevertheless, the response rate to 5-AZA therapy seems to indicate a trend of efficacy and, therefore, the interest in collecting new data to make a significant analysis.

PB1834

CHARACTERISTICS OF BLASTS IN THE PERIPHERAL BLOOD AND BONE MARROW SAMPLES OF THE PATIENTS WITH MDS TREATED WITH 5-AZACITADINE

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Background: Identification of the blast population in the patients with MDS has been improved in the recent years with the application of multiparametric flow cytometry (MFC).
Aims: The aim of our study was to fully characterize the blasts in the peripheral blood (PB) and bone marrow (BM) of the patients with MDS, by MFC before and after the treatment with 5-Azacitadine (5-AZA).
Methods: Patients with MDS, suitable for the treatment with 5-AZA were selected by the participating centers after the approval of the ethic committee. BM and PB samples were analyzed by BD FACSCanto flow cytometer before and after the treatment with 5-AZA. For each sample, detailed analysis with Euroflow leukemia orientation tube and acute myeloid leukaemia panels were performed (Table 1). A minimum of two million events were acquired. Infinicyt software version 1.8 (Euroflow member version) was used to analyze the acquired data files.
Results: Thirteen patients have consented to participate in the study from January 2011 to December 2014. Eight patients had normal cytogenetics and the remainder had one to three abnormal clones with del 5q, del 20q, trisomy 8 and del 11q. All patients had advanced MDS, either RAEB-1 or RAEB2 at 5-AZA treatment and received at least 3 cycles of 5-AZA before the reassessment.There were 13 pre and post 5-AZA BM samples and 8 pre and post 5-AZA PB samples. The pre 5-AZA blast count by flow cytometry ranged from 2.5 to 14% in the bone marrow and post 5-AZA blast count from 0.48% to 13%. By applying the Euroflow panels, the blast population with the same expression pattern as in the marrow was detected in the peripheral blood samples of all patients, with the lowest post treatment peripheral blood blast count being 0,02%. The blasts were uniformly positive for CD117, HLADR, dim CD45, CD13, CD33 and CD34 except one patient with negative CD34 expression. They did not express granulocytic or monocytic maturation markers in all patients, with MPO expression found only in 28% of the tested samples. Dim CD71 and dim CD123 expression was noted in ten patients whereas aberrant CD7 expression in three patients, CD56 in one patient and CD22 in one patient were noted. None of the patients had cCD3 or CD19 expression.

Table 1. Monoclonal antibodies used to analyze the blasts of in bone marrow and peripheral blood samples

Tubes	FITC	PE	PerCP5.5	PECy7	APC	APCH7	PB	PO
Tube 1	CD16	CD13	CD34	CD117	CD11b	CD10	HLA-DR	CD45
Tube 2	CD35	CD64	CD34	CD117	IREM-2	CD14	HLA-DR	CD45
Tube 3	CD36	CD105	CD34	CD117	CD71	CD33	HLA-DR	CD45
Tube 4	cTDT	CD56	CD34	CD117	CD7	CD19	HLA-DR	CD45
Tube 5	CD15	7.1	CD34	CD117	CD22	CD38	HLA-DR	CD45
Tube 6	CD42b/CD61	CD203c	CD34	CD117	CD123	CD4	HLA-DR	CD45
Tube 7	cMPO	cCD79b	CD34	CD19	CD7	cCD3	HLA-DR	CD45

Summary and Conclusions: The myeloblasts in the study patients demonstrated more immature immunophenotypic features with rare expression of maturation markers, consistent with the previous findings in advanced MDS. MFC can accurately identify the blast population of the same immunophenotypic features as that of the BM samples, in the PB samples at diagnosis and post treatment. Assessing the evolution of the characteristic blast population in the PB samples by multiparameter flow-cytometry may be an interesting and useful approach in the patients with MDS during the post-treatment period.

PB1835

SINGLE CENTRE SURVIVAL OUTCOMES IN THE TREATMENT OF INTERMEDIATE AND HIGH-RISK MYELODYSPLASTIC SYNDROME AND ACUTE MYELOID LEUKAEMIA WITH 5-AZACITIDINE

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Background: The hypo-methylating agent, 5-azacitidine, is used to treat chronic myelomonocytic leukaemia (CMML), intermediate and high-risk myelodysplastic syndrome (MDS) and acute myeloid leukaemia (AML) with 20-30% blasts in patients unsuitable for high intensity treatment. Our centre is one of the largest users of azacitidine in the South East of England, reflecting the predominantly elderly population served.
Aims: To evaluate overall survival (OS) of patients treated with 5-azacitidine at our centre. Comparison of OS for MDS and AML groups and according to cytogenetic prognostication. Also to compare local prescribing practice with 2011 guidelines published by the National Institute for Clinical Excellence (NICE).
Methods: A retrospective analysis of medical records for all patients treated with four weekly cycles of 5-azacitidine (75mg/m^2) for 7 days. Patients were grouped according to diagnosis (MDS, CMML or AML) and cytogenetic classification as per the 5-group MDS Revised International Prognostic Scoring System (IPSS-R).
Results: Forty-two patients (*n*=19 AML, *n*=22 MDS, *n*=1 CMML) were identified from a 40 month observation period. Mean age was 71 (range 43-84). The median time from diagnosis to treatment was 17 days. The mean number of cycles received was 6 (range 1-23). Eighteen patients were diagnosed and received 5-azacitidine after treatment for a previous haematological malignancy: relapsed AML (*n*=7); transformed MDS (*n*=4); myeloproliferative disorder (*n*=3);

MDS following AML treatment (*n*=2); chronic lymphocytic leukaemia (*n*=1); myeloma (*n*=1). Kaplan-Meier analysis revealed an OS of 9.77 months for all 42 patients (95% CI 6.55-12.98). OS for MDS patients was 12.11 (CI 7.69-16.5). OS for MDS subgroups according to cytogenetic prognostic groups 'good', 'intermediate', 'very poor' and 'unknown' were 15.7 (*n*= 9, CI 7.7-23.7), 8.25 (*n*=4, CI 6.44-10.05), 3 (*n*=4, CI 0.23-5.77), and 10.6 (*n*=5, CI 2.48-18.72), months respectively. The 'good' cytogenetic group had significantly longer OS compared to 'bad' cytogenetics, P<0.001. OS for AML patients was 6.34 (CI 2.02-10.7) significantly worse when compared to all MDS patients, P=0.001. Twenty-nine patients (69%) met NICE approval criteria. Treatment outside the criteria was due to either no alternative available (9), instruction from regional transplant centre (3) or as part of a trial (1). Treatment within NICE recommendations showed significantly improved survival (P=0.001) with a mean of 13.27 months (CI 9.32-17.23) compared to 2.73 (CI 1.84-3.67).
Summary and Conclusions: Treatment with 5-azacitidine for AML and MDS gave OS of 12.11 and 6.34 months respectively in our elderly population with limited alternative therapeutic options. Favourable prognostic cytogenetics and treatment within national guidelines led to superior survival in this sample.

PB1836

PROGNOSIS IN MDS – CAN SERUM ERYTHROPOIETIN BE OF HELP?
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Background: Myelodysplastic syndromes (MDS) are a heterogeneous entity characterized by dysplasia, hypercellular bone marrow, cytopenias and a risk of transformation to acute leukaemia (AL). Treatment is mainly based in the International Prognostic Scoring System (IPSS). In the last few years a lot has been written about MDS prognosis, with new scores for survival increasing in complexity making their use in everyday practice somewhat difficult. Although other prognostic factors, for instance, bone marrow fibrosis, LDH and β2-microglobulin elevation are readily available to clinical practice, others like gene mutations *TP53*, *EZH2*, *ETV6*, *RUNX1*, *NRAS*, *ASXL1*, *SRSF2*, *U2AF1* and *SF3B1* are not so easy to obtain. Two retrospective studies show a benefit in survival in patients treated with erythropoietin (Epo)±G-CSF, however without an impact on progression to AL.
Aims: We aimed to study the prognostic impact of serum Epo at diagnosis in a cohort of MDS patients.
Methods: We analyzed the records of 102 patients with *de novo* MDS between October 2009 and March 2014. Clinical and laboratorial data were collected and overall survival (OS) was estimated stratified according their serum Epo level: low vs normal vs<100 mUI/mL vs 100-500 mUI/mL vs ≥ 500 mUI/mL.
Results: The patients had median age of 74 years, with a Male:Female ratio of 0.8. According to the WHO classification, MDS subtypes where distributed as follows: Refractory Cytopenia with Multilineage Dysplasia (RCMD) (n=52), Refractory Cytopenia with Unilineage Dysplasia (RCUD) (n=12), Refractory Anemia with Excess Blasts (RAEB)-1 (n=8), (RAEB)-2 (n=8), Refractory Anemia with Ring Sideroblasts (RARS) (n=6), 5q- Syndrome (n=4) and Chronic Myelomonocytic Leukaemia (n=12). Thirty seven patients presented with low IPSS score, 39 with intermediate-1, 10 with intermediate-2 and 1 with high score. The mean erythropoietin was significantly lower in RCMD patients when compared to other MDS subtypes (P<0.05). Eleven patients (7 RAEB-2, 2 RCMD, 1 RAEB-1 and 1 CMML) progressed to AL. Their mean erythropoietin value was higher than in patients who did not evolve (P<0.05). We found that patients with elevated serum Epo had significantly lower survival rates than those with normal Epo levels (P=0.0336). The predictive value of serum Epo was maintained after Cox regression adjustment for LDH, age, IPSS, R-IPSS, hemoglobin, neutrophils, platelets, ferritin, β2-microglobulin and bone marrow blasts. In multivariate analysis, the independent survival predictors were serum Epo (P<0.001), ferritin (P<0.001), β2M (P=0.008) and marrow blast percentage (P<0.001).
Summary and Conclusions: In the present study, serum baseline erythropoietin was identified as in independent poor prognostic factor, with higher values in patients with progression to AL and decreased OS.

PB1837

CHRONIC MYELOMONOCYTIC LEUKEMIA IS CHARACTERIZED BY HYPOADIPONECTINEMIA AND HYPORESISTINEMIA INDEPENDENTLY FROM THE IGF-I SYSTEM: A CROSS-SECTIONAL STUDY
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Background: Accumulating evidence supports a role for obesity in the etiology of hematologic malignancies. Obesity may be also linked to chronic myelomonocytic leukemia (CMML), a hematologic malignancy combining proliferative and dysplastic characteristics, through altered adipokine secretion, one of which, adiponectin, presents a protective role in several malignancies, including leukemia and lymphoma.
Aims: In this cross-sectional study, we investigated the association of circulating adiponectin and resistin with CMML in relation to insulin-like growth factor-I (IGF-I), a hormonal system which is implicated in several human malignancies including leukemia.
Methods: Blood samples were collected from 14 cases with incident, histologically confirmed CMML and 70 healthy controls (1 patient *versus* 5 controls) who came for an annual check-up examination without any neoplastic and infectious conditions, matched on gender, age and year/month of diagnosis (±1 month) between 2004 and 2012. Informed consent was obtained from all participants. Serum adiponectin and resistin were determined respectively by radioimmunoassay (LINCO Research Institute, St Louis, MO). High molecular weight (HMW) adiponectin was measured using ELISA (ALPCO Diagnostics, Salem). IGF-I and IGFBP-3 concentrations were measured using an immunoradiometric assay kit (DSL, Webster, TX) The statistical analysis of the data was performed using IBM-SPSS® version 22 for Windows.
Results: CMML cases presented significantly higher height and weight than control subjects (P<0.001), while differences of body mass index (BMI) were only of borderline significance (P=0.10). Lower serum total or HMW adiponectin and/or resistin levels were independently associated with higher risk of CMML adjusting for age, gender, BMI and serum IGF-I levels (p≤0.05). In particular, CMML was characterized by hypoadiponectinemia (total adiponectin in CMML patients: 12.5±8.8 μg/dL *versus* controls: 16.3±7.9 μg/dL, P=0.05) and hyporesistinemia (resistin in CMML patients: 12.1±6.2 ng/mL *versus* controls: 21.9±32.7 ng/mL, P=0.013). Total adiponectin exhibited a positive correlation with HMW adiponectin both in CMML cases and controls (r=0.83, P<0.001; r=0.88, P<0.001, respectively). Although, total and HMW adiponectin were both significantly reduced in CMML, HMW did not offer any substantial additional predictive value over total adiponectin. Serum IGF-I was not significantly and independently associated with CMML in multivariable analysis adjusting for age, gender, date of diagnosis, BMI and history of lymphohematopoietic cancer (P=0.56).
Summary and Conclusions: Total and HMW adiponectin may present a protective role in CMML by suppressing proliferation of myeloid cell lineage, whereas resistin levels may be decreased via a compensatory mechanism due to an upregulation of other inflammatory factors etiologically and ontologically linked to CMML. Further studies are needed to confirm these associations and to explore the mechanisms underlying adiponectin's role in myelopoiesis and leukemogenesis.

PB1838

THE HETEROGENEITY OF MYELODYSPLASTIC SYNDROME WITH CHROMOSSOME 5 ABNORMALITIES: THE PROGNOSTIC IMPACT OF BLAST COUNT, KARYOTYPE, P53 PROTEIN EXPRESSION AND TRANSFUSION DEPENDENCY
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Background: Deletion of the long arm of chromosome 5 [del(5q)] is the most prevalent cytogenetic abnormality in patients with Myelodysplastic Syndrome (MDS) and is frequently associated with good prognosis. However, MDS with del(5q) is a heterogeneous entity in terms of survival and/or progression to Acute Myeloid Leukemia (AML). The analysis of the proposed Revised International Prognostic Scoring System (R-IPSS) for MDS reinforces the importance of blast count, karyotype and number and degree of cytopenias to define high-risk subgroups with worse prognosis. More recently, the presence of TP53 mutation and/or high P53 protein expression by immunohistochemistry (IHC) has been proposed as additional markers of poorer outcome in MDS with chromosome 5 abnormalities.
Aims: Assess the prognostic impact of blast count, number of additional cytogenetic abnormalities, transfusion dependency and p53 IHC expression, in MDS with del(5q).
Methods: We retrospectively analyzed 18 patients diagnosed with MDS and del(5q) at our centre, between 2005-2014, in which a bone marrow histology was available. All patients were evaluated for R-IPSS, transfusion dependency and P53 protein expression by bone marrow IHC. A high blast count was defined by the presence of >5% of blasts cells in bone marrow smear. A high number of chromosome abnormalities was defined by >=2 abnormalities besides del(5q). A positive p53 IHC expression was defined by the presence of >5% p53 positive cells observed on the histology of bone marrow. Transfusion dependency was defined as no period of eight consecutive weeks without red blood cell (RBC) transfusions.
Results: The median of age was 69 (55-81) years and 11 (61%) patients were male. The MDS WHO-classification was: RCMD n=3 (16,7%), RAEB-1 n=3 (16,7%), RAEB-2 n=5 (27,8%) and MDS with isolated del(5q) n=7 (38,9%). In

terms of R-IPSS evaluation, patients were stratified as: Very Low n=1 (5,6%), Low n=6 (33,3%), Intermediate n=2 (11,1%), High n=2 (11,1%) and Very High n=7 (38,9%). For the relevant prognostic parameters analyzed, frequencies were: n=8 (44,4%) had >5% of blasts, n=4 (22,2%) had >=2 chromosome abnormalities besides del(5q), n=16 (88,9%) had transfusion dependency and n=9 (50%) had >5% of p53 IHC protein expression. Overall Survival (OS) and Progression to AML Free Survival (PFS) were significantly worse for patients with >5% of blast count (OS: 5 months vs 39 months, P<0.05; PFS: 5 months vs 32 months, p>0.05) and for those with >=2 chromosome abnormalities besides del(5q)(OS: 6 months vs 36 months, P<0.05; PFS: 5 months vs 29 months, P<0.05). Those differences were also observed in multivariate analysis (P<0.05). A trend towards a worse OS and PFS was observed for transfusion dependency (OS: 21 months vs 48 months, P=0.18; PFS: 17 months vs 44 months, P=0.12) and >5% of p53 IHC protein expression (OS: 19 months vs 30 months, P=0.37; PFS: 18 months vs 21 months, P=0.75).

Summary and Conclusions: The presence of a high blast count and other chromosome abnormalities besides del(5q) define subsets of worse prognosis in MDS with del(5q), despite of this being classically considered a chromosomal abnormality associated with good prognosis. There is also a trend for a poor prognosis in patients with transfusion dependency and positive p53 IHC protein expression. The absence of a statistical significant difference in OS and PFS for these patients subsets might be due to the low number of patients in this cohort.

PB1839

COMPLEX KARYOTYPE CHANGES ARE THE STRONGEST PREDICTOR OF OUTCOME OF ALLOGENEIC TRANSPLANTATION IN MYELODYSPLASTIC SYNDROME

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Background: Complex karyotype has been recently reported as one of the predictors of poor outcome of allogeneic transplantation in patients with myelodysplastic syndrome (MDS). Current opinions challange transplantation as a curative option in these patients.

Aims: To evaluate impact of complex karyotype changes of MDS in setting of allogeneic stem cell transplantation

Methods: We evaluated all consecutive patients with all stages of MDS transplanted at our center from 2007-2014. Patients parameters, demographic data, disease status, pretransplant remission status, type of transplantation and karyotype has been evaluated as possible factors influencing outcome of transplantation.

Results: Overall 22 patients (12 males, 10 females, median age 56 years, 32-66 years) were analysed. Disease status was as follows: 12/22 were MDS related AML, 4/22 RAEB-2, 3/22 RAEB-1 and 3/22 RA or RCMD. Majority of patients (20/22) underwent transplant from matched unrelated donor, 2/22 had sibling donor. Conditioning was intended myeloablative in 13/22 patients and reduced intensity in 9/22. 14 patients reached hematologic CR before transplant. Five (5/22) patients had complex karyotype changes. The median follow-up was 3 months. Median overall survival was not reached, survival at 5 years was 56%. The strongest predictors of outcome were: CR before transplant (median survival 4.5 months vs. not reached, P=0.0997, trend only), stage of the disease (AML and RAEB-2 vs. others, median survival 18 months vs. not reached, P=0.0361) and komplex karyotype changes (meadian survival 4.5 months *versus* not reached P=0.0044).

Summary and Conclusions: Complex karyotype changes, disease stage and remission status are probably the most singificant factors which influence the outcome of allogeneic transplantation in MDS. The possibility of transplant for these patients is a matter for debate since the outcome remains dismal.

PB1840

AZACITIDINE IN CHRONIC MYELOMONOCYTIC LEUKEMIA (CMML): POSSIBILITY OF IMPROVED RESPONSE AND SURVIVAL

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Background: CMML is a clonal disease characterized by high rate progression to AML. Treatment is not curative traditionally and allo-SCT remains the only option, although in a minority of patients. Recently, significant clinical benefits by hypomethylating agents have been reported.

Aims: To evaluate efficacy and toxicity of azacitidine on CMML.

Methods: Fourteen patients with a median age of 75 (60-85) and several comorbidities were evaluated; 5/14 (37%) aged >80 years. Median PS was 70% (range 50-90). According to WHO, 10, 2 and 2 patients presented CMML-2, CMML-1 with severe and symptomatic cytopenias and CMML-related AML with<30% BM blasts, respectively. Six patients had proliferative CMML, 7 were transfusion-dependent at the start of azacitidine and two had abnormal karyotype. Three patients had CMML secondary to low risk MDS; one patient presented a therapy-related CMML. Previous therapies were: intensive chemotherapy (1), ESA (6) and hydroxyurea (8); 4 patients were treatment-naïve. The interval between diagnosis and the start of azacitidine was 2 months (0.3 – 18) and the median number of courses was 12 (2-33). Treatment was well-tolerated and with no remarkable side effects. Responses were evaluated according to 2006 IWG.

Results: Thirteen patients were evaluable for response after the fourth cycle of azacitidine; the remaining patient developed AML after the second cycle of therapy. Five patients (36%) achieved CR and 6 (43%) PR with an ORR of 78%; two patients presented a primary failure to azacitidine. After a median follow-up of 19 months (5-46), 6 patients (3 in PR, 2 in CR and 1 with AML) are still alive, with an OS from CMML diagnosis and the start of azacitidine treatment of 25 and 17 months, respectively. Among responding patients, 1 is alive and well 120 days after unrelated allo-SCT. Six patients progressed to AML: 1 was primarily unresponsive to azacitidine and 5 progressed after 17 (5-40) months from the start of therapy. Among the 8 deceased patients, 5 died from AML progression and 3 because of complications related to pre-existing cardiac comorbidities (2 patients), or secondary lung cancer (1 patient).

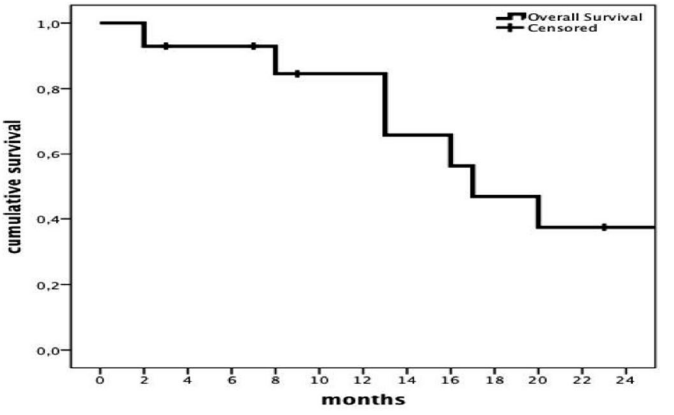

Figure 1. Overall survival from the start of azacitidine.

Summary and Conclusions: Despite the limited number of cases, the results are encouraging, considering age and the complexity of the patients. The ORR of 78% and the unfavorable prognosis of our comorbid patients reinforce the existing evidence, making azacitidine the most appropriate treatment of CMML in the real life setting.

PB1841

CIRCULATING TOTAL LEPTIN, LEPTIN RECEPTOR AND FREE LEPTIN INDEX LEVELS IN CHRONIC MYELOMONOCYTIC LEUKEMIA: A CROSS-SECTIONAL STUDY

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Background: Recent evidence suggests that overweight/obesity may be implicated in the etiology of hematologic malignancies, including myelogenous leukemia and myelodysplastic syndromes. A strong asociation of overweight/obesity with insulin resistance, characterized by hyperinsulinemia, has been well documented. There is evidence that insulin resistance is implicated in several malignancies associated with excess body weight. Leptin and, particularly, free leptin, the biologically significant form of leptin, reflecting accurately the body fat mass, regulate glucose and lipid metabolism by ameliorating insulin sensitivity and decreasing intracellular lipids.

Aims: In this cross-sectional study, we investigated the potential association of leptin and free leptin with chronic myelomonocytic leukemia (CMML), a hematologic malignancy combining proliferative and dysplastic features, after adjusting for a potential confounding effect of age,gender, date of diagnosis (matching factors) a well as withbody mass index (BMI), family history of lymphohematopoietic cancer (LHC) and serum insulin.

Methods: Blood samples were collected from 14 cases with incident, histologically confirmed CMML and 70 healthy controls (in an analogy of one patient *versus* five healthy controls) who came for an annual check-up examination without any neoplastic and infectious conditions, matched on gender, age and year/month of diagnosis (±1 month) between 2004-2012. Informed consent was obtained from all study participants. Serum leptin and insulin were determined by radioimmunoassay (Linco Research Institute St Louis MO, and Milli-

pore Co Billerica, MA respectively). Serum leptin receptor levels (sOB-R) levels were measured using a commercially available ELISA (BioVendor R&D, Brno, Czech Republic). Free Leptin Index (FLI) was calculated as the ratio of leptin to sOBR. The statistical analysis of the data was performed using IBM-SPSS® version 22 for Windows.
Results: CMML cases presented significantly higher height and weight than control subjects (P<0.001), while differences of BMI were only of borderline significance (P=0.10). Serum insulin was significantly higher in cases than controls (P=0.05). CMML cases exhibited a significant total and free hypoleptinemia in comparison to controls (total leptin in patients with CMML: 13.2±16.3 ng/mL *versus* controls 24±21.1 ng/mL, P=0.005; FLI in patients with CMML: 0.81±1.6 *versus* controls: 2.84±5.4, P=0.04). Moreover, CMML cases exhibited significantly lower serum levels of sOB-R than controls (P=0.04). In multivariate analysis, subjects with total and free hypoleptinemia presented significantly higher odds for CMML after adjusting for age, gender, date of diagnosis, BMI, family history of LHC and serum insulin levels.
Summary and Conclusions: This study raises the hypothesis that leptin which reflects overall fat mass and insulin may be associated with CMML. Leptin's major physiological role is to signal inadequate rather than excess energy stores, and hypoleptinemia found in a small but significant percentage of obese humans is associated with hyperinsulinemia and impaired T-cell function. Further mechanistic, interventional and epidemiological studies are needed to confirm these findings and to explore whether leptin may mediate the effect of body fat distribution on insulin resistance and leukemogenesis risk.

PB1842

ASSOCIATION OF THYROID HORMONES AND AUTOIMMUNE THYROIDITIS WITH CHRONIC MYELOMONOCYTIC LEUKEMIA
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Background: Thyroid disease has been associated with increased incidence of leukemia, myelodysplastic syndrome (MDS) and lymphoma. No previous study has investigated whether thyroid disease and especially autoimmune thyroid disease (ATD) is associated with chronic myelomonocytic leukemia (CMML), a hematologic malignancy which has been classified as a subtype of MDS but was recently demonstrated to be a distinct entity combining proliferative and dysplastic features.
Aims: In this case-control study, we investigated the potential association of thyroid hormones and ATD with CMML.
Methods: Our study included 14 cases with incident histologically confirmed CMML and 70 controls (one patient *versus* five controls) who came for an annual check-up examination without any neoplastic and infectious conditions, matched on gender, age and year/month of diagnosis (±1 month) between 2004-2012. All participants were submitted to clinical and ultrasound thyroid evaluation. Informed consent was obtained from all study participants. Serum free T3 (fT3), free T4 (fT4), thyroid-stimulating hormone (TSH), thyroglobulin and thyroperoxidase antibodies (anti-TG and anti-TPO) were determined using electrochemiluminescence on Cobas e411 analyzer (Roche, Basel, Switzerland). The statistical analysis of the data was performed using IBM-SPSS® version 22 for Windows.
Results: Mean serum levels of fT3 and fT4 were significantly higher in CMML patients than in controls (fT3 in patients with CMML: 3.6±0.3 pg/mL *versus* controls: 2.9±0.42 pg/mL, P<0.001; fT4 in patients with CMML: 1.7±0.29 ng/dL *versus* controls: 1.4±0.28 ng/dL, P<0.001). The prevalence of anti-thyroid antibodies (anti-TG and anti-TPO Ab) was significantly higher in CMML patients than in controls (P<0.001). On the contrary, mean TSH serum levels were significantly lower in CMML patients than in controls (TSH in patients with CMML: 1.56±0.6 μIU/mL *versus* controls: 2.9±0.4 μIU/mL, P=0.001). The frequency of family history of thyroid disease (first degree relatives) in CMML patients was similar with that reported by controls (P=0.52). Finally, the prevalence of clinical thyroid disease-especially ATD-was higher in patients with CMML than in controls (P=0.06, though not statistically significant at α=0.05).
Summary and Conclusions: We have found biologically plausible and empirically strong evidence that thyroid hormones, thyroid disease and especially ATD may be associated with CMML. Further larger prospective studies are needed to confirm this association and explore underlying interactive mechanisms between thyroid hormones and thyroid autoimmunity with myelopoiesis and leukemogenesis.

PB1843

EFFICACY OF AZACITIDINE IN MDS AND AML WITH PROLIFERATIVE FEATURES
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Background: Azacitidine is a hypomethylating agent with demonstrated efficacy in high risk myelodysplastic syndromes (MDS), chronic myelomonocytic leukemia (CMML) and acute myeloid leukemia (AML). The trials that have led to its approval for these indications excluded patients with leucocytosis and most observational studies suggest that it is less efficacious in patients with proliferative disease.
Aims: We present a single-centre retrospective analysis of the efficacy of azacitidine in 18 with MDS, CMML and AML with proliferative features and compare these with a similar cohort of patients with non-proliferative disease.
Methods: Data was collected retrospectively from clinical files following ethical committee approval
Results: The cohort with proliferative disease, defined by a leukocytosis >13x10^9/L, had a median age at diagnosis of 67 years; 55% were men. WHO 2008 diagnosis was MDS in 2 patients, AML in 8 patients and CMML in 8 patients. At diagnosis, the median hemoglobin was 8.9 g/dL (range 6.5-13.0), median WBC was 34.7x10^9/L (range 12.7-95.0) and median platelet count 89x10^9/L (range 12-695). Bone marrow blast percentage was >10% in 72% of patients; median 19% (range 1.2-98) Karyotypes were normal in 44%, intermediate risk in 27% and poor risk in 29%. Azacitidine was used as single agent as first line therapy with no prior cytoreduction in all patients. Patients started treatment a median of 2.3 months following diagnosis. Marrow response was assessed after the 6th cycle. Treatment was continued until progression in all cases. A median number of 8 cycles were administered. Marrow assessments were available in 16 patients. Responses were documented in 6 patients, of which 1 was complete and 5 were partial. Stable disease at time of assessment was seen in 4 patients and progression in 6. Transfusion independence was achieved in 8 of 10 patients who were previously transfusion-dependent. Four patients only received 1 cycle of treatment due to progression in 3 and pneumonia in one. Normalization of WBC was achieved in all but the former 4 patients after a median of 2.3 cycles. All patients had died by the time of analysis, 16 due to progression, one due to pneumonia and one due to cerebral haemorrhage. Median overall survival was 15.85 months. The median survival of the comparator non-proliferative group was 11.58 months. This was not significantly different from that of the proliferative group (15.85 vs 11.58 months, respectively; log rank test: P=0.25).
Summary and Conclusions: These results support the use of azacitidine in patients with proliferative disease, increasing treatment options in these patients.

PB1844

CHARACTERISTICS, TREATMENT, OUTCOMES AND SURVIVAL OF IRISH PATIENTS WITH CHRONIC MYELOMONOCYTIC LEUKAEMIA (CMML)
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Background: CMML is classified as a myelodysplastic/myeloproliferative neoplasm (2008, WHO). Compared with other haematological malignancies it is an uncommon disorder, and there are few large studies regarding outcome. Approximately 9.5% of patients registered with the Irish National Registry for Myelodysplastic Syndromes (MDS) between 2007 and 2014 had CMML.
Aims: We evaluated the treatment, outcome and survival for 29 Irish patients with CMML.
Methods: We evaluated presenting symptoms, diagnostic findings, cytogenetics and treatment received including blood transfusions, haematopoietic growth factors, hypomethylating agents, chemotherapy and transplantation.
Results: The median age at diagnosis was 75 years (range 56-89) with male: female ratio of 1.9:1. Nineteen patients (65.5%) were asymptomatic at presentation and 10(34.5%) had constitutional symptoms, bleeding, and/or splenomegaly. Apart from monocytosis in all patients, features at presentation included anaemia (65.5%), thrombocytopenia (55%), leukocytosis (45%), neutropenia (20.5%), raised LDH (68.9%) and splenomegaly (17%). Peripheral blasts were present in 5 patients, all of whom subsequently developed Acute Myeloid Leukaemia (AML). JAK2V617F mutation was negative in all patients tested (n=9). Seven patients (44%) had CMML-2 and 21(72.4%) CMML-1. Ninety three percent of patients had a hypercellular marrow. Evidence of dysplasia (93% dyserythropoiesis, 86% myeloid dysplasia, 72% megakaryocytic dysplasia) and increased reticulin fibrosis (73%) were noted. Only 7 patients (24.1%) had cytogenetic abnormalities, including trisomy 8 (n=2), t(8;21) (n=1), Monosomy 7 (n=1), trisomy 19 (n=2) and –Y (n=2). Seventeen patients (58.6%) received active management including Azacitidine (n=11), intensive AML-type induction therapy (n=2), allogeneic marrow transplant (n=1) and Hydroxycarbamide (n=6). Seven (24.1%) patients received transfusion support only. Erythropoietin was given in 7 patients (24.1%) and G-CSF in 2. Sixteen patients (55%) required transfusion at diagnosis. Twelve patients (72%) required red cells and 13(68%) required platelets during the course of illness. Eight of 11 patients treated with Azacitidine remained transfusion dependent. Median survival was 20 months (0-154 months). Transformation to AML was seen in 7 patients (24.1%). Survival for patients who received Azacitidine was 22 months

(11 to 154) with a median treatment cycles of 18 (3-33). One patient has been on Azacitidine for 7 years and remains alive 13 years after diagnosis. Median survival of patients who received Hydroxycarbamide and AML-type induction were 11.5 and 22 months respectively. Only one patient (with monosomy 7) received allogeneic transplantation but died 12 months later due to AML. Survival was 32 and 15 months respectively for patients with normal/ good risk karyotype and those with adverse cytogenetics and 18 months and 21 months respectively for those who received active management and those managed by best supportive care.
Summary and Conclusions: A significant number of CMML patients are diagnosed incidentally and the majority of patients are asymptomatic at diagnosis. The classical finding of splenomegaly is not always present. All male patients were anaemic at diagnosis. Adverse risk cytogenetics was less frequently encountered but indicated poor outcomes. Most patients were transfusion dependent and two thirds of patients were managed actively, mainly with Azacitidine.

Myeloma and other monoclonal gammopathies - Biology

PB1845

PI3K GAMMA/DELTA AS A NOVEL TARGET FOR THE TREATMENT OF MULTIPLE MYELOMA

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Background: Inhibiting tumor proliferation by targeting specific tyrosine kinases has proved an effective and well tolerated strategy in a number of hematological malignancies. Phosphoinositide-3-kinases (PI3K) are an enzyme group that generates phosphatidylinositol 3, 4, 5-triphosphate which provides a membrane docking site for the tyrosine kinase AKT (also known as protein kinase B). The class 1 PI3K isoforms (p110α/β/γ/δ) are known to be involved in the carcinogenesis and chemotherapy resistance of many cancers types, with p110δ and p110γ specifically being enriched in the hematopoietic system. The aberrant activation of the PI3K-AKT pathway has previously been shown to be active in Multiple Myeloma (MM) where it functions as a pro-survival signaling pathway. IPI-145 (Duvelisib) is a PI3K p110δ and p110γ inhibitor which at 25mg twice a day dosing is reported to achieve a plasma steady-state concentration of 0.9 μM and plasma peak concentration (Cmax) of 2.4 μM.
Aims: Here we investigate the effects of IPI-145 on MM pro-survival signaling, proliferation and bone marrow stromal cell (BMSC) and SDF1 mediated plasma cell migration using drug concentrations achievable in vivo.
Methods: To investigate the role of PI3K p110δ and p110γ subunits in regulating MM survival and migration we used PI3K pharmacological inhibitor IPI-145 (PI3K δγ inhibitor) with LY295002 (Pan PI3K inhibitor) and CAL101/Idelalisib (PI3K p110δ inhibitor) as controls. CellTiter-Glo was used to measure survival and transwell permeable plates with 8.0μM pores for migration assays. Western blotting was used to examine the role of BMSC, SDF-1 and IL-6 in regulating AKT and MAPK activation in MM cells in response to PI3K δγ inhibition.
Results: The PI3K δγ inhibitor IPI-145 reduced survival of MM cells at 1uM. IPI-145 triggered cytoxicity was associated with pAKT inhibition. IPI-145 also inhibited MM cell growth in response to IL-6 and bone marrow stromal cell co-culture. Furthermore we found that IPI-145 inhibited the migration of MM cells responding to BMSC and SDF-1 stimulation. Finally, we report that IPI-145 can inhibit SDF-1 induced AKT phosphorylation and downstream signaling.
Summary and Conclusions: IPI-145 inhibits MM cell survival and migration at concentrations achievable in vivo. The results reported here provide a molecular mechanistic rationale for the clinical evaluation of IPI-145 in MM patients.

PB1846

BORTEZOMIB INHIBITS OSTEOCLASTOGENESIS AND MODULATE CHIT1 AND YKL40 EXPRESSION

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Background: Osteolytic bone disease is a common manifestation of multiple myeloma (MM) that leads to progressive skeleton destruction and is the most severe cause of morbidity in MM patients. It results from increased osteolytic activity and decrease osteoblastic function. Activation of mammalian chitinases CHIT1 and YKL40 is associated with osteoclast (OCs) differentiation and bone digestion.
Aims: In the current study, we investigated the effect of two Bortezomib's concentration (BO) (2.5 nM and 5nM) on osteoclastogenesis by analyzing regulation of chitinase expression.
Methods: In order to obtained the OCs, the conditioned medium was supplemented with 25 ng/mL soluble rhRANK ligand (Peproteck, BDA, Italy) and 20 ng/mL rhM-CSF (Peproteck, BDA, Italy), for 21 days w/o Bortezomib (2.5nM or 5nM). The medium was replaced every 3 days. Cells and supernatants were harvested every 3 days for enzymatic assay, qRT-PCR, immunofluorescence and Western blotting. The supernatants were stored at − 20 °C. To confirm that macrophages achieved OCs differentiation, suitable markers were analyzed by qRT-PCR. Finally, in order to evaluate the ability of MM cell lines (U266) to digest bone, dentine discs were added to the wells before cell seeding. U266 cultured with conditioned medium (without BO) for 24 h were used as a control.
Results: OCs exposition to BO was able to inhibit the expression of different OCs markers such as RANK, CTSK, TRAP and MMP9. In addition BO-treatment reduced CHIT1 enzymatic activity and both CHIT1 and YKL40 mRNA expression levels and cytoplasmatic and secreted protein. Moreover,, immunofluorescence evaluation of mature OCs showed that BO was able to translocate YKL40 into the nucleus, while CHIT1 remained into the cytoplasm. Since MM

cell lines such as U266, SKM-M1 and MM1 showed high levels of CHIT1 activity, we analyzed bone resorption ability of U266 using dentin disc assay. After 3 days of incubation, we observed that U266 cells were able to form resorption pits on a dentin disc. Silencing the chitinase proteins in U266 cell line with specific siRNAs, resulted in pits number reduction on dentine discs
Summary and Conclusions: In conclusion we showed that BO decreases osteoclastogenesis and reduces bone resorption in OCs and U266 cell line by modulating the chitinases CHIT1 and YKL40. These results indicate that chitinases may be a therapeutic target for bone disease in MM patients.

PB1847

POLYMORPHISM WITHIN THE BFGF PROMOTER REGION IS ASSOCIATED WITH DISEASE PROGRESSION AND RESPONSE TO CHEMOTHERAPY IN PATIENTS WITH MULTIPLE MYELOMA

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Background: Vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) play an important role in the initiation of angiogenesis.
Aims: We aimed to assess whether polymorphisms located within the genes coding for these key angiogenic activators (*VEGF* (rs3025039; C>T) and *bFGF* (rs308395, G>C)) contribute to disease susceptibility and/or progression in patients with multiple myeloma (MM) and response to chemotherapy.
Methods: For this purpose, 133 patients with MM and 122 healthy individuals were typed for the *VEGF* and *bFGF* alleles by PCR-RFLP technique.
Results: Patients and controls presented with similar distributions the *VEGF* and *bFGF* alleles and genotypes, thus none of the polymorphisms was associated with the risk of MM. However, it was observed that patients presented with stage I-II of the disease (according to the Durie-Salmon criteria) more frequently carried the *bFGF-923G* allele as compared to patients in stage III of MM (0.31 vs 0.16, P=0.052) and healthy controls (OR=2.010, P=0.040). Progression of the disease after first line chemotherapy was more frequent among patients carrying this allelic variant (6/32 vs 4/88, P=0.022).
Summary and Conclusions: Clinical course of disease in patients with multiple myeloma is associated with a polymorphism within the *bFGF* promoter region.

PB1848

PLASMA CELLS CULTURE OPTIMIZATION FOR CYTOGENETIC STUDY IN MULTIPLE MYELOMA

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Background: Multiple myeloma (MM) is a malignant disease characterized by clonal proliferation of plasma cells (PC) and their accumulation in the bone marrow (BM). Cytogenetics alterations have a remarkable prognostic value in patients with MM. But the main limitation of the karyotype study is that PC have a low proliferative index, and conventional cytogenetics methods allow to detect chromosomal alterations in only 20-40% of cases at diagnosis. For this reason the eligible diagnostic technique is the iFISH in selected PC. Selected PC cytogenetics cultures studies are not found in the literature
Aims: To optimize the conventional cytogenetic study by the selection of PC
Methods: In 32 patients with PC neoplasms (19 MM at diagnosis, three MM after treatment, five MM at relapse, three smoldering MM (SMM) and two monoclonal gammopathy of undetermined significance (MGUS)) a PC negative selection with the commercial kit *RosetteSep® Human Multiple Myeloma Cell Enrichment Cocktail (Stemcell®)* and the culture (SCP) was performed. In parallel, a conventional culture (N-SCP) as a control was performed in 27 patients. To evaluate the technique efficiency, in both cultures, the%CP post-culture and the detection of chromosome alterations were compared
Results: It was observed that the PC negative selection prior to culture significantly increased the%CP post-culture (54% SCP *vs* 16.9% N-SCP) in all PC neoplasms (P=0.02) and significantly increased the detection of chromosome aberrations (75% SCP *vs* 37,5% N-SCP) (P=0.022). It was also confirmed the possibility to perform the iFISH technique in addition to karyotype and the increase in the percentage of interfasic nuclei with genetic alterations in SPC culture(71% SCP *vs* 16% N-SCP)
Summary and Conclusions: The PC selected culture is a viable, simple and effective technique that allows the conventional cytogenetic study with an increase of chromosomal aberrations detection in patients with PC neoplasms (75%) and also allows iFISH study

PB1849

THE ROLE OF FLT3-LIGANT IN THE PROGRESSION OF MULTIPLE MYELOMA: CORRELATION WITH ANGIOGENIC CYTOKINES.

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Background: Background.Multiple myeloma (MM) is a malignant proliferation of monoclonal plasma cells, resulting in a variety of clinical manifestations including osteolytic bone lesions, anemia, hypercalcemia and renal failure. Angiogenesis, the formation of new blood vessels from existing blood vessels,is an essential component in the growth and progression of MM. Fms-like tyrosine kinase 3 (Flt3) ligand (Flt3-L) is a potent hematopoietic cytokine that is probably involved in early B cell development and it is expressed by endothelial cells.
Aims: Aims. The aim of the present study was to evaluate serum levels of circulating FLT3-L in newly diagnosed MM patients and to estimate if there is any relationship between FLT3-L and known markers of angiogenesis such as,TNFa,HGF, and the expression of CD105 on endothelial cells.
Methods: Methods. We studied 56 newly diagnosed MM patients (26 female and 30 male with mean age 64.5±12.3 years), according to ISS.15 had stage-I disease,19 stage -II and 22 stage- III. We also studied 20 healthy persons as control group.TNFa,HGF and FLT3-L serum levels,were determinate by a solid-phase sandwich ELISA,using commercially available kits.CD105 expression was determinate by immunohistochemical methods.
Results: Results.FLT3-L,TNFa,HGF and expression of CD105 values,were significantly higher in MM patients compare to controls(P<0,001 in all cases),TNFa,FLT3-L,HGF and CD105 values,were also significantly higher with advancing disease stage(P<0,001 in all cases). We also found significant correlations between FLT3-L serum levels with expression of CD105 values (r=0,459,P<0,0001),TNFa (r=0,458,P<0,0001) and HGF (r=0,537,P<0,0001).
Summary and Conclusions: Conclusion. There is a growing evidence that FLT3-L has a significant role in the progression of MM.Serum levels of the angiogenic cytokines TNFa,HGF and the expression of CD105 wich reflect bone marrow neovascularisation,are increased in MM patients and correlated strongly with FLT3-L values this findings indicates that FLT3-L serum levels could be used as a potential tumor marker for disease severty and angiogenesis.

PB1850

SYNERGISTIC APOPTOTIC EFFECTS OF BORTEZOMIB AND METHYLSTAT ON DIFFERENT MULTIPLE MYELOMA CELL LINES

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Background: Several treatment strategies are used for multiple myeloma (MM), because of the high heterogeneity of MM, it still remains an incurable disease and it gives different clinical response against the therapeutic agents and drugs. Bortezomib, proteasome inhibitor, is an anticancer agent used for the treatment of multiple myeloma while methylstat is a demethylase inhibitor having anticancer potential. Until now, the effects of methylstat have been reported in breast and oesophageal cancer types. However, there is no study on multiple myeloma cells (MM).
Aims: In this study, we investigated antiproliferative and apoptotic effects of methylstat alone or in combination with bortezomib on U266 and ARH77 cells. We also examined the genes involved in methylstat induced apoptosis.
Methods: Cytotoxic effects of bortezomib and methylstat on U266 and ARH77 cells were demonstrated by MTT cell proliferation assay. To understand the apoptotic effects of these agents, loss of mitochondrial membrane potential and changes in caspase-3 enzyme activity were investigated by using JC-1 dye based-method (Cayman Chemicals, USA) and caspase-3 colorimetric assay kit (BioVision Research Products, USA) while phosphatidylserine localization was investigated by Annexin V assay. Cell cycle analysis in response to Bortezomib and Methylstat alone or in their combination were measured by flow cytometry. Changes in expression profiles of 84 genes underlying apoptosis and cell cycle control, in response to Methylstat were determined by Human Cancer Pathway Finder RT^2 Profiler PCR Array System (SABiosciences Corporation, USA).
Results: There were dose- and time- dependent decreases in cell proliferation in response to bortezomib and methylstat based on the results of MTT assay. IC_{50} values were calculated from cell proliferation plots and found to be 41 nM and 19 nM for U266 and ARH77 cells in respons to bortezomib, respectively while they were 2.2 μM and 4.2 μM for U266 and ARH77 cells in response to methylstat, respectively. On the other hand, combination studies showed that IC_{50} values decreased as compared to bortezomib and methylstat alone and were calculated as 0.5 nM and 0.6 nM for U266 and ARH77 cells, respectively. To evaluate the apoptotic effects of agents, JC-1, caspase-3 and Annexin-V

analysis were performed. Combination of bortezomib with methylstat showed more effect on apoptosis as compared to any agent alone and untreated control group. Also, cell cycle analysis was performed to detect the DNA contents of cells. Combination of bortezomib and methylstat arrested cells at the S phase. The fraction of cells in S phase increased accordingly. Previous data demonstrated that combination of agents caused apoptosis and it could be cell cycle-dependent. Human Cancer Pathway Finder RT^2 Profiler PCR Array System results of this study had shown that 1.1 μM and 2.2 μM methylstat treatment caused upregulation of FASLG, NGFR, TNF, TNFRS10B and TNFRS1B apoptosis- triggering genes in U266 cells and BCL2L11, CSP7, TNFRSF21 and 2.1 μM and 4.2 μM methylstat treatment caused TNFSF8 apoptosis- triggering genes in ARH77 cells in a dose-dependent manner. Furthermore, there were significant decreases in the expression levels of AKT1, AVEN, BAG1 BCL2L2 and RELA anti-apoptotic genes in U266 cells and NFKB1 anti-apoptotic gene in ARH77 cells in response to increasing concentrations of methylstat.
Summary and Conclusions: In conclusion, all results showed that the effects of bortezomib in combination with methylstat on U266 and ARH77 MM cells had significant decreases in proliferation and triggered apoptosis. Lots of genes and pathway in the cell were affected by methylstat treatment so methylstat might be used as candidate agent for the treatment of MM after *in vivo* analyses.

PB1851

MYELOMA OVEREXPRESSED (MYEOV) ONCOGENE: AN ANCIENT TRANSPOSABLE ELEMENT EXAPTATION EVENT ALLOWED FOR THE PRODUCTION OF A HUMAN-SPECIFIC PEPTIDE.

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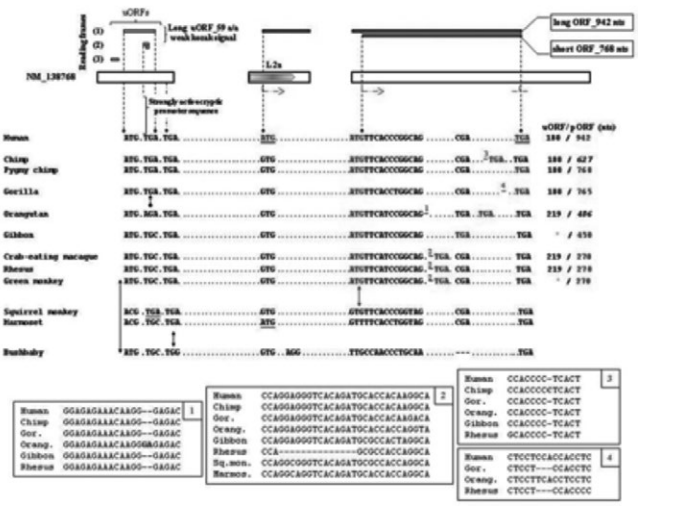
Background: It has formerly been suggested that the high incidence of cancer in human, as compared to non-human primates, could likely be related to maladaptations during the recent evolutionary past. *MYEOV*, an oncogene in multiple myeloma (MM), was recently characterized as a human-specific protein-coding gene of *de novo* origin from long non-coding RNA. In 2010, *MYEOV* expression was documented in primary MM cells and in purified plasma cells from patients with monoclonal gammopathy of undetermined significance however was scarcely detected in bone marrow plasma cells and was absent in normal plasmablasts or memory B cells. In addition, the gene was identified as causally involved in promoting MM cells propagation. *MYEOV* has the potential to encode for two protein isoforms, namely for a 313 amino acid peptide (MYEOV-313) and for a shorter one (MYEOV-255). Solid Western-blot assays support the production of both the proteins while MYEOV-313 expression has been validated in patients with MM. Both the peptides seem to be directed to the membrane nonetheless are of, yet, unknown function.
Aims: Illuminate, via *in silico* analysis, the evolutionary path leading to *MYEOV*. Highlight that a captivating exaptation event of an ancient transposable element (TE) significantly contributed to the creation of *MYEOV* gene structure.
Methods: BLASTN search against the GenBank human genomic database and BLASTP search against the GenBank nonredundant protein database were used to exclude *MYEOV* origination via gene duplication and to verify unambiguous *MYEOV* orthologs. *MYEOV* orthologs annotated in the respective databases from NCBI and Ensembl were identified by searching with the gene name. Whole-genome shotgun (WGS) sequence contigs, including within the DNA segments used to curate the reference genomic sequences of *MYEOV* orthologs, were downloaded from the NCBI Gene database. The WGS sequence contigs were used to build *MYEOV* locus alignment blocks between human and the 11 species with annotated orthologs, via BLASTN. Each alignment block was manually scrutinized for validating the primary open reading frame (ORF) of the annotated orthologs. Search for *MYEOV* upstream ORFs was performed with the ORF Finder program. *MYEOV* splice site analysis was performed with the Human Splicing Finder Version 2.4.1 program. *MYEOV* was scanned for the presence of TEs by Repeat Masker. *MYEOV* locus syntenic alignments of numerous Vertebrates were downloaded from the UCSC Genome Browser Database (UCSC GBD). The automated alignments were used to extract syntenic DNA segments in numerous mammals that flank MYEOV-313 start codon. The DNA segments extracted were scanned by RepeatMasker.
Results: *MYEOV* BLASTN search yields no significant similarity with any other coding sequence in the human genome. Syntenic alignments, extracted from the UCSC GBD, signify that the DNA segment where *MYEOV* locates emerged in Eutherian mammals. An important mutational event occurred in Catarrhini resulting in the *de novo* gain of a translatable ORF in a genomic region that seems noncoding in other species. That is the acquisition of a MYEOV-255 start codon. During evolution in the Catarrhini genome, *MYEOV* acquired a gradually elongated translatable ORF, a gradually shortened translation-regulatory upstream ORF, as well as introns and alternatively spliced mRNA variants. During Homo / Pan separation time, a momentous point mutation was introduced in human resulting in the acquisition of MYEOV-313 start codon. Accordingly, many of the automated annotations of *MYEOV* orthologs, provided by the NCBI and Ensembl automated pipelines, contain inaccuracies. MYEOV-313 start codon is included in a genomic region reported from RepeatMasker to match a TE of the L2 family. The RepeatMasker match appears degenerated however in-depth phylogenetic analysis verified the presence of the L2 repeat in syntenic DNA segments in many mammals.

Table 1.



Summary and Conclusions: *MYEOV* is a Primate Orphan Gene. MYEOV-255 is conserved in Hominines while MYEOV-313 represents a human-specific peptide. *MYEOV* was characterized as a human-specific *de novo* gene because the existence of two protein isoforms was not specifically addressed. MYEOV-313 start codon was evolutionary provided by a L2 TE. Could this rare exaptation event represent a human mal-adaptation, is a tantalizing possibility. Accordingly, MYEOV-313 biological role warrants further research, especially because targeting a human-specific peptide could theoretically cause less adverse effects than targeting components of evolutionary conserved signaling cascades.

PB1852

EXPRESSION OF MULTIDRUG RESISTANCE GENES IN PATIENTS WITH NEWLY DIAGNOSED AND REFRACTORY MULTIPLE MYELOMA

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Background: The myeloma cells have the phenomenon of multidrug resistance (MDR), which reduces the effects of chemotherapy. The better understanding of the molecular genetic mechanisms of development of MDR may lead to the development of the individual criteria of prediction of response to cytotoxic treatment of patients with multiple myeloma.
Aims: To determine the expression of four genes associated with MDR, such as MDR1, MRP1, LRP, and BCRP in relation to treatment status. We studied the aspirate of bone marrow of patients with multiple myeloma before cytotoxic treatment and in the resistant group of patients.
Methods: The study included 33 patients (16 men and 17 women) with a diagnosis of multiple myeloma stage 3 by Durie-Salmon system. The age of the patients ranged from 50 to 78 years. 14 patients were investigated before the start of cytostatic treatment. In 19 patients the bone marrow studied after treatment alkylating-containing combinations at the time of registration of resistance to the given therapy. The mRNA expression of the genes was determined by semiquantitative RT-PCR (polymerase chain reaction with reverse transcription) in the mononuclear fraction of the bone marrow aspirate.
Results: Generally, the incidence of mRNA expression of the studied genes MDR in a group of untreated patients was high. The MDR 1 mRNA expression was detected in 93% (13/14) of the samples, the mRNA of the gene MRP 1 was detected in 79% (11/14). The BCRP mRNA expression was detected in 100% of the investigated samples. The expression of mRNA of the gene LRP was identified in 71% of the studied cases (10/14). In the group of patients after treatment the expression of mRNA of genes MDR1, MRP 1, BCRP was detected in 100% of cases, and the LRP mRNA levels detected in 16/19 (84%) of the samples. The expression level of mRNA of each of the tested MDR genes

in all studied patients was different. The average value of the expression a total of 4 MDR genes in the group of untreated patients was 3.5±0.72 points. In group of pretreated patients value of the expression has increased to 6.3± 0.54points (P=0.004).
Summary and Conclusions: The expression of mRNA of MDR MRP 1, MDR 1, BCRP, LRP was determined in patients with untreated multiple myeloma. The intensity of gene expression increases in resistant patients.

PB1853

IL-4 AND IL-6 GENE POLYMORPHISMS IN PATIENTS WITH MULTIPLE MYELOMA FROM THE NORTHWEST FEDERAL DISTRICT OF RUSSIA
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Background: It is a well known fact that interleukin 6 (IL-6) stimulates the proliferation of neoplastic plasma cells and acts as an osteoclast-activating factor (plays an important role in the development of the bone disease), whereas interleukin 4 (IL-4) suppresses growth of multiple myeloma (MM) by inhibiting the synthesis of IL-6. Single nucleotide polymorphisms (SNPs) in the regulatory regions of cytokine genes may affect on the transcription and production of the cytokines that can effect on the development of various diseases.
Aims: The aim of this study was to identify SNP in different regions of IL-6 (-174G/C, nt565G/A) and IL-4 (-1098T/G, -590T/C, -33T/C) genes associated with the development of MM among the residents of the Northwest Federal District of Russia as well as determination of the bone lesions severity.
Methods: We analysed 43 MM patients with the median age 69 years. Patients were divided into two groups depending on the detected changes in the bones: 1st group (19 patients)-with severe osteolytic bone lesions (III stage of the *Durie-Salmon staging* system); 2nd group (24 patients) – with manifestations of osteoporosis and isolated pockets of osteolytic bone lesions (II stage of the *Durie-Salmon staging* system). The control group consisted of 40 healthy unrelated Caucasoid blood donors (median age 54 years). All analysed people were from the Northwest Federal District of Russia (St-Petersburg). Genomic DNA was extracted from the peripheral blood and both IL-4 and IL-6 gene genotyping was performed by the use of PCR-SSP.
Results: Based on the genotyping results of IL-4 -1098T/G we found that in general cohort of patients with MM genotype frequencies were not significantly different from each other except for the IL-4 -1098GG genotype which was not detected in the group of healthy people but was registered in the group of patients with a frequency of 0.05. When we analysed data for IL-4 -590T/C the genotype frequency of IL-4 -590TC in patients was two times lower than in control group (0.25 vs. 0.53). Although genotype IL-4 -33TT in MM patients with manifestation of osteoporosis occurs three times often than in control group (0.33 vs. 0.10 respectively). Some differences in IL-6 -174GG genotype frequencies in patients with MM were observed: in a total group of patients-0.47 (0.53 in the 1st group and 0.42 in the 2nd group) against 0.13 in the control group. Conversely, the IL-6 -174GC genotype was more frequent in the group of donors than in the group of patients with severe osteolytic lesions (0.70 vs. 0.26 respectively). Similar pattern was observed for IL-6 nt565GA genotype-in control group this genotype was noticed more frequently (0.80) than in patients (from 0.26 in the 1st group and 0.42 in the 2nd group). However, IL-6 nt565GG genotype frequency was less common in healthy people compare to patients with MM (0.13 to 0.49 respectively). Finally, IL-6 nt565GG was significantly higher in patients with severe osteolytic bone lesions than in patients with symptoms of osteoporosis and in the control group (0.53 vs. 0.46 and 0.13 respectively).
Summary and Conclusions: Thus, our results allow to describe some genotypes as markers associated with the development of MM such as IL-4 -33TT, IL-6 -174GG and IL-6 nt565GG. Furthermore, IL-6 nt565GG genotype is associated with the development of severe osteolytic lesions in multiple myeloma.

Myeloma and other monoclonal gammopathies - Clinical

PB1854

HIGH LEVELS OF SERUM ANGIOGENIC GROWTH FACTORS AND MAST CELL DENSITY IN THE DEVELOPMENT OF MULTIPLE MYELOMA
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Background: Bone marrow angiogenesis is involved in the pathogenesis and progression of certain haematological malignancies, like multiple myeloma (MM). Increased mast cell density (MCD) in bone marrow (BM) produces various mediators promoting MM progression with multiple manners.
Aims: The purpose of this study was to evaluate the involvement of BM MCD, and angiogenic cytokines such as angiopoietin-2(angiop-2), angiogenin (ang) and metalloproteinase-9(MMP-9) in MM disease progression.
Methods: We studied 70 newly diagnosed MM patients, 38 males, 32 females, mean age 57±14,5 years.According to ISS 20 were in stage I, 28 in stage II and 22 in stage III. 20 age and sex matched healthy volunteers were used as control group. The immunohistochemical expression stage of mast cell thryptase was measured in BM biopsy samples in order to estimate MCD, while angiop-2, MMP-9 and ang values were measured in serum by a solid phase sandwich ELISA.
Results: There are significant differences in levels of MCD, Angiop-2 and ANG (P<0,001 for all cases) but no significant differences in MMP-9(P=0,538) between patients and controls. All of the measured parameters were also in parallel with ISS stage(P<0,001 for all cases). Moreover BM MCD correlated positively with angiop-2, MMP-9, and ang (P<0,0001 for all cases).
Summary and Conclusions: Mast cells are increased in MM BM and they participate in many aspects of the disease. They release various mediators, increasing directly and indirectly myeloma growth.It has been also established that disease progression in MM is accompanied by increased BM angiogenesis. Overall mast cells and angiogenic growth factors seem to be important factors in MM biology and disease progression.

PB1855

MAST CELL DENSITY AND CIRCULATING BIOMARKERS OF BONE DISEASE INCREASE SIMULTANEOUSLY WITH PROGRESSION OF HUMAN MULTIPLE MYELOMA
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Background: Multiple myeloma (MM) is a neoplastic disorder characterized by clonal proliferation of plasma cells in the bone marrow. Many circulating biomarkers posses variable roles in the pathogenesis of bone disease causing bone distruction. Mast cells (MCs) can produce, store and release many kinds of chemical mediators with angiogenic properties. MCs accumulation has been associated with enhanced tumor growth.
Aims: The purpose of the study is to estimate the participation of RANK-ligand, matrix metalloproteinase 9 (MMP-9), N-terminal telopeptide (Ntx), carboxyterminal propeptide of type 1 collagen (*PICP*) and mast cell density(MCD) in the process of bone disease in MM patients.
Methods: We studied fifty-two newly diagnosed MM patients, 28 males, 24 females, mean age 64 years. The types of monoclonal proteins were 27 IgG, 18 IgA, 7 light chain, according to ISS:15were in stage I, 18 in stage II, 19 in stage III. Twenty age- and sex-matched healthy subjects were used as control group. We measured serum levels of sRANKL, MMP-9 and Ntx in urine samples by a solid-phase sandwich ELISA, and MCD in bone marrow biopsy samples by immunostaining. Radiographic examination including skull, pelvis, long bones and cervical, thoracic, and lumbar spine was carried out in all patients as a routine staging procedure.
Results: MCD, RANKL and Ntx were significant higher in untreated myeloma patients in comparison to healthy controls (P<0,001 for all cases). All values were increasing in parallel with ISS stage (P<0,001 for MCD, RANKL, MMP-9 and Ntx). There are significant correlations between MCD and MMP-9, RANKL and Ntx (P<0,001 for all cases). No correlation between MCD and PICP was found.
Summary and Conclusions: In conclusion, our results underline that MCD is an important factor responsible for the enhancement of bone involvement in MM. The positive correlation between MCD and circulatin biomarkers of bone

disease support the involvement of mast cells in the biology of myeloma cell growth. Moreover MCD could be used as a marker of disease activity.

PB1856

SUCCESSFUL TREATMENT OF PATIENTS WITH NEWLY DIAGNOSED/UNTREATED LIGHT CHAIN MULTIPLE MYELOMA WITH A COMBINATION OF BENDAMUSTINE; PREDNISONE AND BORTEZOMIB (BPV)

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Background: Patients with light chain myeloma have frequently a light chain tubular cast nephropathy, which can lead to severe renal impairment.
Aims: Both bortezomib and bendamustine have been identified as quickly acting, effective and well tolerated drugs and might therefore constitute an adequate combination regimen for patients with newly diagnosed/untreated light chain multiple myeloma.
Methods: Between September 2009 and March 2014, 20 patients with newly diagnosed/untreated light chain multiple myeloma were treated with bendamustine 60mg/qm on days 1 and 2, bortezomib 1.3 mg/qm on days 1,4,8 and 11, and prednisone 100mg on days 1,2,4,8 and 11 once every 21 days. 5 patients (25%) had a moderate or severe renal dysfunction (eGFR 15–59ml/min) and 9 patients (45%) a renal failure/dialysis (eGFR<15ml/min).
Results: The median number of the BPV-treatment was 2 (1-5) cycles. 19 patients (95%) responded after at least one cycle of chemotherapy with 3sCR, 4nCR, 5VGPR, and 7PR. The myeloma light chains decreased rapidly, reaching the best response after the first cycle in 8 and after the second cycle in additional 9 patients. 16 patients discontinued therapy after median 2 cycles of BPV treatment to receive autologous (n=13) or autologous/allogeneic SCT (n=3). All together 10/14 patients with at least moderate renal failure improved their renal function (4CRrenal, 2PRrenal, 4MRrenal). 3 of the 6 dialysis-dependent patients became dialysis-independent.With a median follow up of 23 months of the surviving patients, median PFS and OS for patients at 24 months were 90% and 95%, respectively. The most common severe side effect was grade 3-4 leukocytopenia in 25% of the patients. Grade 3-4 thrombocytopenia was observed in 15% of the patients. Moderate to severe infection were seen in 4 patients.
Summary and Conclusions: We conclude that BPV is effective and well tolerated in patients with newly diagnosed/untreated light chain multiple myeloma.

PB1857

THE FREQUENCY OF CYTOGENETIC ABNORMALITIES AND THEIR IMPACT ON OVERALL SURVIVAL IN PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA.

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Background: Cytogenetic analyses, including fluorescence *in situ* hybridization (FISH) is integral part of stratification in patients with multiple myeloma on risk groups. Detection of specific abnormalities optimizes treatment strategy for life expectancy improvement. The impact of the mixed specific genetic aberrations on the overall survival (OS) is studied.
Aims: To estimate the OS in patients with multiple myeloma, according to risk stratification.
Methods: 133 patients were prospectively assessed (median age 61 years, range 26-93; male: female ratio – 1:1.27) with newly diagnosed multiple myeloma. Cytogenetic and FISH analyses were determined in all patients for search of genetic abnormalities. Cytogenetic studies were performed on bone marrow samples using standard GTG-method. Metaphase FISH analyses were performed according to the manufacturer's protocol with use of DNA probes: LSI 13(*RB1*)13q14, *IGH/CCND1*, *IGH/FGFR3*, LSI *TP53* (17q13.1) (Abbott). Stratification of patients was carried in risk groups according to the molecular classification mSMART 2.0 Patients with nonspecific genetic abnormalities were included in standard risk group. Patients with complex karyotype (3 and more chromosomal aberrations) and who has 2 specific chromosomal abnormalities were included in certain group-mixed specific genetic aberrations risk group.
Results: Genetic abnormalities in newly diagnosed multiple myeloma were detected in 49.5% (56/113). The occurrence frequency of t(11;14) was 26.5% (n=30), del(13q) – 22.1% (n=25), t(4;14) – 7.1% (n=8), del(17p) – 7.1% (n=8). 73/113 (64.4%) patients was in standard risk group, 17/113 (15,0%) – intermediate risk, 8/113 (7,1%) – high risk and 15/113 (13,3%) – with mixed specific genetic aberrations risk. 5-years OS in standard risk group was 70%, in intermediate risk-84% (*P=.42*), in high risk – 36.5% and in group with mixed specific genetic aberrations – 68.7% *(P=.036)*.

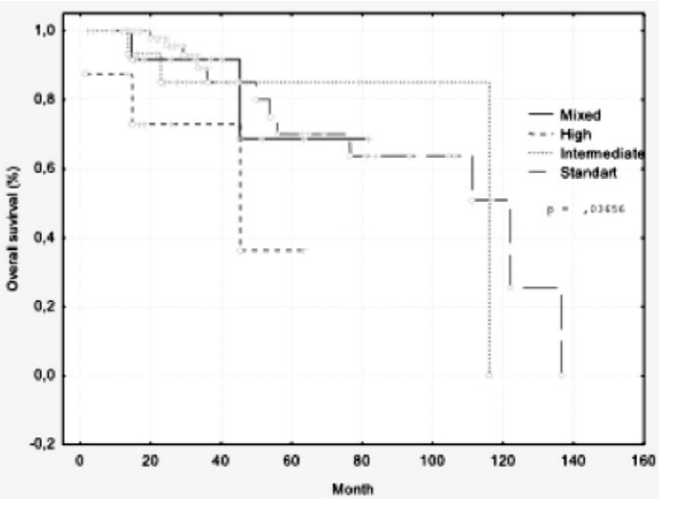

Figure 1.

Summary and Conclusions: Genetic abnormalities are important prognostic factors in patients with multiple myeloma. Identification of specific genetic markers, which included into high risk group, is a reason for search of optimal strategy of therapies. The obtained data need in further analysis taking into account age and variants of anti-myeloma therapies.

PB1858

THE DYNAMICS OF SERUM CROSSLAPS AND SCLEROSTIN LEVELS CORRELATES WITH TREATMENT RESPONSE IN MULTIPLE MYELOMA PATIENTS WITH THE PRESENCE OF MYELOMA BONE DISEASE

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Background: We performed a prospective study in patients with multiple myeloma (MM) and monoclonal gammopathy of undetermined significance (MGUS) aimed at the behavior of 13 selected parameters of myeloma bone disease (MBD).
Aims: The aim of our study was to assess the behavior of selected parameters of MBD with respect to therapeutic response.
Methods: Altogether we assessed a cohort of 51 patients: 31 patients with newly diagnosed active MM who responded to treatment reaching at least partial remission (PR) after 4 cycles of chemotherapy, 10 patients with refractory MM (progression or not reaching PR after 4 months), and two control groups – 5 patients with smoldering MM and 5 individuals with MGUS. The selected parameters of MBD were as follows: serum calcium (Ca), phosphorus (P), procollagen-I N-terminal propeptide (PINP), carboxyterminal telopeptide of type I collagen (ICTP), cross linked C-telopeptide (CTX), osteocalcin (OC), parathormone (PTH), calcitonine (C), 25-hydroxyvitamin D (D2), 1,25 dihydroxyvitamin D (D3), bone fraction of alkaline phosphatase (bALP), sclerostin (Scl) and matrix metallopeptidase 9 (MMP9). In the whole cohort we performed the analysis of MBD parameters at the time of diagnosis (before start of chemotherapy in active MM) and after 4 months. We assessed the behavior of the parameters with respect to treatment response. The patients were treated using bortezomib or thalidomide based regimens. All patients with MBD received bisphosphonates and adequte calcium support. In patients undergoing autologous stem cell transplantation the follow-up results were acquired 4 months after the diagnosis but before the transplantion. For statistical estimation we used Wilcoxon pair test at $p < 0,05$, and McNemara test of symetry at $p < 0,05$.
Results: We detected significant decrease of CTX (M 0,649 vs. 0,413ug/l; P=0,014) and sclerostin (M 44,4 vs. 40,6pmol/l; P=0,030) together with significant increase of parathormone (M 26,0 vs. 60,0pmol/l; P=0,0005) after 4 months of therapy in patients responding to initial treatment. All the other assessed parameters were without significant change. In patients with refractory MM there was no significant change in any of the assessed parameters. Similarly, there was no significant change in any of the assessed parameters in the control groups, *i.e.* in patients with smoldering MM, and there was only boderline significant decrease of Ca (M 2,42 vs. 2,28 mmol/l; p =0,042) in MGUS individuals.
Summary and Conclusions: Despite appropriate anti-resorption therapy, there is no change in the parameters of MBD in patients with MM who failed to respond after 4 months of treatment. Patients who respond to therapy have significant decrease of sclerostin and CTX suggesting their auxiliary clinical potential in the assessment of bone turnover in MM patients. With support of NT14393.

PB1859

POST-AUTHORIZATION SAFETY OF LENALIDOMIDE + DEXAMETHASONE IN PATIENTS WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA: EARLY SAFETY REPORT OF TURKISH PASS STUDY

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Background: Prognosis of patients with multiple myeloma (MM) has improved with the use of novel agents in the past decade. The data on real-world safety of these agents is limited. Following its regulatory approval in 2010, lenalidomide is now commonly used in the relapsed/refractory multiple myeloma (RRMM) setting in Turkey.
Aims: This post-authorization observational study is designed to define the safety profile of lenalidomide + dexamethasone therapy under routine real-world clinical practice by characterizing and identifying the incidence of adverse events (AE) of special interest in RRMM patients in Turkey.
Methods: Patients aged ≥18 years with RRMM and started on lenalidomide + dexamethasone treatment were included in the study. Patients who had previously received lenalidomide and discontinued or who had a treatment interruption for ≥4 weeks were excluded from the study. Thromboprophylaxis was allowed but not required. AEs were graded according to NCI-CTCAE (version 4.03) grading.
Results: As of January 30, 2015, 119 patients across 24 institutions in Turkey were enrolled. Of those, results of 111 patients with available data are going to be presented in this early safety report. Median follow-up was 19.9 weeks (range, 1.4–56.4). Median age was 62 years (range, 29–85) and 56.8% were male. Among 98 of the patients whose ECOG status were recorded at entry, 81 (82.7%) had good performance status (ECOG score 0–1) and 17 (17.3%) had an ECOG score of 2–4. Median number of prior therapies was two (29.7% had one prior therapy, 53.2% had two prior therapies and 17.1% had ≥3 prior therapies). Fifty-four (48.6%) patients had received autologous stem cell transplantation. The overall incidence of peripheral neuropathy at the baseline was 30.6%. Starting dose of lenalidomide was 25 mg in 85 (76.6%) patients, while 15 mg, 10 mg, and 5 mg were the initial dose in 10 (9.0%), 11 (9.9%), and 5 (4.5%) patients, respectively. Dose modifications were required in 13 (11.7%) patients. Prophylaxis for thromboembolism was performed in total of 37 (33.3%) patients with aspirin (n=21), heparin (n=15) and warfarin (n=1). Overall, 46 Grade 3-4 AEs were observed in 41 (%36.9) patients and 70 (63.1%) patients were free of Grade 3-4 AEs. Grade 3–4 neutropenia, pneumonia, anemia, and thrombocytopenia developed in 8.1%, 5.4%, 3.6%, and 1.8% of patients, respectively. VTE was reported only in one patient (Grade 2). Among 111 patients, 69 (62.2%) are still receiving lenalidomide + dexamethasone while 42 (37.8%) patients discontinued treatment (AEs : 12 [10.8%], death: 10 [9.0%], progression: 6 [5.4%], and other reasons: 14 [%12.6]). No second primary malignancy was reported.
Summary and Conclusions: Early safety data confirmed known AEs of lenalidomide plus dexamethasone therapy in RRMM patients in Turkey. Longer-term follow-up of AEs in this study will provide better characterization of AEs.

PB1860

LIFE-THREATENING BOWEL PERFORATION WHILE ON THALIDOMIDE-BASED TRIPLET REGIMEN FOR MULTIPLE MYELOMA: A RETROSPECTIVE CASE SERIES

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Background: Thalidomide is an oral agent which has demonstrated significant anti-myeloma activity resulting in improved progression-free and overall survival rates in newly diagnosed multiple myeloma (MM) patients. The mechanisms of action of Thalidomide are complex and thought to include both antiangiogenic and immunomodulatory properties. However, as well as targeting neoplastic plasma cells, Thalidomide can affect other organ systems resulting in toxicities. These have been well documented and supportive measures are currently incorporated into our daily clinical practice to counteract these. However, there are other adverse effects which have been recorded as uncommon, including diverticular perforation. Recent figures have suggested that<1% patients treated between 2004-2012 reported this to the Food and Drugs Administration (FDA). However, contradictory to these findings, we have noticed an increasing number of patients within our trust presenting with this life-threatening complication. Furthermore, this complication has not been observed in patients receiving other types of anti-myeloma treatment, including Bortezomib, Lenalidomide and Bendamustine.
Aims: To study the association between Thalidomide and diverticular perforation in patients with Multiple Myeloma.
Methods: A retrospective case series was performed examining all MM patients who received Thalidomide, either as a single agent or in a combination regimen, between 1st January 2007 and 31st December 2014 at Epsom and St Helier Hospital. Patients who developed diverticular perforation while on Thalidomide were identified and further data collected. This included demographics, comorbidities, disease response (according to the International Myeloma Working Group Response Criteria), time interval between current cycle of treatment and diverticular perforation, and patient outcome.
Results: A total 118 patients received Thalidomide of which 6 (5%) developed diverticular perforation. Interestingly, 3 of these patients had presented in the last 12 months. The median age for this group was 69 years (range 56-86 years) with equivalent numbers of males and females. None of the patients were known to suffer with gastrointestinal disease, although one patient did have associated renal amyloidosis. All 6 patients received Thalidomide as part of a triplet regime with Dexamethasone and either Cyclophosphamide or Bortezomib for newly diagnosed multiple myeloma. The median number of cycles received was 4 (range 2.5 – 6) with 5 out of the 6 patients achieving a disease response. Response rates included 1 Complete Response (CR), 1 Very Good Partial Response (VGPR) and 3 PRs. Five patients received 100mg Thalidomide with 20mg Dexamethasone, while one patient was taking 200mg Thalidomide with 40mg Dexamethasone. All 6 patients were receiving thromboprophylaxis throughout their treatment. Median time to presentation with diverticular perforation was 24 days (range 14-37) from day one of their current cycle. At presentation the median nadir neutrophil count was $5.1x10^9$/L (range 1.1-$14.4x10^9$/L). All 6 patients were managed surgically with subsequent bowel biopsies confirming diverticulitis. Five patients made a good post-operative recovery and were discharged. Three later received further treatment of which one patient was rechallenged with Thalidomide while the other two patients received Bortezomib instead. Two patients did not receive further treatment and are currently under active surveillance.
Summary and Conclusions: Our case series demonstrates that Thalidomide-associated diverticular perforation is far more prevalent than previously reported. Given the increasing use of this drug caution should be exercised by clinicians, particularly in patients with a known background of diverticular disease. Further research to establish the molecular mechanism by which Thalidomide induces diverticular perforation should be performed.

PB1861

HYPERCOAGULABILITY AND ITS ASSOCIATION WITH METABOLIC AND PRO-INFLAMMATORY MARKERS OF CARDIOVASCULAR RISK IN PATIENTS WITH MULTIPLE MYELOMA

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Background: Patients with multiple myeloma (MM) have an increased risk of thrombotic complications. The incidence of venous thromboembolism in patients with MM ranges from 3% to 10%. Hypercoagulability is one of prognostic biomarkers of venous and arterial thrombosis.
Aims: To identify associations between blood markers of coagulation activation, systemic inflammation, lipid and protein metabolism in patients with MM to elaorate prognostic markers of thrombotic events.
Methods: Our study involved 22 patients with MM, 14 men and 8 women aged 46-81 years, and a control group of 16 healthy people. Parameters of hemostasis, systemic inflammation, protein and lipid metabolism were studied. In statistics, Mann-Whitney test and Kendall's tau correlation were used. Medians and interquartile intervals were used to describe parametric data.
Results: In the group of patients with MM, markers of hypercoagulability, such as increased levels of fibrin monomer and D-dimer were found in 77.3% of cases. Frequencies of other cardiovascular risk factors were the following: overweight/obesity – 81.8%, hyperuricemia – 45.5%, atherogenic dyslipidemia with higher than optimal levels of low density lipoprotein cholesterol (LDL-Ch) and low levels of high density lipoprotein cholesterol – 68.2%, increased inflammatory markers CRP and IL-6 – 77.3%. There were significantly higher levels of fibrinogen – 5,35 (3,93-6,87) g/L vs 3,80 (3,49-4,40) g/L (P=0,005), fibrin monomer – 6 (4-10) mg/dL vs 3 (3-3,5) mg/dL (P=0,002) and D-dimer – 90,05

(32,9-487,8) ng/mL vs 21,55 (18,96-35,50) ng/mL (P<0,001) in comparison with the control group. Fibrinogen levels correlated positively with body mass index (BMI) (τ=0.63, p=0.001), levels of total cholesterol (τ=0.44, p=0.004), LDL-Ch (τ=0.42, p=0.006), albumin in percentage (τ=0.43, p=0.007), and correlated negatively with total protein (τ=-0.41, p=0.01) and M-protein levels (τ=-0.35, p=0.029). Fibrin monomer levels correlated positively with fibrinogen (τ=0.51, p=0.001), IL-6 (τ=0.51, p=0.001), triglycerides levels (τ=0.47, p=0.002), albumin in percentage (τ=0.38, p=0.018), and correlated negatively with M-protein (τ=-0.34, p=0.034) and IgA levels (τ=-0.56, p=0.034). D-dimer levels correlated positively with triglycerides levels (τ=0.32, p=0.035). Thus, in myeloma patients, increased BMI, systemic inflammation and atherogenic hyperlipidemia promotes hypercoagulability. Laboratory data of more severe disease stage according to parameters of protein metabolism were associated with less significant features of hypercoagulability.
Summary and Conclusions: To predict the risk of arterial and venous thrombosis in patients with MM it is advisable to determine BMI, blood levels of fibrinogen, fibrin monomer, D-dimer, CRP, IL-6, and lipid fractions.

PB1862

BORTEZOMIB IN THE FIRST-LINE TREATMENT OF MULTIPLE MYELOMA: THE EXPERIENCE OF A CENTER
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Background: The therapeutic approach of Multiple Myeloma (MM) has been marked by two great advances: the possibility of hematopoietic stem cell transplantation in eligible patients and the use of new active drugs, such as Talidomide, Bortezomib and Lenalidomide.
Aims: Analyze the follow-up of a group of patients with MM submitted to regimens including Bortezomib in the first line, evaluate the response to the treatment, analyze the overall survival (OS), duration of response (DOR) and time to progression (TTP).
Methods: Retrospective analyses of the clinical files of patients with MM, diagnosed between January 2009 and December 2014, undergoing treatment with protocols including Bortezomib in the first line. Response criteria after treatment according to the International Myeloma Working Group (IMWG). Statistic analyses using SPSS 22.
Results: Between January 2009 and December 2014, 65 patients were diagnosed with MM, 34 of which (52,3%) were treated with first line Bortezomib (CyBorDexa in 29; BorDexa in 3; VMP in 2). The median number of cycles administered was 8,5. The median age at diagnosis was 65,6 years (min=42; max=79), 18 were females (52,9%) and 16 males (47,1%). The mean follow-up time was 1,9 years. At diagnosis 31 patients (91,2%) presented anemia, 24 bone disease (70,6%), 19 renal impairment (55,9%) and 11 hypercalcemia (32,4%). According to the ISS, 10 patients were low risk (ISS 1) (29,4%), 10 patients intermediate (ISS 2)(29,4%) and 14 high risk (ISS 3)(41,2%). Six patients were eligible for transplant consolidation (17,6%). In 5 patients disease progression occurred during treatment (14,7%) and 3 are still under treatment. Response after treatment completion was accessed in 26 patients: PR=13 patients (50%), CR=9 (36,4%), VGPR=3 (11,5%) and sCR=1 (3,9%). Eleven patients progressed (42,3%) with a median TTP of 15,81 months and mean DOR of 9,09 months, 2 of them had reached VGPR and 9 PR. Five patients died. The 5-year overall survival was 85,3%.
Summary and Conclusions: The use of Bortezomib in the regimens CyBorDexa, BorDexa and VMP was effective in the majority of the patients, with response rates and overall survival mimicking the literature. In 58,8% of patients the ISS risk was low and intermediate; all the high risk patients presented renal impairment. Most of the patients completed treatment, 29/34 with CyBorDexa protocol, 86,4% reaching PR or CR. Among the responding patients, 15 (57,7%) have not progressed after a mean follow up time of 14 months. Although being widely used for its good tolerance and low toxicity, in several studies the CyBorDexa protocol showed inferior results compared with other multiple-drug protocols, such as VTD (Bortezomib, Talidomide and Dexa) or VRD (Bortezomib, Lenalidomide and Dexa). Currently, ongoing clinical trials will hopefully define the new standard of care in Multiple Myeloma, namely for the transplant eligible patients.

PB1863

BORTEZOMIB, LENALIDOMIDE AND DEXAMETHASONE FOLLOWED BY AUTOLOGOUS TRANSPLANTATION AS FIRST LINE TREATMENT IN MULTIPLE MYELOMA. SINGLE CENTER EXPERIENCE
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Background: A three drug induction regimen followed by autologous transplantation is considered the best approach in multiple myeloma patients who can endure that procedure. Despite scarce evidence available, the combination of lenalidomide, bortezomib and weekly dexamethasone (VRd) has been widely adopted, probably due to more favourable toxicity profile as compared to bortezomib, thalidomide and dexamethasone
Aims: To review our center's experience in using VRd protocol and autologous transplantation as first line therapy in multiple myeloma patients
Methods: Multiple myeloma was diagnosed according to IMWG criteria, as well as response assessment. All patients fit enough to undergo transplantation were treated with VRd combination, as previously described by Richardson et al, except for weekly administration of 40 mg dexamethasone. Four cycles were initially scheduled, but two additional cycles could be administered if less than partial response was achieved. Only patients with creatinine clearance below 50 mL/min were excluded. At least partial response was required to proceed to transplantation. Postransplant consolidation with two cycles of bortezomib and dexamethasone was administered in case of partial response, as well as lenalidomide maintenance in order to achieve complete response
Results: From April 2011 to February 2014 a total of 19 patients were treated according to this protocol. Median age at diagnosis was 58 years, ranging 32 to 70. ISS score was 1 in 7/19, 2 in 7/19 and 3 in 5/19. One patient required second line therapy due to lack of response to VRd induction, but proceeded to transplant once a partial response to DT-PACE was achieved. Other patient did not receive transplantation because of significant cardiac amyloid deposition. Stem cell mobilization yielded enough cells for the procedure in all patients despite lenalidomide usage. Responses after VRd induction therapy were complete response (CR) in 5/19, very good partial response (VGPR) in 4/19 and partial response (PR) in 10/19. A total of 18 transplantation procedures were performed, conditioned with melphalan 200 mg/m2 in 15 patients; the other 3 received 140 mg/m2 because of renal impairment (one patient) and poor tolerability to previous therapies (2 patients). Time to engraftment, infectious and non-infectious complications were similar to those previously reported. No deaths occurred during the procedure or immediately thereafter. Response after transplantation was CR in 13/18 (strict in 11/13), VGPR in 2/18 and PR in 3/18. Transplant upgraded the response in 9 out of 18 patients (50%). Only three patients required posttransplant consolidation and four received lenalidomide maintenance. After a median follow up of 28 months, a total of 11 patients (61%) have experienced relapse after a median of 18 months from start of treatment. Only 7 of them, (39%) have experienced clinical or paraprotein relapse requiring second line therapy. Median progression free survival is 22 months. No second neoplasms have been detected so far
Summary and Conclusions: VRd induction followed by autologous transplantation is a safe and effective therapy for newly diagnosed multiple myeloma. Half of the patients upgraded their response after transplantation, making it an essential part of therapy. Despite excellent responses achieved, relapse is still the main cause of failure

PB1864

BONE MARROW PLASMA CELLS ASSESSMENT AFTER INDUCTION THERAPY DOES NOT IMPACT ON PERIPHERAL BLOOD STEM CELLS MOBILIZATION IN MULTIPLE MYELOMA PATIENTS IN THE NOVEL AGENTS ERA
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Background: Over the past 10 years, novel agents (NA), including immunomodulatory drugs and proteasome inhibitors, have been increasingly incorporated in front line multiple myeloma (MM) treatment. Induction with NA did not negatively affected the efficacy of stem cell harvest in some studies. Few data are available on the prognostic role of residual bone marrow plasma cells (BMPC) assessment on peripheral blood stem cell harvest after induction therapy with NA in MM patients undergoing autologous stem cell transplant (ASCT).
Aims: The aim of this retrospective single-center study was to determine the impact of BMPC, quantified before peripheral blood stem cell (PBSC) mobilization, on the efficacy of $CD34^+$ cells collection and number of leukapheresis.
Methods: This retrospective study concerned 132 newly diagnosed MM patients who underwent ASCT in the Hematology Unit in Florence from January 2006 to January 2015. Induction therapy included dexamethasone with thalidomide (TD) and/or bortezomib (VTD, VD). Patients who were refractory to induction therapy were excluded from the study. Bone marrow biopsy was performed before PBSC mobilization and residual plasma cells were evaluated after CD138, kappa and lambda immunostaining. All patients were mobilized with cyclophosphamide (EDX) 3 or 4 gr/m^2 followed by G-CSF 5-10 μg/kg daily subcutaneously from day 2 until the end of the collection procedure. Leukapheresis was scheduled to start on day 10 and be performed until at least 2.0×10^6 $CD34^+$ cells/kg were collected. Patients who failed G-CSF mobilization received plerixafor. $CD34^+$ cells counts were assayed using a single platform method. Statistical analisys was made by Mann-Whiney test.
Results: Median age was 59 (range, 37-68), 48 patients (37%) were females; 81 patients (61%) received VTD as induction therapy (3-7 cycles) while 39 (30%) received TD (3-8 cycles) and 12 (9%) received VD (4-6 cycles). After induction therapy 18 (14%) patients obtained a complete response, 62 (47%) had a very

good partial response, 49 (37%) a partial response, and 3 (2%) a minimal response. BM biopsy was performed in all patients: in 67 (51%) the BMPC were<5% of the total cellularity, whereas in 40 (30%) the BMPC were between 5% and 20% and in 25 (19%) the BMPC were over 20%. All patients proceeded to chemo-mobilization: 16 (12%) with EDX 3 gr/m^2 and 116 (88%) with EDX 4 gr/m^2. No grade ≥3 toxicities were recorded. In 98 patients (74%), the mean number of CD34$^+$ cells collected was 12.2×10^6/kg (range 2.8-57.2x10^6/kg) within 1 leukapheresis; 23 (17%) patients needed a second procedure to collect a mean CD34$^+$ cells count of 7.3×10^6/kg (range, 2.0-16.2x10^6/kg), whereas 7 patients (5%) required 3 or 4 procedures and collected 4.4×10^6/kg (range, 2.3-7.4x10^6/kg) (P=ns). A total of 4 (3%) patients, all females, aged 49-61 years (median age 60 years), received plerixafor. In 2 patients, the mean number of CD34$^+$ cells was 5.8x10^6/kg collected in 1 day, in 2 other patients leukapheresis was repeated 3 times to collect 6.9x10^6 cells/kg. The BMPC percentage was not significantly correlated with the mean CD34$^+$ cells counts or with the numbers of leukapheresis. The CD34$^+$ cells count was significantly higher in patients younger than 55 (n=47; mean 12.7×10^6/kg, range 4.9-36.8) than in the older patients (n=85; mean 9.6×10^6/kg, range 2.0-57.2) (P<0.001). Furthermore the mean CD34$^+$ cells count was significantly higher in patients treated with VTD (n=81; mean 11.8×10^6/kg, range 2.7-57.2) vs those treated with TD (n=39; mean 8.2× 10^6/Kg, range 2.0-21.2).

Summary and Conclusions: The residual BMPC percentage did not adversely impact on CD34$^+$ collection in our study. Younger age and VTD induction were associated with higher CD34$^+$ cells counts.

PB1865

CURRENT MANAGEMENT OF MULTIPLE MYELOMA TREATMENT IN SPAIN: MEETINGS OF EXPERTS FROM 41 HOSPITALS

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Background: The outcome of patients with multiple myeloma (MM) has improved significantly due to the introduction of autologous hematopoietic stem cell transplant (ASCT) and, subsequently, with the introduction of new drugs. However, the optimal sequence of treatments is unknown and, in Spain, there are no national MM treatment guidelines.

Aims: The purpose of the expert meetings was to obtain updated information about the most commonly used treatment strategies in Spain outside clinical trials.

Methods: A total of 5 expert meetings attended by 43 hematologists were held in November-December 2014. The treatment algorithms published by Ludwig *et al.* (The Oncologist 2014) were used as the basis for the discussions. Information was obtained on the most commonly used treatments in 3 different scenarios: 1) front line in patients who are candidates for ASCT, 2) front line in patients who are not eligible for ASCT, and 3) first relapse.

Results: Most patients are not candidates for ASCT (50-70%). The most frequently used induction regimens before transplant (4-6 cycles) are triple therapies (86%), bortezomib/thalidomide/dexamethasone (VTD) being the most common (79%), followed by bortezomib/doxorubicin/dexamethasone (PAD) (36%), bortezomib/cyclophosphamide/dexamethasone (VCD)(32%), lenalidomide/bortezomib/dexamethasone (RVD)(14%), and cyclophosphamide/thalidomide/dexamethasone (CTD)(4%). When triple therapy cannot be used due to access restrictions or patient characteristics, bortezomib/dexamethasone (VD) is generally administered. Most centers (53%) do not administer consolidation after ASCT. If consolidation is administered, the regimen is usually the same as in induction. Maintenance treatment after transplant is used only in selected cases and centers and usually consists of lenalidomide for 2 years (47%)(exceptionally until progression), thalidomide for 1 year (26%) or bortezomib (21%). In non-transplant candidates, the front-line regimen most commonly administered is (VMP)(97%). Maintenance is not usually administered. After the first relapse, a second transplant is considered if the remission lasted ≥18 months and if patients have good performance status. Retreatment with the same initial regimen (20-60% cases) is reserved for patients with a treatment-free interval >12-18 months or in whom the progression-free survival is greater than the median for the regimen. Otherwise, physicians prioritize inclusion in clinical trials or treatments with authorized drugs with a different mechanism of action, such as lenalidomide and dexamethasone (50-60%). If no new drugs are used in induction (8%), bortezomib-based regimens are usually administered (77%).

Summary and Conclusions: Although there is considerable inter-center and inter-region variability in the treatment of MM in Spain, partly explained by different patient characteristics or restrictions on access to some drugs, pre-ASCT induction and front line treatments without ASCT are quite consistent. Following the first relapse, treatment is more variable and individualized.

PB1866

OUTCOME IMPROVEMENT IN IGD MULTIPLE MYELOMA WITH AGGRESSIVE CLINICAL FEATURES: IMPACT OF NOVEL DRUGS

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Background: IgD Multiple Myeloma (MM) is one of the rarest type of MM, representing almost 2% of all myeloma cases. It has been reported a poorer outcome of this variant when compared with other subtypes, with an aggressive course, high incidence of bone complications, extramedullary disease and resistance to chemotherapy. These data come from small retrospective studies mainly before the introduction of bortezomib and immunomodulatory drugs.

Aims: We describe aggressive and unusual clinical course of IgD MM, with a good outcome after hematopoietic-stem-cell transplantation (HSCT) and "novel-agent" therapy.

Methods: We present four female patients with aggressive IgD MM diagnosed in our Department; they received chemotherapy with novel agents alone or followed by autologous hematopoietic stem cell transplantation (HSCT).

Results: Mean age was 56 yrs. All patients had multiple bone lesions, two of them a serum creatinine >2 mg/dl and hypercalcemia; two patients showed extramedullary involvement and multiple lymphadenopathy, two of them showed chromosomal abnormalities. Bone-marrow biopsy revealed more than 40% clonal plasma cells. Three women showed LDH superior to normal values. Three young patients underwent autologous HSCT upon conditioning chemotherapy with melphalan 200 mg/m^2: in two cases it was preceded by bortezomib-thalidomide based induction therapy; the third patient received VAD induction and she was treated with bortezomib for recurrent disease. The fourth older patient receveid melphalan, bortezomib and steroids based regimens. A relapse was observed in this patient with progressive paraplegia; MRI revaled an extramedullary tumour of 68x 57 mm extended from D5 to D7, with need of irradiation plan-treatment. Chemotherapy consisted of Lenalidomide and Dexamethasone. All these four patients presented an atypical clinical onset, but they all obtained a CR after treatment, which is still present after various months (median overall survival 55 months). Despite heterogeneity of the population studied, use of the novel drugs both in first line therapy and relapsing disease has been shown to achieve an improvement in overall survival.

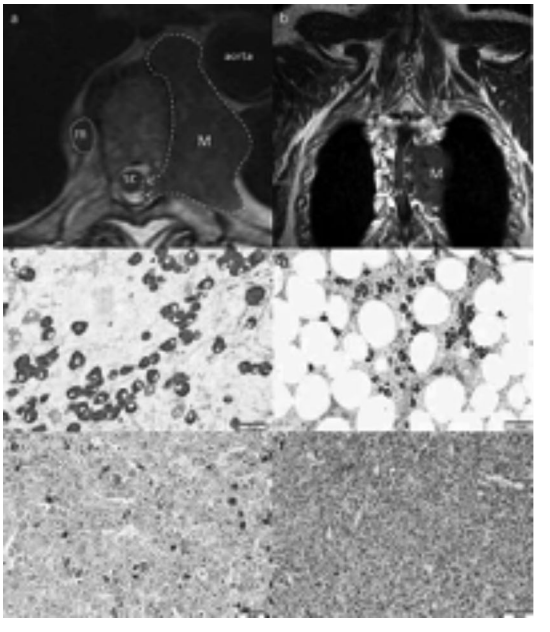

Figure 1.

Summary and Conclusions: The IgD MM is really different from IgG and IgA MM; it could be interesting to discover if the clinical picture and the course of illness could be somehow related to the class of M-component. IgD MM could be considered a different kind of neoplasia, with high kinetic proliferation rate and peculiar biologic aspects. Probably new cytogenetic aberrations, as presented in our population (trysomy of chromosome 5 and 9 in two patients) could explain this different biology and behavior. Nowadays IgD myeloma is still a challenging matter. Half of our patients with IgD MM presented extramedullary plasmacytoma, a rare and poorly understood entity. It is not known which mechanisms are involved in the hematogenous myeloma spread and how can myeloma cells growth and survival at extramedullary sites. Therefore in a patient with fever, lymphoadenopaties and high LDH levels we should consider this kind of aggressive myeloma as differential diagnosis with lymphoproliferative disorders. Nevertheless, after the introduction of different novel agents the clinical course and prognosis of IgD MM seems to have improved in particular with novel drugs in combination with autologous HSCT. All our patients are in CR after treatment and are currently being monitored at our centre. Our results were obtained in a few patients and an international collaborative trial is needed to confirm these findings.

PB1867

USE OF FLC AND HLC RATIOS FOR DIAGNOSIS AND MONITORING OF MONOCLONAL GAMMOPATHIES

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Background: Monoclonal Gammopathies are characterized by the presence of a serum monoclonal component (MC), either an intact immunoglobulin, a free light chain, or a combination of both. Measurement of FLC with Freelite® is standard practice and it is recommended by IMWG guidelines. Recently, Hevylite™ heavy light chains (HLC) assays were introduced to specifically target junctional epitopes between the heavy and light chains of intact immunoglobulins, allowing the independent quantification of the involved Monoclonal Component and uninvolved, polyclonal background.
Aims: In our Department we have used FLC and HLC for diagnosis and monitoring of Monoclonal Gammopathies, particularly in Multiple Myeloma patients this method is useful for monitoring of disease.
Methods: from January 2012 to March 2014 we collected 300 samples from 90 patients and assessed the diagnostic and monitoring performance of Hevylite® A and G assays in 7 transplanted patients, 17 non eligible patients and 18 relapsing patients, receiving a combination therapy with VTD; PAD; MPV or Len/Dexa. We selected 3 emblematic case studies
Results: Hevylite® absolute values and ratio demonstrated high sensitivity and specificity with respect to Freelite and serum protein electrophoresis, serum immunofixation. The combined use of Hevylite® A and G with Freelite® was particularly useful in dubious cases with more than one MC or with co-migrating components, and in the course of monitoring to assess the independent change of FLC and HLC, possibly reflecting the presence of clonal heterogeneity in our cohort.
Summary and Conclusions: Freelite and Hevylite are independent, useful markers to monitor the MC and to assess with greater specificity and sensibility the effect of therapy, thereby providing clinical support. More studies are needed to assess the prognostic potential of Hevylite in SMM and MGUS patients.

PB1868

IMPACT OF PG-SGA SCORE ON SURVIVAL IN PATIENTS WITH MULTIPLE MYELOMA

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Background: Disease-related weight loss is relatively common in patients with newly diagnosed multiple myeloma, but there are limited data on the impact of nutritional status on survival.
Aims: Using the Patient-Generated Subjective Global Assessment (PG-SGA) score, we retrospectively explored the effect of malnutrition on the survival of multiple myeloma patients. We also investigated the relationship between PG-SGA score prior to chemotherapy and clinical manifestation, in patients with multiple myeloma.
Methods: Analysis was performed on a retrospectively assembled cohort of 216 multiple myeloma patients diagnosed between October 1, 2002, and October 31, 2013. A total of 216 myeloma patients were subdivided into three groups based on PG-SGA scores.
Results: Twenty-three percent (50/216) patients had a PG-SGA score 9 or over, indicating severe malnutrition requiring specialist nutrition intervention. BMI and serum LDH were independently associated with PG-SGA scores (P<0.05). The median survival times were not reached in nourished patients with PG-SGA score 0-3, 58.7 months in moderately malnourished patients with PG-SGA score 4-8 and 35.0 months in severely malnourished patients with PG-SGA score ≥9 (P=0.001). Multivariate analysis showed that PG-SGA score ≥9 patients compared with PG-SGA score 0-3 patients (HR 2.347, 95% confidence interval [CI] 1.271-4.334; P=0.006), International Staging System (ISS) stage III compared with ISS stage I (HR 2.360, 95% CI 1.271-4.379, P=0.007) and autologous stem cell transplantation (HR 0.388, 95% CI 0.248-0.606, P<0.001) were associated with overall survival.
Summary and Conclusions: In conclusion, higher PG-SGA score prior to chemotherapy was associated with reduced survival among patients with multiple myeloma. Nutritional evaluation should be integral part of the clinical assessment of MM patients and PG-SGA score would be an appropriate tool to evaluate nutritional status.

PB1869

BING NEEL SYNDROME, ABOUT A CASE REPORT AND REVIEW OF LITTÉRATURE

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Background: Waldenstrom's Macroglobulinaemia (WM) is is defined by the World Health Organization as an IgM secreting "lymphoplasmacytic lymphoma, characterized by BM infiltration, and occasionally lymph nodes and spleen.
The most frequent neurological complication of Waldenström's macroglobulinemia is IgM-mediated polyneuropathy. Direct tumor cell infiltration of the nervous system is extremely rare and better known as the "Bing and Neel Syndrome" (BNS), that was first described in 1936.
Aims: We describe a patient with a long history of WM presenting Bing and Neel syndrome revealed by convulsion.
Methods: 68-year-old man was followed up in our department since September 2002 for a typical WM. A first complete remission was obtained with CHOP. In March 2007, he relapsed, and a second complete remission was obtained with chlorambucil. FCR therapy regimen (fludarabine, cyclophosphamide and rituximab) was used on July 2011. In March 2014, the patient presented with persistant headaches, episods of convulsion, blurry vision and disorders of memory. Computerized tomography of the brain showed a tumor mass multifocal supratentorial with edema. Brain magnetic resonance imaging displayed pachymeningeal regions of enhancement with associated FLAIR hypertensity on T2-weighted sequences. Examination of the cerebrospinal fluid (CSF) showed a lymphocytic meningitis with an increase of the CSF protein, and a normal CSF glucose. The patient did not undergo biopsy. The staging including full body scan, bone marrow aspiration, and bone marrow biopsy was normal, no organomegaly, meaning stable WM. Our patient underwent successful treatment with MPV-A regimen chemotherapy of Methotrexate 3,5 g/m² D1/ Vincristine 1,4 mg/m² max 2,8 D1/Procarbazine 100 mg/m² D1to D7(cycles 1, 3, & 5)-5 cycles repeated every 15 days, at final a closing course with AraC 3g/m² D1 and D2 without radiation therapy.
Results: The evolution was characterized by disparition of clinical symptoms, a marked regression of lesions on MRI and normalization of CSF analysis. The patient is still alive with free-symptoms in December 2014, last scheduled visit.
Summary and Conclusions: BN syndrome is a very rare complication of WM that should be considered in patients with neurologic symptoms and a history of WM. A brain MRI and histologic analysis may be a good supportive tool to diagnose Bing-Neel syndrome.There is still no consensus on the treatmentstrategies to use in BNS.

PB1870

DAY-100 BONE MARROW TREPHINE FOLLOWING AUTOLOGOUS STEM CELL TRANSPLANTATION FOR MULTIPLE MYELOMA: HOW USEFUL ARE THE RESULTS?

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Background: Bone marrow trephine samples are routinely assessed from patients following autologous stem cell transplantation (SCT) for Multiple Myeloma (MM) 100 days after the procedure
Aims: The aim of this study is to assess the clinical value of the day 100 trephine biopsy and to see if it offers additional information when compared to serological markers.
Methods: We analysed the pre-transplant and day 100 (d100) post-transplant bone marrow trephines for a series of consecutive patients (n=64) who underwent autologous stem cell transplant between June 2013 to August 2014. Only patients who had adequate pre- and post-transplant trephine biopsies taken were included in the study (n=46). Pre-transplant state was categorised as either complete response (CR) or as partial response (VGPR/PR groups) as defined by the International Myeloma Working Group (Uniform Response Criteria for MM). At d100 the bone marrow trephine results and corresponding serum paraprotein and light chain values were analysed. The concordance between these sets of results was determined
Results: Prior to transplant, 34 patients were in VGPR/PR and 12 in CR. Overall, the bone marrow trephines showed no change in 34/46 patients. There was an improvement in 11/46, while poor regeneration was noted in 1/46. In all patients with CR (n=12), there was no change in the d100 trephine results. However, paraprotein became detectable in 7/12. 5/12 remained in CR. In the VGPR/PR patients (n=34), d100 trephine showed no change in 22/34, 11/34 showed an improvement, 1/34 showed poor regeneration. Out of the 11 that showed improvement, 4/11 showed concordant improvement in the serum markers (paraprotein and/or light chain levels), 1/11 showed worsening of serum markers, 5/11 showed no change and 1/11 had no d100 serum markers available. In the VGPR/PR group, 2/34 had progressive disease according to serum markers; of these, one had no change in the trephine while the other showed improvement. 10/34 showed improvement in the serum markers; of these, 4/10 showed concordant improvement in the trephine samples. 21/34 showed no change in serum markers, of which 15/21 showed no change in the trephine, 5/21 showed improvement, 1/21 had poor regeneration. There were no d100 serum markers available for one patient. Overall, no progression was seen in all d100 trephines compared to pre-transplant sample, while progression was seen in 9/46 serum markers at d100 compared to the pre-transplant levels
Summary and Conclusions: Data from this study demonstrates that based on the bone marrow trephine results in isolation, patients who had received

SCT for MM did not require a change to clinical management. There was no progression noted in any d100 trephine biopsy sample, although 9/46 patients showed progression in serum markers. Although this study is too early to assess any longer-term predicative value of d100 marrow assessment, we would propose routine day 100 bone marrow trephine biopsies for MM patient's post autologous SCT contributes little additional clinical value and could be omitted with little risk to the patient. It may be more appropriate to follow up such patients using serum paraprotein and/or light chain levels alone.

PB1871

OUTCOME OF POMALIDOMIDE THERAPY IN RELAPSED / REFRACTORY MYELOMA: A UK MULTI-CENTRE EXPERIENCE

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Background: Pomalidomide is licensed in Europe for patients with relapsed / refractory myeloma, who have received at least two prior therapies including lenalidomide and bortezomib and who have progressed on their last therapy. In the phase 3 NIMBUS study, pomalidomide and dexamethasone was associated with longer progression free (PFS, 4.0 vs 1.9 months) and overall survival (OS, 12.7 vs 8.1 months) compared to dexamethasone alone (San Miguel et al 2013).
Aims: To assess the clinical efficacy of pomalidomide in a real-world setting in several large treatment centres in the UK.
Methods: Patients had measurable disease (IMWG criteria) and received at least 1 cycle of pomalidomide with dexamethasone. Disease response was assessed as per IMWG and high risk disease defined as ISS II/III plus t(4;14) +/- del(17p) (IMWG criteria). PFS and OS were measured from start of pomalidomide therapy and estimated using Kaplan-Meier method.

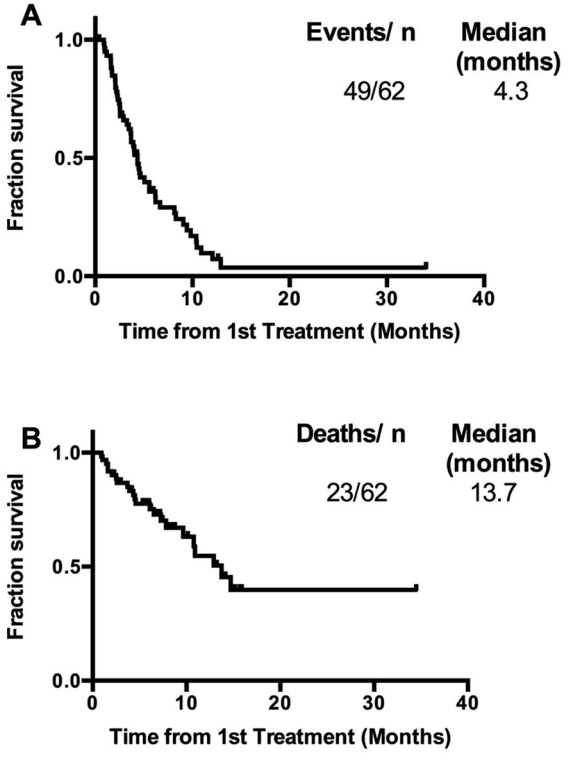

Figure 1. A) Progression-free survival and B) overall survival of all patients.

Results: Seventy-nine patients were identified, of whom 62 (78.5%) were suitable for inclusion in response analyses. All patients received pomalidomide (2-4mg days 1-21) with weekly dexamethasone, 30/79 (38%) received another agent(s) [clarithromycin (23), cyclophosphamide (9), carfilzomib (1), bortezomib (1)]. Patient characteristics were as follows: median age 67 years (range 40-89); isotypes were: 43 (55%) IgG, 19 (24%) IgA, 15 (19%) LC only. Median time from diagnosis was 4.9 years (range 0.5 to 18); median prior lines of therapy was 4 (range 1 to 8). Prior therapies included lenalidomide (100%), bortezomib (98%), thalidomide (84%), and autologous stem cell transplantation (61%). Seventy-three patients (92%) were refractory to their last therapy, and 58 (73%) were refractory to both bortezomib and lenalidomide. Median follow up was 6.4 months (0.92-34.5). Median number of cycles was 4 (range 1-32), and median daily dose was 4 mg. Fifteen patients (19%) had dose reductions. In those with a GFR<45ml/min at baseline, 50% (7/14) started at a dose<4mg. Overall response (≥ PR) was 53%, VGPR 5%, and at least stable disease was achieved in 58/62 (94%). PFS was 4.3 months (Figure 1A), and OS was 13.7 months (Figure 1B). Impaired renal function (GFR<45ml/min, 14 patients) did not appear to influence PFS (4.0 months vs 4.5 months, P=0.44), or OS (10.8 vs 13.7 months, P=0.80). High risk FISH was present in 11/40 (28%) patients, who had comparable outcomes to standard risk patients: PFS 3.6 months vs 4.5 months, (P=0.70) and OS 11.3 vs not reached (P=0.19). Inclusion of a third agent at start of therapy (14 patients) did not appear to confer benefit (PFS 4.3 vs 4.0 months, P=0.40, OS 7.8 vs 13.7 months P=0.37). In eight patients with biochemical or clinical progression on pomalidomide/dexamethasone, a third agent was added, and 7 achieved SD/PR. Grade 3/4 non-haematological toxicities occurred in 27/79 (34%) patients: pneumonia, 15 patients (19%) and neutropenic sepsis, 9 patients (11.4%) being the most common. Grade 3/4 neutropenia occurred in 28 patients (35%) and thrombocytopenia in 17 patients (22%).
Summary and Conclusions: Pomalidomide is an effective treatment in relapsed / refractory myeloma, with survival outcomes comparable to reported results from the phase 3 NIMBUS study. Impaired renal function and adverse genetics do not appear to influence outcomes. The addition of a third agent should be explored prospectively.

PB1872

THE IMMUNOPHENOTYPIC CHARACTERISTICS OF CD19 POSITIVE MYELOMA AND ITS PROGNOSTIC IMPACT

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Background: Clonal plasma cells (CPCs) in multiple myeloma were usually characterized by negative CD19 expression by multiparametric flow cytometry (MFC). However, positive CD19 expression in CPCs was noted in some cases with the reported incidence ranging from 0 to 8%. Positive CD19 expression in CPCs could complicate the minimal residual disease (MRD) assessment by high sensitivity MFC as the majority of polyclonal plasma cells (PPCs) retained CD19 expression. In addition, CD19 positive myeloma patients were reported to have a poorer clinical outcome in the group of patients treated with induction chemotherapy followed by autologous stem cell transplantation.
Aims: We aimed to review the immunophenotypic features of CD19 positive myeloma patients in comparison with that of CD19 negative cases to determine specific immunophenotypes which could be useful for MRD assessment. The prognostic impact of positive CD19 expression on clinical outcome in patients treated with the regimens including novel therapies was also evaluated.

Table 1.

Table 1. Patients characteristics, clinical and biochemical profile

		CD19 positive	CD19 negative	p-value
Sex				
	Male (%)	4.90	95	0.79
	Female (%)	4	96	
Heavy Chain				
	Ig G (%)	6	94	0.921
	Ig A (%)	6.50	93.50	
Light Chain				
	Kappa (%)	5.20	94.80	0.867
	Lambda (%)	4.50	95.50	
ISS				
	1 (%)	4.80	95.20	0.934
	2(%)	6.10	93.90	
	3(%)	3.10	96.90	
FISH				
	Standard risk (%)	5.10	94.90	0.85
	High risk (%)	4.20	95.80	
Age (mean)		69.86	71	0.466
Creatinine (mean, mg/dl)		1.52	1.51	0.278
Bone disease				
	Yes (%)	4.1	95.9	0.373
	No (%)	8.1	919	
Hypercalcaemia				
	Yes (%)	10.7	89.3	0.08
	No (%)	2.9	97.1	
Haemoglobin (g/dl)		10.65	11.38	0.043
LDH (mean, U/L)		241	266	0.26
Albumin (mean, g/L)		3.49	3.69	0.221
B2 microglobulin (mean, mg/L)		4.34	5.61	0.494
Plasma cells count by flow cytometry in %		16.77	12.8	0.526
Plasma cell count by microscopy in %		38	28.96	0.520

Methods: A retrospective review of newly diagnosed myeloma patients in a single centre was performed for the period of Jan 2006 to Jan 2015. The clinical data was retrieved from the patient information system of the institution as well as by reviewing the clinical records. Multiparametric flow cytometry analysis was performed by BD FACSCalibur™ for four colour analysis and BD FACSCanto™ flow cytometer for eight colour analysis. CD19, CD45, CD38, CD138 and CD56 expression was determined for all patients and for those cases where eight colour analysis was applied, CD27, CD28, CD117 and CD81 expression was studied. All the FCS data was analysed using infinicyt software. Plasma cells were identified by primarily gating on CD38 and CD138 positive cells. Mean fluorescent intensity of CD19 expression on plasma cells was compared with that of B-lymphocytes to determine positive or negative expression. It was considered a positive expression if 20% of the CPCs were positive.
Results: A total of 157 newly diagnosed multiple myeloma patients were reviewed. The incidence of CD19 positive myeloma cases was 4.46%. Out of 7 patients who were CD19 positive, four patients had homogeneous strong positive expression while the remainder had heterogeneous dim positive expression. Baseline demographics of the patients, ISS, immunoglobulin subtypes and clinical parameters at presentation *i.e.* related organ and tissue impairment, LDH, albumin,beta2 microglobulin levels and cytogenetic risks were similar in both CD19 positive and CD19 negative myeloma patients (Table 1). CD56 expression was 100% and 74% positive in CD19 positive and negative myeloma patients respectively (P 0.12). There was a higher percentage of positive CD81 expression (80% vs. 37.3%, P 0.056) and higher percentage of negative CD27 expression (60% vs. 35.9%, P 0.276) in CD19 positive cases though it did not reach statistical significance. No statistically significant difference in overall survival was found with a median OS of 56 and 75 months for CD19 positive and negative cases respectively (P 0.847).
Summary and Conclusions: In our study, CD19 expression was associated with a 100% positive CD56 expression, a higher positive CD81 expression and a higher negative CD27 expression rate even though statistical significance was not reached due to few numbers of patients in the cohort. This could be a useful finding for MRD assessment of the patients as well as to differentiate CPCs from PPCs in MGUS or smoldering myeloma by MFC. In the current cohort, we did not demonstrate a poorer prognostic impact of CD19 positive expression. This could be explained by the difference in the treatment era of the study patients where almost all the patients eligible for treatment received one or more novel therapeutic agents, compared to previous cohorts. Prospective studies with larger cohorts may further elucidate the significance of CD19 positive myeloma.

PB1873

BURDEN OF MULTIPLE MYELOMA IN THE UNITED KINGDOM

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Background: The introduction of novel agents for the treatment of multiple myeloma (MM) in recent years has been shown to lead in improvements in response and overall survival. However, real world evidence studies on the burden of disease are limited and outdated.
Aims: To describe patient characteristics of newly diagnosed MM patients in the United Kingdom (UK) and to assess disease burden in terms of healthcare resource use.
Methods: Retrospective primary care data from the Clinical Practice Research Datalink (CPRD) linked to the Hospital Episode Statistics (HES) was used. Adult patients with a first diagnosis of MM between Jan 2008-Dec 2012 were identified. Patient characteristics including comorbidities were described at date of diagnosis (index date). Disease-related symptoms, complications and adverse events were described during follow up, as well as primary and secondary care resources, including GP visits and hospitalisations.
Results: In the CPRD cohort, 1,457 newly diagnosed MM patients were identified and followed up on average for 2.1 years. Of those, 910 (62.5%) were linked to HES (CPRD-HES cohort). Mean age in the CPRD cohort was 71 years (SD: 11.6) and 54.8% were male. Body mass index (BMI) recorded in the 12 months prior diagnosis for 48.5% of the patients was on average 27, classifying them as obese or overweight. The most common comorbidities identified in whole medical history prior diagnosis were renal disease (25.5%), chronic pulmonary disease (19.6%), other malignancies excluding MM (17%) and diabetes without complications (13.1%). During the follow–up period, renal insufficiency was the most common disease symptom (annual incidence: 176.5 per 1,000 patients). Side effects including constipation, vomiting, diarrhoea, syncope, skin rash, and venous thromboembolism were observed at a rate greater than 10 per 100 patients in the first year after diagnosis. GP visits around diagnosis increased from a rate of 4.5 visits/patient (95% Poisson Confidence Intervals [CI]: 4.4-4.6) in 12 to 6 months prior to 8.4 (CI: 8.2-8.5) in the 6 months post diagnosis, and remained high for the entire follow up period (rate: 6.4; CI: 6.4-6.5). Demographic and clinical characteristics of the CPRD-HES cohort were comparable to the overall CPRD cohort; mean age at diagnosis was 71 years (SD: 11.7). A majority (86.6%) of patients in the CPRD-HES cohort incurred at least one hospitalisation post diagnosis (Figure 1). Most hospitalizations were day case admissions (85.6%), with inpatient admissions (14.4%) accounting for the remainder. On average, 4.4 day case admissions per patient per year (SD: 8.8) were encountered in the post-diagnosis period, while the average length of stay for inpatient admissions was 9.8 days (SD=11.85).

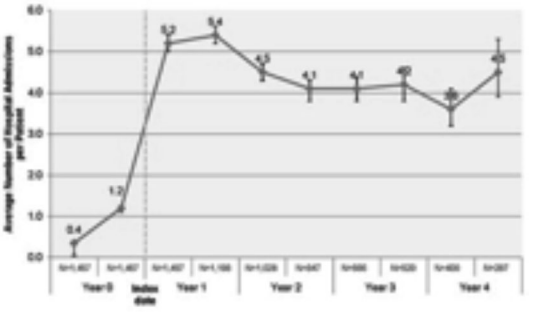

Figure 1. Average number of hospital admissions (inpatient and day cases) per patient during 6-month periods prior and post index date (Poisson CI)

Summary and Conclusions: Renal disease and respiratory conditions are common presenting comorbidities among MM patients. The management of disease symptoms, complications and adverse events as well as severe comorbidities leads to increased resource use in both primary and secondary care settings.

PB1874

TIME TO ONSET AND DURATION OF INDUCTION THERAPY AND ASSOCIATED FACTORS AMONG NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS

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Background: While early start of induction chemotherapy may improve treatment outcomes for patients with cancer, little is known about time to treatment onset and duration of induction therapy in newly diagnosed multiple myeloma (NDMM) patients.
Aims: We examined onset and duration of induction therapy and associated factors including age, gender, race, index year, stem cell therapy, and drug regimen in NDMM patients in the United States.
Methods: Using Medicare 20% data, we created a cohort of adult (≥18 years) NDMM patients (2008-2010) who initiated therapy with defined medications within 1 year of cancer diagnosis. Induction therapy regimens were categorized as bortezomib-based, immunomodulator (IMiD)-based, bortezomib-IMiD-based, or corticosteroid-based. Median number of days from diagnosis to therapy onset and of therapy duration were examined overall and for associated factors in a univariate approach using the Kruskal-Wallis test.

Table 1.

		Treatment onset		Treatment Duration	
	% of sample	Median(IQR)	P	Median(IQR)	P
Overall	100	26 (12-64)		195 (113-349)	
Age (years)					
18-64	8.1	25 (14-67)	0.7	187.5 (99-275)	0.2
65+	91.9	26 (12-64)		197 (114-358)	
Gender					
Male	44.8	25 (12-58)	0.5	201 (117-357.5)	0.3
Female	55.2	27 (13-68)		187 (106-343)	
Race					
White	78.9	25(12-64)	0.7	195 (113-357)	0.9
Black	15.8	27 (13-75)		201.5 (113-333)	
Other	5.3	30 (13-57)		185 (116-339)	
Index year					
2008	34.0	29(14-84)	<0.0001	187.5 (107-331)	0.06
2009	34.7	28 (14-72)		190 (112-329)	
2010	31.3	21 (10-42)		213.5 (117-366)	
Receipt of SCT					
No	93.6	26(12-65)	0.5	202 (114-366)	<0.0001
Yes	6.4	25(14-55)		162 (106-215)	
Regimen					
Bortezomib-based	25.2	21 (11-45)	<0.0001	203 (143-292)	<0.0001
IMiD-based	27.4	24 (13-50)		257 (151-366)	
Bortezomib-IMiD-based	9.2	20 (10-35)		258 (169-366)	
Corticosteroid-based	12.4	31 (11-108)		129 (91-276)	
Others	25.8	38 (15-114)		131 (91-366)	

Results: We identified 1455 NDMM patients**.** Overall, median (IQR) number of days to therapy onset from diagnosis and of therapy duration were 26 (12-64)

and 195 (113-349), respectively. Neither days to onset nor duration varied significantly by age, sex, or race. Days to onset decreased from 29 to 21 days from 2008 to 2010, respectively (P<0.0001), but therapy duration was similar across years ranging from 188 to 214 days (P=0.06). Although days to onset were similar for patients who did (25 days) and did not (26 days) receive stem cell therapy (SCT; P=0.52), median therapy duration was 25% longer at 202 days for those who did not (P<0.0001). Onset was more rapid (21, 24, and 20 vs. 31 days) and duration longer (203, 257, and 258 vs. 129 days) for patients treated with bortezomib-, IMiD-, and bortezomib-IMiD-based regimens compared to those treated with corticosteroid-based regimens, respectively. Duration of therapy was longer for patients who did not receive SCT compared to those who did for bortezomib-based (209 vs. 156 days), IMiD-based (284 vs. 124 days), and bortezomib-IMiD-based (291 vs. 142 days) regimens, respectively.
Summary and Conclusions: We observed differences in time to onset and duration of induction therapy by index year, SCT status, and choice of regimen, and found no racial or sex differences. The clinical implications of these findings are unknown. Further studies are warranted to better understand the observed differences.

PB1875

SALVAGE THERAPY WITH BORTEZOMIB AND DEXAMETHASONE IN VERY ELDERLY PATIENTS WITH RELAPSED REFRACTORY MULTIPLE MYELOMA

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Background: Despite recent advances in therapy, multiple myeloma (MM) patients eventually relapse and become refractory to treatment with a median survival ranging from 6 to 9 months. Over the last decade, the introduction of new front-line agents, such as the immunomodulatory drugs (IMIDs) thalidomide and lenalidomide and the proteasome inhibitor, have led to an increase in survival in MM by achieving deeper levels of responses and prolonging the duration of remission. Bortezomib (bort)–dexamethasone (dex) is an effective therapy for relapsed/refractory (R/R) MM, but few data are available for elderly patients. Although MM affects people of all ages, more than 60% of MM patients are older than 70 years of age (median age at diagnosis 70 years), and the number of new patients is expected to double worldwide by 2030
Aims: To evaluate efficacy and toxicity of bort-dex treatment in elderly R/R MM patients.
Methods: From 2006 to 2015 we performed a retrospective, observational study to detect the efficacy and tolerability of bort-dex in a cohort of 81 elderly patients with R/R MM. Patients were treated with bortezomib at the dose of 1.3 mg/m^2 on days 1, 4, 8, 11 every 3 weeks up to 6–8 cycles alone or in association with dexamethasone at the dose of 20 mg on the day of bortezomib administration and on the subsequent day. Responses were assessed according to the International Myeloma Working Group (IMWG) criteria
Results: Median age of study population was 73 years (range 65-89 years). Fifty-three (65. 4%) of patients achieved at least a partial response, including 8 patients (11%) with Complete Response (CR) and 9 patients (12.5%) with very good partial response (VGPR). The median number of prior lines of therapy was 2. The median number of cycles of bort-dex was 6 (range 1-11). Thirty-nine patients (48%) received bortezomib intravenously, 42 (52%) received bortezomib subcutaneously. Fifty patients (61.7%) had received immunomodulatory drugs. The median duration of response, time to next therapy and treatment free intervals were 8, 11 and 5 months. The duration of response was significantly longer for patients achieving CR/VGPR than for those achieving PR (7.3 vs. 3.8 months, P=0.03). After a median follow up of 24 months, 78 patients showed disease progression and 70 patients died. Median Time to progression (TTP), progression free survival (PFS) and overall survival (OS) of the whole population were 8.9, 8.7 and 22 months respectively. The most relevant side effect was peripheral neuropathy (PN), occurring in 38 patients.
Summary and Conclusions: The depth of response to bortezomib and clinical benefit are known to be strictly related in younger MM R/R patients. Our data highlight that bort-dex is highly effective and tolerable in fit elderly patients, thus justifying the efforts to obtain deeper responses to therapy also in this subset of patients. However, awareness of the risk of short living haematological responses to bort-dex salvage therapy (both in young and in old patients) should lead to thorough evaluation about the need of a maintenance therapy with other agents such as lenalidomide or pomalidomide in order to achieve sustained responses.

PB1876

LEVELS OF UNINVOLVED IMMUNOGLOBULIN PREDICT CLINICAL STATUS AND PROGRESSION FREE SURVIVAL FOR MULTIPLE MYELOMA PATIENTS

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Background: The levels of monoclonal immunoglobulins (M-Igs) are used to monitor multiple myeloma (MM). HevyLite® +(HLC) assays are able to discriminate between uninvolved and M-Ig levels.
Aims: We evaluated the levels of involved and uninvolved HLC, their ratios and differences and their relationship to outcomes among 189 MM patients.
Methods: Serum samples were analyzed using the HLC assays, and results were correlated with clinical status (complete response (CR), ≥ partial response (PR),<partial response, and progressive disease (PD)). Comparisons were made using student's t, Mann-Whitney, and Fisher's tests. PFS was calculated using Kaplan-Meier analysis. All tests were double-tailed and P-values determined.
Results: The patients were 62% IgG and 38% IgA with a median age of 66 years, β2 microglobulin 3.27 mg/L, albumin 3.8 g/dl, and median follow up of 72.5 months. Patients with PD had higher involved HLC levels, lower uninvolved HLC levels, higher ratios of involved/uninvolved HLCs and greater differences between them compared with patients with ≥ PR (all with P<0.0001). A higher proportion of patients in ≥ PR had normal uninvolved HLC levels than patients with<PR (P<0.0001). Additionally, patients in CR were more likely to have normal uninvolved HLC than those with below normal levels (P<0.0001). Similarly, patients in CR were more likely to have normal HLCs than in PR (P=0.0040). Patients with normal uninvolved HLCs showed a longer PFS (45 months) than those with less than normal levels (11 months; P=0.0019). Patients with normal involved HLC levels had longer PFS (33 months) than patients with involved HLC above the normal range (11 months; P=0.0405).
Summary and Conclusions: We show that involved/uninvolved HLC ratios, differences between them, involved and uninvolved HLC levels correlate with clinical status for MM patients. Patients with normal uninvolved levels or normal involved HLC levels have a longer PFS.

PB1877

SUBCUTANEOUS BORTEZOMIB IS AS EFFECTIVE AND LESS NEUROTOXIC THAN INTRAVENOUS BORTEZOMIB: ANKARA University EXPERIENCE

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Background: Bortezomib is a proteasome inhibitor which has proven potent activity against malignant plasma cells.Peripheral polyneuropathy (PNP)is one of the most frequent, non-hematologic adverse effects (AE) of Bortezomib often requiring dose modification or discontinuation.
Aims: Although the initial report by Moreau et al demonstrating the advantages and safety of subcutaneous use which led to the approval,this route of application is not being widely used in Turkey.Here we aimed to present our experience on the incidence of Bortezomib induced neuropathy (BiN)comparing two approved routes of administration.
Methods: After 2012 s.c.administration has become an accepted approach in our clinic for patients presenting with BiNin addition to dose reduction as recommended in the package.Weekly administration is the second step that follows if PNP does not improve.After 2013 s.c.has become the preferred approach for NDMM too.We retrospectively analyzed 129 consecutive patients diagnosed with Multiple Myeloma at Ankara University School of Medicine Department of Hematology between 2008-2013 and had completed at least three cycles of a Bortezomib containing protocol either at diagnosis or at relapse. Hospital records were screened to evaluate the incidence ofBiN, dose adjustments arising from AEs of Bortezomib and outcome measures.We compared the frequencies of AEs and treatment outcomes between patients who started treatment with s.c.*versus* i.v.administration of Bortezomib.We also analyzed outcomes following conversion from iv to sc too.Statistical analysis were performed using the SPSS for Windows,Version 16.0
Results: Baseline demographics,disease characteristics or number of previous lines of therapy were similar between patients receiving s.c.or i.v. treatment (Table-1). *De novo* subcutaneous Bortezomibwas given to 58 patients during induction;16 patients converted from i.v.tos.c route following diagnosis of BiN(n:9) or due to change in adminstration policy(n:7).s.c.groupconsisted of 74 patients.55 Patients completed Bortezomib treatment only i.v.The median cumulative dose of Bortezomib was 15.6 (range 5.2-44.8) and 18.3 (range 5.2-62.4) mg/sq.m. among sc and iv groups (P=0.72).Neurologic adverse effects are summarized in Table-1.The median cumulative Bortezomib dose to onset of any grade of PN was 20.8 (range 3.9-41.6) and 20.8 (range 5.2-44.8)

mg/sq.m. to onset of PN with sc and iv group (P=0.69)respectively. Although statistically not significant dose reduction was required more often among patients in the iv group.Eight of the nine patients who converted to sc following onset of BiN had a improvement in symptoms with change in route and weekly administration.None of the patients experienced severe AEs related to s.c. administration or discontinued treatment. Rash at injection site was the most frequent AE which was lasting for 3 days maximum and easily tolerable.The progression-free survival (PFS) and OS between arms were also similar.

Table 1.

	Subcutaneous n=74	Intravenous n=55	P
Median age, range, years	57 (26-75)	57 (37-83)	0.41
Gender (M/F)	45/29	28/27	0.26
ISS I/II/III	24/18/32	14/20/21	0.35
Treatment phase Induction Relapse Induction+Relapse	 59 4 11	 29 8 18	 0.005
Lines of previous treatment 1/2/3/4/5/6	59/10/4/0/0/1	29/14/6/6/0/0	0.11
Number of cycle	4 (1-11)	4 (1-12)	0.46
Response of induction CR/VGPR/PR/Stabile/ Progress	18/35/15/0/3	10/20/18/1/6	0.15
Diabetes at baseline	26 (35.1%)	12(30%)	0.10
PNP at baseline	6 (8,1%)	9 (16,3%)	0.15
Sensorial neuropathy Grade≥2	23 (35,9%)	11 (22%)	0.11
Autonomic neuropathy +/-	47 (66.1%)	44 (88%)	0.006
Polyneuropathy+/-	27 (36.4%)	30 (54.5%)	0.04
Median cumulative dose until PNP (mg/sq. m)	20.8(3.9-41.6)	20.8 (5.2-44.8)	0.69
Median cumulative dose (mg/sq. m)	15.6 (5.2-44.8)	18.3(5.2-62.4)	0.72
Dose reduction due to neurotoxicity	14 (19%)	17(31%)	0.12
Orthostatic hypotension	18 (25.7%)	13 (26%)	0.97
Gastointestinal symptoms Grade≥2	16(23%)	9 (18%)	0.52
Discontinuation due to toxicity*	4 (5,4%)	5 (9%)	0.41

Table 1 Clinical characteristics and comparision of neurological side effects
*among only s.c. route group

Summary and Conclusions: These results confirmed the safety and equal efficacy of sc Bortezomib compared to iv administration.Based on our results we recommend s.c. administration to avoid dose reduction requirement and discontinuation arising from neurotoxicity.

PB1878

CANCER-TESTIS ANTIGEN SLLP1 REPRESENTS A PROMISING TARGET FOR THE IMMUNOTHERAPY OF MULTIPLE MYELOMA

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Background: Most patients with Multiple Myeloma (MM) will relapse after an initial response and eventually succumb to their disease. This is due to the persistence of chemotherapy-resistant tumor cells in the patients' bone marrow (BM) and immunotherapeutic approaches could contribute to eradicating these remaining cells. We evaluated SLLP1 as a potential immunotherapeutic target for MM.
Aims: Our goal was to evaluate cancer-testis antigen SLLP1 as a potential target for the immunotherapy of Multiple Myeloma.
Methods: We determined SLLP1 expression in myeloma cell lines and 394 BM samples from myeloma patients (n=177) and BM samples from healthy donors (n=11). 896 blood samples and 64 BM samples from myeloma patients (n=263) and blood from healthy donors (n=112) were analyzed for anti-SLLP1 antibodies. Seropositive patients were evaluated regarding SLLP1-specific T cells.
Results: Most cell lines showed SLLP1 RNA and protein expression while it was absent from normal BM. Of 177 patients 41% evidenced SLLP1 expression at least once during the course of their disease and 44% of newly diagnosed patients were SLLP1-positive. Expression of SLLP1 was associated with adverse cytogenetics and with negative prognostic factors including the patient's age, number of BM-infiltrating plasma cells, serum albumin, β_2-microglobulin, creatinine, and hemoglobin. Among patients treated with allogeneic stem cell transplantation those with SLLP1 expression showed a trend towards a reduced overall survival. Spontaneous anti-SLLP humoral immunity was detectable in 9.5% of patients but none of the seropositive patients evidenced SLLP1-specific T cells. However, antigen-specific T cells could readily be induced *in vitro* after stimulation with SLLP1.
Summary and Conclusions: SLLP1 represents a promising target for the immunotherapy of MM, in particular for the adoptive transfer of T cell receptor-transduced T cells.

PB1879

ASSESSMENT OF INDIVIDUAL THROMBOSIS RISK ON THE CAPRINI SCALE IN MULTIPLE MYELOMA PATIENTS UNDERGOING PERIPHERAL BLOOD STEM CELL MOBILIZATION.

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Background: Patients (pts) with multiple myeloma (MM) face an high risk of thrombosis. We are talking about pts-related, disease-related and therapy-related risk factors. Meanwhile no specific scale exists to assess the risk of thrombotic complications for MM pts. We suppose that the Caprini scale is the most comprehensive instrument for validation of individual risk factors and it can be used not only for surgery pts.
Aims: The study was aimed to investigate the blood coagulation status in MM pts depending on the individual risk assessment on the Caprini scale before and during peripheral blood stem cell (PBSC) mobilization.
Methods: The study's sample consisted of 30 pts-candidates for high dose chemotherapy. There were 19 males and 11 females at the age of 27 – 64 years (median 55). After bortezomib-containig induction therapy 7 of them achieved PR, 16 – VGPR and 7 – CR. PBSC mobilization was performed by using cyclophosphamide (CY, 4g/m^2) and granulocyte colony-stimulating factor (G-CSF, 5mcg/kg/day). According to the internal protocol all pts had prevention heparin-sulphate continuous infusion (in initial dose 500 IU/hour), starting the day before CY introduction and stopping the next day after finish last PBSC collection. All pts were assessed with Caprini model. Hemostasis analysis was performed 5 times and included validation the results of activated partial thromboplasin time (APTT, 25 – 38 sec) and concentration of D-dimer (0 – 500 mkg/l). Hypercoagulation was considered in cases when APTT<25 sec and D-dimer >500 mkg/l. And hypocoagulation was estimated by data APTT >38 sec. Statistical analysis was performed with SAS 9.1 (using the GLM procedure).

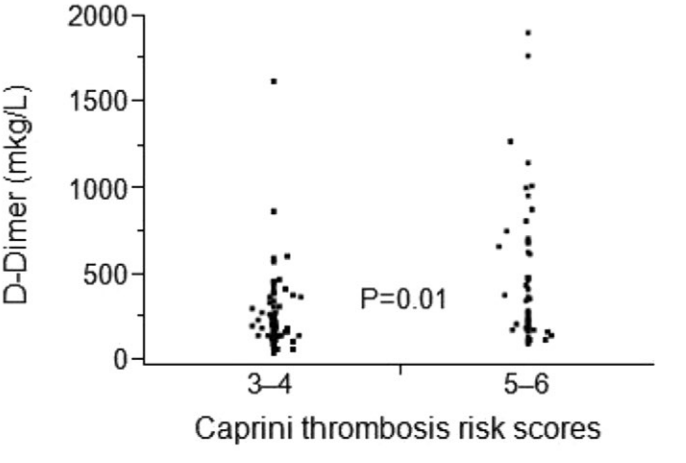

Figure 1.

Results: This group of pts had 3-6 (mean 4,4±0,96) scores on the Caprini scale. Initially (before heparin administration) APTT evaluation showed normal coagulation in 26 pts and hypocoagulation in 4 pts (mean 34 sec; 95% CI 32 – 36 sec). D-dimer was normal in 25 pts and increased in 4 cases (mean 309,8 mkg/l; 95% CI 170,0– 449,5 mkg/l). APTT had significantly (P<0,05) changed to hypocoagulability on the next day after starting thromboprophylaxis and in a state of agranulocytosis became 39 sec (95% CI 36 – 46 sec) and 40 sec (95% CI 36 – 50 sec) respectively. On the next day after CY infusion, APTT was statistically significantly decreased (P<0,05) reaching 34 sec (95% CI 32 – 37 sec). On the day of PBSC collection the mean APTT was 38 sec (95% CI 37 – 43 sec). Concentration of D-dimer was not modified in response to heparin or CY infusion or in a state of agranulocytosis and it icreased (P<0,05) reaching 476 mkg/l (95% CI 326-626 mkg/l) on the day of PBSC collection. Thrombotic complications were detected in 2 pts from a very high risk group (>5 score) on the Caprini scale. The analysis of our results showed statistically significant increase of (P=0,01) concentration of D-dimer in pts with the score of 5 or 6

compared pts with the score of 3 or 4 score (figure 1). Pts in CR demonstrated no significant difference in coagulation status compared to pts in VGPR or PR.
Summary and Conclusions: The possibility to use the Carpini scale to assess an individual risk of thrombotic complications for patients with MM has been confirmed clinically and by results of laboratory tests.

PB1880

REAL-WORLD CLINICAL CHARACTERISTICS AND TREATMENT PATTERNS IN US PATIENTS WITH RELAPSED/REFRACTORY (R/R) MULTIPLE MYELOMA (MM)

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Background: The introduction of proteasome inhibitors (PIs) and immunomodulatory drugs (IMiDs) has improved survival of patients (pts) with R/R MM. However, there is little real-world data on PI, IMiD, and autologous stem cell transplantation (ASCT) use.
Aims: The aim of this study was to describe recent US multiple myeloma treatment patterns through August 2014.
Methods: Pts with R/R MM were selected from a longitudinal, nationally-representative electronic medical record (EMR) database (Flatiron Health). MM diagnoses were confirmed by physician (MD) notes. Pts were required to progress after 1st line (1L), ≥ 1 visit after 2010, and ≥ 3 months follow-up post-progression. Data were integrated from structured and unstructured EMR sources: disease class from laboratory results and confirmed in MD notes and ASCT status from MD notes.
Results: We identified 607 pts with R/R MM (median age, 70 years; 32%<65 years). Most pts were male (57%), Caucasian (63%), and had IgG myeloma (66%). Of the 117 pts who received an ASCT, the most common 1L regimens were RVD 44%, VD 14%, RD 11%, and CyBorD 9%. In non-ASCT pts, the most common 1L regimens were VD 27%, RVD 16%, RD 14%, and CyBorD 7%. Overall, 29% of pts received both mechanisms of action (MOAs; PIs and IMiDs) in 1L. Of pts exposed to either a PI or IMiD in 1L who went on to receive 2L, 62% switched from PI to IMiD or IMiD to PI, and 30% continued with the same MOA. MOA switching was highest in ASCT pts who received 1L PI (73% received an IMiD in 2L, 23% continued on PI). By 3L, 79% of non-ASCT pts had been exposed to both MOAs vs. 96% in ASCT pts. Treatment utilization is shown in Table 1.

Table 1.

		Had Bortezomib, %			Had Carfilzomib, %			Had Lenalidomide, %			Had Pomalidomide, %			Had Thalidomide, %		
	Line number	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
All lines	ASCT	83	50	41	0	11	23	66	68	45	0	3	14	4	5	5
	No ASCT	69	56	36	0	6	23	35	49	43	0	2	9	8	6	4
Starting in 2013-2014	ASCT	72	45	36	0	16	29	61	54	33	0	5	16	0	4	7
	No ASCT	74	56	30	0	9	35	39	48	35	0	4	15	4	5	4

Summary and Conclusions: PIs and IMiDs are the most prevalent MOAs used in R/R MM in the US. Most patients receiving a single MOA in 1L go on to receive the other MOA in 2L, suggesting a perceived clinical benefit of switching MOA. Most patients have been exposed to both MOAs by 2L (67%) or 3L (83%), suggesting a possible need for treatment options with novel MOAs.

PB1881

REAL-WORLD BORTEZOMIB USAGE PATTERNS AMONG PATIENTS WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA

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Background: The 2012 FDA approval of subcutaneous (SC) bortezomib (BTZ) was based on non-inferior efficacy and reduced toxicity vs intravenous (IV) administration of BTZ. While differences in BTZ toxicity are attributed to route, dose, schedule, and duration, little is known about BTZ use in recent real-world practice in the US.
Aims: This study aimed to examine the real-world use of bortezomib in relapsed/refractory multiple myeloma patients in the US.
Methods: Patients (pts) with relapsed/refractory (R/R) multiple myeloma (MM) were selected from a longitudinal, nationally-representative electronic medical record (EMR) database (Flatiron Health). MM diagnoses were confirmed by physician (MD) notes. Pts were required to have disease progression following 1st line of therapy (LOT), ≥1 visit after 2010, and ≥3 months follow-up post-progression. Pt-level data were integrated from structured and unstructured EMR sources: transplant status abstracted from MD notes and IV/SC administration data merged with oral data from MD notes. We evaluated BTZ usage among these pts with R/R MM.
Results: We identified 607 pts with R/R MM; among these pts, 542 (89%) received BTZ. There were 830 LOTs containing BTZ. Initiation with SC vs IV BTZ increased over time (2011: 13% SC; 2012: 64% SC; 2013: 76% SC; 2014: 71% SC). Among BTZ initiations after 2012, 34% of those starting IV BTZ later switched to SC (7% of SC BTZ initiations later switched to IV). Across all BTZ initiations, 47% started at a schedule of >once per week (1×/wk); the rate was higher in earlier LOTs and transplant recipients (Table 1). BTZ initiations starting at 1x/wk were associated with longer duration (4.9 vs. 4.0 months, P=0.02), fewer dose reductions (26% vs. 40%, P<0.01) and similar cumulative total dose (median of 20.8 vs. 22.3 mg/m2, P=0.24) than BTZ initiations starting at >1x/wk.

Table 1.

Status	Line of Therapy	No. of Administrations in wk 1	% of Patient Starts
No transplant	1	1	45
		>1	55
	2	1	70
		>1	30
	3	1	70
		>1	30
Transplant	1	1	22
		>1	78
	2	1	56
		>1	44
	3	1	57
		>1	43

Summary and Conclusions: To our knowledge, this is the first real-world study evaluating evolving patterns of BTZ use in pts with R/R MM in the US. There was increased SC administration after 2012, longer duration of treatment among those starting at 1x/wk, and fewer dose reductions among those starting at 1x/wk; all which may be associated with toxicity management.

PB1882

RISK OF THROMBOTIC EVENTS IN PATIENTS WITH NEWLY DIAGNOSED (NDMM) AND RELAPSED REFRACTORY MULTIPLE MYELOMA (RRMM) TREATED WITH LENALIDOMIDE BASED THERAPY: A SINGLE CENTRE EXPERIENCE

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Background: Thrombotic events (TEs) are a known complication of lenalidomide (LEN) therapy in patients with multiple myeloma. Venous thromboprophylaxis has been recommended to reduce this risk. There is paucity of information regarding the risk of arterial TEs in this group of patients. Furthermore the difference in the risk and pattern of TEs between the NDMM and RRMM patients exposed to LEN therapy has not been assessed in clinical trials.
Aims: To compare the incidence and nature of arterial and venous TEs between NDMM and RRMM patients receiving LEN based therapy. Identification of patients at risk by correlation with thromboprophylactic measures, LEN dose, duration of treatment and additional anti-myeloma therapy.
Methods: We conducted a retrospective analysis of patients receiving LEN based therapy between September 2009 to January 2015 in Singleton Hospital, ABMUHB, UK. Patients were identified from our electronic prescribing system (ChemocareÓ) and correlated with clinical notes.
Results: A total of 81 patients were identified (NDMM: n=34; RRMM: n=47). The mean age at the start of treatment was 64.3 years (range 45-85 years) and 69.4 years (range 48-86 years) for NDMM and RRMM groups respectively. Thromboprophylactic measures were identified in 33 (97.1%) and 39 (83.0%) of NDMM and RRMM patients respectively. A total of 12 TEs were noted (NDMM: n=6, 17.6%; RRMM: n=6, 12.8%). Arterial TEs were noted in 3 patients (8.8%) of the NDMM group compared to only 1 (2.1%) patient in the RRMM group. The arterial TEs in the NDMM group were 2 TIA's and one popliteal artery thrombosis. One of the NDMM patients developed the arterial TIA whilst on LEN monotherapy in the absence of additional risk factors for arterial thrombosis. In the RRMM group, one patient who had a previous history of TIA, developed an ischaemic stroke resulting in dense hemiplegia despite dual antiplatelet therapy. There were 3 (8.8%) venous TEs (all below knee DVTs) in the NDMM group compared to 5 (10.6%) in the RRMM group (2 PEs, 2 proximal lower limb DVT and 1 distal DVT). The mean LEN dose of those having TEs was 22.5mg and 18.3mg in NDMM and RRMM groups respectively. The mean time to TEs was 3.0 months in NDMM patients and 5.8 months RRMM groups from the start of LEN therapy. All but one patient was on dexamethasone at the time of TE.
Summary and Conclusions: Our analysis confirms the thrombotic risk of LEN therapy both in NDMM and RRMM patients. There was a trend towards an increase in arterial TEs (8.8% vs 2.1%) in NDMM compared to RRMM patients. Hence LMWH in combination with anti-platelet therapy may be warranted in

NDMM patients. Larger prospective studies are required to define optimal strategies of TEs prophylaxis in patients with multiple myeloma receiving LEN therapy.

PB1883

PRESENTING FEATURES AT DIAGNOSIS OF MULTIPLE MYELOMA IN AN ISRAELI COHORT: PATIENTS YOUNGER THAN AGE 50 YEARS REPRESENT A SPECIAL GROUP WITH A HIGH FREQUENCY OF T (11; 14).

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Background: Multiple myeloma is uncommon in persons younger than the age of 50. Conflicting reports have been published concerning the presenting features in this age group. The question regarding characteristics in presentation is clinically relevant since significant differences in biological features have been demonstrated in several malignancies.
Aims: We analyzed the presenting features of patients with multiple myeloma aged younger than 50 years currently treated in our center.
Methods: Patients fullfilling diagnostic criteria for multiple myeloma currently treated in our center and younger than the age of 50 were included for analysis. The following information was available: age, sex, date of birth, date of diagnosis, type, percentage of BM inflitration, FISH, serum levels of immunoglobulins free light chains, hemoglobin, creatinine, albumin,and b2 microglobulin,ISS stage, bone lytic lesions, proteinuria, and plasmocytoma
Results: Of the total of 23 patients studied, median age was 42 years (37.5-47), with 36.3% younger than 40. In accordance with previously published studies[1], the patients were more frequently male (81.8%), had favorable features such as low International Staging System (ISS) (78.6% ISS I-II) as well as relatively small frequency of adverse prognostic factors including hemoglobin <10gr/dL (33.3%), serum creatinine >2mg/dL (16.6%), albumin <3.5m/dL (44.4%), b2 microglobulin >3.5mg/dL (42.8%), and calcium >10mg/dL in only 27.7% of cases. However, most of the cases (71.42%) showed bone marrow plasma cell infiltration >30% and bone lytic lesions were present in 88.2% of cases. As usually described, the most common isotype was IgG (47.6%) while that of light chain myeloma was higher than frequently reported (40.9%). FISH analysis was available in 22 patients. Surprisingly, t (11; 14) was present in 13 cases (59.1%). This is significantly higher than the normally reported frequency of this translocation (15 to 20%) in myeloma patients[2-3] (binomial test with P<0.001, equal than 0.02, respectively). Patients carrying such abnormality were younger (mean age of 40 year vs. 46 year), had lower b2 microglobulin values (mean 3.1mg/dL vs 5.2mg/dL), lower serum FLC kappa (38.6 vs 150mg/dL), lambda (8.3 vs 47mg/dL), and FLC ratio (4.82 vs 24.75 mg/dl). Moreover, the group revealed more plasma cell infiltration (75% vs. 47.5%), higher proteinuria (2.9gr/day vs 0.19gr/day), and only 20% of these patients suffered from plasmocytomas. Patients younger than 40 did not show differences in any of parameters evaluated.
Summary and Conclusions: In summary, our cohort of young myeloma patients from a single center showed at presentation a less aggressive disease (low ISS stage, and low frequency of b2 microglobulin higher than 3.5mg/dL, hypercalcemia, anemia and renal failure), as previously suggested[1]. Interestingly, we found a higher frequency of t(11;14) by FISH analysis in this age group. The data may support the fact that t (11;14) is present at the earliest stage of plasma cell dyscrasias[4]. Further studies are needed to evaluate the different biological nature of Multiple Myeloma in this age group.

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PB1884

Abstract withdrawn

PB1885

EPIDEMIOLOGIC AND CLINICAL CHARACTERISTICS OF MULTIPLE MYELOMA IN FIVE REGIONS OF RUSSIAN FEDERATION

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Background: Multiple myeloma (MM) is an incurable hematological disease previously associated with poor prognosis and survival rates. MM accounts for 15% of all hematologic malignancies and 2% of all malignancies. In Europe it affects about 4 per 100 000 individuals each year. The total number of patients in Russian Federation is still unknown. Epidemiological cancer registries are institutions for the collection, storage, processing, analysis and interpretation of data on the incidence and prevalence of cancers within defined registration areas. The data from the cancer registries also forms an indispensable basis for further studies into the assessment of early detection measures and population-based care of tumour patients.
Aims: To define epidemiologic and clinical characteristics of MM in 5 different regions in Russian Federation.
Methods: 170 patients with multiple myeloma from 5 different regions of Russian Federation (Tul'skaya region, Vologodskaya region, Tverskaya region, Mordoviya republic and Amurskaya region) diagnosed from January 01, 2009 to January 01, 2012 were included in the study. Data regarding these patients were extracted from prospectively maintained databases and review of medical records. Follow-up information on these patients was collected prospectively and entered at the time of each visit. Response was defined according to IMWG criteria. Kaplan-Meier analysis was used for analyzing overall survival. The cut-off date for the interim analysis was January 22, 2014.
Results: Among 170 pts, there were 68 men and 102 women, aged from 42 to 86 years (median 61 year). 48,9% of pts were less than 60 years. There were 55% pts with myeloma G, 19% with myeloma A, 13% with light-chain myeloma, 1% with myeloma D, 3% with myeloma M, 2% with non-secretory myeloma, 5% pts with diclonaly myeloma and in 2% of pts the type of MM was not defined. 85 (50%) pts were in stage III, 77 (45%) pts were in stage II and only 8 (5%) of pts in stage I. At the time of diagnosis 36 (21%) pts had signs of renal failure (serum creatinine more 177 mmol/l). Information about treatment and treatment responses was available in 153 cases. 97% of pts proceeded induction therapy: 84% pts – bortezomib-containing treatment, 13%-conventional therapy and 3% refused of treatment. High-dose therapy with autologous stem cell transplantation was performed only in 14 cases due to remoteness from transplant centers. Overall response was achieved in 68% of pts, among them complete remission – in 26%. Absolute 5-year overall survival rate in our group was 37%±6,3% (median 41,7 months). No difference was seen in overall survival between men and women. There was a trend of increase of 3-year overall survival in pts diagnosed in 2011 in compare with pts diagnosed in 2009 (65,5% vs 46,4%). 71of 170 pts are still alive. The main reasons of deaths were disease progression (70%), concomitant illnesses (21%) and inflectional complications (5%).
Summary and Conclusions: Our study showed that the median of age of pts with newly diagnosed MM in Russian Federation is 10 years less than in Europe and USA. Absolute 5-year overall survival rate in our pts is 37% that is comparable with MM pts in Germany (39%).

PB1886

BENDAMUSTINE IN THE MANAGEMENT OF RELAPSED AND REFRACTORY MULTIPLE MYELOMA: A REGIONAL REAL-LIFE EXPERIENCE

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Background: Bendamustine is a bifunctional alkylating agent, with low toxicity, that produces both single- and double-strand breaks in DNA, and shows incomplete cross resistance with other alkylating drugs, proved to be effective either in relapsed or refractory and in new diagnosed MM as single agent or combined with steroid and has also additive/synergistic activity with bortezomib.
Aims: Here we evaluate the efficacy and tolerance of bendamustine in combination with bortezomib-dexamethasone in patients with relapsed and refractory multiple myeloma, whose prognosis is severe, so that there is a strong need for new options for the management of these patients. A retrospective analysis of patients with relapsed and refractory MM, who had received bendamustine as salvage therapy, has been performed.
Methods: 39 patients, 21 males, 18 females, with advanced multiple myeloma, treated with several lines of treatments, and refractory to all the lines previously performed, received a schedule Bendamustine-based. Median age at diagnosis was 57 years (r.36-82) while age at start of treatment was 62 years (r.37-83), and median number of prior lines of treatment was 6 (r.2-11). ISS was equally distributed, and cytogenetic characteristics were evaluable in 9 patients, only two of whom had cytogenetic abnormalities, and in particular one of them had del13q and in the other one was observed t(11;14). All the patients had previously been treated with schedule containing bortezomib and lenalidomide, while 90% of them had been treated with melphalan, 77% with cyclophosphamide and 34% with anthracyclines, and 30% had also received radiotherapy. 58% of patients had undergone at least to a single autologous stem cell transplantation. Last treatment before bendamustine was a bortezomib-based regimen in 39%, an IMIDs- based regimen in 49% (a combined bortezomib/IMIDs-based regimen in 27%), while 12% of patients had received other chemotherapies. All patients were relapsed and refractory to last therapies received.

Results: Only patients completing at least two courses of Bendamustine were considered for analysis. A total of 128 cycles was administered (median 3, range 2-9). Bendamustine was variously associated to bortezomib (66%), or IMIDs (25%) and only in 9% it was combined only with dexametasone. In our schedule, Bendamustine was given, at a median dose of 90 mg/sqm (range total dose : 120-180) on day +1 and +2 every 28 days. Median OS from diagnosis was 56.5 months (range 6-145), while median OS from start of Bendamustine was 6.5 months (range 2-30). 16/39 patients went in progressive disease during treatment, 2/39 patients died for other causes (one for cardiovascular disease and the other one had a gastric cancer). Grade 3 transfusion-dependent anemia occurred in 31% while in 46% grade 3 neutropenia occurred. No severe extrahematologic toxicity was observed, only grade 1 gastrointestinal side effect (nausea), treated by common antiemetic drugs. According to IMWG response criteria, after a median follow up of 6 months (r.2-30), 1 patient achieved a complete response, 1 patient achieved a VGPR and 21 out of 39 evaluable patients achieved a partial response after a median time of 2 months with an overall response rate of 59% and 12 patient were considered in stable disease, which can be considered an impressive result in this subset of patients. In particular, for 4 patients of this study, Bendamustine treatment was, after having achieved a PR, a bridge to second autologous stem cell transplantation, and for one patient a bridge to allogenic stem cell transplantation.
Summary and Conclusions: In conclusion, Bendamustine has shown significant efficacy in a particularly severe setting of patients, relapsed and refractory to almost all available therapeutic resources, and in particular cases it could be considered as a bridge to a second autologous or to allogeneic BMT.

PB1887

ROLE OF HEAVY/LIGHT CHAIN ASSAY, ALONE AND IN COMBINATION WITH FREE LIGHT CHAIN ASSAY AND MINIMAL RESIDUAL DISEASE, IN MYELOMA PATIENTS WHO ACHIEVE COMPLETE RESPONSE AFTER FIRST LINE TREATMENTS

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Background: The International Myeloma Working Group (IMWG) guidelines recommend the use of protein electrophoresis, serum/urine immunofixation and free light chain (FLC) assay for assessing response to treatment in multiple myeloma (MM). In this setting, flow cytometry (FC) is emerging as a useful, though difficult to standardize, additional tool to detect minimal residual disease (MRD). Furthermore, the novel heavy/light chain (HLC) assay has recently become available to improve quantification of IgG and IgA MM isotypes.
Aims: To evaluate the effect of HLC ratio, FLC ratio and MRD on clinical outcome of MM patients achieving immunofixation negative complete response (CR) after first line therapy, according to IMWG criteria.
Methods: We followed a prospective cohort of 25 patients with MM (14 males, 11 females; median age 63.2, range 43-82; 14 IgG and 11 IgA) who had achieved CR after initial treatments including novel agents, with (n. 11) or without (n. 14) autologous stem cell transplantation (AuSCT). CR was identified after 6 induction cycles in patients not eligible for AuSCT and after AuSCT in those undergoing such a procedure. Sera samples were tested for HLC and FLC ratios by commercially available immunonephelometry kits, while bone marrow samples were analyzed by multiparametric FC for assessing MRD, using a home-made method based on a combination of CD138, CD38, CD56, CD19, CD45, CD20, and CD117 monoclonal antibodies. MRD was defined negative if< 10 neoplastic plasma cells were detected in a 100.000-event file. The Kaplan-Meier method was used to plot and calculate progression free survival (PFS) and overall survival (OS). Variables were analysed by log-rank test and p-value<0.05 was considered statistically significant.
Results: At CR detection time, 18 patients showed normal HLC ratio, 11 had normal FLC ratio (thus they were in "stringent" CR), and 13 had negative MRD. After a median follow-up of 42 months, 12 patients remain in CR and 13 have relapsed, 11 of whom are deceased. Overall, though neither HLC or FLC assays, nor MRD evaluation, alone or in different combinations, showed a statistically significant influence on the clinical outcome, a trend toward a better PFS (81% vs 50%) was observed in patients with sCR, particularly in those with IgA subtype (100% vs 35%), where normal HLC also identified a subgroup with more favorable outcome (PFS 67% vs 41%). Interestingly, PFS was lower in patients with both abnormal HLC and FLC ratios than in those with only abnormal FLC (40% vs 55%, respectively).
Summary and Conclusions: Our preliminary and still numerically limited experience suggests that HLC might have a prognostic role in patients with IgA MM in CR and might enhance the negative effect of abnormal FLC on PFS estimate. A larger number of patients and an extended follow-up are required for achieving more robust data.

PB1888

IMPACT OF INITIAL CHEMOTHERAPY RESPONSE AND PRETRANSPLANT DISEASE STATUS BEFORE AUTOLOGOUS TRANSPLANTATION ON SURVIVAL IN PATIENTS WITH MULTIPLE MYELOMA

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Background: High dose chemotherapy with autologous stem cell transplantation (ASCT) has been considered the standard of care for multiple myeloma patients and has been shown to increase both overall survival (OS) and progression free survival (PFS). However, the significance of initial treatment response and the significance of pretransplant disease status are not clear whether the depth of response influences the OS or PFS.
Aims: In this retrospective study we aimed to evaluate the significance of initial chemotherapy response and pretransplant disease status on PFS and OS.
Methods: We identified 127 myeloma patients who underwent ASCT between 1997- 2014 at Istanbul University Cerrahpasa Medical Faculty. Five of them were excluded from the study due to missing first line response data. Remaining 122 were grouped according to the first line chemotherapy responses, as suboptimal and optimal. Standard International Myeloma Working Group criteria were used for classifying disease responses and defining progression of multiple myeloma. Suboptimal response to first line pretransplant therapy was defined as a failure to achieve at least a VGPR following the first line chemotherapy. Demographic variables and disease related variables were given in Table 1. PFS and OS of patients in the 2 study cohorts were compared using Logrank test (Table 3)
Results: 127 patients treated with high dose chemotherapy and ASCT between 1997-2014 were included in the study. Median follow up time is 56 months. Of these patients, 56% (n:68) failed to achieve an optimal response following the first line chemotherapy. Most of these patients received second line chemotherapy before proceeding to ASCT. Subsequent to additional chemotherapy, 37% (n:46) underwent ASCT. The overall survival of patients responded as CR or VGPR is 53 months.

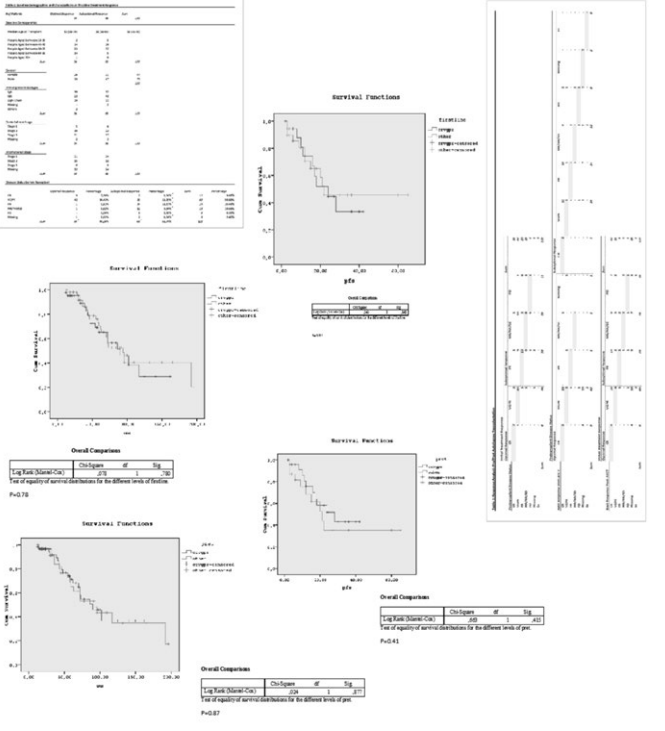

Figure 1.

Summary and Conclusions: In conclusion, for patients achieving less than a VGPR response following initial chemotherapy, additional chemotherapy improved depth of response, but was not associated with survival benefit. Furthermore we found no impact of initial treatment response on PFS and OS. This might be explained by the increased efficacy of new agents.

PB1889

NEUTROPHIL TO LYMPHOCYTE RATIO (NLR) IMPROVES THE RISK ASSESSMENT OF ISS STAGING IN NEWLY DIAGNOSED MM PATIENTS TREATED UPFRONT WITH NOVEL AGENTS

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Background: Recent reports identify the ratio between absolute neutrophils count (ANC) and absolute lymphocyte count (ALC), called NLR,as predictor of progression free survival (PFS) and overall survival (OS) in cancer patients.
Aims: We retrospectively tested NLR in a cohort of 309 newly diagnosed MM patients treated upfront with novel agents.
Methods: NLR was calculated using data obtained from the complete blood count (CBC). PFS and OS were evaluated.
Results: Median NLR was 1.9 (range 0.4-15.9). Higher NLR was not due to ISS stage, plasma cell infiltration or cytogenetics. The 5-year PFS and OS estimates were, respectively, 18.2% and 36.4% for patients with NLR ≥ 2 *versus* 25.5% and 66.6% in patients with NLR<2. NLR ≥ 2 reduced PFS for ISS stage I and OS significantly for stages I and III, but not stage II. Among younger patients (age<65 years, N=179), NLR ≥ 2 had a negative prognostic impact on both PFS and OS, in all ISS stages. By combining ISS stage and NLR in a model limited to young patients, we found that 19% of the patients were classified as very-low risk group, and 70% and 11% were in standard-risk and very-high risk groups, respectively. The 5-year estimates were 39.3%, 19.4% and 10.9% for PFS and 95.8%, 50.9% and 23.6% for OS for low, standard and high risk groups, respectively.
Summary and Conclusions: We found NLR as predictor of PFS and OS in MM patients treated upfront with novel agents. NLR can be combined with ISS staging system allowing a better identification of patients with dismal outcome.

PB1890

MULTIPLE MYELOMA IN ELDERLY AFRO-CARIBBEAN PATIENTS TREATED WITH MEPHALAN PREDNISONE AND THALIDOMIDE IN MARTINIQUE;A RETROSPECTIVE STUDY

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Background: In African-Americans (AA), the incidence of multiple myeloma (MM) is twice as high as in Caucasians. Only few studies have focused on overall survival. A recent population-based study comparing white and AA subjects showed a better survival in AA patients over a entire study period ranging from 1973 to 2005. However, significant survival improvement after the current era of intensive treatment or new therapies was only seen among whites, with smaller, non-significant change seen among blacks.This study hypothesized that the disparities in improvement in outcome observed could be related to a lower response to new therapies in AA patients. In Martinique, a French overseas department, the vast majority of the population is of Afro-Caribbean origin.
Aims: Our aim was to assess the characteristics, progression-free (PFS) and overall survival (OS) of this ethnicity subgroup.
Methods: We conducted a retrospective study on transplant-ineligible patient, newly diagnosed with MM who were treated in the Hematology Department of Fort de France in Martinique between 2007 and 2012.

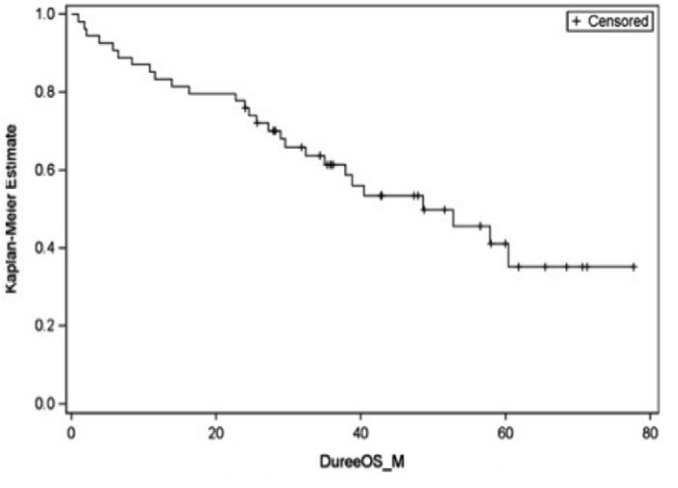

Figure 1.

Results: During this period, the Martinique Association for Epidemiological Research on Cancer revealed an incidence of 8/100,000 inhabitants per year. Fifty four patients were not eligible for autologous stem cell transplantation and had been treated with melphalan, prednisone and thalidomide as part of frontline therapy.The patients had a median age of 80 years, with ISS stage III documented in 48%. The overall response rate was 76%. With a median follow up of 35 months, PFS and OS were 28.9 months and 48.6 months.
Summary and Conclusions: Compared to metropolitan France, our results show a higher incidence and a similar outcome in this population, despite an advanced age and disease status at diagnosis. No data have been published focusing only on the outcome of AA or African-Caribbean elderly patients treated with combination therapies that include novel agents. Our study highlights findings which had so far not been described and do not support the hypothesis that AA patients had a lower response to new therapies.

PB1891

WALDENSTROM'S MACROGLOBULINEMIA EXPRESS B-CELL MATURATION ANTIGEN AND SERUM LEVELS CORRELATE WITH DISEASE STATUS AND CONVENTIONAL M-PROTEIN AND IGM LEVELS

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Background: Waldenström's macroglobulinemia (WM) is an incurable B-cell lymphoplasmacytic lymphoma. B-cell maturation antigen (BCMA) is expressed on malignant B cells and we have previously shown that serum BCMA levels are elevated in multiple myeloma (MM) patients and correlate with disease status in multiple myeloma (MM) patients.
Aims: Our objective was to determine whether BCMA is present in WM; and, furthermore, whether its serum levels also correlate with disease status and track with conventional tumor markers for patients with WM.
Methods: Data was obtained on 20 WM patients who received treatment in a single clinic specializing in monoclonal gammopathies. Mann-Whitney analysis was used to measure statistical significance (P<0.05). Immunohistochemical staining and flow cytometric analysis was used to determine the expression of BCMA on WM cells.
Results: First, we found that BCMA is highly expressed on the surface of tumor cells from the bone marrow of WM patients using both immunohistochemical and flow cytometric analysis. Next, we compared serum BCMA levels from untreated WM patients (n=9) to healthy individuals (n=14), and the levels were markedly higher in WM patients (P<0.0001). Next, BCMA levels in WM patient sera were also correlated with disease status at the time during which the sample was collected. Serum from patients achieving ≥partial response (n=7) showed significantly lower levels of BCMA than samples from patients with stable disease (n=4) or progressive disease (n=7; P=0.003 and P=0.0003, respectively). Additionally, the BCMA levels of seven WM patients were correlated with the serum M-protein and IgM levels of these patients during the course of their therapy. Changes in serum BCMA levels correlated with changes in serum M-protein as well as IgM levels.
Summary and Conclusions: These results indicate that BCMA is present on the malignant cells from WM patients and serum levels of this protein can be used as a potential marker for tracking the course of their disease.

PB1892

EXTRA MEDULLARY RELAPSE AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION FOR MULTIPLE MYELOMA (MM)

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Background: The role of allogeneic stem-cell transplantation (allo-SCT) in treatment of myeloma patients is still controversial. Allo-SCT consolidates treatment by virtue of its graft-*versus*-myeloma (GvM) effect and results in a higher rate of molecular remission and lower risk of relapse. However, in our experience we observed extra-medullary relapse (EMR) which can be reversed by new post-transplantation strategies.
Aims: The goal of our study is to evaluate the frequency, aspects and the prognosis of EMR after a tandem auto-allogeneic bone marrow transplantation (allo-BMT) for MM.
Methods: Our study is retrospective about 25 patients with MM who underwent allo-BMT between February 1998 and December 2013 in the "Centre National de Greffe de Moelle Osseuse de Tunis". The median age at transplant were 42 years (range; 31-49). Conditioning regimen were oral or IV Bu 14-Cy (n=23), Fludarabine-TBI (n=2). All patients received cyclosporine with methotrexate as graft-*versus*-host disease (GVHD) prophylaxis; one patient received in addition mycophenolate mofetil. Responses were assessed using the IMWG criteria. EM relapse was defined as the presence of plasmocytoma evidenced by medical imaging techniques and secreting the same monoclonal component while the myelogram is normal and hematopoiesis was of donor type.

Results: After allogeneic –BMT, eight patients (32%) are alive after a median follow up of 36 months (range 2- 168 months). Nine patients (36%) died from toxicity mainly related to GVHD and its complications. Eleven patients (44%) relapsed (n=9) or progressed (n=2) after ABMT. In eight patients (8/11; 72%), relapse was extra medullary with histological confirmation in 2 patients. Component monoclonal was IgG lambda (n=4), IgA lambda or kappa (n=2), IgG kappa (n=1) and light chains lambda (n=1). The median time between allogeneic- BMT and EM relapse was 29 months (range 3-132 months). The status of the disease before allograft was CR (n=4), PR (n=4). Six patients had a tumor mass adjacent to bone, 2 patients have soft tissue involvement or multiple organs involvement (pancreas, kidney and abdomen). There were no evidences of medullary involvement at the time of relapse. Molecular and cytogenetic chimerism was performed in 5 patients and they were of donor type. Seven patients were treated by chemotherapy (bortezomib or lenalidomid; n=4) and / or radiotherapy (n=6). Three patients from them (3/8; 37.5%) are alive after a median follow-up of 156 months (range; 132-168) with partial (n=1) or complete remission (n=2).
Summary and Conclusions: After allogeneic BMT, patients are at risk of TRM and extra medullary relapse. The effect GvM seems to be limited to the bone marrow. New treatments options (lenalidomide/bortezomib) in combination with radiotherapy seems controlling the disease.

PB1893

CLINICAL PROGNOSTIC FACTORS OF LONG TERM SURVIVAL IN MULTIPLE MYELOMA

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Background: Multiple myeloma prognosis may correlate with the patient's clinical characteristics as well as biologic variables of the malignant clone.
In the mid 90's with the systematic use of high dose melphalan and ASCT the average survival rate was 3-4years,while after 2007 with the indroduction of immunomodulatory agents and proteasome inhibitors, life expectancy exceeded the limit of 7-8 years.At the same time the impact of the molecular features of the malignant cell in prognosis was strongly validated and acknowledged.
Aims: In this cohort we present patients diagnosed with multiple myeloma before 2006, who remain alive at least eight years after the initial diagnosis.The purpose of the study was to identify common clinical characteristics of favorable prognosis among long term survivors independently of cytogenetic or molecular abberations.
Methods: The present study included 26 patients(19males,7 females) diagnosed with multiple myeloma between 2000-2005 in our institution.The median age at the time of diagnosis was 57(44-75)yrs.Among the cases reported 10 were IgGk, 4 IgGλ,3IgAk, 3IgAλ,3 light chain(λ), 2 non secretory and one had tripple M-component(IgGk/IgAk/IgDk). The majority of patients (25/26) were eligible of treatment initiation according to the current criteria (presenting with CRAB).23 patiens were diagnosed with bone disease,14 had anemia,11 presented with renal insufficiency and 3 with hypercalcemia.In 6/26 patients MGUS preceeded the diagnosis for more than 12 months. At staging by Salmon Durie 15/26 were at stage III,6 at stage II,5 at stage I,while according to ISS 6/26 were at stage III.Classical cytogenetic analysis was performed in bone marrow speciments for 20/26 patients and all had a normal karyotype. 23/26 received VAD or VAD-Caelyx regiments.Two received melphalan -steroids combination and one bortezomib-steroids.Palliative radiotherapy was applied to all patients having bone disease (23/26).19/26 received high dose melphalan and autologous transplant while 5 of them were submitted to a second transplantation (tandem) and 15 reached complete remission after transplantation.22/26 followed a maintenance schedule with thalidomide.
Results: 15 out of 26 patients remain in complete remission 9-15 years after diagnosis,while 3 are alive with relapsed or progressive disease.8 patients died >8-14 years after diagnosis(7 died from disease complications and one from an irrelevant cause).Although no statistical analysis can be performed in this sample,two different subgroups are revealed:the first subgroup consists of 15 patients of younger age (median 54),no comorbidities,with bone disease as the main clinical feature,who were treated with high dose melphalan and ASCT and achieved CR according to the contemporary response criteria.The latter subgroup included 9 patients of older age with previous MGUS diagnosis (mostly IgGk),whose survival exceeded the average life expectancy.
Summary and Conclusions: A clinical profile of younger age,lower ISS, prolonged PFS is compatible with long term survival. ISS appears to have higher prognostic value than Salmon Durie.Even in the era of novel treatment agents clinical characteristics of the patient may indicate survival benefits although the use of cytogenetic or molecular analysis when available has become mandatory.

PB1894

THE ROLE OF THE UPTAKE PATTERN OF 18F-FDG PET/CT AND 99MTC-MIBI IN THE DIAGNOSTIC EVALUATION OF PATIENTS WITH MULTIPLE MYELOMA

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Background: for staging of myeloma it is important not only to measure the total tumor mass but also to localize the lesions in order to treat special localizations
Aims: to compare the image patterns of ^{18}F-FDG positron emission computed tomography/ computed tomography (PET/CT) with those of ^{99m}Tc-MIBI single photon emission tomography/CT (SPECT/CT) at diagnosis in plasma cell neoplasms (MM) and evaluate the relation of this pattern with risk factors, therapy response and patients' progression-free survival (PFS).
Methods: Eighteen newly diagnosed MM patients were enrolled. PET/CT and SPECT/CT were included in the diagnostic work-up, reporting focal lesions and diffuse bone marrow involvement. One patient had a solitary plasmacytoma and was not evaluated for response. Patients gave informed consent.
Results: Eleven patients (61%) were male. Median age: 57 years (37-84); albumin 3.4 g/dL (2.16-4.2), beta-2-microglobulin: 4.2 mg/L (0.4-29.4), calcium 9.8 mg/dL (7.8-15), serum creatinine 0.85 mg/dL (0.6-2.4). No patient was on dialysis. According to ISS, 2 patients were stage I; 6 were stage II, and 10 were stage III. One case fulfilled criteria for plasma cell leukemia and 1 had a solitary plasmocytoma. Patients received CTD (12 patients); MPT (3 patients); CVD and Dexa-Tal (one case each). PET/CT and SPECT/CT, were concordant for presence (11) or absence (4) of a diffuse pattern. In the last ones, two showed positive foci in PET/CT. PET was able to detect >84 (extramedullary in six cases) while SPECT/CT detected only 10 focal lesions (extramedullary in one case). Intensity of ^{99m}Tc-MIBI uptake as well as the number of foci seen in PET/CT had an inverse correlation with hemoglobin values but not with ISS. A very good partial remission was seen in 5 patients and 2 had a complete remission. After a median follow-up of 12 months (1-19), 4 patients had progressed and 3 patients (17.6%) died during the follow-up. The 12 month progression free survival for diffuse pattern of PET/CT and SPECT/CT was not statistically different, although none of the patients with a negative SPECT/CT had progressed.
Summary and Conclusions: in this preliminary study, ^{18}F-FDG PET/CT detected more focal lesions (including extramedullary lesions) and ^{99m}Tc-MIBI was able to measure the intensity of diffuse BM involvement. Both techniques should be used together, especially in clinical studies, as they give complementary information. Concerning therapy response, the time of observation is rather short, and no relation with PFS and OS was found yet.

PB1895

BENDAMUSTINE-BASED THERAPY RELAPSE/REFRACTORY MULTIPLE MYELOMA PATIENTS: FIRST IMPRESSIONS IN RUSSIA

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Background: Relapsed and refractory (R/R) multiple myeloma (MM) constitutes a specific and unmet medical need. Median survival ranges from as little as 6 to 9 months, and responses to treatment are characteristically short. In patients with R/R MM after therapy of bortezomib and/or immunomodulators (IMiDs) a bendamustine-based treatment can be used as "salvage".
Aims: Examine efficiency of bendamustine-based chemotherapy in R/R MM patients.

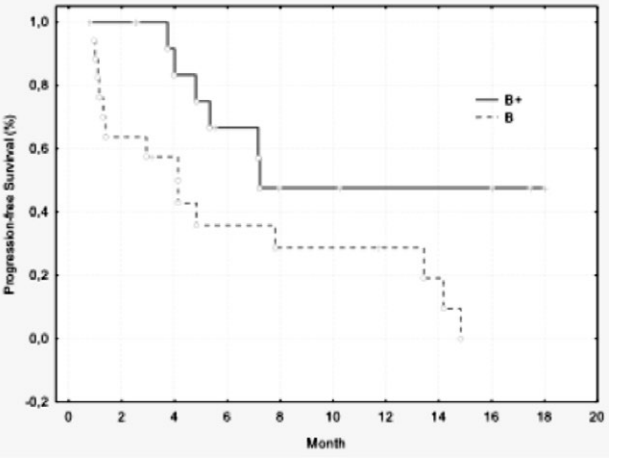

Figure 1.

Methods: In this retrospective analysis we have identified 32 patients with R/R MM by means of case research, who have been bendamustine-based treated at Hematological Clinics of Russian Federation since 2011. Median age was 67 years, the female/male ratio was 2.5:1. After in median 2 lines of prior therapy (range: 1-7) patients received in median 3 (range: 1-9) cycles of bendamustine-based therapy. Bendamustine dosage was 70-120 mg/m^2/day on days 1 and 2 of a each 28-day cycle until progressive disease or intolerability. Bendamustine was administered as monotherapy in 12% of patients, whereas 88% received concomitant steroids (group Bendamustine (B), n=17). Bendamustine with dexamethasone and bortezomib or IMiDs (BBD, BRD, BTD) was evaluated in 15 patients with R/R MM (group Bendamustine-plus (B+)). Primary end point was overall response rate (ORR). Secondary end points were time to progression (TTP), overall survival (OS), and toxicity.
Results: ORR in both group was 59.2%: 22.2% partial response (6.7% in group B vs. 41.7% in group B+, p<0.05), stable disease 37.0% (26.7% vs. 50.0%, accordingly, p>0.05). Median TTP was 9.7 months (4.1 mo in group B vs. 7.2 mo in group B+, p=0.02)) (pic). Median OS was 25.4 months in group B, but in group B+ median OS is not reached. Hematologic toxicity was in 25.0% of patients: grade 3/4 anemia was noted in 9.4%, grade 3/4 thrombocytopenia was noted in 6.3%, and grade 3/4 infections were noted in 21.9%.
Summary and Conclusions: Bendamustine-based therapy is active and well tolerated in patients with relapsed/refractory multiple myeloma.

PB1896

THE IMPORTANCE OF IMMUNOPHENOTYPIC COMPLETE REMISSION AFTER INDUCTION THERAPY IN PATIENTS WITH MULTIPLE MYELOMA ON PROGRESSION/RELAPSE FREE SURVIVAL

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Background: Multiple myeloma (MM) is a malignant, essentially incurable desease caused by malignant transformation of B-lymphocytes. Depth of achieved remission appears to correlate with the duration of desease control. Therefore there are cumulative data supporting the importance of the molecularly or imunophenotypically defined remission (absence of minimal residual disease-MRD). MRD in bone marrow may be detected by flow cytometry (with a threshold sensitivity at a level of 1x10-4). Immunophenotypic complete remission (iCR) is defined by fulfilling requirements of conventional CR along with negative test of free light chains in serum and absence of myeloma cells in the bone marrow by examination of at least 10□ cells using >4-color flow cytometry.
Aims: To verify the importance of iCR after induction therapy in patients with MM on progression/relapse free survival (PFS).
Methods: Retrospective analysis of 25 patients with MM consecutively diagnosed in 2010-2013, who achieved conventional CR after induction therapy and were examined by flow cytometry to evaluate eventual presence of MRD in bone marrow (according to EMN – European myeloma network).
Results: Our cohort of patients comprised 14 men and 11 women, the mean age was 60 years. Patients were treated with induction chemotherapy containing thalidomide in 12/25 (48%) and bortezomib in 13/25 (52%) and all patients subsequently received highdose chemoterapy (HD-Melphalan 200 mg/m2) supported by autologous stem-cell transplantation. A total of 8 patients (32%) in conventional CR after induction therapy achieved also iCR (the MRD-ve), while 17 patients (68%) had still by flow cytometry detectable MRD in bone marrow at a level of 1x10 to 1x10-2-4 (MRD +ve). Immunofenotypic data after autologous transplantation are not available. Between the two groups was no statistical difference in sex (P=0.34), cytogenetic risk group (P=0.63), or type of induction chemotherapy (P=0.73). The median PFS for MRD+ve was 23 months (12-48) and for MRD-ve 35 months (15-57), however difference in the median PFS between the two groups was not statistically significant (P=0.73).
Summary and Conclusions: Our data indicated a trend towards a longer PFS for patients MRD-ve, however absence of statistical signification may be caused by the relatively low number of patients and eventually by the efect of subsequent HD-chemotherapy. The study with longer follow-up of more patients with flow cytometry-monitoring in all stages of treatment (also after HD-Melphalan) is thus waranted.

PB1897

RAPID EFFICACY OF LENALIDOMIDE PLUS DEXAMETHASONE FOR POEMS SYNDROME: ASSESSMENT OF FOUR PATIENT'S CLINICAL OUTCOME

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Background: The POEMS syndrome (coined to refer to polyneuropathy; PNP), organomegaly, endocrinopathy, M protein, and skin changes) is a rare disorder and characterized by elevated vascular endothelial growth factor (VEGF) levels. The treatment for POEMS syndrome is mostly derived from regimens that have shown efficacy in other plasma cell dyscrasia, such as lenalidomide which reduces proinflammaotory and proangiogenic cytokines.
Aims: We report the efficay of a combination of lenalidomide and dexamethasone (Ld) in four patients with POEMS syndrome.
Methods: Four patients (M : F, 3 : 1)(age ranf-ge: 37-56 years) had been diagnosed as having POEMS syndrome. Serum VEGF level elevation could be documented only in one patient. The common main complain were fatique and weight loss which defined the reason that Internal Medicine speciaist were the first physicians. Two of the patients had severe PNP which inhibits normal daily activity and led to need assistance for walking. Two patients had severe extravascular volume overload as pleural effusion and ascite. As endocrinopathy, all of them had hypogonatorophic hypogonadism and hypocortisolemia among hypothyroidism and mild diabetes. Hyperpotasemia, mild increased serum creatinine level and hyponatremia were noted as biochemical changes. Only two patients had hyperpigmentation and acanthosis nigrigans. Treatment was started with lenalidomide (25mg, days 1-21) and dexamethasone (40mg weekly) following approvement for reimbursement. All patients received venous thrombosis prophylaxis.
Results: Massive ascite and also pleural effusion dramaticaly diminished and disappeared with the time. All of the patients put on weight and biochemistry was normalized. With intensive physical and occupational therapy, there was also steady improvement of the pateint's motor function. Two patients were agree to collect autologous peripheral blood stem cell harvesting but decide for delayed transplantation. One patient had needed radiotherapy for the pain related with bone lesions. The patient's serum free light chain ratio became normalized but M-protein was still detectable with immunfixatipn.
Summary and Conclusions: Our experience suggest that Ld therapy is not only realtively safe but also a promising option for POEMS syndrome.

PB1898

CASE REPORT: CEREBRAL LIGHT CHAIN DEPOSITION DISEASE (LCDD)

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Background: Light chain deposition disease (LCDD) is a rare monoclonal gammopathy characterized by the systemic deposition of non-amyloid immunoglobulin light chains causing a progressive accumulation of extracellular material with varying degrees of organ impairment. Although LCDD mainly affects the kidneys (it manifests as proteinuria and/or renal insufficiency) the involvement of other organs (*e.g.*, heart, central nervous system and peripheral nerves) is very common. We present the case of a patient with a newly diagnosed cerebral restricted λ LCDD.
Aims: We present the case of a patient with a newly diagnosed cerebral restricted λ LCDD.
Methods: A 49 -year-old female was admitted to our hospital with progressive loss of coordination in her right hand and some in her left leg for 3 months, associated with mayor depression, headaches and constitutional symptoms (asthenia, weight loss and anorexia). Clinical examination revealed a decrease in the strength and the sensitivity in the right hemisphere 4/5. Her complete blood count showed a mild anaemia (Hb 114 g/L) and trombocytopenia (122.000/uL) with total leukocyte in the normal range. Calcium was normal. Liver and renal function was also normal. Serum and urine protein electrophoresis did not reveal any monoclonal protein spike. Immunofixations were negative. Nephelometry was performed showing a decreased of IgA and IgM levels with a normal IgG. Serum λ free light chains were 6.98 mg/L with a normal or K/λ ratio (K/λ:). The MRI study was made to the patient in order to discard a central nervous system (CNS) neoplasm. The MRI revealed a space occupying diffuse lesion in left front parietal. Histological examination with hematoxylin and eosin of the resected tumor demostrated an amorphous eosinophilic deposits in white and grey matter of the brain without plasmacytic cells. These deposits were positive for λ-light chain antibody by immunohistochemistry. To rule out other plasma cell dyscrasias, bone marrow aspiration (BMA) and fine-needle aspiration of abdominal fat pad (FPFNA) were assessed. BMA showed a 3% of plasma cells (PC). Flow cytometry did not evidence monoclonal PC. FPFNA was negative to amyloid. With these data, Multiple Myeloma (MM) and Primary Systemic Amyloidosis (PSA) were discarded and the diagnosis of λ cerebral LCDD was made. The patient received conditioning regimen consisted of BEAM: BCNU 300 mg/m2 (d-6) cytosine arabinoside 200 mg/m2/d and VP-16 200 mg/m2/d (d-5 to d-2) and melphalan 200 mg/m2 (d-1) followed by autologous stem cell transplantation (ASCT). Currently (After 3 months of ASCT) the patient has been improved her strength and sensitivity.

Results: LCDD is a rare monoclonal gammopathy and it is less common involving CNS. As in other neurological disorders, the clinical presentation (epilepsy, cognitive impairments, hemiparesis,headaches.) depends mainly on the location of deposition and not on the histological finding. Although chemotherapy including an alkylating agent, radiotherapy or steroids have been reported in a few cases, the standard treatment has not yet been clearly established. ASCT has been reported as a feasible strategy in LCDD. We choised BEAM conditioning regimen in order to cross the blood-brain barrier. The median overall survival is approximately of 4 years. Prognostic factors for LCDD include age, presence of concomitant MM or PSA. Nowadays, there is not enough data about the management of brain LCDD and more information is needed describing the efficacy of the different treatments.
Summary and Conclusions: We choised BEAM conditioning regimen in order to cross the blood-brain barrier. The median overall survival is approximately of 4 years. Prognostic factors for LCDD include age, presence of concomitant MM or PSA. Nowadays, there is not enough data about the management of brain LCDD and more information is needed describing the efficacy of the different treatments.

PB1899

EXTRAMEDULLARY BREAST PLASMACYTOMA ACCIDENTALLY DIAGNOSED PRECEDING AGGRESSIVE SYSTEMIC RELAPSE: AN UNUSUAL AND RARE MANIFESTATION OF RECURRENCE OF MULTIPLE MYELOMA

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Background: Extramedullary (EM) plasmacytoma is a plasma cell neoplasm of the soft tissues that may occur with or without bone marrow and systemic involvement. EM disease is an uncommon manifestation in Multiple Myeloma (MM) and can either accompany newly diagnosed disease or more frequently develop with relapse. It seems to have a different pathogenesis from the medullary involvement and represents an independent poor prognostic factor with high rate of mortality. Myeloma of the breast is a rare entity not completely understood, with only a few reported cases in the literature not providing any statistical information.
Aims: We present a patient with extramedullary breast plasmacytoma diagnosed incidentally during breast plastic surgery for long-term history of fibrocystic disease. This unexpected diagnosis was shortly thereafter followed by a severe systemic medullary and extramedullary recurrence, treated with chemotherapy and radiotherapy.
Methods: A 44 year-old woman was diagnosed with IgG lambda MM stage IIIA ISS3, showing deletion of 17 and 13 chromosome detected by FISH and without bone or EM lesions. She was affected by MGUS from 14 years and had undergone right nephrectomy for renal cancer five years before. VTD therapy was started (total 4 cycles) followed by double autologous HSCT obtaining a VGPR. She remained in remission of disease for 14 months until mastopexy breast surgery. Surprisingly the histopathological examination of the breast tissue was consistent with diagnosis of plasmacytoma. Strangely, both the ultrasound and the breast MRI showed no nodular areas. Molecular examination confirmed the presence of monoclonal lambda positive plasma cells achieved through analysis of the rearrangement of the immunoglobulin heavy chain genes by PCR.
Results: A subsequent disease restaging showed an unexpected massive skeletal bone marrow and extramedullary involvement. PET/CT revealed pathological accumulation in the femoral heads, left iliac bone, gluteal muscles, pelvis. X-rays demonstrated multiple lytic lesions of right and left humerus, pelvis and D2 vertebral wall. MRI showed massive effusion in the left iliac bone with multiple lytic lesions extending up to gluteal muscles. She presented high level of LDH and 30% of atypical bone marrow CD56 negative plasma cells. She underwent radiotherapy on her pelvis and was treated according with PAD regimen (4 cycles). Nowadays patient is currently undergoing therapy; autologous and allogenetic HSCT are being evaluated.
Summary and Conclusions: EM is prevalent in genomically defined high-risk MM with shorter overall survival. Some autopsy studies have shown extraskeletal involvement in approximately 50-70% of patients with MM. EM disease seems to be prevalent in patients treated with novel agents, although this finding is not confirmed and could be due to the increased survival of patients with new drugs. The mechanisms of extramedullary spread are poorly understood; a decreased expression of integrins and loss of CD56 have been described, which could cause disease dissemination. Several papers have described relapses of MM occurring from different subclones of plasma cells from those of initial diagnosis. Recently, it has been speculated the presence of Circulating Myeloma Tumor Cells (CTCs), that constitute a subpopulation of clonal plasma cells associated with aggressive disease. They are characterized by downregulation of adhesion molecules, might transit in the peripheral blood and colonize other sites during the patients' resting and remission period. One might ask some questions: some recurrent MM could be firstly an awakening of dormant MM circulating clones? Which are the mechanisms for hematogenous spread and EM involvement? These and others are unanswered questions; international collaborative trials are necessary to better understand EM dissemination and discover optimal treatment.

Myeloproliferative neoplasms - Biology

PB1900

TAZ GENE FROM HIPPO PATHWAY IS OVEREXPRESSED IN POLYCYTHEMIA VERA AND ESSENTIAL THROMBOCYTHEMIA PATIENTS

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Background: Polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibosis (MF) are myeloproliferative neoplasms (MPN) characterized by the increase of mature hematopoietic cells of one or more of the myeloid series proliferation. MPN may present theJAK2V617F mutation which occurs in 95% of PV patients and 50% of ET and MF patients. The JAK2V617F mutation constitutively active STAT3 transducer protein, which is related to apoptosis resistance and myeloproliferation in MNP. Although all the molecular knowlegde about MNP pathogenesis, these diseases do not have an efficient therapy. Thus, more studies related to MPN pathophysiology and description of new therapeutic targets, such as the molecules from Hippo pathway are relevant. The Hippo signaling pathway has been defined as a tumor suppressor pathway responsible for regulating the proliferation, differentiation and cell death. This pathway is composed of *MST1/2, WW45, Lats1/2, Mob1* and *YAP/TAZ* proteins present in mammals.
Aims: To compare the *TAZ* gene expression among PV, ET, MF patients and controls (CTRL) subjects.
Methods: The leukocytes were obtained from peripheral blood of 23 PV patients (median age=60 years; 13 men and 10 women), 21 ET patients (median age=62 years; 10 men and 11 women), 21 MF patients (median age=64 years; 16 men and 5 women) and 23 CTRL (median age=58 years; 6 men and 17 women). The leukocytes were separated by Voluven gradient method. The RNA were extracted by Trizol® method and the cDNA were synthesized using High Capacity cDNA reverse transcription kit®. The gene expression was assessed by a real time PCR and the results were expressed as relative units of expression (RUE).
Results: Moreover, in PV patients the *TAZ* expression is higher in those JAK2V617F-negative patients.
Summary and Conclusions: The results indicate that *TAZ* gene may contribute to PV and ET pathogenesis since *TAZ* is well known for their regulation by the Hippo pathway acting as a transcriptional co-activator inducing expression of cell-proliferative and anti-apoptotic genes via interactions with specific transcription factors. Supported by FAPESP process 2014/04234-9

PB1901

ABCB1 AND SLC22A1 MRNA EXPRESSIONS ARE INCREASED IN A HOMOZYGOUS JAK2V617F CELL LINE TREATED WITH JAK INHIBITOR

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Background: Pharmacokinetics of many anticancer drugs is affected by drug transporters as ABCB1, ABCG2 and SLC22A1. These molecules are responsible for cell uptake (SLC22A1) or efflux (ABCB1 and ABCG2) of severalxenobiotics and are widely distributed in many tissues. Several studies have shown their role in resistance and toxicity to chemotherapeutic agents. Dysregulation of JAK-STAT pathway is considered a hallmark of myeloproliferative neoplasms (MPN), and disruption of this pathway may result from mutations such as $JAK2^{V617F}$. Therefore, therapies targeting the inhibition of JAK proteins have been developed. However, the impact of JAK-STAT pathway inhibition over ABCB1, ABCG2 and SLC22A1 gene expression in MPN cells has not been investigated yet.
Aims: To evaluate the effect of JAK-STAT pathway inhibition in the expression of drug transporters ABCB1, ABCG2 and SLC22A1 in $JAK2^{V617F}$ positive cell line HEL92.1.7.
Methods: Erythroleukemia homozygous $JAK2^{V617F}$ cell line HEL92.1.7 was treated with 1 µM of *JAK Inhibitor I* (Merck/Calbiochem, Darmstadt, Germany), an ATP-competitive inhibitor of JAK proteins, for 24h. Western blot was used for verifying JAK-STAT pathway inhibition by inactivation of STAT-5. Quantitative real time-PCR and flow cytometry analysis were used to assess *ABCB1*, *ABCG2* and *SLC22A1* mRNA and protein expression, respectively, in JAK inhibitor-treated cells and in vehicle-treated control cells.
Results: JAK-STAT pathway was inhibited by treatment with 1 µM of *JAK Inhibitor I* for 24h, confirmed by non-detection of phosphorylated STAT-5. *ABCB1* and *SLC22A1* mRNA expressions were increased after treatment (median of 1.58 and 1.64 fold change, respectively), however, no difference was observed between ABCB1, ABCG2 and SLC22A1 protein expression in cells treated with 1 µM of *JAK Inhibitor I* for 24h and controls (P>0.05 for all).
Summary and Conclusions: Our data suggest that JAK-STAT pathway inhibition could modulate mRNA expression of drug transporters ABCB1 and SLC22A1 in MPN cells. Further studies with longer periods of treatment may confirm this proposition and help to elucidate the effect of JAK-STAT pathway in ABCB1, ABCG2 and SLC22A1 protein expression.

PB1902

INCIDENCE OF CALR MUTATIONS IN COHORT OF MPD PATIENCE FROM RUSSIAN FEDERATION

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Background: Since discovery of mutation in 9th exon of the CALR gene in 2013, molecular diagnostics of ET and PMF by analysis of JAK2V617F, CALR and MPL515L/K mutations became standard in many of the research centers in the world. In this study we are for the first time reporting incidence of abovementioned mutations in patients from Russian Federation.
Aims: This study aimed to reveal incidence of mutation in calreticulin encoding gene in patients with myeloproloferative deseases: PV, ET, PMF.
Methods: Patients: DNA from peripheral blood of 103 patients diagnosed with PV, ET, and PMF tested for the mutations. Patients group was sex balanced 54F/49M. Average age was 52 years. For JAK2V617F and MPL515L/K detection allele specific real-time PCR with specific primers set were used (Genotechnology, Russia). CALR mutations were detected by Sanger sequencing of the C-end coding region, as described earlier (1). 1. Nangalia J, *et al.*, Somatic CALR mutations in myeloproliferative neoplasms with nonmutated JAK2. N Engl J Med. 2013 Dec 19;369(25):2391-405.
Results: Results of patints test presented in table. We did not found any cases with simultaneous appearance of two mutations in one patient. PMF patient with MPL515L mutation also have co appearence of BCR-ABL chimera. Calreticulin mutations were mostly type1 -deletions of 52 bp (15 cases), type 2-insertions of 5bp (4 cases) and deletions other than 52bp (2 cases). CALR mutated patients were no younger than average – mean 57 years old (from 20 to 77). Also, we did not observe any significant correlation between type of the mutation and age/sex/complications.

Table 1.

	JAK2V617F	CALR	MPL	all negative
PV	25 (100%)	0	0	0
ET	12 (46%)	6 (23%)	1 (4%)	7 (27%)
PMF	28 (54%)	15 (29%)	1 (2%)	8 (15%)

Summary and Conclusions: Incidence of the JAK2V617F, CALR and MPL515l/L in studied patient group is similar to the one reported in European centers. We did not find correlation of CALR mutation status with younger age, as described previously. No thrombotic events were found in ET patients with CALR mutations.

PB1903

CALR GENE MUTATIONS IN MOROCCAN PATIENTS IN ESSENTIAL THROMBOCYTHEMIA: FIRST REPORT

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Background: Since 2005, the discovery of the somatic mutation V617F in exon 14 of the Janus Kinase 2 gene (JAK2) was the front-line molecular tool for the diagnosis of Myelproliferative neoplasms (MPN) of which essential thrombocythemia (ET). In addition, detection of genetic alteration in the 10th exon of the myeloproliferative leukemia gene (MPL) plays a significant role in the pathogenesis of ET. However, about 40% of patients did not carry any of these markers and the diagnosis of ET remained a major challenge. Towards the end of 2013, somatic mutations in the 9th exon of the gene encoding cal-

reticulin (CALR) were revealed to be responsible of MPN in around 80% of ET patients who don't carry the JAK2 or MPL mutations.
Aims: This discovery have improved and simplified the accuracy of diagnosis of patients with ET worldwide, and opened the door to a better understanding of the disease pathogenesis. In this study, we present the 1st mutational profiles in CALR gene assessed in Moroccan Patients.
Methods: 25 Moroccan patients from Clinical Hematology Department of the Military Hospital Mohammed V were reviewed for having ET. All patients had a CBC with elevated platelet count; We searched magakaryocytes proliferation at the bone marrow biopsy for only 8 patients. The bcr-abl transcript was negative for all patients. The mutational status for exon 10 of MPL gene has not been evaluated in any of our patients. We evaluate somatic mutation V617F in JAK2 gene and mutations in 9th exon of CALR.
Results: Patients ages were between 23 and 75 years, 13 of them were female. Discovery circumstances ranged from fortuitous discovery to thrombotic or hemorrhagic manifestations. Median platelet countat diagnosis was 1 027 578 /mm3. Molecular assessment of the somatic mutation V617F in JAK2 gene was positive in eight, negative in 13 patients, 4 patients were not evaluated. Among the 13 "JAK2 negative" patients mutations in 9th exon of CALR were evaluated in 11 patients and were detected in four of them (36.3%). All the variants found were insertion/deletion mutations; All mutations were found between positions 1142 and 1157 in the coding DNA sequence (cDNA). Patients with intermediate and high risk received therapy such as hydroxyurea (13 patients), anagrelide (2 patients), pipobroman (4 patients) to lower the platelet count.
Summary and Conclusions: CALR gene mutations discovery represented a major milestone in the diagnosis of ET that would be highly considered in the revision of the WHO classification of MPN. Up to date, comparative studies on the treatment of CALR mutated patients with ET were not yet reported and further studies should help to explore this area.

PB1904

ET BUT NOT PV ARE ASSOCIATED WITH POLYMORPHISM ARG399GLN XRCC1 IN PATIENTS OF THE KRASNOYARSK TERRITORY
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Background: The associations between the Arg399Gln polymorphism in X-ray repair cross-complementing gene 1 (XRCC1) gene and the risk of hematological malignancies have been extensively investigated. In the subgroup analysis by ethnicity and cancer types, significant association was found in Asians but not in Europeans (Du L e.a. 2015 PMID: 25619474). No data on any it association with Ph-negative myeloid neoplasms.
Aims: The purpose of this study was to evaluate the association between XRCC1 Arg399Gln and Ph-negative myeloid neoplasms risk in patients of the Krasnoyarsk Territory.
Methods: In this study was included 134 cases with ET -75 V617F JAK2 (+), 31 CALR(+) and 5 MPL(+), 91 patients was included with PV -79 V617F JAK2(+). The control group (n= 114) consisted of healthy individuals. The XRCC1 polymorphism was detected in DNA isolated from peripheral leucocytes using PCR-based Taqman assay using a "iQ iCycler" 5.0. We used two pairs of primers (F: GTA-AGG-AGT-GGG-TGC-TGG-ACT-GT; R: GTC-TGA-CTC-CCC-TCC-AGA-TTC-C) and two probes (A allele: FAM-CTG-CCC-TCC-CAG-AGG-TAA-GGC-CTC-BHQ1; G allele: HEX-CTG-CCC-TCC-CGG-AGG-TAA-GGC-C-BHQ1).
Results: Our data are shown in the table. The additive model of Arg399Gln was associated with Essential Thrombocytemia (ET) risk. Conversely, no statistical association was found with Polycythemia vera (PV).

Table 1.

	Arg/Arg	Arg/Gln	Gln/Gln	n
healthy	47%	41%	11%	114
PV	45%	47%	8%	91
ET	33%	52%	14%	134
	OR (95% CI)			p
ET vs healthy	0,56 (0,33-0,93)	1,56 (0,94-2,59)	1,31 (0,61-2,78)	< 0,05
ET vs PV	0,50 (0,20-1,23)	0,82 (0,48-1,40)	1,64 (0,95-2,84)	< 0,04

Summary and Conclusions: The differences were found in the association between the polymorphism PV and ET to suggest Arg allele of the Arg399Gln polymorphism prevents the loss of homozygosity typical to PV.

Myeloproliferative neoplasms - Clinical

PB1905

Abstract withdrawn

PB1906

A PRESENTING NEUTROPHIL COUNT MORE THAN 75% IS A PREDICTOR OF THROMBOSIS AT DIAGNOSIS BUT IS ASSOCIATED WITH LESS THROMBOSIS SUBSEQUENTLY IN PATIENTS WITH POLYCYTHAEMIA VERA
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Background: Polycythaemia vera (PV) is a myeloproliferative neoplasm (MPN) characterised by elevated haemoglobin and thrombotic tendency. Leukocytosis has been demonstrated to be a risk factor for thrombosis in PV. However, the white cell subtype implicated in thrombosis in PV has not been evaluated.
Aims: In this study we examined the role of presenting neutrophil levels in PV patients with thrombosis.
Methods: We retrospectively reviewed the case records of 163 patients with PV for thrombotic events in our institution's MPN registry. This is an institutional review board approved registry that utilises REDCap, an electronic data capture tool for research studies. Thrombotic events considered were ischaemic heart disease, cerebrovascular accident, peripheral arterial disease, retinal artery occlusion, thrombosis of the splanchnic circulation and venous thromboembolism.
Results: Median age of diagnosis was 58years; 58.3% were male. 32 patients presented with a thrombotic event at diagnosis of PV. 12.5% of events were venous. Patients with a thrombosis at diagnosis were more likely to have risk factors for atherosclerotic disease (31.3% vs. 8.4%, P=0.012), have a higher presenting white cell count (15.2x 10^9/L vs. 13.6x 10^9/L, P=0.04) and to be treated with cytoreduction (90.6% vs. 71.8%, P=0.026). Patients with a thrombosis at diagnosis had a higher presenting neutrophil percentage of 82% (vs. 76.4%, P=0.002) and median absolute neutrophil count (ANC) (12.1x 10^9/L vs. 10.1x 10^9/L, P=0.017) than patients who did not. Based on receiver operating characteristic (ROC) analysis, a cut-off neutrophil percentage of 75% was derived for further analysis. 80.6% of patients who presented with thrombosis at diagnosis had a neutrophil count ≥ 75%, whereas 57.3% of those who did not, had a presenting neutrophil count ≥ 75% (P=0.016). On logistic regression, adjusting for gender, age ≥ 60 at diagnosis, prior thrombosis and presence of atherosclerotic disease risk factors, a presenting neutrophil percentage ≥ 75% (Odds ratio (OR) 3.36, 95% CI 1.25-9.03, P=0.016) and presence of atherosclerotic disease risk factors (OR 4.43, 95% CI 1.21-16.18, P=0.024) were predictors for thrombosis at diagnosis. Over a 5.4year median follow up, 31 thrombotic events occurred in 28 patients. Six patients who presented with thrombosis had subsequent events. Paradoxically, less patients who had a thrombotic event on follow up had a presenting neutrophil percentage ≥ 75% than patients who did not have thrombosis on follow up (34.6% vs 67.4%, p =0.002). Of patients who had a thrombosis after diagnosis, 57.1% were on cytoreduction compared to 74.8% of those who did not develop thrombosis (P=0.059). 10 and 20year thrombosis-free survivals were better in patients with presenting neutrophil percentage ≥ 75% than those with lower presenting neutrophil counts: 91.4% vs. 47.6% and 64.9% vs. 35.7% (P=0.001) respectively. On multivariate analysis, in a model including gender, age ≥ 60 at diagnosis, atherosclerotic disease risk factors, prior thrombosis, use of cytoreduction, thrombosis at diagnosis and presenting neutrophil ≥ 75%, a presenting neutrophil level ≥ 75% was significant (Hazard ratio 0.25, 95% CI 0.11-0.6, P=0.002).
Summary and Conclusions: More patients who presented with a thrombotic complication at diagnosis had a presenting neutrophil count ≥ 75%. A presenting neutrophil count ≥ 75% and presence of risk factors for atherosclerotic disease were predictors of thrombosis occurring at diagnosis. However, for follow up thrombotic events, a presenting neutrophil ≥ 75% was associated with less thrombosis.

PB1907

WT1 EXPRESSION IN PH-NEGATIVE MYELOPROLIFERATIVE DISEASES
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Background: Expression of Wilms tumor antigen (WT1), which encodes a transcription factor, is significantly increased in tumor cells of the kidney, testes, ovaries, breast and certain hematological malignancies. WT1 is overexpressed in many patients with acute myeloid leukemia and in the absence of more specific molecular markers can be used for monitoring of minimal residual disease.

The rate and significance of WT1 overexpression in chronic myeloproliferative diseases (MPDs) currently remains unclear

Aims: The aim of this study was to evaluate the incidence of overexpression of WT1 gene in MPDs.

Methods: Peripheral blood samples from 52 (male n= 33, female n=19) patients with polycythemia vera (PV), essential thrombocythemia (ET) and primary or post-PV/post-ET myelofibrosis were evaluated for WT1 expression. Patients's characteristics at the time of evaluation is presented in the table 1. WT1 expression was performed by quantitative PCR, using a standard set ProfileQuant Wt1 kit, PCR analyzer «Rotor- Gene 6000". Normal reference range in peripheral blood considered is 0.00-50.00 WT1/10^4 ABL copies.

Results: WT1 overexpression was observed in 25/52 (48%) patients. It was found in all patients with myelofibrosis (primary or post-PV/post ET). No patients with PV or ET have WT1 overexpression. The mean level of WT1 gene expression was 714 WT1/10^4 ABL copies in MF (range, 171-6000), 4.0 WT1/10^4 ABL copies in PV (range 1.1-17), and 8.32 WT1/10^4 ABL copies in ET(range, 1.8-14). We did not find any significant correlation of WT1 expression level with either mutation status of Jak2, MPL and CALR genes or clinical data (age, gender, DIPSS risk groups, median time from MF diagnosis).

Table 1.

	PV n=16	ET n=11	PMF n=20	post-PV/post ET MF n=5	All MPDs n=52
Age, median (years)	50	50	65	60	60
JAK2V617F +	16 (100%)	9(82%)	8 (40%)	5 (100%)	38(78%)
MPL + in Jak2V617F negative pts	NA	0/2 (0%)	1/12 (8%)	NA	1/14 (7%)
CALR + in Jak2V617F negative pts	NA	2/2(100%)	7/12 (58%)	NA	9/14 (64%)

Summary and Conclusions: WT1 gene was overexpressed in all MF, including post-PV/post-ET cases, but was normal in PV and ET patients. It seems that WT1 gene overexpression could be potential marker for prediction of bone marrow fibrosis development in patients with PV/ET. Whether the level of expression of WT1 is correlated to grade of fibrosis is a subject of our investigation.

PB1908

MULTICENTER-RETROSPECTIVE ANALYSIS OF TURKISH PATIENTS WITH CHRONIC MYELOPROLIFERATIVE NEOPLASMS

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Background: Chronic Myeloproliferative neoplasms (CMPN) are Philadelphia-negative malignancies characterized by a clonal proliferation of one or several lineages. According to WHO classification, CMPNs include Polycythemia Vera (PV), Essential Thrombocythemia (ET) and Primary Myelofibrosis (PMF).

Aims: The aim of this report was to determine the demographic features, disease characteristics, JAK mutational status, treatment strategies, and survival rates of 708 patients with CMPN from 8 centers in Turkey.

Methods: Across all of Turkey, 8 centers were enrolled in the study. We retrospectively evaluated 708 patients' results with CMPN.

Results: The JAK2V617F mutation was found positive in 75.11% of patients with PV, in 51.5% of patients with ET and in 50.4% of patients with PMF. Thrombosis occurred in 20.65% of patients with PV, arterial in 21 (47.7%) cases and venous in 23 (52.2%). Thrombosis was observed in 15.12% of patients with ET, arterial in 30 (50.8%) cases, venous in 27(45.8%) and two (3.4%) patients suffered both arterial and venous thrombosis. Approximately ten percent of PMF patients suffered from thrombosis, arterial in 7 (6.7%) cases and venous in 3 (2.9%). Bleeding at diagnosis occurred in 7.5% of PV patients, in 9% of ET patients and in 10.4% of PMF patients. Six hundred and eight patients (85.9%) had a cytoreductive therapy. The most common used drug was hydroxyurea (75.1%). Hydroxyurea was used 79.3% of PV patients, 57.1% of PMF patients, and 77.7% of ET patients in the first-line treatment. Interferon was used 5.1% of PV patients, 1.9% of PMF patients, and 5.1% of ET patients. Cytoreductive therapy was changed in 198 (28% of all) patients. In the second-line treatment, the most common used drug was anagrelide (147 of 198 patients). Anti-platelet therapy was used in 553 (78.1%) patients. Splenectomy was performed in 10 (1.4%) patients. Eight PMF patients (1.1% of all CMPN patients) were treated with allogeneic stem cell transplantation. Progression to acute leukemia and secondary myelofibrosis were observed in 0.6% and 11.3% of all patients, respectively. The median follow- up was 38 months (0-322) and overall survival (OS) was 86.7% at 10 years in all patients. Among the 213 PV patients, the median follow- up was 3.5 months (0-322) and the OS was 89.7% at 10 years. In 390 ET patients, the median follow- up was 35 months (0-280) and the OS was 98.5% at 10 years. Among the 105 PMF patients, the median follow-up was 15.5 months (0-229) and the OS was 82.5% at 10 years.

Summary and Conclusions: Our patients results is compatible the literature except the frequency of JAK2V617F mutation in PV patients. Hydroxyurea was the most common used cytoreductive therapy in our country.

PB1909

INCREASED PLATELET COUNTS AT DISEASE DIAGNOSIS IS RELATED WITH EARLY INTRODUCTION OF CYTOREDUCTICTION AND REDUCED EVENT-FREE SURVIVAL IN YOUNG ADULT PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA.

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Background: ET is not such an innocent disease in young adults. Most thrombotic events occur during diagnosis and almost half of the initially asymptomatic patients will have an event during the course of their disease

Aims: Herein, we analyzed retrospectively the medical records of 33 ET patients diagnosed below the age of 45 years and followed in a single institution from 1992 to 2014.

Methods: Management was left at the discretion of the treating physician but antithrombotic agents were used from disease diagnosis in all patients. Among 33 young adult patients 11 were males and 22 females. 18 patients were followed for a median period of 94,5 (range 45-178) months and left untreated and they were included in group A. While cytoreductive treatment was introduced in 15 patients included in group B. Among 15 treated patients cytoreduction was introduced from disease diagnosis in seven patients and in eight during the course of their disease after a median period on observation of 36 months (ranging from 6 to 168).

Results: We separated our patients into two groups according to the need to start any cytoreductive treatment and try to discover patient's characteristics at diagnosis that can predict the early use of cytoreductive treatment. Splenomegaly of any grade was palpated in 3/18 (16.5%) patients in group A and in 2/15(13%) in group B, P=0,796. Although hematocrit levels were equal between the two groups [42,7%(34,7-47,6) in group A and 40,7%(37,3-46) in group B, P=0,84] as were also and the leucocyte counts [7,85 (5,6-13,2) X 10^3 pcm in group A and 9,73 (4,7-11,7) X 10^3 in group B P=0,6], the median values of platelet counts at diagnosis was significantly higher in group B compared to group A [805(490-2300) X 10^9 pcm vs 604,5(490-921) X 10^9 pcm, P=0,012]. Fibrosis (grade I) at the initially performed bone marrow biopsy was met at higher percentages in patients allocated in group B 40% (6/15) in group B and11% (2/18) at group A, P=0,03. JAK2V617F was detected in 40% (6/15) in group B and 50% (9/18) in group A, P=1. According to the IPSET-thrombosis scoring system in group A most patients had low thrombotic risk 10/18, 7/18 had intermediate thrombotic risk and 1/18 had high thrombotic risk. In group B 7/15 had low thrombotic risk, 3/15 intermediate and 5/15 had high thrombotic risk. In the total cohort of 33 young ET patients platelet counts at disease diagnosis can predict reduced Event free Survival (EFS). As an event were considered thrombohemorhagic complications, development of myelofibrosis and the need to start cytoreductive treatment. 10 year EFS was for patients with platelet counts<600x 10^9 pcm 89% for platelet counts between 600-800 X 10^9 pcm 66% and for platelet counts>800 X 10^9 pcm was 22%, P=0.003, Kaplan-Mayer, LogRANK test. On the contrary the presence of grade-I fibrosis was not associated with reduced EFS.

Summary and Conclusions: In young ET patients increased platelet counts at disease diagnosis can predict an eventful disease journey with early need for the use of cytoreductive treatment and reduced EFS.

PB1910

INCIDENCE AND CLINICAL CORRELATION OF CALR MUTATIONS IN PATIENTS WITH JAK2/MPL NEGATIVE ESSENTIAL THROMBOCYTEMIA (ET) AND PRIMARY MYELOFIBROSIS (MF). A SINGLE INSTITUTION COHORT REVIEW.

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Background: The recent description of *CALR* mutations occurring in ET and MF greatly contributed to the diagnostic evaluation of *ph-* Myeloprolyferative Neoplasms (MPN). The *CALR* gene encodes the endoplasmic reticulum Ca^{2++}-binding chaperone-Calreticulin. Exon 9 *CALR* somatic insertions and deletions have been reported and the most frequent variants are Type-1, 52-bp deletion (p.L367fs*46), and type-2, 5-bp TTGTC insertion (p.K385fs*47), representing

about 80% of the cases. These mutations have also been reported as favorable prognostic factor on ET thrombosis-free patients, particularly the type-1.
Aims: To evaluate *CALR, JAK2* and *MPL* mutations in a group of ET and MF patients and to correlate clinical and analytical data.
Methods: We actualized a retrospective analysis of 115 patients (pts) diagnosed MPN: 88 ET and 27 MF (according to WHO2008 criteria), with a mean follow-up time of 8 years (3 moths-31 years). *JAK2V617F* mutation was assessed by ASO-PCR and real-time quantitative PCR, mutations in exon 10 *MPL* and exon 9 *CALR* by PCR-sequencing in blood or bone marrow samples.
Results: In ET group (39males/49females), 59,1% (52) were *JAK2* positive; 20,5% (18) *CALR* 4,5% (4) *MPL* and 15,9% (14) were negative for *JAK2/CALR/MPL*- "triple negative". The *CALR* type-1 mutation was detected in 11/18, the type-2 in 6/18 and one patient harbored the p.K375Rfs*49 mutation. Comparing *JAK2*, *CALR* and triple negative pts, *CALR* had a male dominance and higher mean platelet (PLT) counts (P=0.02), *JAK2* pts had higher hemoglobin (Hb) levels (P=0.04) and triple negative pts presented at younger age (P=0.002). Analyzing the *CALR* subgroups, there is a trend to an older age and a higher PLT count in type-2 mutation group and an incremental difference on PLT counts comparing to *JAK2* (P<0.001). There were no differences in leukocyte (WBC), serum ferritin and erythropoietin levels. Regarding hematological response to cytoreduction we did not observe differences within *CALR* pts. Thrombosis occurred in 24/88 ET, 18 previously or at diagnosis; 21/52 *JAK2*: 2 of splanchnic veins both associated to prothrombotic factors (1 HTZ Factor V Leiden (*FVL*), 1 HTZ PRT20210G/A/FVL); 3/18 *CALR* all type-1 and 2 associated to prothrombotic factors *FVL* (1) and HTZ *PRT20210G/A* (1). One *CALR* (type-2) and 4 *JAK2* pts progressed to MF, one of them to AML. Eleven patients deceased: 6 *JAK2*, 3 *CALR (type-1)* and 2 triple negative. The MF pts (15M/12F) were 55,6% (15) *JAK2*, 33,3% (9) *CALR* : 6/9 type-1, 3/9 type-2; 7,4% (2) *MPL* and 3,7%(1) triple negative. About 70% of *JAK2* pts had an allelic burden >50%. We observed a higher WBC count in *JAK2* compared to *CALR* (P=0.04) and no differences in Hb, PLT, serum ferritin and EPO values. Thrombosis occurred in 3 JAK and 1 *CALR* type-1. There were 3 deaths 2 *JAK2* and 1 *CALR* type-1.
Summary and Conclusions: In this cohort of MPN, 58,2% (67/115) have *JAK2V617F* mutation, 23,5% (27/115) *CALR* and 5,2% (6/115) *MPL*. In 13,1% (15/115) we found no *JAK2/MPL/CALR* mutation. Exon 9 *CALR* mutations, the second most prevalent marker in ET pts, are associated to higher PLT counts and lower Hb levels comparing to *JAK2*. Comparing *CALR* subgroup, ET pts with type-1 mutations had more thrombotic events, most of them in association to prothrombothic factors while type-2 presented with higher PLT counts and only a type-2 pt progressed to MF. There were no differences in cytoreduction response. The impact of the different type of *CALR* mutations on natural history of ET and MF pts are not yet completely elucidated. Nevertheless, these mutations constitute a molecular marker of ph- NMP and offer an important tool in the diagnostic pathway ET and MF.

PB1911

CALRETICULIN V/S JAK2 MUTATION CHARACTERISTICS IN ESSENTIAL THROMBOCYTOSIS IN ASIAN AND NON-ASIAN POPULATION: A META-ANALYSIS

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Background: 2014 was marked in myeloproliferative neoplasms by the discovery of Calreticuline mutation in essential thrombocytosis and primary myelofibrosis. Many papers were published describing the clinical, biological and epidemiological characteristics of this mutation compared to JAK2 mutation in ET. A meta-analysis of these studies was necessary to confirm the different characteristics of this new mutation in Asian and non-Asian populations.
Aims: The aim of this study is to evaluate the epidemiological, clinical and biological characteristics of patients with essential thrombosis having JAK2 or Calreticuline mutation and to compare the particularity of these mutations in Asian and non-Asian population.
Methods: 8 studies published from December 2013 to October 2014 met the inclusion criteria of this meta-analysis: three of them in Asian population and five in non-Asian population. More than 2307 patients diagnosed having ET either with Calreticuline or JAK2 mutation were included. The epidemiological, clinical and biological characteristics of those patients at diagnosis (age, sex, hemoglobin level, white blood cells count, platelet counts, risk of thrombosis and hemorrhage, transformation to myelofibrosis or leukemia) were compared according to the positivity of JAK2 or Calreticuline mutation and to the race (Asian and non-Asian).
Results: The incidence of Calreticuline is 25% in ET patients in Asian and non-Asian subgroups. JAK 2 mutated ET patients are older than Calreticuline mutated patients with a mean difference of 6.9 years in all included patients, 5.3 years in Asians and 8.5 years in non-Asians. JAK2 mutated patients are less likely to be men when compared to calreticuline mutated patients in non-Asians with an OR =0.49; this OR was 1 in Asians. Hemoglobin level and white blood cell count was higher in JAK2 mutated patients compared to Calreticuline mutated patients in all sub-groups, while platelet count in JAK2 mutated patients was significantly lower. The risk of thrombosis was higher in JAK2 mutated patients compared to Calreticuline mutated patients at diagnosis and during the follow-up in all sub-populations with an OR>2 in all groups. The transformation to primary myelofibrosis was lower in JAK2 mutated patients, while there was no-significant difference in leukemia transformation, presence of splenomegaly at diagnosis and risk of hemorrhage.
Summary and Conclusions: With those specific characteristics, Calreticuline mutation will probably be added as diagnostic criteria of ET and will be considered a new prognostic factor affecting the treatment and the management of ET.

PB1912

TWO NOVEL CALRETICULIN MUTATIONS IN A TURKISH PATIENT WITH PRIMARY MYELOFIBROSIS: C.1116DELA AND C.1120A>C

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Background: With the discovery of somatic frameshift mutations in exon 9 of the calreticulin (CALR) gene, approximately 90% of myeloproliferative neoplasm (MPN) patients have gained a chance of genetic diagnosis. *CALR* mutations were first identified exclusively in JAK2-MPL negative essential thrombocytemia (ET) and primary myelofibrosis (PMF) at a rate of 60-88%, accounting for 1/4 to 1/3 of all patients with ET and PMF. *CALR* mutations that reported in the literature, consist of somatic insertions, deletions or both which always leading to an one base pair shift in the open reading frame and produce a novel C-terminal amino acid sequence that impair the function of calreticulin. As of today, more than 50 different types of mutations have been reported. The 2 most common mutations accounting for 85% of mutated cases are either a 52-bp deletion (Type 1; c.1099_1150del; L367fs*46; 44%–53% of cases) or a 5-bp insertion (Type 2; c.1154_1155insTTGTC; K385fs*47;32%–42% of cases). The remaining 15% include various other infrequent mutations that are often unique or found in a few patients.
Aims: Here we present another two *CALR* mutations in one patient with PMF that is not reported before.
Patient: The patient is a 46 year old man who is suffered from low back pain that have started 6 months ago. MRI scan of lumbosacral region revealed sacroileitis at the left side and he referred to a rheumatologist for further investigations. Anemia (hgb 10,8 g/dl) and thrombocytosis (700x10^9/L) with high LDH level (351 U/l) were found in initial tests. The other tests for a possible rheumatologic disease including HLA-B27, were all negative and the patient was admitted to hematology clinic. Physical examination was almost normal with no sign of organomegaly. Spleen size was also normal in the abdominal ultrasound. Peripheral blood smear showed dacrocytes, occasional myelocytes (1%) and metamyelocytes (1%). Bone marrow biopsy showed diffuse, grade 3-4 reticulin fibrosis with atypical proliferation of megakaryocytes and increased cellularity consistent with PMF. BCR-ABL, JAK-2 V617F, and MPL 515L/K tests were found to be negative.
Methods: Genomic DNA was extracted from whole blood using a commercial kit (HibriGen Biotech R&D, Istanbul, Turkey). Exon 9 of the *CALR* gene was amplified using Forward ACAACTTCCTCATCACCAACG and Reverse GGCCTCAGTCCAGCCCTG primers. PCR reaction was performed using 2X PCR master mix (HibriGen Biotech R&D, Istanbul, Turkey), primers and 25-30 ng genomic DNA. Direct Sanger sequencing of amplified fragments were performed using BigDye Terminator Cycle Sequencing kit v3.1 and an ABI3130xl sequencer (Applied Biosystems, Foster City, CA, USA). Our results were evaluated using the BLAST (http://www.ncbi.nlm.nih.gov/BLAST) and SeqScape™ v2.0 (Applied Biosystems, Foster City, CA, USA) software packages. Mutation was confirmed on a second DNA sample isolated from a duplicate tube of blood followed by sequencing in both forward and reverse directions. All nucleotide numbers refer to the wild type cDNA sequence of *CALR* (NM_004343) as reported in Ensembl.
Results: Electropherogram of *CALR* exon 9 of the patient was shown in figure 1. Due to a 1bp deletion in codon 372 c.1116delA (D373fs*57) and c.1120 A>C, reading frame has changed in codon 373 and there after.

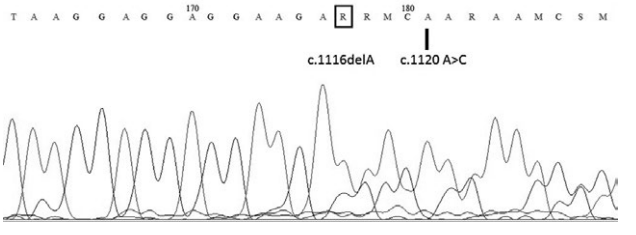

Figure 1.

Summary and Conclusions: To date 55 different CALR mutations have been described. Here we report two new *CALR* mutations [1bp deletion; c.1116delA (D373fs*57) and c.1120 A>C] in a same patient with PMF. These mutations have occured in exon 9 of the *CALR* gene, and changed the aminoacid sequence of C domain starting with aminoacid residue 372; which will interfere

with calcium binding capacity of the molecule. The identification of new *CALR* mutations will improve our understanding of the pathophysiology of MPN, and will help to find new theraupetic targets.

PB1913

ESSENTIAL THROMBOCYTEMIA IN CHILDREN – CLINICAL, HEMATOLOGICAL, MOLECULAR AND THERAPEUTICAL ASPECTS – SINGLE CENTER EXPERIENCE

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Background: Essential thrombocytemia (ET) is an extremely rare disease in children, with an incidence of 1-4 cases/10 million people/year. ET can be present as sporadic or familial disease and exclusion of secondary thrombocytemia is mandatory. There are few data about ET in children. Some studies showed a lower incidence of JAK2 mutation in children compared to adults.
Aims: The purpose of this study is to evaluate the clinical manifestations and follow-up of children with ET.
Methods: We performed a retrospective study of 5 children with ET, followed between 2008-2014 in the Fundeni Clinical Institute, Bucharest. Diagnosis consisted of: a) tests to exclude secondary thrombocytosis; b) tests to exclude other malignant thrombocytosis and c) specific tests: JAK2V617 PCR analysis, calreticulin, C-MPL, bone marrow examination, cytogenetic analysis. The WHO 2008 criteria for ET were used. Thrombopilia screening was performed with – PC, PS, AT, APC-R analysis; in case of positive screening for thrombophilia, PCR assay for MTHFR 677, FVL, FII was recommended.
Results: Five patients, 4F/1M were diagnosed with ET at ages 7- 15 years (median 8 years). One case was familial ET and 4 were sporadic ET. The platelet count at diagnosis was 700-2500x 10^9/L. Three cases showed extreme thrombocytemia (>900x109/L), 2 cases moderate thrombocytemia (700-900x10^9/L), one case with mild thrombocytemia (450-700x10^9/L). Clinical manifestations at diagnosis: 2 cases had cephaleea, 1 case cephaleea and severe abdominal pain, 1 with cerebral thrombotic event and hemiparesis and 1 asymptomatic case. Three out of 5 cases were JAK2 positive. BM biopsy revealed in all cases hypercellularity, giant megakaryocytes, absence of reticulin fibrosis. The karyoype was normal in all cases. One case was MTHFR heterozygote. Treatment consisted of α-Interferon followed by anagrelide, due to severe symptoms persistence, in a JAK2 negative case, first-line anagrelide in all 3 JAK2 positive cases and hematologic monitoring in the ET familial case. Anagrelide treatment reduced the platelet count in all cases, and controlled the symptoms, but 1out of 4 patients developed ischemic stroke despite treatment. No secondary effects were recorded.
Summary and Conclusions: We observed severe clinical symptoms in 4/5 pediatric ET cases. Some pediatric studies suggested that JAK2 positive cases have a severe course and could be considered distinct disorders. It is necessary to asses which child with ET really needs cytoreductive therapy, as already defined for adults.

PB1914

TYPE 1 CALR MUTATION IMPROVES DIAGNOSIS OF PREFIBROTIC IDIOPATHIC MYELOFIBROSIS (MF0)

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Background: CALR mutation discovery improves essential thrombocythemia (TE) and idiopathic myelofibrosis (MI) diagnosis. Over 36 CALR type mutations have been found, but only two types (type 1: del52bp and type 2: ins5bp) are present in 80% of the patients. It seems that type 1 mutation is frequently associated to fibrosis and that it has a worse impact on overall survival than type 2 mutation. Diagnosis of a prefibrotic state (MF0) presents histological diagnostic difficulties. MF0 has a worse prognostic impact than TE with regard to thrombotic risk, and a higher risk towards MI and acute leukemia evolution. For this reason a new type of identification of a group of patients is very useful.
Aims: The aim of this study was to evaluate CALR mutations in patients with MI and TE JAK2-negative and MPL-negative and the type of CALR mutations frequently associated to MF0.
Methods: We studied 47 patients CALR-positive, 21 with TE and 26 with MI. 12 out of 26 with MI were classified as MF0 on the basis of the histological examination of bone marrow biopsy. Debut features have been evaluated for WBC, Hb, PLT, LDH, peripheral CD34, spleen. Fragment analysis of PCR exone 9 CALR gene was performed.
Results: Type 1 mutation was present in 6 out of 21 patients (30%) with TE and in 19 out of 26 patients (73%) with MI. In the subgroup of MF0, 9 patients out of 12 (75%) were positive to del52bp. Conversely, type 2 mutation was present in 15 patients out of 21 (70%) with TE and in only 7 patients out of 26 (27%) with MI. Of the 12 patients with MF0, 3 patients (25%) were positive to ins5bp. Statistical analysis was negative for WBC, Hb, PLT, LDH, peripheral CD34 and spleen and not statistically significant between these 3 groups of patients.
Summary and Conclusions: It was recognized that CALR mutation has a better impact with respect to the complications of myeloproliferative neoplasm Ph1 negative. It is still unclear the role of del and ins mutations of CALR gene. In our experience, type 1 mutation is more representative in "fibrotic" phenotype as it is demonstrated the higher frequence of del 52bp in MF0 patients than in MI patients. This allowed us to better identify MF0 clinical features, in that a particular clinical attention for MF0 is required, being different from TE.
Keywords: MF0, CALR, CALR mutations, TE

PB1915

TET2 MUTATIONS IN ESSENTIAL THROMBOCYTHEMIA AND PRIMARY MYELOFIBROSIS

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Background: Myeloproliferative neoplasms other than CML are grouped as policytemia vera (PV), essential trombocythemia (ET) and primary myelofibrosis (PMF). By the identification of JAK2617f mutation in 2005; the explanation of the clonal proliferation in 90-95% of PV, 50% of ET and 50% of PMF have been possible. Thereafter JAK exon-12 mutations for PV and MPL mutations for ET and PMF have been defined. However the clonal proliferation in approximately half of the ET and PMF cases have been undetermined. One of the many mutations which could have a role in the pathogenesis is TET2 mutations. TET2 mutations were found positive in 15% of PV, 4-11% of ET and 19% of PMF patients. Some studies also showed that TET2 mutations have prognostic value.
Aims: Our aims are the determination and clinical relevance of TET2 mutations in patients with ET and PMF which are following in our clinic.
Methods: 35 ET ve 10 PMF patiens are included according to WHO criteria. Patients' demographic informations, clinical and laboratory findings were registered. DNAs were isolated from venous blood samplings and all exons of the TET2 gene were analyzed by Sanger based sequencing method. Informed consent was obtained from all patients. In addition to descriptive statistics, datas were evaluated for the comprasion of two groups with t test, chi-square test and Odds Ratio.
Results: TET2 mutations were found in 47,5% (16/35) of ET patients and 70% (7/10) of PMF patients. Six mutations were defined: I1762V, M1907T, L1721W, H1778A, C1298Y and Q1828X. Of these, L1721W and I1762V have possibility of being a genetic polymorphism. The frequency of TET2 mutation was not statistically different between sex and age. We demonstrated a correlation between initial spleen size and TET2 mutations for patients with ET (P=0.011). Increased bone marrow fibrosis is also found in bone marrow biopsy examination in TET2 mutation-positive ET patients (P=0,011). Furthermore, complications including arterial thrombosis and bleeding are found more frequent in TET2 mutations positive ET and PMF patients (for ET relatively OR:2,59, for PMF relatively OR:1,7, OR:1,6). Togetherness of JAK2V617F and TET2 mutations associates bigger spleen size (P=0,001) and increased risk of arterial thrombosis and bleeding in patients with ET and PMF (for ET, relatively OR:3,85, OR:2,8; for PMF, relatively OR:3,67, OR:3,67).
Summary and Conclusions: We herein found higher rates of TET2 mutations in patients with ET and PMF. Positivity of TET2 mutations are correlated with especially spleen size and the rate of fibrosis for patients with ET; and these mutations increase the risk of arterial thrombosis and bleeding. For patients with PMF, positivity of TET2 mutations increase spleen size and the risk of arterial thrombosis and bleeding. Togetherness of JAK2V617F and TET2 is associated with increased risk of complications. We conclude that to know the TET2 mutations in ET and PMF can give information about disease progression and complications thus may identify a high-risk subgroup of MPN patients.

PB1916

VORINOSTAT MAY OFFER LONG TERM CONTROL OF DISEASE IN THERAPY RESISTANT ESSENTIAL THROMBOCYTHAEMIA

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Background: Vorinostat, a histone deacetylase (HDAC) inhibitor has shown promise in a number of haematological malignancies including myeloma, lymphoma and myelodysplastic syndromes. Inhibition of histone deacetylases leads to arrest of tumor cell growth, differentiation and increased apoptotic cell death. The role of HDAC inhibitors in myeloproliferative neoplasms were described by Guerini *et al* (2008) who demonstrated the arrest of proliferation in polycythaemia vera (PV) and essential thrombocythaemia (ET) haemopoietic cells.

Aims: We describe our experience using Vorinostat as an alternative agent in therapy resistant ET.
Methods: A phase II study evaluating vorinostat in ET and PV reported early discontinuations due to adverse events, and poor tolerability (Andersen *et al* 2013). Two patients at our institution continued vorinostat in a compassionate use programme following this phase II study. We describe herein their on-going responses.
Results: Patient 1, a 54 years old male with *CALR* positive ET since 2001 complicated by a left internal capsule infarct, chronic kidney disease and leg ulcers whilst on hydroxycarbamide (HU). Despite dose escalation he was unresponsive to standard therapy with HU and anagrelide (ANA). On initiation of vorinostat 400 mg daily, his Haemoglobin (Hb) concentration was 103g/L (130 – 150g/L), leukocyte count 12.3x 10^9/L (4.0 – 11.0x 10^9/L), platelet count was 978x 10^9/L (150-400x 10^9/L), and creatinine was 151 umol/L (59-104 umol/L) (EGFR 42). Shortly following commencing on Vorinostat, there was a notable decline in his renal function to 201umol/L (EGFR to 29), thus a dose reduction to 300 mg daily was made with satisfactory control of platelet count. The only side effect he reported was dysgeusia, which did not impact on his activities of daily living. He has now been on Vorinostat for 51 months; over this time, the vorinostat dose was gradually reduced with overall stable platelet count control. His current maintenance dose is 100 mg/200 mg alternate days with a platelet count of 367x10^9/L at last clinic visit. Renal function has remained stable throughout therapy; the most recent creatinine level was 171umol/L (EGFR 36). The second patient, a 53 year old male also had CALR positive ET, diagnosed in 2004. He had a history of a previous cerebrovascular accident and was symptomatic from his ET; his main complaints were profound pruritus and fatigue. Both blood counts and symptoms were resistant to treatment with busulphan, HU, interferon and ANA. When vorinostat was initiated his Hb concentration was 128g/L, leukocyte count 5.3x10^9/L and an unstable platelet count that ranged between 315 – 947x10^9/L. To date the patient has been on vorinostat 52 months with minimal side effects. Vorinostat 300mg/400mg alternate days has reduced his symptom burden and maintained the platelet count below 800x 10^9/L with no thrombotic or haemorrhagic events. Renal function has remained stable; creatinine level on commencing therapy was 77umol/L (EGFR 92) increasing to 104umol/L (EGFR 64) at last clinical visit.
Summary and Conclusions: Although displaying two contrasting clinical courses on vorinostat, both patients have demonstrated a prolonged clinical response with symptom and platelet count control. Therefore, we conclude that vorinostat may offer selected patients resistant to standard therapy an alternative agent in ET when options are often extremely limited.

PB1917

UTILITY OF NEXT-GENERATION SEQUENCING FOR INTEGRATED MOLECULAR PROFILING OF PRIMARY MYELOFIBROSIS USING ION TORRENT PGM SYSTEM

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Background: In the last decade a number of genes have been reported to be recurrently associated with primary myelofiborsis (PMF). While some mutations such as *JAK2* V617F and *MPL* exon 10 mutations are easily detectable by conventional molecular genetics methods other mutations are more difficult for screening because of lower frequency and being scattered along large genomic ranges. On the other hand, newly developed approaches for next-generation sequencing (NGS) provide an affordable solution for targeted multiplex resequencing of up-to several hundreds of amplicons.
Aims: Here, we aimed at the development and validation of a novel custom panel for targeted resequencing of PMF samples using the Ion PGM™ System (Ion Torrent, USA).
Methods: We designed a pool of 424 primers for amplification of 212 amplicons covering 99.46% of the exonic regions of 9 human genes as follows: *ASXL1*, *EZH2*, *CALR*, *RUNX1*, *SETBP1*, *SF3B1*, *SRSF2*, *TET2* and *U2AF1*. Initial testing of the panel performance was done on Ion PGM™ machine using PGM™ 316 v2 chips on 8 DNA samples from PMF patients (6 males, 2 females, median age 61.5 years). Sequences alignment, variants calling and annotation were performed using Ion Reporter software.
Results: We identified a total of 22 nonsynonymos somatic coding variants in 7 out of 8 samples. The most frequently mutated gene was *TET2* (6 mutated samples out of 8). Two patients had mutations in *SRSF2*, and *CALR*, *EZH2* and *SETBP1* were found mutated in one sample each. No mutations in *RUNX1*, *SF3B1* and *U2AF1* were found. Overall, mutations rate was consistent with previous reports on PMF molecular profiling.
Summary and Conclusions: This small proof-of-concept study confirms the feasibility of Ion Torrent systems for resequencing of clinically relevant mutations in myeloid malignancies such as PMF. It can be particularly useful in cases without the most frequent clonal markers in PMF such as *JAK2*, *MPL* and *CALR* mutations.
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PB1918

HYPEREOSINOPHILIA: A CENTER'S EXPERIENCE

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Background: Hypereosinophilias comprise a very heterogenous group of haematologic alterations. They are usually secondary to diseases affecting various organ systems, although they may also have clonal or idiopathic aetiologies. The differential diagnosis is crucial and has significant implications that affect the therapeutic decision. According to the World Health Organization Classification (WHO) 2008, clonal hypereosinophilias are included in lymphoid and myeloid neoplasms associated with eosinophilia and platelet-derived growth factor receptor alfa (PDGFRA), platelet-derived growth factor receptor beta (PDGFRB) or fibroblast growth factor receptor 1 (FGFR1) abnormalities. Screening for rearrangements of these genes to document clonal eosinophilias, in which the tyrosine kinase inhibitor Imatinib has demonstrated effectiveness as a first-line agent, is fundamental since the treatment of reactive hypereosinophilias and idiopathic Hypereosinophilic Syndrome (HS) is, respectively, that of the underlying condition and systemic corticosteroids.
Aims: Analysis of patients with peripheral blood (PB) or bone marrow (BM) hypereosinophilia screened for PDGFRA and PDGFRB gene rearrangements and evaluation of response to treatment.
Methods: Retrospective analysis of patients with PB (>1500/uL) or BM (>5%) hypereosinophilia screened for PDGFRA and PDGFRB gene rearrangements, between 2008 and 2014.
Results: PDGFRA and PDGFRB gene rearrangements were screened in 23 patients with hypereosinophilia: of these 12 had secondary aetiologies and 11 were primary. Of the secondary eosinophilias, 4 were due to parasitic infestation, 4 allergic, 2 lung diseases, 1 systemic mastocytosis and 1 T cell lymphoma. Of the primary eosinophilias (11): 4 patients had PDGFRA rearrangements, 2 of them associated with T-ALL; 2 had PDGFRB rearrangements; 3 had HS; 1 had chronic eosinophilic leukemia and 1 myeloproliferative neoplasm. Out of the 6 patients with PDGFRA or PDGFRB rearrangements, 1 died before starting treatment, 1 had no criteria for therapy iniciation and all 4 treated with Imatinib (100-400mg/day) had good tolerance and hematologic response with 2 achieving molecular response. The median eosinophil count at presentation was 1780/uL (Q1 382.5; Q2 1787.5).
Summary and Conclusions: Our data is in agreement with the literature regarding the incidence of primary hypereosinophilias and the therapeutic efficacy of Imatinib, with response rates >80%.
Disclosure of Interest: none declared

PB1919

HOW DIAGNOSTIC AND THERAPEUTIC APPROACHE CHANGED IN THE LAST DECADES IN THE PHILADELPHIA NEGATIVE MPN PATIENTS OF THE REGISTRO ITALIANO TROMBOCITEMIE (RIT)

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Background: The last decade starts for the patients with Philadelphia Neg-MPN after the JAK2 mutations discovery (2005), the Italian guidelines for Essential Thrombocythemia therapy (2004), and the WHO classification (2001)
Aims: To evaluate how the diagnostic and therapeutic approach changed in the clinical practice in the last decade.
Methods: The patients of the Registro Italiano Trombocitemie (RIT) diagnosed before and after 2005 were compared by considering demography, clinico-biological characteristics at diagnosis, and the initial treatment approach.
Results: The 2418 analyzed patients were diagnosed (PVSG or WHO criteria) before 2005 (Group I, n 1230, 51%) and after 2005 (Group II, n 1188, 49%). The patients of Group II, as compared with those of Group I, had at diagnosis older age (median 60 vs 55 y, p60 y in 50% vs 41%, P<0.001), higher rate of comorbidities (56% vs 49%, p 0.002), and lower median PLT count (748 vs 786x109/L, P< 0.001). No significant differences were found for gender (males 38% vs 38%), prior thrombosis (20% vs 20%), prior hemorrhage (4.9% vs 5.6%), CV general risk factors (71% vs 68%), symptoms (41% vs 41%), splenomegaly (25% vs 23%), hepatomegaly (22% vs 24%), WBC count (median 8.7 vs 8.5x109/L; >10x109/L 28% vs 27%), and HCT level (median in males 44.8% vs 44.1%; median in females 41.5% vs 41.0%). The bone marrow biopsy in the first year was performed more frequently (76% vs 67%, P<0.001). The test for JAK2 mutation was performed in 89% of Group II patients, and, a posteriori, in 72% of Group I patients (P<0.001). The patients of Group II had a higher rate of JAK2 V617F mutation (62% vs 56%, p 0.015), and of allele burden<25% (65% vs 51%, P<0.01). Moreover, the patients of Group II had a higher rate of standard high thrombotic risk (57% vs 49%, P<0.001).During the first year after diagnosis, an antiplatelet (+ or-cytoreductive) treatment (almost always low dose aspirin) was started more frequently in the Group II (65% vs 48%, P<0.001), particularly (P<0.001) in patients at low thrombotic risk (standard and IPSET-Th). Similarly, a cytoreductive (+ or-antiplatelet) treatment was started more frequently in the Group II (57% vs 50%, p 0.002), particularly (74% vs 68%, p 0.013) in patients with high thrombotic risk (standard and IPSET-Th). In the Group II, as compared with Group I, the rate of hydroxycarbamide use was higher for patients over 60 y (71% vs 63%, P<0.01) and lower for those below 40 y (8% vs 14%, P<0.05), while the rate of anagrelide use was higher for patients below 40 y (6.4% vs 2.7%, P<0.05).
Summary and Conclusions: The patients of the RIT diagnosed after 2005, compared with those diagnosed before, although were older, had more frequently a bone marrow biopsy at diagnosis. They had lower platelet count, higher rate of JAK2 V617F mutation, and higher rate of high thrombotic risk. No significant differences were found for gender, prior thrombosis, CV general risk factors, symptoms, splenomegaly, WBC count. During the 1st year, they were treated more frequently with antiplatelet drugs (particularly if at low thrombotic risk), and with cytoreductive drugs (particularly if at high thrombotic risk). The hydroxycarbamide use increased in older patients, while the anagrelide use increased in younger ones. In conclusion, in the real life of the RIT centers, a significant improvement of the diagnostic and therapeutic approach was documented in the last decade.

PB1920

ATHEROSCLEROSIS AND RELATED FACTORS IN PATIENTS WITH PHILADELPHIA-NEGATIVE CHRONIC MYELOPROLIFERATIVE NEOPLASMS

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Background: Cardiovascular diseases,thrombohemorrhagic complications, bone marrow failure due to myelofibrosis and leukemic transformation are the major causes of mortality and morbidity in patients with Philadelphia negative chronic myeloproliferative neoplasms (MPNs). Chronic inflammation is also suggested to play a role in the development of atherosclerosis in this group of patients.
Aims: We aimed to evaluate the prevalance of subclinical atherosclerosis in patients with essential thrombocythemia and polistemia vera with the terms of conventional risk factors like lipid profile and carotid intima media thickness (CIMT).
Methods: 100 patients with Philadelphia negative chronic MPN and 50 age and gender matched healthy controls were enrolled in our study. We determined the lipid profile, fasting blood glucose, body mass index and CIMT of both groups.
Results: Mean age of patient group was 58.46 years (26-86 years). 44 were female (44%) and 56 were male (56%). In the control group, mean age was 58.68 years (37-67 years), 24 were female (48%) and 26 were male (52%). Duration of disease was 1 to 14 years (mean 5.81). In the medical history of patients venous thromboembolism was present in 10 patients (10%) and arterial thromboembolism was present in 28 patients (28%). 1 patient had diabetes (1%) and 34 patients had hypertension (34%). Levels of cholesterol, LDL, HDL, triglyceride, fasting blood glucose and body mass indexes were similar in both groups. Over 1 cm thickness was accepted as plaque. Plaque was observed on the right in 3 patients (%3) while on the left in 8 patients (%8). No patients had plaque on both carotid arteries. In the control group, 3 patient had plaque on the right carotid artery (6%) and 2 patient had on the left carotid artery (4%, total 10%). CIMT was related with age, disease duration, body mass index, levels of total cholesterol, LDL and absolute leucocyte count while not related with gender, fasting blood glucose, levels of HDL, triglyceride, hemoglobin, hematocrite and thrombocytes. Logistic regression analysis of age, disease duration and presence of plaque showed a relation with disease duration and presence of plaque (P=0.001). In patients with a history of arterial thrombosis, 6 had plaque (21.4%) while in patients with a history of venous thrombosis, 5 was observed to have plaque (50%), though no relation was observed with logistic regression analysis.
Summary and Conclusions: During the course of chronic MPNs, a chonic inflammatory process with thromboembolic complications is certain. Development of subclinical atherosclerosis is related with traditional risk factors like hypercholesterolemia as well as disease duration independent of age.

PB1921

RELATIONSHIP BETWEEN HUMAN PLATELET ANTIGEN-1 GENE POLYMORPHISM AND ASPIRINATED PLATELETS IN PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA

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Background: Essential thrombocythemia (ET) is a myeloid neoplasm characterized by platelet activation and thrombotic risk. Aspirin (ASA) is the standard therapy to prevent the thrombosis. It is reported that thrombocythaemic patients have ASA insentivity. It is debated if inherited thrombophilia induces the thrombocythemic platelet activation and, hence, the ASA platelet insensitivity.
Aims: Therefore, we evaluated human platelet antigen-1 (HPA-1) gene polymorphism, as thrombophilic molecular mutation associated with platelet hyperfunction, platelet count, b-thromboglobulin (b-TG) and platelet factor 4 (PF4), as markers of platelet activation, the platelet functional activity (PFA) as indicator of ASA platelet sensitivity and the maximum clot firmness (MCF), as indicator of aspirinated platelet contribution to clot firmness. We studied 48 patients (4 men, 8 women; mean age 51 years, range 32-70) with ET according to WHO criteria.
Methods: The mean duration of disease was 11 years. All patients were on ASA 100 mg once daily. Fifty subjects served as controls. The genotype HPA-1 was determined using a commercialized polymerase chain reaction kit with sequence-specific primers. Platelets were measured by automated analyzer. b-TG and PF4 were determined by ELISA. ASA platelet sensitivity and MCF were measured by Platelet Function Analyzer (PFA-100) and by ROTEM delta, respectively.
Results: Of 48 patients, 36 were HPA-1a/1a and 12 were HPA-1a/1b. The mean platelet count was 462±240x109/L. All patients had high b-TG and PF4 (244±15 IU/mL vs 20±11 IU/mL and 162±56 IU/mL vs 6±2 IU/mL, respectively) (P<.0001 and P<.0001, respectively), prolonged C/EPI closure time (CT, unit: s, n.v. 84-160 s) (252±48 s) and normal MCF (MCF, unit: mm, n.v. 50-72 mm) (71±2 mm).
Summary and Conclusions: These findings suggest that HPA-1 gene polymorphism does not inhibit aspirinated platelets sensitivity in patients with ET.

PB1922

BIOMARKERS FOR PREDICTION OF THROMBOSIS IN PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA (ET)

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Background: Arterial or venous thrombotic events occurs in 15% of essential thrombocythemia (ET) cases at presentation and in 10% to 20% during the disease course. The pathogenesis of thrombosis in ET is multifactorial and not yet clear. Prevention of thrombohaemorrhagic events is the important goal of the ET therapy.
Aims: The aim of this study was to examine biomarkers for prediction of possible prothrombotic condition in patients with essential thrombocythemia.
Methods: Diagnosis of ET based on the WHO criteria (2008). The study was approved by the Ethics Committee of the Medical University of Wroclaw and was performed in accordance with the Declaration of Helsinki. Each patient has been tested using Ceveron alpha (Technoclone) for fibrinogen (F) (Fibrynogen Reagent, Technoclone), factor VIII (Siemens test), D-dimer (Technoclone), pro-

tein C (PC) (TECHNOCHROM® Protein C, Technoclone), activated protein C resistance (APCR) (APC Restance Kit Technoclone), thrombin generation and microparticles (MPs) (TECHNOTHROMBIN® TGA (Technoclone). The activation markers thrombin-antithrombin complexes (TAT) and prothrombin fragment F_{1+2} (F1+F2) were determined by ELISA using kits from Enzygnost micro, Simens). Testing for the factor V Leiden mutation (FVL mutation) and prothrombin gene mutation was accomplished by the PCR on peripheral blood leukocyte DNA.
Materials: The study group consisted of 45 patients with ET (13 males and 32 females; mean age 61) and 45 healthy subjects (28 males and 17 females; mean age 62). The *JAK2V617F* mutation was present in 25 ET patients. Twenty-one patients experienced thrombotic events, 12 had arterial thrombosis (myocardial infarction in 6, TIA in 3, and peripheral arterial disease in 3 and 8 had deep venous thrombosis in 8 patients. Out of 45 ET patients 35 were treated with anagrelide and 8 with hydroxyurea.
Results: In ET patients the median level D-dimer was significantly higher compared to control (183.2 vs 18.5 ng/mL; P=0.00018), but not fibrinogen level, TAT, F1+F2, FVIII activity and protein C activity. Neither thrombin generation (TG) nor microparticles (MPs) were elevated in ET patients. Five of 45 ET patients had the activated protein C resistance (APCR), and in two of them the heterozygous type of FVL was confirmed. Prothrombin gene mutation was not present in the ET group.
Summary and Conclusions: Conclusions: D-dimer has been shown as a valuable biomarker for prediction of thrombotic events in patients with essential thrombocythemia, and the knowledge of carriership of FVLeiden can be used in the individual thrombosis risk assessment.

PB1923

CLINICOPATHOLOGIC CHARACTERISTICS OF UNCLASSIFIABLE MYELOPROLIFERATIVE NEOPLASMS

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Background: According to WHO classification 2008, myeloproliferative neoplasm, unclassifiable (MPN-U) is defined as neoplasms that has MPN features but fails to meet the criteria of any specific MPN entities, or that has two or more characteristics of MPN entities. Accurate diagnosis of each MPN subgroup is important because each subgroup could show different clinical courses; leukemic transformation rate at 20 years is estimated at<10% for polycythemia vera (PV) and<5% for essential thrombocythemia (ET) and fibrotic transformation rates are slightly higher (Tefferi A. Am J Hematol 2015;90:162). The prognosis of primary myelofibrosis (PMF) is significantly poor, compared with those of PV or ET.
Aims: The clinicopathologic characteristics of MPN-U are needed to be cleared so that we could assign MPN-U to specific subtypes, if possible. The purpose of this study was to identify characteristics of laboratory findings, bone marrow pathology, genetic aberrations and clinical course in MPN-U.
Methods: The study group included 8 patients diagnosed as having MPN-U based on WHO 2008 criteria from 2000 to 2014. Median follow up period was 59 months (range 8-91 months). The mutation analysis of JAK2, MPL and CALR was performed by real time PCR or direct Sanger sequencing. Whole genome single nucleotide polymorphism array (SNP-A) (SNP 6.0, Affymetrix, CA) based karyotyping and target exome sequencing (Ion AmpliSeq Comprehensive Cancer panel, Life Technologies) were performed according to manufacturer's instructions.
Results: Five patients with thrombocytosis (450-991x10^6/L) and normal or mild leukocytosis(7.6-14.9x10^6/L) showed upper normal hemoglobin (16.1-17.4 g/dL in 2 men, 15.9-16.1 g/dL in 3 women), but, not met PV criteria of WHO 2008 (Hb >16.5 g/dL for women and 18.5 g/dL for men). Hypercellularity with trilineage proliferation for ages was found in 4 patients and megakaryocytic atypia was found in 1 patients. All patients showed normal karyotype and *JAK2 V617F* mutation. Of them, two patients showed one or more mutations in addition to *JAK2* : 1 patient had *JAK2, DNMT3A, and C-KIT*; 1 patient *JAK V617F* and *CSF1R*. In the other hand, a 73-year old female patient with high hemoglobin (Hb 16.3 g/dL) had neither *JAK2 V617F* nor *CALR* mutation and the laboratory findings were as follows; WBC 15x10^6/L, platelet 1,290x10^6/L, low levels of erythropoietin and panhyperplasia with megakaryocytic atypia. There was no one progression showing hemoglobin levels which met the criteria for typical PV during follow up period. Taken together, these patients didn't meet the hemoglobin criteria for PV, but we can't exclude the possibility of pre-PV phase. In two patients developed myelofibrosis, the initial laboratory findings met neither the criteria of PMF nor ET fully. One patient (55-year old female) developing myelofibrosis at 33 months after initial diagnosis showed *CALR* mutation and copy neutral-loss of heterozygosity of 19p13.3-p13.12 region (including *CALR* gene) by SNP-A based karyotyping. The other laboratory findings were as follows: Hb 13.8 g/dL, WBC 6x10^6/L, platelet 1,626x10^6/L, increased LDH, normocellularity for ages and megakaryocytic atypia. The other patient (62- year old female) developing myelofibrosis at 6 months showed dual mutations of *MPL* and *SF3B*1, Hb 13.0 g/dL, WBC 17x10^6/L, platelet 930x10^6/L, increased LDH, hypercellularity for ages, but not significant megakaryocytic atypia. These findings suggested that these two patients may be prefibrotic stage of PMF at initial diagnosis.
Summary and Conclusions: This study suggests that MPN-U could include pre-PV or prefibrotic-PMF. Therefore, the pathologists need to assign a more specific diagnosis in MPN-U and further follow up study is recommended to provide a more precise classification in MPN-U patients.

PB1924

IS THERE A ROLE FOR A NEW REGISTRY IN PV? EARLY ASSESMENT OF A NEW MPN REGISTRY IN HUNGARY

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Background: A new MPN registry,based on the database of available patient data and a disease specific questionnaire were conducted and used in several Hungarian hematology centers.
Aims: The aim of the survey was to collect data of Philadelphia negative MPN patients, in order to establish a Hungarian MPN registry.
Methods: There was close cooperationbetween hematologists and information technicians (IT) experts to create an easily evaluable questionnaire. Data of classical MPN patients were collected from Hungarian centers. The thrombotic risk and the risk adapted treatment characteristics were stratified according to the Landolfi criteria. In this review the data of 408 patients (57% male) registered as having PV,were analysed. The data entry (Table 1)started at 2014 and the centers entered their patients data, diagnosed between 1989-2014.
Results: There were 820 evaluable MPN patients till the initial assesment deadline(15th Feb 2015) In the 408 evaluable PV pts the average time from diagnosis was 7.6 years, and about 50% of them were diagnosed more than 5 years ago. The number of evaluable cumulative patient years was 3016.
Among the 408 PV patients there were 80%(327) JAK2 V617F positive,13%(52) of them were JAK2 V617F negative. JAK status was missing in 7%(29)of patients.The detailed analysis of the JAK negative group revealed that 6 of them had an elevated hemoglobin + 2 positive minor criteria, fulfilling the 2008 WHO criteria, therefore they had proven PV.The other 67 patients did not fulfill the WHO diagnostic criteria of PV,they were identified as …insufficient" and therefore their data have not been included into the present evaluation. According to the Landolfi thrombotic risk stratification there were 73 patients stratified as low/moderate risk. They have to be treated with phlebotomy and ASA only. 44 (60%) of them were treated with phlebotomy and only 25(34%) with ASA. 45(62%) of them were treated with cytoreductive drugs-mainly with hydroxyurea 36pts(49%), 1 with a JAK inhibitor,1 with anagrelide,and 1 with HU+anagrelide+ASA combination. Seven pts.(10%) received interferon-alfa. Among them 8 thrombotic events and no transformation was observed, thus, according to the Landolfi risk treatment criteria, patients in this group were overtreated. In the high /extremely high risk group there were 260 patients. 35% had thrombotic events prior to diagnosis. 32% had a thrombotic event after the diagnosis. Disease transformation has been found in 10% of the patients (MF 22 pts, AML 2 pts, lymphoproliferative neoplasia in two patients.) 6% of registered PV patients had bleeding episodes

Table 1: **PV patients, year of diagnosis**

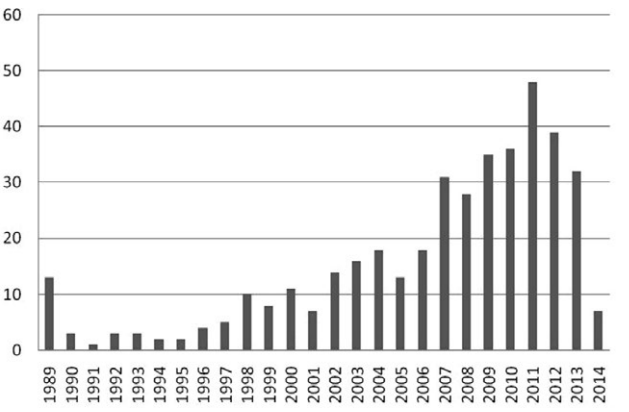

Figure 1.

Summary and Conclusions: The main aim of the questionnaire was to create a national registry of MPN patients and to help our colleagues how to stratify the thrombotic risk and to choose the proper treatment. To date it remains unanswered whether low risk patients need cytoreductive treatment to prevent

thrombotic events? Our data show an increase of thrombotic events in low risk patients treated with cytoreductive drugs (8 pts treated with cytoreductive drugs *versus* no patient treated with phlebotomy and ASA), thus arguing against treating these patients with cytoreductive drugs.Our results show the risk of thrombotic and hemorrhagic complications in PV to be comparable to published results. Based on our data we are convinced that the new national registry helps to give a good feedback about our diagnostic and treatment habits in PV.

PB1925

EARLY EXPERIENCE WITH THE ESTABLISHMENT OF A REGISTRY FOR PHILADELPHIA-NEGATIVE CHRONIC MYELOPROLIFERATIVE NEOPLASMS (MPN) IN HUNGARY

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Background: Considering and realising the remarkable heterogeneity of MPN care in Hungary an MPN Working Group has been established in year 2012.
Aims: The Hungarian MPN Working Group created our MPN Registry in 2013, the aims were: (1) to gain epidemiological, diagnostic, therapeutic data, to follow up complications and disease transformations. (2) to investigate the adherence to the WHO/2008 diagnostic criteria and to the Landolfi therapeutic guidelines, to gain insight into vascular and haematological complications. (3)Try to identify crucial issues and possible gaps, and promote internationally accepted, standard care in MPN.
Methods: The basis of the Hungarian MPN registry was the former Regional Centre of the Hungarian Academy of Sciences, Veszprém. The questionnaire had been thoroughly updated regarding the 2008 WHO diagnostic criteria (morphology, mutations, etc.)with focus on complications, risk stratification and treatment. The electronic platform can be continuously updated as needed by our steering committee (new molecular results *e.g.* calreticulin mutations can be included). All haematologists using the system are entitled to initiate search and association analysis. Our MPN Registry is legally permitted by our authorities (ETT-TUKEB).
Results: During the two active years of the Hungarian MPN Registry 15 of our major or smaller haematological centers provided patient data, alltogether reaching the evaluable patient number of 820. Even if some major centers data are still missing probably our data are representative enough to characterize MPN patient care in our country. The most important data are summarised in Table. I. The complex multiparametric analysis of vascular events (venous, arterial, minor, major) correlation with haematocrit, cell counts (platelet, leukocyte, monocyte), Landolfi score, traditional vascular risk factors, treatment modalities will be presented in details. We detected some shortcomings regarding the diagnostic evaluation of some JAK2V617F negative PV and some myelofibrosis cases, this will be shown in a separate poster (M. Egyed *et al.*) Treatment modalities have been analysed in depth, both regarding treatment modalities (*i.e.* phlebotomy, ASA, cytoreductive drugs, interferons, JAK inhibitors,etc.) and in the context of cell counts, vascular complications and risk stratification. Even if the observation was short, we found interesting transformation patterns in PV (408 patients): myelofibrosis 22, MDS-AML in two patients, secondary lymphoproliferative disease in two patients.

Table 1.

Table 1: **Data of the registry for Philadelphia-negative chronic myeloproliferative neoplasms (MPN) in Hungary**

Diagnosis	Number of registered patiens	Gender female/male	JAK2 V617F positive *	JAK2 V617F negative *	Thrombotic event before diagnosis JAK2+/JAK2-	Vascular event during follow up JAK2+
PV	408	176/232	327	52	90/12	81
ET	337	229/108	218	103	42/21	27
MF	30	15/15	14	12		
Complex, overlap	45	28/17				

* Till the initial assesment deadline (15,Febr.2015) some data are missing

Summary and Conclusions: 1. We have created a national MPN Registry covering a large part of Hungary. The database is operational, online, user-friendly, easily adjustable to the new professional needs. It is convenient for complex search, correlation and other multiparametrical analysis. The data collected so far are in concert with the international epidemiological data. 2. In addition, it gives a valuable reflection on the adherence to the diagnostic and therapeutic standards, revealing some heterogenities. The Hungarian MPN Working Group decided to help small centers to render better diagnostic possibilities (rare genetic alterations and determination of von Hippel Lindau mutation). The Steering Committee tries to facilitate patient enrollment to new MPN drug multicenter trials, too. 3. The early results of our database clearly show that important correlations could be made or further confirmed regarding vascular events, cell counts, monocyte number, etc. In spite of the short observation period patients with non-myeloid transformation were also found deserving further attention.

PB1926

A HOMOZYGOSITY DEVELOPMENT IN THE PATIENT WITH THE JAK2 EXON 12 N542-E543 DEL MUTATION DETECTED BY «PYROMARK Q24» PYROSEQUENCING

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Background: An increase of allele burden and development of homozygosity with the JAK2 gene V617F mutation is well-known in polycythemia vera (PV) patients (Vannucchi AM et al, Leukemia 2008). However, the homozygosity development of specific for PV mutations in JAK2 exon 12 has been much less studied.
Aims: The aim of this study was detection and quantification of JAK2 exon 12 allele burden in JAK2 V617F- negative PV patients using a pyrosequencing method.
Methods: The 10 DNA samples from JAK2 V617F- negative patients with confirmed clinical diagnosis and bone marrow morphological features have been selected. The informed consents from these patients were obtained. The nucleotide sequencing of the JAK2 exon 12 fragment was performed by pyrosequencing method as described in (Mironov K et al, Spravochnik zav clin lab, 2011). The routine sample preparation and PCR kits («AmpliSens», Russia) we used. Pyrosequencing was carried out with «PyroMark Q24» using «PyroMark Gold» kit («Qiagen», Germany). To verify the presence of mutations, the DNA sequences extracted from the clinical samples were cloned into pGem-T vector using standard protocol («Promega», USA), and obtained clones were sequenced using reagents and equipment of the «Applied Biosystems» (USA).
Results: Among 10 of JAK2 V617F-negative PV patients, a N542-E543del mutation of JAK2 in exon 12 has been detected in one 62-year old male patient. On the onset of the disease in July 2013 he had a pronounced plethoric syndrome, Hb 204 g/l, HCT 49.9%, RBC $6.43x10^{12}$/l, WBC $8.0x10^{9}$/l, PLT $250x10^{9}$/l. The level of JAK2 exon 12 N542-E543del allele burden was 19%. The patient was treated phlebotomy only and did not received any cytoreductive treatment during 18 months. After these 18 months, the patient characterized with presence of moderate splenomegaly, level of Hb 171 g/l, HCT 61.3%, RBC $9.03x10^{12}$/l, WBC $7.47x10^{9}$/l, PLT $500x10^{9}$/l. The level of JAK2 exon 12 N542-E543del allele burden was increased up to 68%.
Summary and Conclusions: A pyrosequencing with «PyroMark Q24» was able to identified mutations in JAK2 exon 12 and it demonstrated a possibility that N542-E543del mutation is evolving into homozygous form in the same manner as described for the JAK2 V617F mutation.

PB1927

SCREENING PCR FOR DETECTION OF CALR MUTATIONS IN PATIENTS WITH THROMBOCYTOSIS

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Background: *JAK2* and *MPL* mutation play an important role in diagnosis of myeloid proliferative neoplasm (MPN). Another key marker in the molecular diagnosis of MPNs, mutations in *CALR*, was recently reported. Known mutations are all located at exon 9 and they are somatic mutations of deletion or insertions; the two commonest abnormalities account for 80-90% of *CALR* mutations, whereas lesions are highly heterogeneous in the remaining 10-20%.
Aims: Almost previous reports analyzed *CALR* mutations using Sanger sequencing. However, sequencing method can't be used easily in every laboratory condition. Authors want to make an easy system to detect *CALR* mutations which is available in small laboratory without sequencing machine. We investigated the screening PCR to detect *CALR* mutations in Korean patients with thrombocytosis and compared the results with Sanger sequencing.
Methods: Bone marrow DNA samples were obtained from 83 patients with thrombocytosis. Screening PCR primer sets were designed to detect the wild

type (product: 357 bp), type 1 mutation (product: 302 bp) and type 2 mutation (product: 262 bp) in one reaction (Figure 1). And Sanger sequencing was done in hot spot region.

Results: *JAK2V617F* was detected in 36 patients (33 ET, 1 PMF, 1 MPN, U, and 1 MDS/MPN, U) (Table 1). Two kinds of *CALR* mutation, type 1 (c.1092_1143del) and type 2 (c.1154_1155insTTGTC) were detected in 16 ET and 1 PMF patients. In ET group, mutational frequencies were 62.3% for *JAK2V617F* and, 30.2% for *CALR*, 5.7% for dual positive and 13.2% for dual negative. Among the ET patients without *JAK2V617F*, frequency of *CALR* mutation was 80%. The frequency of type 2 *CALR* mutation (8/16, 50.0%) was higher than type 1 (6/16, 37.5%). Interestingly, one patient with both mutations (1/16, 6.3%) was identified and novel mutation pattern (c.1123_1132delinsTGC) was detected by only Sanger sequencing. Any mutation was not detected in reactive disease group.

Table 1. The frequency of JAK2 and CALR mutations in different patient groups

Disease	ET	PMF	MDS/MPN, U	MPN, U	HES	Reactive	Total
	N=53	N=3	N=3	N=2	N=1	N=21	N=83
JAK2 mutation, N (%)	33 (62.3%)	1 (33.3%)	1 (33.3%)	1 (50.0%)	0 (0.0%)	0 (0.0%)	36 (43.4%)
CALR mutation, N (%)	16 (30.2%)*	1 (33.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	17 (20.5%)
Dual positive, N (%)	3 (5.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	3 (3.6%)
Dual negative, N (%)	7 (13.2%)	1 (33.3%)	2 (66.7%)	1 (50.0%)	1 (100.0%)	21 (100.0%)	34 (41.0%)
CALR mutation (%) in *JAK2* (-) patients	80%	50%					

**Type 1: 6 patients (37.5%); Type 2: 8 patients (50.0%); Type 1 & Type 2: 1 patient (6.3%) and novel mutation: 1 patient (6.3%)*

Abbreviation: ET; essential thrombocythemia, PMF; primary myelofibrosis, MDS/MPN,U; myelodysplastic syndrome/myeloproliferative neoplasm, unclassifiable, MPN,U; myeloproliferative neoplasm, unclassifiable, HES; hypereosonophilic syndrome, Reactive; reactive disease group with non-hematologic malignancy.

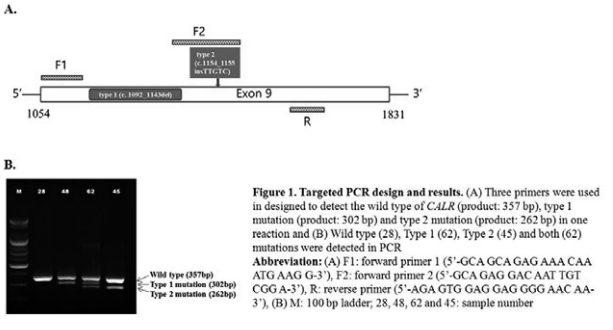

Figure 1. Targeted PCR design and results. (A) Three primers were used in designed to detect the wild type of *CALR* (product: 357 bp), type 1 mutation (product: 302 bp) and type 2 mutation (product: 262 bp) in one reaction and (B) Wild type (28), Type 1 (62), Type 2 (45) and both (62) mutations were detected in PCR
Abbreviation: (A) F1: forward primer 1 (5'-GCA GCA GAG AAA CAA ATG AAG G-3'), F2: forward primer 2 (5'-GCA GAG GAC AAT TGT CGG A-3'), R: reverse primer (5'-AGA GTG GAG GAG GGG AAC AA-3'), (B) M: 100 bp ladder; 28, 48, 62 and 45: sample number

Figure 1.

Summary and Conclusions: Screening PCR could detect the 94.1% (16/17) of mutation cases, and this method showed the concordant result with Sanger sequencing in the case of type 1 and type 2 mutations. One novel mutation was only identified with Sanger sequencing. Our system can't detect single base pair substitution or small base insertions like the mutation of this case; however, the frequency of these mutations is very rare. Therefore, this Screening PCR would be useful in small laboratory condition as a screening test for detection of *CALR* mutations.

PB1928

MYELOPROLIFERATIVE NEOPLASMS: TREATMENT APPROACH AND OUTCOMES, THE DREXEL University EXPERIENCE

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Background: Myeloproliferative neoplasms are a group of clonal disorders that arise from a transformation in a hematopoietic stem cell. These disorders consist of essential thrombocytosis (ET), primary myelofibrosis (PMF) and polycythemia vera (PCV). Several therapeutic agents have been used in the past to treat these disorders. Treatment strategies for these patients must consider the possibility of long-term survival, morbidity from thrombotic complications, transformation into myelofibrosis with myeloid metaplasia or acute myeloid leukemia, and the effect of specific therapies on the incidence of leukemic transformation and on pregnancy.

Aims: At Drexel University, a significant number of patients were treated with busulfan and were thought to have a more favorable clinical course and increased survival in comparison to other agents. In our study we analyzed the outcomes of patients treated in the practice of I. Brodsky Associates diagnosed with ET, PCV and PMF, who received a variety of treatment modalities, and compared their clinical courses to determine if there is a superior treatment.

Methods: This study is a retrospective cohort study in which we examined the medical records of patients treated for the diagnoses of ET, PCV and PMF at Hahnemann Hospital-Drexel University College Medicine in the practice of I. Brodsky Associates from January 1960 to December 2013. The following variables were measured and compared: age of the patient; sex of the patient; demographics; cytogenetics; family history; baseline hemoglobin level, hematocrit level, platelet count and WBC count; JAK 2 V617F mutation status; initial erythropoietin level, red cell mass, oxygen saturation, presence of splenomegaly and B12 level; Bone marrow biopsy results; thrombohemorrhagic complications; transformation to acute leukemia, progression to myelofibrosis and development of secondary malignancies; and treatment with busulfan, aspirin with or without clopidogrel, anegralide, hydroxyurea, and phlebotomy
Results: One hundred ninteen patients charts were reviewed. Twenty-four patients were given aspirin. One progressed to myelofibrosis (4%), seven patients were noted to have recurrent thrombotic episodes in the form of TIA, CVA, DVT/PE, and MI (29%). Thirty four patients were given Busulfan. Four patients progressed to myelofibrosis with MM (11%) and one patient progressed to CML (3%). Six patients were noted to have thrombotic events in the form of CVA, MI, subclavian vein thrombosis and carotid artery stenosis (17%). Twenty six patients were given hydroxyurea. One patient's course was complicated by Hodgkin's lymphoma (4%), four patients progressed to acute leukemic blast crises (15%), and three patients progressed to myelofibrosis (11%). Eight patients were noted to have multiple thrombotic events in the form of CVA, DVT, TIA, splenic vein thrombosis, and renal artery stenosis (31%). Twenty four patients were given Busulfan and Hydroxyurea together during their course of treatment. Fourteen patients were noted to have multiple thrombotic events in the form of PVT, SMV thrombosis, PE, TIA, Budd Chiari syndrome and LE arterial thrombosis (58%). Two patients progressed to CML blast crises (8%) and three patients developed myelofibrosis with MM (12%). Eleven patients were treated with only phlebotomy. Five patients progressed to myelofibrosis with MM (45%). Two patients were noted to have thrombotic events that included MI or TIA (18%)
Summary and Conclusions: Our study set out to discover if this unique treatment correlated with improved survival and less treatment toxicity. Busulfan and hydroxyurea given together proved to have the lowest rate of progression to leukemia and myelofibrosis when compared to other standard therapies. Patients treated with hydroxyurea and intermittent busulfan, were shown to have the best long-term outcomes. This suggests that physicians should include the use of busulfan in treating myeloproliferative neoplasms

PB1929

THROMBOTIC AND BLEEDING PROFILE IN PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA (ET)

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Background: ET is a chronic myeloproliferative neoplasm, characterised by the presence of thrombohemorrhagic events (THE), the evolution to myelofibrosis and acute myeloid leukemia. The mutation of JAK2 exon 14 V617F is detected in 70% of the patients, and it is related to more THE and a higher number of leukocytes and platelets.
Aims: To analyze the thrombotic (T) and bleeding (B) clinical and analytical profile in patients with ET.
Methods: Clinical files of a sample of patients diagnosed of ET from 1990 to 2014 in our hospital, and that were evaluated in our center consultations for 12 months were reviewed. Demographic and analytical variables, type and THE's location, and the treatment received were analyzed.

Table 1.

		JAK 2		
		NEGATIVE	POSITIVE	NOT AVAILABLE
THROMBOSIS				
PRE DIAGNOSIS	YES	3	15	5
	NO	39	36	3
POST DIAGNOSIS	YES	5	9	2
	NO	37	42	6
HEMORRHAGES				
	YES	4	3	0
PRE DIAGNOSIS	NO	38	48	8
POST DIAGNOSIS	YES	7	2	0
	NO	35	49	8
ARTERIAL*				
PRE DIAGNOSIS		1	11	3
POST DIAGNOSIS		5	7	2
VENOUS*				
PRE DIAGNOSIS		2	4	2
POST DIAGNOSIS		0	2	0

Table 1. (*) Ratio arterial/venous 2,9

Results: 101 patients were valued, 34 ♂ (33.7%) and 67 ♀ (66.3%). *Mean age at diagnosis* 59.32 (♂ 56.53 and ♀ 60.73). A higher prevalence of male (38.2% *vs* 19.4%) was o*bserved in patients between 45 and 65 years,* in contrast to the group of ≥65 years where prevalence in women was higher (65,7% *vs* 52,9%).

39 events T in 35 patients, 29 arterial (74.3%) and 10 venous (25.7%) which is shown in the data table. T pre diagnosis: 6 in legs, 10 brain, 5 coronary, 1 thrombosis in lower cava, 1 central retinal vein, 78 without thrombosis. Post-diagnosis: 1 in legs, 5 brain, 9 coronary, 1 thrombosis in lower cava, 85 without thrombosis. 51 patients (50.5%) were JAK2 positive (+) and 24 of those presented T (61.5% of the total T), with regard to 7 in group JAK2 negative (-) (results were not available in 8 patients). 16 T (41.02%) were observed during the evolution of disease in 12 patients (11.9%), 4 of these patients didn´t received cytorreductive treatment, 14 antiplatelet therapy and 2 anticoagulantion. B was observed in 16 patients (5 JAK2 +/11 JAK2 -) mainly located in ORL mucous membranes and genitourinary. At diagnosis *no significant differences* were *observed* between hematologic values and development of T, however, patients who had developed B events had a higher platelet counts (>900x 10^9/L).
Summary and Conclusions: THE define the natural evolution of ET. B events, as shown in literature, are more frequent on mucous membranes levels and in patients with higher platelet counts. We observe a higher frequency of these events in patients JAK2-. A third of the patients developed T events in our series reaching values around 50% in JAK2+, with an incidence of arterial T similar to prospective studies. Despite antithrombotic prophylactic treatment after diagnosis and during the evolution of the disease, 10% of the patients developed T which suggests that a better stratification and control the T risk is needed.

PB1930

JAK-INHIBITORS COULD INDUCE HEPCIDIN DOWNREGULATION IN PATIENTS WITH MYELOFIBROSIS

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Background: Iron homeostasis is dysregulated in primary myelofibrosis (PMF), given the high prevalence of anemia, need for red blood cell (RBC) transfusions, and disease-associated inflammatory state. Recent data published by Pardanani *et al.* (Am. J. Hematol. 88:312–316, 2013) show that hepcidin levels in patients diagnosed with Myelofibrosis (MF) are considerably elevated (MF patients median 156,3 pg/mL, Healthy patients median 13,5 pg/mL) and are strongly correlated with serum ferritin levels and even increased hepcidin levels were associated with shortened survival.
Aims: To appraise serum hepcidin levels in MF patients treated with JAK inhibitors.
Methods: Plasma hepcidin levels were measured in six patients diagnosed with Myelofibrosis, all of them with intermediate-2 or high IPSS-risk, were on treatment with a JAK-1/JAK-2 inhibitor (Ruxolitinib) at least 6 months prior. All patients with transfusion dependence were receiving iron chelation therapy (except patient 1). The methodology for hepcidin measurement was DRG Hepcidin-25 (bioactive) ELISA kit. It is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the principle of competitive binding (DRG Instruments GmbH, Germany). The microtiter wells are coated with a monoclonal (mouse) antibody directed towards an antigenic site of the hepcidin-25 molecule. Endogenous hepcidin-25 of a sample competes with a hepcidin-25-biotin conjugate for binding to the coated antibody. After incubation, the unbound conjugate is washed off and a streptavidin-peroxidase enzyme complex is added to each well. After incubation, unbound enzyme complex is washed off and substrate solution is added. The blue color development is stopped after a short incubation time, turning the color from blue to yellow. The absorbance of each well is determined at 450 nm with a microtiter plate reader. The intensity of color developed is reverse proportional to the concentration of hepcidin in the sample. A standard curve is calculated using a 4PL (4 Parameter Logistics) curve fit. The concentrations of the samples can be read directly from this standard curve. Data from serum ferritin level, transferrin saturation index and transfusion dependence was collected from their medical records.
Results: The results are summarized in the table.

Table 1.

	Serum Hepcidin	Serum Ferritin	Transferrin Saturation Index	Transfusion requirements
Patient 1+	94 ng/mL	8825 ng/mL	107.2%	4 units / month
Patient 2	12 ng/mL	223 ng/mL	45.4%	
Patient 3	40 ng/mL	1235 ng/mL	95.6%	3 units / month
Patient 4	24 ng/mL	116 ng/mL	20.9%	
Patient 5+	138 ng/mL	2091 ng/mL	60.4%	4 units / month
Patient 6	23 ng/mL	862 ng/mL	43.9%	

**median serum Hepcidin in control cohort: 9.8 ng/mL.*
+Dead.

Summary and Conclusions: In our cohort of patients diagnosed with Myelofibrosis we can observe elevated levels of hepcidin and its direct correlation with serum ferritin levels. In turn, we realize the hepcidin levels represent a negative prognostic factor in survival of these patients. Comparing hepcidin levels observed in our patients with the data published by Pardanani et al we can infer that Ruxolitinib plays a role in lowering serum hepcidin level.

PB1931

LEUKOCYTOSIS AS A RISK FACTOR FOR THROMBOTIC COMPLICATIONS IN PATIENTS WITH PHILADELPHIA (PH)-NEGATIVE MYELOPROLIFERATIVE NEOPLASMS

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Background: Myeloproliferative neoplasms (MPNs) are a group of clonal hematopoietic stem cell malignancies, characterized by overgrowth of one or more blood lines with normal or nearly normal maturing of those cells in the bone marrow and extramedullary hematopoietic organs. MPNs are acquired prothrombogenic conditions. The mechanism of increased predisposition to thrombosis in myeloproliferative neoplasms is not clear enough. Elevated leukocyte count is an independent prognostic factor for the development of thrombosis. It is considered that activated neutrophils bind platelets influencing the increased expression of tissue factor and activation and damage of endothelial cells, especially in JAK2V617F-positive patients.
Aims: The aim of this paper is to monitor the leukocyte count as a potential risk factor for the development of thrombotic complications with patients with Philadelphia-negative Chronic Myeloproliferative Neoplasms.
Methods: During the five-year period we followed up the occurrence of thrombotic complications and the leukocyte count in 109 patients of both sexes aged between 30 and 78 years, with a diagnosis of Ph-myeloproliferative neoplasms. Patients were classified into the following groups: 1. Group with Polycythemia Vera (PV) (51); 2. Group with Essential Thrombocythemia (ET) (28); 3. Group with Idiopathic Myelofibrosis (IMF) (21); 4. Group with myeloproliferative neoplasm unclassified (MPNs) (29). We used methods of clinical, laboratory, ultrasound and CT scans.
Results: The leukocyte count ranged from 2,2-17,1x10^9/L. The highest leukocyte count was recorded in the group of patients with PV and MPNs ($P<0.001$) and the lowest in the group of patients with the IMF ($P<0.01$). Between the group of patients with PV and MPNs there were not any statistically significant differences noticed in the leukocyte count. The highest percentage of thrombotic complications (arterial and venous) is in a group of patients with ET and MPNs ($P<0.01$) and then in the group with PV ($P<0.05$). Thrombotic complications in those groups were more frequent in percentage with patients with leukocytosis, but statistical significance was found only in the group with MPNs.
Summary and Conclusions: Leukocytosis may be considered as a potential risk factor for thrombosis in patients with myeloproliferative neoplasms, especially with PV, ET and MPNs. It is necessary to continue follow-up of these patients and to increase the number of subjects as well. Follow-up of the patients with neoplasms unclassified is of special importance, which showed a high prevalence of leukocytosis and thrombotic complications, and all with the aim to continue their further differentiation.

PB1932

TREATMENT WITH RUXOLITINIB IN PATIENTS WITH PRIMARY OR SECONDARY MYELOFIBROSIS: SINGLE CENTER EXPERIENCE

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Background: Myelofibrosis is one of the classical Philadelphia negative myeloproliferative neoplasms (MPNs), either appearing de novo (primary MF[PMF]) or following a previous ET or PV (post-ET or post-PV MF). The disease is essentially the same. The therapeutic landscape of MF has changed with the introduction of JAK inhibitors.
Aims: To evaluate the efficacy and safety of ruxolitinib treatment in patients with MF.
Methods: Eighteen patients with MF (12 male and 6 female), median age 62.5 (33-75) years old were evaluated: 7 with PMF, 5 post PV, 3 post ET, 3 post MPN-U. Median follow up since ruxolitinib initiation was 4 (1-33) months, whereas total follow up since diagnosis was 5 (2-19) years. JAK2 mutational status was available in 14/18 patients, 12/14 being positive for the V617F mutation. Cytogenetic study was available in 12/18 patients, 8 of them having normal karyotype, 2 with deletion of long arm of chromosome 20 [del(20)(q13.1)], one with trisomy 8 and one with complex karyotype [48,XX,+8,+9,t(12:14)(q24.1:q24)]. According to dynamic international prognostic scoring system (DIPSS) upon ruxolitinib initiation, risk group was low in 2, intermediate-1 (int-1) in 4, intermediate-2 (int-2) in 7 and high in 5 patients.
Results: Spleen volume (SV) reduction was observed in 11/18 (61%) patients with a mean SV reduction of 33.7%. Constitutional symptoms subsided in all patients within 2 weeks of ruxolitinib initiation. Fifteen out eighteen patients had weight gain and quality of life improvement. Hematologic toxicity involving anemia and thrombocytopenia was observed in 6/18 (33%) and 2/18 (11%) patients respectively. Red cell transfusion was required in one patient. Dose adjustment with dosage de-escalation was required in 2 patients due to platelet count decrease or preexisting thrombocytopenia, while upgrading and dosage escalation in 3 patients was performed due to platelet count increase (6^{th}, 8^{th} and

12th week). Low dose hydroxyurea administration was continued as concomitant medication in post-PV and post-MPN-U MF patients.
Summary and Conclusions: JAK inhibitors, such as ruxolitinib, have significantly improved the treatment of MF. The clinical experience of the drug has shown that it is generally well tolerated. Long term effects and impact on disease natural history remain to be further studied through diligent monitoring.

Non-Hodgkin & Hodgkin lymphoma - Biology

PB1933

OUT OF EPIGENETIC CONTROL: REGULATION OF MIR23A~27A~24-2 IN B CELL NON-HODGKIN LYMPHOMA AND CLASSICAL HODGKIN LYMPHOMA

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Background: Recent publications suggest an important role for microRNAs in B-cell differentiation, maturation and malignant transformation. Epigenetic and genetic alterations are supposed to contribute to the deregulation of microRNA expression in lymphoma.
Aims: We sought to identify microRNAs which are deregulated in B-cell lymphoma due to epigenetic alterations and aimed to test these for subtype-specific DNA methylation patterns.
Methods: Using a microRNA expression array, we screened for microRNAs inducible by the DNA demethylating agent 5-Aza-2'-deoxycytidine (Aza) in B cell non-Hodgkin lymphoma (B-NHL) cell lines. Putative Aza-induced microRNAs were shortlisted by harboring CpG islands in their 5'-region. Array data were validated with quantitative real-time PCR on the level of primary, precursor and mature microRNAs. To determine any correlation of DNA methylation and histone acetylation cell lines were treated with suberoylanilide hydroxamic acid (SAHA) or Trichostatin A (TSA) in combination with Aza. DNA methylation was analyzed by methylation specific PCR and bisulfite sequencing of respective CpG islands in a panel of B-NHL and classical Hodgkin lymphoma (cHL) cell lines together with primary B-NHL samples and healthy controls.
Results: Of the 872 microRNAs represented on the microarray 15 (1.7%) were induced >1.5-fold by Aza in follicular lymphoma (FL) cell line SC-1, 31 (3.6%) were activated in mantle cell lymphoma (MCL) cell line JEKO-1. Altogether 11/15 miRNAs are located downstream of a CpG island in SC-1 and 20/31 in JEKO-1, rendering them potentially susceptible to silencing via DNA methylation. Transcriptional validation of mature and precursor microRNAs identified the microRNA cluster MIR23A~27A~24-2 which was inducible by both DNA demethylating agents and histone deacetylase inhibitors. Analysis of MIR23A~27A~24-2 in a panel of B-NHL and cHL cell lines revealed that its expression was inversely correlated with the DNA methylation status of its 5'-region. Burkitt lymphoma (BL) and FL cell lines were methylated and mainly negative for miR-23a, miR-27a and miR-24 expression. In contrast, most MCL and all cHL cell lines analyzed expressed these microRNAs and were not methylated. Diffuse large B cell lymphoma (DLBCL) cell lines showed a more heterogeneous picture. Notably, these *in vitro* data are in accordance with the methylation status of the MIR23A~27A~24-2 5'-region in primary samples: 23/29 BL patient samples were methylated, compared to 4/10 DLBCL samples and only 1/9 MCL samples. Peripheral blood mononuclear cells and tonsils from three healthy controls were all methylated at the MIR23A~27A~24-2 5'-region, indicating that loss of epigenetic silencing might be the aberrant event in lymphomatous entities like MCL and cHL.
Summary and Conclusions: The microRNA cluster MIR23A~27A~24-2 is frequently downregulated due to DNA methylation in BL, FL, and some DLBCL, but is demethylated and highly expressed in MCL and cHL. Future investigations need to test the eligibility of this microRNA cluster as a potential diagnostic or prognostic biomarker in B-NHL and cHL.

PB1934

PRELIMINARY STUDY ON THE MECHANISM OF CCL5/RANTES IN DIABETIC PATIENTS WITH DIFFUSE LARGE B CELL LYMPHOMA

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Background: The chemokine RANTES/CCL5 is an inducible, secreted inflammatory cytokine of small molecular weight, and has functions of chemotaxis and activation of T cells and mononuclear cell. The protein among them, containing 68 amino acid residues and its molecular weight being 8kd, is mainly formed by NK cells and T lymphocytes, and plays an important part in immunity to the tissue damage, infection and tumor.
Aims: The purpose of this experiment is to investigate the mechanism of chemokine CCL5 in diabetes mellitus with diffuse large B cell lymphoma (DLBCL), and to investigate the action of CCL5 gene from molecular level, cell level and animal level and partial molecular mechanism, in order to provide experimental data with reference value for the function mechanism of CCL5 in diabetes with DLBCL.
Methods: Normal human B cells and DLBCL cells were cultured *in vitro*, and

RT-PCR was used to detect the expression of CCL5 mRNA; human diffuse large B lymphoma cell lines and rat diffuse large B cell lymphoma cell lines A20 were cultured in two kinds of sugar concentration of 5mmol/L and 30mmol/L, RT-PCR was applied for the detection of the expression of CCL5 mRNA respectively; BALB/ c mice were intraperitoneal injected streptozotocin (STZ) of small dose to construct diabetic rats model, and cell lines with a stable low expression of CCL5 and high expression of CCL5 were established via lentiviral transfection technology. The three kinds of cell lines of low expression of CCL5, high expression of CCL5 and un-transfection were injected subcutaneously in the diabetic BALB/c mice and normal blood glucose BALB/c mice, then were observed about the rate and the time of tumor formation and tumor size and texture; the expression of CCL5 in each group was detected by tumor tissue routine HE staining and immunohistochemistry; peripheral blood from mice in each group was extracted to detect the expression of CCL5 in peripheral blood by ELISA.

Results: 1. DLBCL cell lines of Ly1, Ly8, Ly10 are cultured *in vitro*, the expression level of CCL mRNA in each group is higher than the normal B cell(P<0.05); 2. Glu30mmol/L cultivate human DLBCL cell strain Ly1, Ly8, Ly10 and mice DLBCL cell strain A20, after culturing for 2W, the CCL5mRNA are all higher than the one cultured by Glu5mmol/L P<0.05 ; 3. Through injecting the diabetic mice by lower expression CCL5, high expression CCL5, none transfection cell lines A20, the diabetic mice's tumor formation rate is A1: 93.3%; A2: 60%; A3: 66.6%, the tumor forming time are respectively A1: 7.0±0.85d; A2: 9.5±2.8d; A3: 9.0±1.8d. Higher than the normal blood sugar mice's tumor forming rate B1 20% B2: 20%; B3: 46.6%, the tumor forming time are respectively B1: 12±1.3d; B2: 14±2.5d; B3: 12 ±4.2d; 4. Using the immuno-histochemistry to detect the diabetic mice tumor forming tissue CCL5's expression, and the result is higher than the normal blood sugar mice's tumor tissue;5. The result of ELISA detecting the diabetes mice's CCL5 expression is higher than the mice with normal blood sugar in the peripheral blood.

Summary and Conclusions: 1. Human DLBCL cell strain's expression of CCL5mRNA are higher than the normal B cell; 2. High concentrated glucose cultivate human DLBCL cell strain and mice DLBCL cell strain's CCL5mRNA is higher than the low sugar cultivate one, this shows in the high glucose environment, the DLBCL tumor cell can produce more CCL5; 3. The high expression CCL5's cell strain is more easily to form tumor in the body than the low expression CCL5's cell strain, the tumor grow faster; and at the same condition, the diabetes mice are more easily to form the tumor than the normal mice; 4. The expression of CCL5 in the tumor forming tissue of the diabetic mice is higher than the tumor forming tissue of the normal mice; 5. The expression of CCL5 in the serum of the diabetic mice is higher than the normal mice.

PB1935

INFILTRATING T-CELLS IN HODGKIN LYMPHOMA LYMPH NODES: A NEW BIOLOGICAL MARKER RELATED TO DISEASE OUTCOME

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Background: Hodgkin lymphoma (HL) is the most frequent cancer in adolescent and young adults (AYA). It is considered a curable tumor, but still many patients do not favorably respond or quickly relapse after responding to the current chemotherapy. Most widely used prognostic guides still fail to accurately identify those patients with very poor prognosis. Recently, microenvironment has been a main focus of interest for define risk and disease outcome. Few studies have analyzed the role of $CD4^+$ T-helper cells (THs) and macrophages. Results about cytotoxic T cells remain controversial.

Aims: To analyze the role of the amount of CD8 assessed by flow cytometry in the outcome of patients diagnosed of HL.

Methods: Fresh samples from lymph the node biopsy were obtained during diagnostic process in 80 patients. Single cell suspensions were prepared after homogenizing these samples. Immunophenotyping was made by flow cytometry with standard methods as a tool to help in the diagnosis, which was finally got by standard pathological methods. Clinical data were collected retrospectively from clinical courses and statistical comparisons were performed using SPSS 20.0.

Results: Ratio male/female was 52/46, and median age 37 year (12-85). Forty-six patients (60%) patients had advanced-stage disease (III, IV, B or Bulky disease). Histologic diagnoses were: nodular sclerosis n=58 (73%), mixed cellularity n=10 (12%), lymphocyte rich n=5 (6%), and lymphocyte depletion n=2 (3%), and finally nodular lymphocyte predominance n=5 (6%).The distribution of cases according to IPS prognosis was: good 16%, intermediate 49%, and poor 35%. Considering the total lymphocyte number, CD8+ T cells median value of was 15% (2-75). 76 patients received therapy with ABVD+/-radiotherapy (RT) and 2 with RT alone. With a median follow up of 5 years, freedom from treatment failure (FFTF) was longer for females (P=0.05), patients achieving complete response to 1st line therapy (P<0.001) and cases with >15% CD8+ cells (P=0.018). This superiority was maintained when only advanced-stage cases were evaluated (P=0,035), as well as in patients with Bulky disease, where all patients with >15% CD8+ remained free from treatment failure (P=0.08). In multivariate analysis, response to therapy and having >15% CD8+ were the parameters with independent statistically significance.

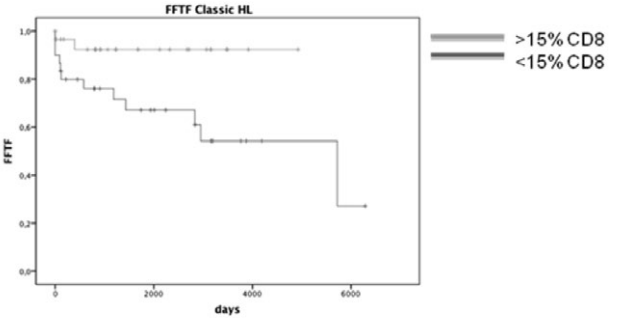

Figure 1.

Summary and Conclusions: An increased number of infiltrating CD8+ cells in the microenvironment of HRS cells assessed by FCM is associated with superior FFTF in classic HL and could be considered as a new biomarker for risk stratification. Prospective studies must be performed to confirm these results and further understanding of their role in microenvironment.

PB1936

ROLE OF HLA SPECIFICITIES IN FOLLICULAR LYMPHOMA

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Background: Follicular lymphoma (FL) is the most common indolent lymphoma, accounting for ~20-30% of all non-Hodgkin (NHL). It is an incurable disease with frequent relapses and shorter response to further treatments, developing drug resistance. Recently, using GWAS, polymorphisms in 6p21.3 region were related to susceptibility to develop NHL, including FL. The role of the HLA system in antigen presentation could be related to susceptibility and disease control. Previous studies show an association between HLA alleles and/or haplotypes and NHL. However, information regarding FL is little.

Aims: To analyze the role of HLA specificities in development and prognosis of FL.

Methods: A total of 149 consecutive patients from a single centre diagnosed of FL between 2000 and 2012 were included in the study. Grade 3b FL or those cases with DLBCL areas were not considered. Healthy donors (n=1940) from the CyL Bone Marrow Donors Registry as control group. HLA typing of class I (–A, –B y –C) and II (–DRB1 y –DQB1) at low resolution level were performed according to the EFI methodology. Allelic frequencies were estimated by direct counting. Phenotypic frequencies between groups were compared with the Fisher test, considering P<0.05 as statistical significant. P-value were corrected (Pc) according to the number of valid comparisons (Bonferroni correction). Survival analyses were carried out using Kaplan-Meier. Differences between curves were estimated using the log-rank test (SPSS 20.0).

Results: There were no statistical differences in specificities frequencies of HLA class I. However, a higher incidence of HLA-DRB1*01 was observed in patients than in donors (46% vs. 19,5%, P<0,0001, Pc<0,0013). The median follow-up of the series was of 75 months. A total of 82% of the patients received treatment for FL, 68% of them with Rituximab. Those patients receiving a CHOP-like plus Rituximab regimen and carrying the HLA-DRB1*13 had worse 10-year OFS (54% vs. 85%, P=0.05) compared with patients without this specificity.

Summary and Conclusions: The present study suggest an association between HLA-DRB1*01 and FL development, in line with previous studies. However, this study should be considered as preliminary, requiring higher sample size.

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* *Equal contribution*

PB1937

DIFFERENTIAL EXPRESSION OF ROBO1 IN MANTLE CELL LYMPHOMA, CHRONIC LYMPHOCYTIC LEUKEMIA AND ACUTE MYELOID LEUKEMIA.

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Background: Mantle Cell Lymphoma (MCL) is a B-cell neoplasm that represents 6%-9% of malignant lymphoma in Western Europe. MCL is characterized genetically by the translocation t(11;14) leading to overexpression of the cell cycle protein Cyclin D1 distinguishing MCL from other lymphomas. The outcome is extremely heterogeneous, supporting the need for new molecular markers to clarify the disease cause as well as assisting in diagnosis and prognosis. Chronic lymphocytic leukaemia (CLL) is the most common leukaemia of adults in Western countries. The mutational status of the IgH-V genes envisages here the prognosis along with mutations in a number of genes including *p53*, *notch1*, *sf3b1*, and *birc3*. Nevertheless, there is still a need for new markers for prediction of survival and treatment strategy in CLL. Recent next generation sequencing (NGS) studies have pinpointed a number of candidate genes that harbor mutations in clonal B cell neoplasms among others, the *ROBO1* gene (1). We likewise identified mutations in the ROBO genes in a pair of monozygotic twins with monoclonal B-cell disorders (2). The ROBO gene family consists of the genes *ROBO1-4* which encode single pass transmembrane proteins involved in neuronal migration across the midline and fetal brain development. These genes have previously been associated with several malignancies though only a single study, to our knowledge, -directly associated *ROBO1* with lymphomas (3).
Aims: The aim of this project is to investigate *ROBO1* as a new molecular target in patients suffering from MCL, CLL and acute myeloid leukemia (AML). We explored the mRNA expression patterns of the gene and compared the expression to a cohort of healthy individuals.
Methods: Blood samples from 20 patients with MCL, 20 patients with CLL and 20 patients with AML were collected at diagnosis. In addition blood samples from 20 healthy individuals were employed. The qPCR assay Hs00268049_m1 (Applied Biosystems, Foster City, CA, USA) targeting the specific ROBO1 mRNA was applied in triplicates on a MX3005 (Agilent Technologies, Santa Clare, CA, USA) and the quantitative range of the assays was determined. β-glucoronidase (GUS) and β-2-microglobulin (B2M) were employed as reference genes and the cell line K562 was used as positive control. ΔCq was calculated as Ave(Cq, target gene) – Ave(Cq, reference genes). The non-parametric Mann-Whitney U test was used to evaluate significant difference between the groups (Figure).
Results: We identified a significant difference between the expression of *ROBO1* in MCL *versus* healthy individuals (P=0.0002), between CLL and healthy individuals (p =<0.0001) and between AML and healthy individuals (p =<0.0001). Interestingly, three MCL patients displayed remarkably high expression of *ROBO1* compared to the rest of the cohort (Figure).

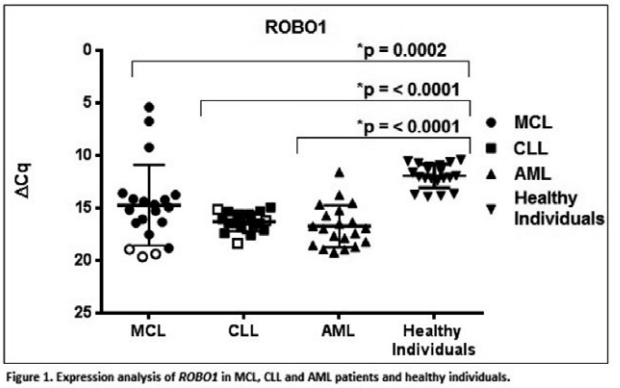

Figure 1. Expression analysis of *ROBO1* in MCL, CLL and AML patients and healthy individuals.

Figure 1.

Summary and Conclusions: Using a qPCR assay, specifically targeting *ROBO1* mRNA we found a significantly difference in the expression level in the MCL, CLL and AML cohorts compared to the healthy individuals. There has previously been a report on promoter methylation of *ROBO1* in MCL with impact on prognosis (3). Methylation of the promoter CpG islands could explain the significant difference in expression that we observed. However, whether the difference in expression of *ROBO1* solely derives from promoter methylation or is due to mutations in the gene needs to be further explored. Perspectives of using the expression profile of genes like *ROBO1* as additional tool for prognosis are interesting in these B-cell neoplasms with very heterogeneous outcome.

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PB1938

CXCR4 WHIM-LIKE MUTATIONS ARE A RARE EVENT IN DIFFUSE LARGE B-CELL LYMPHOMA

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Background: *MYD88* p.L265P mutation has been recently reported to have predictive value for worse response and survival in diffuse large B-cell lymphoma (DLBCL). In lymphoplasmacytic lymphoma (LPL), that is characterized by a high presence of the *MYD88* p.L265P change, different mutations in the C terminus of the *CXCR4* gene have been lately described. These alterations, that affect almost a 30% of LPL cases, are similar to those found in the WHIM syndrome and virtually all of them coexist with the *MYD88* p.L265P alteration, modifying the clinical presentation and outcome of the disease.
Aims: To study the occurrence of *CXCR4* WHIM-like mutations in adult patients with DLBCL and their possible association with other frequent mutations affecting the *MYD88* (p.L265) and C*D79B* (p.Y196) genes.
Methods: We analysed the mutational status of *CXCR4 and CD79B* in a series of 101 DLBCL (19 of them harbouring the *MYD88* p.L265P mutation) diagnosed in our institution according to the WHO 2008 diagnostic criteria for lymphomas. DNA samples extracted from FFPE tissue were used and macrodissection was performed in those cases where tumoral tissue represented less than the 50% of the sample. The presence of the *MYD88* p.L265P mutation was assessed by allele-specific real time-PCR. The mutational status of *CXCR4* was determined by direct Sanger sequencing of the C terminus of the gene (aminoacidic positions Glu288 to Ser352, according to the NCBI reference sequence NM_003467.2). Mutations affecting the tyrosine 196 at the ITAM motif of *CD79B* were studied also by direct Sanger sequencing of the targeted region.
Results: A total of 101 DLBCL cases (54 male; 47 female) with a median age of 71 years (range 25-88) were included in the study. According to the Hans' immunochemistry-based algorithm, 48% of the cases were ABC-type and 52% were GCB-type. *CD79B* p.Y196 mutations were found in 10 patients. Consistently with previous studies, most of the cases of the *MYD88* p.L265P positive group (n=19) were ABC-type lymphomas (74%) and presented a primary extranodal origin (68.4%) besides of being more enriched in *CD79B* Y196 mutations than the *MYD88* wild-type group (36.8% vs 3.7%, *P*<0.001).
Only one case with a mutation in the *CXCR4* gene was detected in the *MYD88* p.L265P positive group (5.26%) whereas no *CXCR4* mutations were found within the *MYD88* wild-type cases. The alteration consisted in the c.C1000T heterozygous substitution, leading to a nonsense mutation at the arginine 334 (p.R334*) and coexisted with a mutation in the *CD79B* ITAM motif (p.Y196C). Noteworthy, this mutation represents a rare *CXCR4* WHIM-like alteration also in LPL, where the most frequent altered position is, by far, the serine 338. Regarding the clinical features of the mutated case, the patient was a 71 years old male that presented an ABC-DLBCL with primary testicular origin. After 26 months of follow-up, the patient has not presented monoclonal component, bone marrow affectation or any other meaningful alteration.
Summary and Conclusions: Our study shows that the *CXCR4* WHIM-like mutations are a rare event in DLBCL, even within those patients bearing the *MYD88* L265P mutation, despite the recently shown correlation between these two molecular alterations in patients with LPL. Although these findings require further validation in larger studies, these results suggest that the pathogenic events driven by the *MYD88* L265P mutation might differ among both diseases.

PB1939

THE B-CELL RECEPTOR PATHWAY IS HIGHLY ACTIVED IN CANINE DLBCL CELL LINE CLBL-1

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Background: Diffuse large B-cell lymphoma (DLBCL) is the most common subtype of canine lymphoma and shows significant similarities in its clinical and biological presentation to human DLBCL. Since the majority of canine DLBCL appears to have genetic parallels to the human Activated B-Cell (ABC) subtype of DLBCL, analysis of the biology and evaluation of possible treatment targets in canine DLBCL could supply valuable insights about the disease.
Aims: Here we demonstrate the expression of hallmark kinases of the B-cell

receptor pathway in the canine DLBCL cell line CLBL-1. Further we investigated the treatment with B-cell receptor pathway interacting compounds such as BTK inhibitor Ibrutinib and PI3K inhibitor Idelalisib and substances lately known for their anti-lymphoid activity.
Methods: Established canine DLBCL (CLBL-1) and T-cell lymphoma (CL-1) cell lines were cultivated under standard conditions in RPMI and exposed to ascending doses of Idelalisib, Ibrutinib, Temsirolimus, BX-912, Ku-63764, Enzastaurin and Bortezomib. Cell proliferation was determined after 48 hours based on WST cell proliferation assay. Western blotting was performed after 24h. All experiments were performed at least in triplicates.
Results: In Western blot analysis kinases hallmarking the B-cell receptor- PI3K-AKT pathway (AKT, PDK, PI3K, mTOR) and their phosphorylated isoforms were detected in CLBL-1 cells. Furthermore untreated CLBL-1 cells expressed p42/44, p38, MEK, GSK alpha and GSK beta and their phosphorylated isoforms as well as the cyclins CDK2, CDK4, CDK7, CDK9 but no cyclin D1. Significantly, treatment with only 1,25nM Ibrutinib induced in WST analysis a growth reduction to 45%, with 1µM arresting growth thoroughly. The PI3K-delta inhibitor Idelalisib showed dose dependent effects: 0,6µM reduced cell growth to 41%, whereas 5µM reduced proliferation to 13%. The mTor inhibitor Temsirolimus showed high efficacy: 1,25nM Temsirolimus reduced cell proliferation to 38%, while the mtorc1/mtorc2 inhibitor Ku-63794 induced at the dose of 0,25µM a reduction to 49%. CLBL-1 was also sensitive towards other compounds with anti-lymphoid activity such as the PDK-1 inhibitor BX-912 (0,25µM; 20%), the PKC inhibitor Enzastaurin (1,25µM; 52%) and the proteasome inhibitor Bortezomib (5nM; 50%). To identify B-cell receptor pathway specific mechanisms the T-cell lymphoma cell line CL-1 was treated as control. Increasing doses of Ibrutinib, Idelalisib and Enzastaurin (up to 5µM) and Temsirolimus (up to 20nM) did not have significant effects.
Summary and Conclusions: The detected activated B-cell receptor pathway in CLBL-1 and the sensitivity towards small molecule inhibitors targeting this pathway indicates the similarity to human ABC DLBCL. This data strongly supports the relevance of canine DLBCL as model for its human counterpart.

PB1940

CANCER-TESTIS GENES ARE EXPRESSED IN T-CELL LYMPHOMAS
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Background: Several types of T-cell lymphomas are characterized by a high risk of relapse after standard chemotherapy. It continues to be reasonable to investigate potential targets for immunotherapy in T-cell lymphomas. CTG are expressed in some hematologic malignancies. Nowadays there are a few information about the CTG expression in T-cell lymphomas.
Aims: To investigate the expression profile of CTG in T-cell lymphomas.
Methods: We used RQ PCR to evaluate mRNA expression of CTG's *HAGE*, *NY-ESO-1*, *MAGEA1*, *PASD1*, *SCP1*, *SEMG1*, *SPANXA1*, *SSX1* and *PRAME* relatively gene *Abl*. Systems of specific primers and fluorescent probes were used for each gene. mRNA has been extracted from peripheral blood (PB), marrow (BM) and lymph nodes (LN). mRNA has been extracted from 4 samples of LN, 4 PB and 5 BM of 6 patients with Angioimmunoblastic T-Cell Lymphoma (AITL); 2 samples of LN, 3 PB and 3 BM of 6 patients with T-cell lymphoma, not otherwise specified (PTL-NOS); 4 samples of LN, 5 PB and 3 BM of 5 patients with Anaplastic Large Cell Lymphoma ALK-positive (ALTCL ALK+); 2 samples of PB and 3 BM of 3 patients with Anaplastic Large Cell Lymphoma ALK-negative (ALTCL ALK-). Statistical analysis was carried out using χ^2.
Results: We observed the *PRAME* (in 1 of 4 cases) and *SSX1* (1/4) expression in LN of patients with AITL. *SCP1* (1/4), *SEMG1* (1/4) and *SSX1* (2/4) were expressed in PB of these patients. *SCP1* (1/5), *SEMG1* (1/5), *SPANXA1* (1/5) and *SSX1* (2/5) were expressed in BM of patients with AITL. We detected *GAGE* (1/2) *SEMG1* (1/2) *SSX1* (1/2) and *PRAME* (2/2) in LN, *GAGE* (1/3), *SEMG1* (1/3), *SSX1* (1/3) and *PRAME* (2/3) in PB of patients with PTL-NOS. It is interesting that *GAGE*, *SEMG1*, *SSX1* and *PRAME* were expressed together in LN and PB of one patient. There wasn't any CTG expression in BM of PTL-NOS patients. *PASD1* (1/4), *SCP1* (2/4) and *PRAME* (4/4) mRNA were expressed in LN; *NY-ESO-1* (1/5), *PASD1* (1/5), *SCP1* (1/5), *SEMG1* (2/5), *SPANXA1* (1/5) and *PRAME* (2/5) were expressed in PB, *GAGE* (1/3), *NY-ESO-1* (1/3), *PASD1* (1/3), *SCP1* (2/3), *SEMG1* (2/3), *SPANXA1* (1/3) and *PRAME* (1/3) were expressed in BM of ALTCL ALK+ patients. *SCP1* (1/2) and *SPANXA1* (1/2) were expressed in PB and *GAGE* (1/3), *SCP1* (1/3) and *SEMG1* (1/3). Expression of CTG occurred only in BM of patients with histologically proven tumor involvement. *PRAME* expression was observed in LN more frequently, than in PB (P=0,0841) and BM (P=0,0194). All other CTG were expressed with similar frequency in LN, PB and BM.
Summary and Conclusions: T-cell lymphomas have a different CTG expression profile. *PRAME* expression was predominantly observed in lymph nodes and could be considered like a potential target in relapsed or resistant cases of T-cell lymphomas.

PB1941

CD26 AND CD39 IN THE REGULATORY MICROENVIRONMENTS OF LYMPHOMA AFFECTED LYMPH NODES
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Background: In previous reports we have described that lack of CD26 on the surface of activated $CD4^+$T ($CD38^+$) in the microenvironment of Hodgkin lymphoma (HL) suggest a T-reg immunosuppressive activity. Other studies have shown that low expression of CD26, associated with adenosine deaminase (ADA), and the presence of CD39, ectoenzyme associated with hydrolysis of extracellular ATP, might be responsible for the generation of adenosine recognized as a major mechanism of T-reg for the suppression of anti-tumor immune responses and for successful tumour escape.
Aims: Significant amount of non-clonal cells infiltrating HL is an excellent model to analyze the para-neoplastic cellular elements of the tumor microenvironment. The scope of this study was to perform lymphocyte immunophenotyping by flow cytometry (FC) from lymph node samples suspected of lymphoma with the scope of verify biomarkers of enzymatic activity favorable to the proliferation of neoplastic cells.
Methods: We have examined the relationship between the expression of CD26 and CD39 by FC on $CD4^+$T and measured its expression on $CD4^+$ T subset correlating to the CD26 and CD38 in 52 samples of lymph nodes (6 HL, 30 non Hodgkin lymphoma (NHL) and 16 non malignant nodes (BN).
Results: HL samples show statically differences compared to BN in the expression of CD26 (12% vs 42%; P<0,0002), CD38 (72% vs 20%; P<0,0005), CD39 (42% vs 14%; P<0,05). When compared to NHL there is a confirmation significance in CD26 (12% vs 37%; P<0,0007), in CD38 (72% vs 25%; P<0,0009) in contrast to CD39 (42% vs 26%; P<0,3). Among NHL cases, there were 6 relapses, that we have compared to HL: CD26 (42% vs 12; P<0,02); CD38 (23% vs 72%; P<0,04); CD39 (30% vs 42%;P<0,7).
Summary and Conclusions: Data confirm the activated profile ($CD38^+$) that distinguishes HL-infiltrating cells from NHL and BN. It is more evident in HL, but in NHL microenvironment there is a tendency to a reduction of the expression of CD26 on $CD4^+$T cells compared with BN; this, correlated with CD39 increase, might suggests an enzymatic activity with a significant adenosine accumulation in NHL too. Although the small size of the cohort, the pattern of relapsed seems similar to HL. This mimics the regulatory microenvironments that strengthens the neoplastic cells to manipulate its surrounding to avoid host immune attack suggesting a possible resistance to treatment.

PB1942

DETECTION OF MYD88 L265P IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA
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Background: The mutation of the myeloid differentiation primary response gene 88 (MYD88) at amino acid position 265 leading to a change from leucine to proline (L256P) is present in approximately one-third of patients (pts) with activated B-cell–like types of diffuse large B-cell lymphoma (ABC-DLBCL) and rarely occurs in pts with other subtypes of DLBCL. Considering the published data, patients with MYD88 DLBCL treated by standard chemotherapy (CHOP and CHOP-like regimens), was associated with an unfavorable outcome.
Aims: To identify the clinical and prognostic value of the MYD88 L256P mutation in pts with ABC-DLBCL who received treatment in the National Research Center for Hematology during 2008-2014.
Methods: Thirty-two newly diagnosed pts with ABC-DLBCL were selected for the study. The diagnosis of ABC-DLBCL was established according to the World Health Organization 2008 classification. DNA from 28 cryopreserved and 4 formalin-fixed paraffin-embedded tumor samples were available for our study. The screening for MYD88 L256P mutation was performed by Sanger sequencing and allele-specific PCR.
Results: Thirty-two pts with ABC-DLBCL (median age 54,5 years (range 18-74), 18 males and 14 females) were tested for MYD88 mutation. MYD88 L265P mutation was found in 28,2% (9/32) of pts. There was just a slight predominance of the female sex for MYD88 L265P positive group (5:4) as compared to MYD88 negative group (9:14). According to the Ann Arbor classification, approximately 55% pts had stage IV of the disease in both groups. Sixty-six percent of the pts with MYD88 mutated DLBCL were classified as the high risk group by the international prognostic index (IPI) vs 8 (34,8%) pts MYD88 unmutated DLBCL. In addition, 17 of 23 pts (73,9%) with MYD88 wild type DLBCL had elevated lactate dehydrogenase (LDH) levels *versus* 100% of pts with MYD88-positive DLBCL. Among MYD88 positive group 5/9 (55,6%) pts had extranodal lesions: central nervous system, skin, gastrointestinal tract, adrenal gland, bone and soft tissue, as compared to cases without MYD88 DLBCL 8/23 (34,8%). The clinical characterictics of the pts are summarized in Table 1. All pts received high-dose chemotherapy, 6/9 MYD88 mutated pts achieve complete remission after initial

treatment, one of them experienced disease relapse with nasopharynx, bone marrow, vertebral involvement 45 months after the diagnosis, median follow-up was 41 (range 11-83) months. Three pts had died of progressing lymphoma. Out of 23 pts with MYD88 wild type DLBCL, 17 pts achieved complete remission (median follow-up 54 months, range 11-90), 2 pts had early relapse and 4 pts were refractory. Overall survival (OS) was better in pts with MYD88 unmutated DLBCL than pts with MYD88 positive DLBCL (5-year *OS*, 72% vs 42%).

Table 1.

Patients Characteristics n=32 (%)		
	MYD88 mutation	**MYD88 WT**
Number of patients	9 (28,2)	23 (71,8)
Sex (male:female)	4:5	14:9
Age at diagnosis, years.		
Median	62	53
Range	18-70	23-74
Over 60years old	5 (55,6)	8 (34,8)
Serum LDG Level > normal	9 (100)	17 (73,9)
International Prognostic Index		
Low	-	4 (17,4)
Low-intermediate	2 (22,2)	6 (26)
High-intermediate	1 (11,1)	5 (21,8)
High	6 (66,7)	8 (34,8)
Lymph node involvement	4 (44,4)	15 (65,2)
Extranodal sites:	5 (55,6)	8 (34,8)
Gastrointestinal tract	1	2
Brain	1	2
Bone	1	-
Skin	1	2
Adrenal	1	-
Bone marrow	2 (22,2)	9 (39,1)
5-year OS rate	(42)	(72)

LDG: lactate dehydrogenase; OS: overall survival

Summary and Conclusions: Our study has demonstrated that the presence of MYD88 L256P mutation in pts with ABC-DLBCL is associated with worse OS probability. An analysis of treatment outcomes revealed no statistically significant differences in OS because of the small number of pts enrolled in study. MYD88 L265P DLBCL presented with higher number of extranodal involvement, IPI high risk and older age as compared to MYD88 wild type DLBCL. We plan to increase patient recruitment in our research, continue to analysis MYD88 mutation in ABC DLBCL and try to improve treatment strategy in patients with poor-prognosis DLBCL.

PB1943

T REGULATORY CELLS IN CHILDHOOD MALIGNANCIES

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Background: T regulatory cells (T regs) participate in the maintenance of immunological self-tolerance and according recent studies play an essential role for the control of antitumor immune responses. The utility of T regs as prognostic factors and targeted treatment strategies in different cancers is under immense investigation.
Aims: The study of T regulatory cells' subpopulations CD4+CD25high and CD4+CD25highFOXP3+ in the peripheral blood of children with malignancies (lymphomas and soft tissue sarcomas) at diagnosis, during therapy and after treatment completion as well as of children with autoimmune cytopenias.
Methods: Mononuclear cells were isolated from peripheral blood (PBMCs) of children with malignancies at diagnosis (Md, n=9), during therapy (T, n=8) and at the end of treatment (ET, n=26) as well as of children with autoimmune cytopenia (CP, n=6) by density gradient centrifugation. A multi-color flow cytometry panel was used for the validation of T reg subpopulations using CD4-PC5, CD25-PE and the intracellular FOXP3-FITC monoclonal antibodies. The panel for the estimation of CD4/CD8 ratio included CD45-PC7, CD3-FITC, CD4-PE and CD8-PC5.
Results: The findings of our study revealed that the levels of CD4+CD25high and CD4+CD25highFOXP3+ subpopulations of T regulatory cells were low at diagnosis of malignancies. According to our analysis an increase of CD4+CD25high and CD4+CD25highFOXP3+ levels (0.5±0.094 vs 1.08±0.187, P=0.015 and 0.390± 0.087 vs 0.84±0.173, P=0.049 respectively) were observed during treatment administration and a new reduction of the above subpopulations after treatment completion (1.08±0.187 vs 0.62±0.062, P=0.034 and 0.84±0.173 vs 0.46±0.063, P=0.031 respectively). T reg CD4+CD25high-FOXP3+ cells were also elevated during treatment compared to cytopenias (0.84±0.173 vs 0.36±0.065, P=0.041). No statistically significant differences were estimated from the comparison between the group of cytopenias and the diagnosis of malignancies as well as the end of treatment. In addition CD4/CD8 ratio was significantly higher at diagnosis of malignancies compared to treatment period and the end of treatment group (2.13±0.299 vs 1.29±0.207, P=0.043 and 2.13±0.299 vs 1.4±0.085, P=0.019 respectively).
Summary and Conclusions: In childhood malignancies studied the levels of T regulatory cells were estimated low at diagnosis and after treatment completion whereas were elevated under treatment as a result related to immunosuppression.

PB1944

ASSOCIATION OF SELECTED GENE POLYMORPHISMS WITH PROGNOSIS AND TREATMENT RESULTS IN FOLLICULAR LYMPHOMA PATIENTS

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Background: Biological studies suggest that a host immunologic environment plays a major role in follicular lymphomagenesis, which is partly determined by host genetic background. Cytokines are key regulators of immune function and regulation, they are highly polymorphic, and have been implicated in lymphoma etiology and prognosis. To date, few studies addressing the association of polymorphisms with prognostic indicators and results of the treatment in follicular lymphoma have been carried out.
Aims: We analyzed the effect of polymorphisms of selected genes on some indicators of prognosis, response to treatment regimen and survival rate.
Methods: We genotyped 4 single nucleotide polymorphisms (SNPs) from 44 candidates' cytokine and immune genes in 64 follicular lymphoma patients who had participated in our study. Baseline clinical data and survival rates were obtained from cancer registry files. Genotyping of polymorphisms (IL2 rs 2069762, IL12B rs 3212227, FCGR2A rs 1801274, C1QA rs172378) was performed by the method of analysis of melting curve according to real time PCR by using Eco Real-Time PCR system. Statistical analysis was executed by using statistical software SPSS with the use of following tests: chi-kvadrat test, Fisher exact test, Kaplan-Meier analysis and Cox regres model. P=0.05 was considered as level of statistical significance. Following genetic models were tested: codominant, recessional and dominant.
Results: The median age at diagnosis was 53 years (range, 23–76), 41% men, 37% of patients were in high risk, 8 (12.5%) patients died during the follow-up, with a median follow-up of 59 months (range, 29 – 79 months) for surviving patients. The dominant genetic model, IL12B allele carriers rs 3212227G had achieved in comparison to TT homozygotes significantly more complete remissions after first-line treatment (95.5% vs 67.6%, P= 0.018, Fisher exact test). Other 3 polymorphisms were not associated with 1.line treatment (R-CVP, R-CHOP). Statistical significance limit was observed for the association with mortality rate for the recessional genetic model, while the carrier homozygotes CC polymorphism rs 2069762 IL2 showed a pattern of higher mortality rate, compared to carriers of allele A (40% vs. 12%, P=0.144). In the Kaplan-Meier survival analysis, we observed significantly shorter time for overall survival (OS) in the group CC homozygotes compared to carriers allele polymorphism IL2rs2069762 (34 vs 166 months; P<0.001); this difference between genotypes remained statistically significant after adjustment to age and sex in the Cox regression model (P=0005).
Summary and Conclusions: In summary, the results of the project have shown an association of several polymorphisms to response to treatment, mortality and survival rates. For the most important prognostic consider polymorphism IL12B rs3212227, where the G allele was associated with higher frequency of achieving complete remission in the group followed. Given the small number of patients in the study, for the final analysis and possible use of the results in practice it is necessary to evaluate larger population of patients with follicular lymphoma.

PB1945

BRAF-V600E AND P53 MUTATION AS A POTENTIAL PROGNOSTIC MARKERS OF REMISSION DURATION

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Background: Langerhans Cell Histiocytosis (LCH) is an orphan disease of clonal dendritic cells which may affect any organ of the body.It is most often diagnosed in childhood, but can occur at any age. Clinically, LCH manifestations range from isolated bone lesions to multisystem disease, with outcomes ranging from spontaneous remission to progressionon therapy. Morphologically, LCH cells are for CD1a and/or CD207 (Langerin), S-100 positive. Recent identification of cancer-associated mutation BRAF V600E and p53 in LCH cases provided molecular evidence of the neoplastic nature of LCH.
Aims: To investigate the frequency of BRAF V600E and correlation with p53

mutations in histiocytosis, and it clinical significance as a potential biomarker of early relapse or refractory disease.
Methods: Formalin-fixed, paraffin embedded (FFPE) tissuegenotyped for BRAF-V600E mutations with allele-specific, real-time PCR assays and immunohistochemistry (IHC) with moAb p53 in order to study an expression of tumor protein p53.
Results: During last 5 years in our Institute we diagnosed 5 cases of LCH. All patients have multisystem LCH and were immunohistochemically positive for S-100, CD1a. Patients received the same therapy: 6 cycles of chemo according to LCH – 1 study protocol: etoposide – 150 mg/m^2 i.v. (day 1-3), methylprednisolone – 30 mg/kg i.v. (day1-3). We assessed BRAF-V600E status in FFPE samples from all 5 patients (4 men and 1 woman, age between 23-55 years old, median age 39 y.o). BRAF-V600E mutation was found only in one patient. P53 was found at the same patient with positive BRAF-V600E. Compared with patients in which mutation of BRAF-V600Eand p53 have not been identified, this patient demonstrated early relapse (in 6 months after treatment). Two patients without mutation had late relapse (in 46 and 24 months – median 35 months), 1 patient had transformation in Hodgkin's lymphoma (in 12 months) and 1 patient is still in remission (for 36 months).
Summary and Conclusions: BRAF V600E and p53 mutations were found in 1 out of 5 patients with LCH. This patient had short remission duration in comparison with patients without BRAF and p53 abnormalities. Probably BRAF and p53 could be considered as a potential prognostic markers of remission duration.

PB1946

LYMPHANGIOGENESIS AND ANGIOGENESIS IN DIFFUSE LARGE B-CELL LYMPHOMA: RELATIONSHIP WITH CLINICAL FEATURES AND TREATMENT OUTCOME

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Background: Formation of new blood and lymphatic vessels, called angio- and lymphangiogenesis, is essential for the development and dissemination of malignant tumors, including diffuse large B-cell lymphomas (DLBCL). It was demonstrated that the main lymphangiogenesis-involved cytokines:VEGF-C,-D and their receptor VEGFR-3 are expressed in the majority of DLBCL cases. Our previous studies revealed that VEGF-D serum concentration has a negative impact on the overall survival of DLBCL patients, however the influence of lymphangiogenesis on the clinical picture and treatment outcome assessed directly by a mean vascular density (MVD) and mean lymphatic vessels density (LVD) has not been studied so far.
Aims: The aim of our study was to evaluate the angiogenesis and lymphangiogenesis in DLBCL lymph nodes in relationship to the clinical features and the serum VEGF-C,-D, VEGFR-3 and bFGF levels at diagnosis, in order to assess the predictive value of those cytokines as to the complete remission (CR) achievement after standard R-CHOP treatment.
Methods: The study was performed in 31 (21 M, 10 F) DLBCL patients NOS type, aged 26-84 yrs (mean 67,5±13,6) from the Holycross Cancer Center, Kielce, Poland. 14 patients were classified as GCB, and the remaining 17 were non-GCB. For each patient blood count, serum LDH activity, b-2microglobulin, albumin and CRP concentrations were determined. The clinical stage of the disease and the general condition were determined according to the Ann Arbor classification, ECOG and the IPI staging system. The patients received 6-8 courses of the standard 21-R-CHOP regimen, and 10 of them achieved CR, in one case no data on remission were not obtainable. The cytokines serum concentration was established by ELISA method. The angio- and lymphangiogenesis were assessed in the DLBCL lymph nodes by immunohistochemical staining with anti-CD34 and D2-40 (a marker of lymphatic endothelium) antibodies. MVD and LVD was estimated in three selected hot spots by two independent investigators. As a control, MVD and LVD in a group of 11 inflammatory lymph nodes were determined. Statistical analysis was performed using Mann-Whitney test and the Spearman rank correlation coefficient. The p value less than 0.05 was assumed as statistically significant.
Results: For the whole group of patients, the mean MVD and LVD were 126,7±51,1/mm^2 and 99,6±81.6/mm^2, respectively. For the reactive lymph nodes the respective values were 197.1±43,0/mm^2 and 84.4±19,2/mm^2. MVD was significantly higher in reactive than in neoplastic lymph nodes (P=0.002). There was also a significant difference in MVD between complete as opposed to not complete remission patients (144.0±37.6/mm^2 and 117.9±56.7/mm^2; P=0.03). We did not find any relationship between LVD and the clinical parameters studied. MVD displayed no relationship either with clinical features or with treatment issue. We also found no correlation between the cytokines serum concentration and the extent of either angiogenesis or lymphangiogenesis.
Summary and Conclusions: Although the extent of lymphangiogenesis seems to be similar in DLBCL and non-neoplastic lymph nodes, the LVD may be a negative predictive factor with regard to the treatment of DLBCL with R-CHOP immunochemotherapy. Although our results do not support the usefulness of serum concentration of VEGF-C, -D, VEGFR-3 and bFGF as surrogate markers of angiogenesis and lymphangiogenesis in DLBCL, further studies on this issue would be worthwhile. The work was supported from a grant of Medical University of Lodz (503/1-093-01/503-01).

PB1947

OPTIMISATION OF RNA EXTRACTION AND RT-QPCR FOR THE QUANTIFICATION OF AID IN ARCHIVED FFPE BIOPSY SAMPLES: ONLY TWO SECTIONS OF 4 MM TISSUE ARE REQUIRED

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Background: Activation induced cytidine deaminase (AID) induces genomic alterations required for immunoglobulin SHM degradation and CSR in normal B cells. The mutagenic effects of AID may also contribute to genomic instability and adverse outcome in B-cell malignancies. We sought to address this question by measuring levels of AID mRNA and protein in formalin fixed paraffin embedded (FFPE) biopsy samples from patients with follicular lymphoma (FL). Since measuring specific mRNAs in FFPE samples is notoriously challenging owing to RNA
Aims: in this study, we aimed to first optimise the conditions for mRNA extraction, cDNA synthesis and qPCR amplification.
Methods: 8 FFPE tissue samples stored in a local FL tissue bank for 0.5 – 14 years were used for this optimisation. Using 2 sections of 4µm-thick tissue, we found that a better yield of RNA was obtained with the Qiagen RNeasy FFPE Kit as compared to the Promega ReliaPrep™ FFPE Total RNA Kit (2 µg vs 0.9 µg in average).
Results: The yield was further increased (65%) by modification of the deparafinization procedure. Using random primers for cDNA synthesis combined with a pair of qPCR primers targeting a 100-bp sequence spanning two exons, we found that AID mRNA was consistently and reproducibly detected in both recently obtained and old FFPE samples. Applying our method to artificially degraded RNA extracted from B-cell line cells, we found that AID mRNA levels were not changed when the RNA integrity number (RIN) varied between 2.1 and 10. Applying this criterion to archived FFPE samples from patients with FL that had been stored for between 0.5 and 5 years allowed us to successfully quantify AID mRNA levels in 33/56 (59%) cases. To validate our findings, levels of AID mRNA were compared to levels of AID protein detected in all of the 33 samples by IHC; a significant positive correlation (P<0.001) was observed.
Summary and Conclusions: In summary, we have shown that a sufficient quantity of mRNA for RT-qPCR can be extracted from only two sections of 4mm-thick FFPE tissue if an optimised protocol is applied. Our findings are relevant not only to the measurement of AID but to all situations where mRNA quantification is required and tissue availability is a limiting factor.

PB1948

TRANSAMINASES AND THE INFLUENCE OF SILYMARIN IN EPIRUBICIN CHEMOTHERAPY REGIMENS IN MICE

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Background: Epirubicin is a chemotherapeutic drug used in the haematological malignancies regimens. Epirubicin forms a complex with DNA, with consequent inhibition of nucleic acid (DNA and RNA) and protein synthesis. Epirubicin is cytotoxic *in vitro* to a variety of established murine and human cell lines and primary cultures of human tumors. It is extensively and rapidly metabolized by the liver, one of the reported toxicities being the hepatotoxicity. Serum aminotransferase elevations occur in up to 40% of patients on antraciclin therapy. There are some natural compounds that have been investigated for their hepatoprotective effects.
Aims: The scope of this study was to evaluate the hepatotoxicity of epirubicin as seen through the elevation of transaminases and in parallel to evaluate the protective effect of silymarin in mice treated with epirubicin and silymarin.
Methods: The study included 6 mice experimental lots (first lot with no treatment, the second lot treated with epirubicin alone, the third and the forth lots treated with epirubicin and silymarin 50 and 100mg/day, the fifth and sixth lots treated with silymarin alone, 50 and 100mg/day).
Results: The results showed that in the epirubicin treated lot, the level of ASAT was increased compared to the lots treated with epirubicine and silymarine or silymarine alone (P=0,0111 for the lot treated with epirubicin and also 50mg silymarine/day, P= 0.0374 for the ot treated with epirubicin and also with 100mg silymarine/day). Concerning the ALAT, we haven't found any statistical significant results in the compared lots.
Summary and Conclusions: As a conclusion, silymarine could have a hepatic protective effect when taken in the same time with the epirubicin included regimens in mice.

Non-malignant hematopoietic disorders

PB1949

ERYTHROPOIETIC RESPONSE TO A LIGAND TRAP OF ACTIVIN RECEPTOR II IN CULTURES FROM B-THALASSEMIA PATIENTS

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Background: The hallmark of β-thalassemias is ineffective erythropoiesis leading to anemia and tissue hypoxia. Activin has been shown to affect the erythropoiesis in the late-stage of maturation. Sotatercept [ACE-011; Celgene Corporation (Summit, NJ, USA)], a recombinant activin receptor type IIA ligand trap, binds with high affinity activin A/B and other members of the transforming growth factor β-superfamily**.** In animal models, RAP-011, a murine ortholog of ACE-011, reverses bone loss and increases hemoglobin and hematocrit by acting indirectly on the late stages of erythropoiesis and in a mouse model of β-thalassemia, RAP-011 was shown to improve anemia and the quality of erythroid cells.
Aims: The aim of our study is to further explore the molecular mechanisms underlying the effect of RAP-011 on erythropoiesis in erythroid progenitor cell cultures from β-thalassemia patients at different stages of differentiation and maturation.
Methods: $CD34^+$-enriched cells were isolated from peripheral blood of β-thalassemia patients and healthy donors by immunoselection. $CD34^+$ cells were cultured in the presence or absence of RAP-011 (50 and 100 μg/mL) for 14 days in two conditions: liquid standard cultures and HS5 stromal cell line co-cultured with $CD34^+$ cells. $CD34^+$ liquid cultures in medium from HS5 cells conditioned with RAP-011 (CM) were also set up. Conditioned medium was assayed for apoptosis activity and cytokine content by ELISA. In the co-cultures, the erythroid cells were rescued as non-adherent cells in supernatant (NAC), phase-bright cells adhering to the surface of HS5 cells (PBC) and phase-dim cells beneath the stromal cells (PDC). At day 14 erythroid cells were evaluated for cell number and viability, differentiation (Glycophorin/CD71/CD34) and gene expression profile.
Results: At day 14, there were no significant differences in cell number, viability and immunophenotype between untreated liquid cultures and those treated with RAP-011 (either derived from β-thalassemia and control subjects). In β-thalassemia co-cultures, no relevant differences in cell number and viability of the three cell fractions, in presence or absence of RAP-011 were observed; whereas the cell surface marker Glycophorin was more highly expressed in NAC (1.5-fold, $P<0.05$) and PDC (3.6-fold, $P<0.001$) treated with RAP-011 in comparison to untreated fractions. Similar results were observed in controls. In CM cultures, RAP-011-treated erythroid precursors from β-thalassemic patients expanded significantly compared to untreated cells (6.5-fold vs 3.1-fold). No significant differences were found in controls. High levels of anti-inflammatory, anti-apoptotic cytokines (SICAM-1, Bcl-2 and Bcl-xL) and factors that favour erythroid differentiation (MCP-1 and GRO-a) were detected in CM. At day 14 in presence of RAP-011, GATA1 expression increased ($P<0.005$) while GATA2 and α-globin expression decreased in erythroid thalassemic cells. In control subjects, no significant differences were observed. In β-thalassemic CM and co-cultures treated with RAP-011, GATA1 mRNA production was strongly induced ($P<0.001$), while the levels of GATA2 and α-globin mRNA were significantly lower ($P<0.005$). Similar results were observed in controls.
Summary and Conclusions: Our results suggest that RAP-011 didn't directly affect erythroid maturation, but probably acted through bone marrow-derived factors. Furthermore, RAP-011 seemed to recruit quiescent $CD34^+$ cells with more primitive properties (NAC and PDC), stimulating them to differentiate with an especially marked effect on erythroid maturation.

PB1950

DACRYOCYTES ARE A COMMON MORPHOLOGIC FEATURE OF AUTOIMMUNE AND MICROANGIOPATHIC HEMOLYTIC ANEMIA

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Background: Dacryocytes are teardrop- or pear-shaped erythrocytes which are usually found in peripheral blood smears of patients with primary or secondary myelofibrosis as well as infiltrative neoplastic disorders of the bone marrow. Such a teardrop poikilocytosis has rarely been observed in blood smears of patients with autoimmune (AIHA) and microangiopathic hemolytic anaemia (MAHA). The prevalence of dacryocytes in AIHA and MAHA has not been examined to date.
Aims: The aim of this study was to determine the prevalence of dacryocytes in peripheral blood films of patients with AIHA and MAHA.
Methods: We compared the dacryocyte counts in blood smears stained according to the May-Grünwald-Giemsa technique between 20 subjects with AIHA (n=9) and MAHA (n=11) with those from 21 controls having normal peripheral blood counts and lacking signs of hemolysis. The dacryocytes, defined as erythrocytes tapered to a point at one end, were counted as cells per 20 high power fields (HPF) at 630-fold magnification.
Results: In AIHA, MAHA and controls, dacryocytes were found in 89% (8/9), 91% (10/11) and 19% (4/21) of the blood smears, respectively. The medians and ranges of the dacryocyte counts per 20 HPF were 24 (0-89), 11 (0-50) and 0 (0-1). The rates of dacryocyte positivity and the dacryocyte counts between the samples of hemolytic anemias and controls differed statistically highly significant ($P<0.0001$).
Summary and Conclusions: The results of this study indicate that dacryocytes are commonly apparent in blood smears of patients with AIHA and MAHA. Knowledge of this frequent feature may be beneficial in clinical routine diagnosis.

PB1951

SCREENING OF GAUCHER DISEASE TYPE 1 IN NON-ASHKENAZIC POPULATION – A PILOT PROJECT IN MORAVIAN-SLESIAN REGION

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Background: The patients with Gaucher disease (GD) type 1, although it is the most common lysosomal storage disease, have probably not met the primary care doctors not even once in 10 years. This may cause that GD patients have been passing through the years in a specialized clinic under different diagnosis and they are not being treated properly. With regard to the incidence of GD 1: 60000 in our Moravian-Silesian Region (with approx. 1,3 mil. people), should be 20 persons with GD type 1. But there was only one patient with GD in National Center for the treatment of GD in the Czech Republic till December 2013. Based on a successful pilot project of GD diagnosis that was solved by our colleagues in Italy, under the leadership of prof. Cappellini (*), we decided to implement similar in our conditions. *(*) Motta I. et al., A Multicenter Observational Study For Early Diagnosis Of Gaucher Disease In Patients With Splenomegaly and/Or Thrombocytopenia, Poster ASH 2013*
Aims: Our aim is to highlight this rare diagnosis, on which we should think more in the differential diagnosis of hepatosplenomegaly and thrombocytopenia, particularly in all the unclear cases.

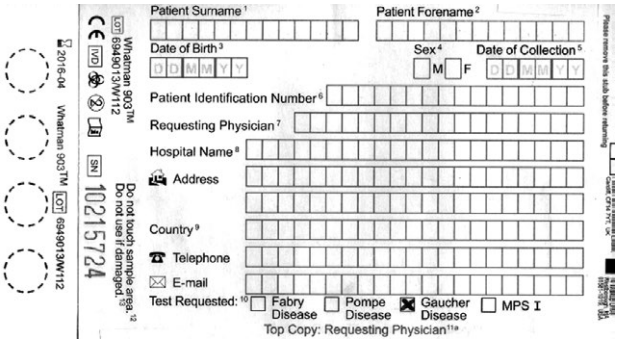

Figure 1.

Methods: We have used Dried Blood Spot (DBS) cards (attached) to detect beta-glucosidase enzyme activity in patients with splenomegaly (palpable spleen or ultrasound examination demonstrated over 12 cm) and/or thrombocytopenia (platelets below 130x 10/9/l). In the case of thrombocytopenia, at least 1 more condition must be met: the current anemia, MGUS or polyclonal gammopathy, bone pain history, or past history of splenectomy. We excluded individual with criteria: A) known hematological malignancy, B) proven cirrhosis of the liver or hemolytic anemia incl. hemoglobinopathy.
Results: For 10 months (from May 2014 to February 2015) we enrolled 15 patients (9 male, 6 female) from hematology clinics in Moravian-Silesian Region. The reasons for testing were: 27% (4/15) splenomegaly, 27% (4/15) thrombocytopenia and 46% (7/15) both of them. During this period all 15 DBS test were negative, none patient was diagnosed with GD.
Summary and Conclusions: We are continuing to search for the patients with GD to may put these patients the chance of timely and adequate treatment to prevent serious complications and restrictions on their quality of life.

PB1952

GAUCHER DISEASE LATE COMPLICATIONS IN AN ITALIAN FAMILY

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Background: Gaucher disease is a genetic disorder, autosomal recessive, due to the lack of an enzyme in the metabolic pathway of phospholipids. The deficiency of glucocerebrosidase leads to accumulation of glucocerebroside in macrophages in the spleen, liver, bone marrow, bone, and other tissues/ organs. Its manifestations are still highly subject to different penetrance.
Aims: We know in Gaucher patients there is major incidence of neoplastic diseases and of Parkinson's disease.
Methods: We describe an Italian family, composed of 5 brothers, where we can observe all described complications.
Results: In this family only one member is healthy. The first brother, suffering from Gaucher (diagnosis 58 years old), never treated, at the age of 64 years developed acute lymphoblastic leukemia with a rearranged karyotype, was treated with supportive care and he died of infection after a few months in 2004. The sister, born in 1941, received diagnosis in 2004 with her brothers.She was already affected of a neurological form of juvenile-onset Parkinson's disease. Treated for about 2 years with enzyme replacement therapy, she manifested improvement in haematological parameters but simoultaneously she showed a rapid deterioration of psychophysical performance. Brother n° 3 (b. 1949) received the diagnosis of Gaucher disease in 2004. He was treated with enzyme, in 2011 he developed acute myeloid leukemia, and he died in 2012 after chemotherapy. The last brother, born 1952, is currently in treatment without complications, with stable disease.
Summary and Conclusions: The history of this family confirms the data of higher incidence of neoplasms and Parkinson's disease reported in the literature. It confirms, also, the reduced expectance of life of Gaucher patients.

PB1953

PRIMARY HEMOPHAGOCYTIC SYNDROME IN PEDIATRIC

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Background: Hemophagocytic syndrome (HS) is a clinical-biological entity characterized by exaggerated and uncontrolled immune response. It can be primary or secondary. Serious illness that can develop life-threatening and imposing the initiation of urgent specific treatment. The existence of a family history, consanguinity and an early start is very suggestive of primitive forms and reports to various forms of immunodeficiencies. We relating 6 cases of primary HS hospitalized in our department over a 4 year period.
Aims: Records of patients with HS
Methods: Retrospective study from December 2009 to December 2014, 6 children with primary HS were collected, the diagnosis was made on the criteria of histiocyte society, 2007.
Results: There are 4 boys and 2 girls with an average age of 23 months (range 2 months-6 years). The clinical picture is dominated by fever and poor general condition (100% of cases), organomegaly (hepato splenomegaly) control tissue infiltration by histiocytes, was found in all cases. Neurological involvement is present in 3 patients. The pigmentation abnormality is found in 3 cases. Very contributive biology revealed a healthy cytopenia at least 2 bloodlines (100% of cases), fibrinogen is decreased in 83% of cases associated with a lower TP. Ferritin sensitive and specific test HS is greater than 500 microg / l in 5 cases. Medullary smear showed hemophagocytosis and confirms the diagnosis in all cases. Genetics is made in 3 cases. The underlying etiologies were: 3 cases of syndrome Chediak-Higashi (CHS), 2 cases of familial hemophagocytic lympho- histiocytosis probable, probable case of Purtilo syndrom type 2. These surges haemophagocytosis were triggered by EBV infection in 3 cases. The combination of corticosteroids and immunosuppressive have been established in all cases, and in 03 cases an anti CD20, no child has received marrow transplant. The evolution was fatal for 4 children in an array of multi-visceral failure (2 CHS and 2 family HLH). A case has developed autoimmune cytopenia.
Summary and Conclusions: Primary HS remains a serious disease with a poor prognosis, 70% mortality was observed in our series. Hope lies in the realization of a bone marrow transplant for our patients in Algeria

PB1954

CONTRIBUTION OF BLOOD SMEAR IN THE DIAGNOSIS OF STORAGE DISEASES: REPORT OF A CASE OF NIEMANN PICK'S DISEASE

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Background: Lysosomal storage diseases are rare constitutional diseases due to deficiency of a lysosomal protein that causes the blockage of a catabolic pathway of macromolecules. These conditions result in the accumulation of non-degraded metabolites in various tissues including the hematopoietic tissue. Their classification is based on the nature of the accumulated substance (mucopolysccharides, sphingolipids, neutral lipids, oligosaccharides...) and the protein in question anomaly. There are: the Gangliosidoses, Gaucher disease and Niemann-Pick disease. Niemann Pick's disease is associated with abnormal transport of cellular lipids inducing an accumulation of cholesterol and glycosphingolipids in the brain (type A +++) and other tissues. There are 3 types according to the presence (type A) or absence (type B), progressive presence (type C) neurological manifestations.
Aims: We report the case of a 50 days old infant hospitalized at the pediatric department for exploration of a fever in the long term with hepatosplenomegaly.
Methods: The blood count shows a bicytopenia: aplastic anemia hypochromic microcytic to 6 g / dL with moderate thrombocytopenia 118 G / L. The examination of the blood smear then highlights a population of abnormal lymphocytes containing large and multiple vacuoles. The anomaly appears vacuolated lymphocytes with enough evidence to we can assert its pathological nature and distinguish visible microvacuoles sometimes in normal lymphocytes.
Results: These clinical characteristics (hepato splenomegaly) and biological (presence of vacuolated lymphocytes) are evoke a lysosomal storage disease. Bone marrow smer confirms the pathological origin of these vacuoles and shows the presence of foam cells and bulky loads that oriented towards the diagnosis of the Niemann Pick's disease.
Summary and Conclusions: The storage diseases include the often poorly understood diseases. In this context, the detection of inconstant cytologic anomalies in blood and bone marrow smears, permitting a rapid screening, is an important step in the diagnostic approach.

PB1955

ETIOLOGICAL EVALUATION OF PATIENTS WITH VENOUS THROMBOEMBOLISM

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Background: Etiological Evaluation of Patients with Venous Thromboembolism.
Aims: Venous thromboembolism (VTE) is the common name for all pathological thrombosis in venous circulation. It commonly occurs deep veins of lower extremities; and more rarely in upper extremities, pelvis and in other veins. The most important component of VTE which poses a threat to life is pulmonary embolism. It is determined that pulmonary embolism is deadly disease, and 30% of the patients with pulmonary embolism die within the 30 days, and exactly the same proportion of patients (30%) die within following 8 years due to chronic complications such as recurrent attacks or pulmonary hypertension. Hospitalization is one of the most important factors which acutely trigger the pulmonary embolism. The purpose of this study is to perform etiological examination of the patients who apply to the department of hematology of our hospital and who are diagnosed with venous thromboembolism.
Methods: In this study, we prospectively evaluated the factors, which can play a role for etiology, in outpatients of our hospital, who were diagnosed with VTE and who received hospitalized treatment during 2009 – 2012.
Results: The patients with venous thromboembolism, 83 in total, ranged in age between 18 and 50 years with an average age of 38,1±10,1. 35 of the patients were men (42,2%) and 48 (57,8%) were women. According to the classification of thrombosis localization in the patients, pulmonary embolism was detected in 34 patients (41,0%), DVT in 29 patients (39%), SVT (sinus ven thrombosis) in 9 patients (10,8%), RVT (retinal ven thrombosis) in 7 patients (8,4%), and intraabdominal ven thrombosis in 4 patients (4,8%). In the classification of thrombosis localization of the patients with venous thrombosis according to the age groups, there was a statistically significant difference among the age groups in terms of pulmonary embolism (P=0,028), and localization of pulmonary embolism was more frequent in the age group of >40 compared to ≤30 (P=0,008). No statistically significant difference was determined among the age groups for the frequency of localization for DVT (P=0,325). There was a statistically significant difference among the age groups for sinus thrombosis (P=0,005), and frequency of sinus thrombosis was higher for the age groups of ≤30 and >40 compared to the age group of 31-40 (P=0,010 and P=0,017). When the findings were evaluated as normal/heterozygote+homozygote for FV Leiden, there was a statistically significant difference among the age groups (P=0,026), and the rates of heterozygote/homozygote was lower for the age groups of ≤30 and >40 compared to the age group of 31-40 (P=0,013 and P=0,035). No statistically significant difference was detected for prothrombin gene mutation (P=0,090). When inherited and acquired thrombophilia factors were studied in the patients with recurrent abortus history, FV Leiden heterozygote (16,7%) was determined in 8 patients, FV Leiden homozygote (6,3%) in 3 patients, prothrombin gene mutation heterozygote 1 (2,1%) in 1 patient, AT3 deficiency (2,1%) in 1 patient, high level of D-dimer (6,3%) in 3 patients, high level of fibrinogen (8,3%) in 4 patients, high level of homocysteine (8,3%) in 4 patients, ANA positive (8,3%) in 4 patients, AntiDsDNA positive (4,2%) in 2 patients, AKAIGM positive 4 (8,3%) in 4 patients, AKAIGG positive (4,2%) in 2 patients.

Summary and Conclusions: Venous thromboembolism is a frequently seen at the clinic practice with high mortality and morbidity. The patients must be evaluated with appropriate methods, and their diagnosis must be made at an early stage. Moreover, inherited and acquired risk factors must be systematically evaluated in an appropriate manner. This evaluation proposes anticoagulant therapy for risky cases and determines duration of the therapy.

PB1956

HEMATOLOGY MEETS ONCOLOGY: AN INTERESTING CASE OF SPLENOMEGALY AND CYTOPENIA AFTER SURGICAL RESECTION AND CHEMOTHERAPY FOR COLORECTAL CANCER

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Background: Hematology and oncology are increasingly becoming different specializations with own experts in their fields.
Aims: The current case from a rural oncologic practice in Germany shows an interesting cross-link between surgery, oncology and hematology.
Methods: A 38 year old woman underwent R0 resection of an adenocarcinoma of the ascending colon in 2001. Afterwards she was treated with 4 cycles of adjuvant chemotherapy with 5-fluorouracil/folinic acid/oxaliplatin. In 2005 recurrent disease with liver metastasis in the right liver lobe was diagnosed and was treated by a right hemihepatectomy. One year after the partial liver resection, a splenomegaly of 18cmx 5cm became evident, but did not cause any symptoms. The blood count then showed a slight tri-cytopenia with leucocytes 2.8 G/L, hemoglobin 11.3 g/dl and thrombocytes 85 G/L, which was interpreted as associated with previous chemotherapy. In frequent follow-up visits, there has been no sign of relapse of colorectal cancer up to now. The splenomegaly remained constant with recovered values for leucocytes and persistent thrombocytopenia around 100G/L. The patient was fully active without any problems due to splenomegaly. In 2013, the patient increasingly suffered from left-sided abdominal pain and fatigue, the spleen then was 20cm in size. Thrombocyte count had occasionally dropped below 50 G/L. Bone-marrow puncture was performed showing no signs of dysplastic syndrome or other malignancy. In August 2014, the spleen was 23 cm in longest diameter. A CT-scan showed signs of portal hypertension with pronounced collaterals, possibly due to constriction of the inferior vena cava and/or left hepatic vein, as a potential complication of the liver-resection in 2005. A gastroscopy confirmed oesophageal varices. The patient was transferred to a university hospital for further diagnostics and potential placement of a portosystemic shunt. Left hepatic vein stenosis could not be substantiated by venous catheter pressure measurements. As a definite reason for portal hypertension was not clear with no evidence for liver cirrhosis or fibrosis, the patient was advised for splenectomy for symptom relief.
Results: To avoid splenectomy, as preferred by the patient and the treating hemato-oncologist, the case was again discussed with the surgeon. It was concluded to initially perform a side-to-side spleno-renal shunt. After constructing the shunt, a significant reduction of blood-flow through the portal vein directly after surgery was observed. Only three months after the intervention, the spleen had shrunk from 24cm to 16cm accompanied by an increase in thrombocytes above 100 G/L and significant improvement of symptoms.
Summary and Conclusions: Splenectomy could finally be avoided and the patient showed a significant improvement in symptoms after construction of the shunt. The current case illustrates the necessity of close networking between different disciplines and especially the treating physicians in rural areas and specialists at highly specialized centers.

Platelets disorders

PB1957

FLOW CYTOMETRY ANALYSIS OF BONE MARROW HEMATOPOIESIS IN PATIENTS WITH IMMUNE THROMBOCYTOPENIA (ITP)

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Background: Immune thrombocytopenia (ITP) is a clinically and pathogenetically diverse autoimmune disorder resulted from antibody and/or T-cell mediated destruction of platelets and insufficient platelet production by immunologically affected megakaryocytes. The studies of bone marrow (BM) hematopoiesis in ITP show morphologically normal marrow with increased number of megakaryocytes in many patients. Abnormal levels of plasma thrombopoietin, interleukin 6 and some other hematopoietic cytokines were recently reported in patients with ITP, and these suggested a disorder of cytokine regulation with possible consequences for BM hematopoiesis. BM examination is usually unnecessary for patients with ITP, but flow cytometry (FC) study of BM is needed in some thrombocytopenic patients to exclude non-immune causes of cytopenia. The data of multi-parametric FC analysis in these patients represent a good opportunity to determine morphologically undetectable abnormalities in BM hematopoiesis at ITP.
Aims: The **aim** of current study was to examine hematopoiesis in patients with ITP by FC analysis of their BM.
Methods: FC records of eleven adult and seven pediatric patients with non-treated primary ITP from our database were enrolled for retrospective analysis. Different types of hematopoietic progenitor cells, B-lineage, myeloid and nucleated erythroid cells of different maturity were counted in BM of patients with ITP using several eight-color staining protocols and multi-parametric FC analysis. The data from ITP patients were compared with the data from hematologically normal adult (n=29) and pediatric (n=7) BM.
Results: Differential count of BM cells by FC detected the elevated numbers of total CD34+ hematopoietic progenitors, CD34+/cytoCD79a+/nuTdT+ B-lineage progenitors, CD34+/CD33+ committed myeloid progenitors and probable increase of early erythroid progenitors (erythroblasts) in adult patients with ITP when compared with normal adult BM and BM from patients with idiopathic cytopenia of undetermined significance as pathological control. FC showed an increased fraction of CD10+/CD20+/nuTdT- immature B cells and increase of total population of B cells in BM of adult patients with ITP. In contrast to adult patients, a reduced number of B cells was found in BM of pediatric patients with ITP, and it resulted from decrease of B-cell progenitors and immature B cells. FC revealed no other significant differences between BM of pediatric patients with ITP and age-related controls.
Summary and Conclusions: The present data suggest an acceleration of hematopoiesis in adult patients with ITP and address the question of its potential role in the pathogenesis of disease. Opposite changes in the composition of BM B cells in adults and children may reflect a different pathogenetic role of B cells and BM lymphopoiesis in the adult and pediatric ITP.

PB1958

IDENTIFICATION OF INCIDENT CASES OF GAUCHER DISEASE IN PATIENTS CONSULTING FOR SPLENOMEGALY AND/OR THROMBOCYTOPENIA IN SPECIALIZED MEDICAL SERVICES IN COLOMBIA THROUGH THE USE OF A SELECTION ALGORITHM

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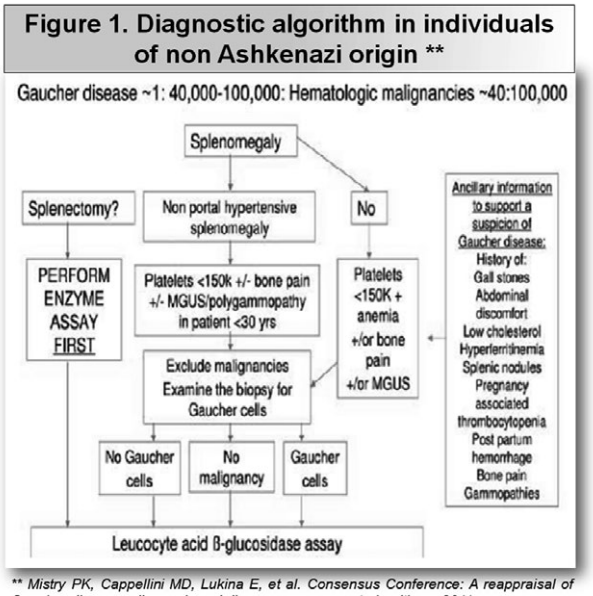
Background: Gaucher disease (GD), categorized by its low frequency as rare disease is an autosomal recessive lysosomal storage disorder, characterized by deficiency of the enzyme acid beta-glucosidase. The prevalence is 1:40,000/100,000 live births in the general population and 1:500 live births among Ashkenazi Jewish (Grabowsky G.,2004). Data from the International Gaucher Registry (GR), 2010 have included 5828 patients, reporting that 15.6% (911) of the patients are from Latin America. GD has great variability in the severity and organ involvement, being usually of nonspecific clinical characteristics, leading to late diagnosis, with irreversible complications. Splenomegaly and thrombocytopenia are the two most common manifestations, revealed in 2008 on the GR, which reported 86% cases with moderate to severe splenomegaly and 60% thrombocytopenia at the time of diagnosis,

and these are the reasons why patients are referred in the first instance to hematology. Considering the diagnosis of GD after other diagnostic hypotheses have been ruled out. The consensus of international experts in the management of patients with GD, established a diagnostic algorithm that is specially intended for specialists (Mistry et al 2010). The diagnostic algorithms of straightforward implementation, to support medical specialties in Latin America for early diagnostic suspicion of GD is required.

Aims: To identify new cases of GD in a selected population referred to Hematology, Pediatric Hematology, Pediatrics, Genetics and Internal Medicine services with a history of splenomegaly and/or thrombocytopenia, which are parameters of the diagnostic algorithm for the general population, based on the recommendations of the article by Mistry, et al (Figure 1).



** Mistry PK, Cappellini MD, Lukina E, et al. Consensus Conference: A reappraisal of Gaucher disease - diagnosis and disease management algorithms. 2011.

Figure 1.

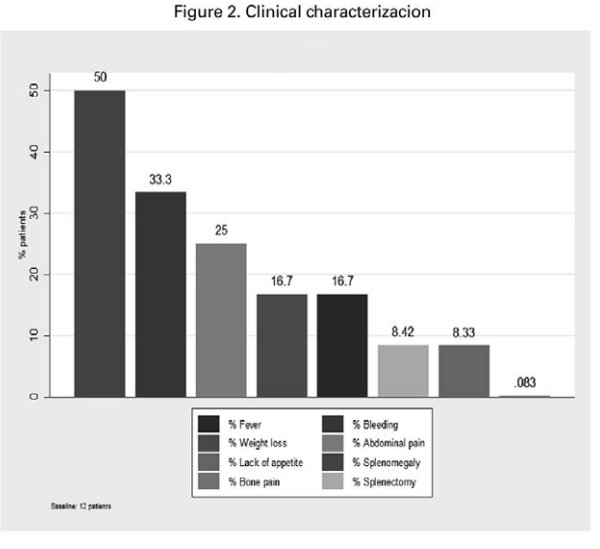


Figure 2.

Methods: Multicenter, descriptive study, in active recruitment process with non-probabilistic sampling by convenience. Currently, the study has 16 specialized medical centers in Hematology, Pediatrics and Internal Medicine in Colombia. Eligible patients are those with documented thrombocytopenia<150,000 mm3 according to Mistry algorithm, or splenomegaly defined as palpable spleen ≥1 cm below the costal rim or diagnosed by ultrasound, Nuclear Magnetic Resonance and Computed Tomography. Patients with prior diagnosis of GD, splenomegaly due to portal hypertension, documented hematologic malignancy or hemolytic anemia/ thalassemia were excluded. All patients were compiled information from their medical history and the determination of the activity of the enzyme beta-glucosidase, was performed. The study has an expected duration of 12 months of recruitment for each center.

Results: Since February 2014 (many of the patients were admitted in November and December), 27 patients have been recruited, of which analysis of enzymatic results report was obtained in 12 patients (7 women and 5 men) with median age of 9 years in a range of 1.33 to 79 years. All patients of non-Ashkenazi origin reported 50% of splenomegaly, among other symptoms and signs (Figure 2). It was recorded from the total sample analyzed, 91.7% of thrombocytopenia, and an enzymatic report of a patient with evidence of a positive correlation with chitotriosidase levels (Figure 3).

Figure 3. Laboratory report

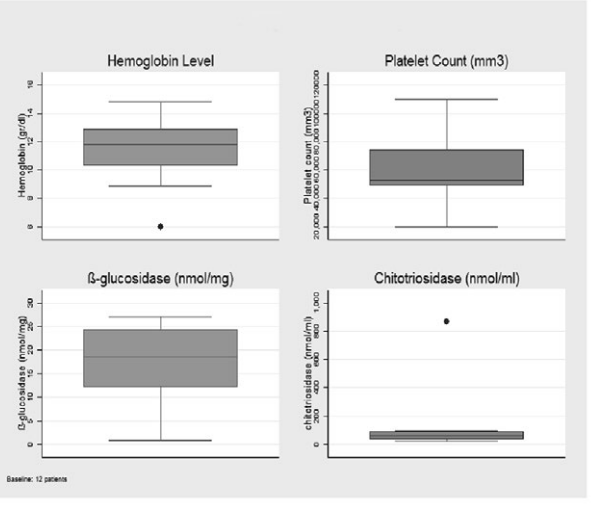


Figure 3.

Summary and Conclusions: The results of this study are in early stages of development, and its analysis is based on a limited sample. Nevertheless there is evidence that the algorithm offers support to specialists in our environment to detect new cases of GD. We will expect to recruit more patients and sites during year 2015 to provide more power to the results of this clinical study.

Acknowledgements: We thank Genzyme Colombia for financial support and CAIMED Colombia for coordination of the study.

PB1959

CORRELATION OF CLINICAL FEATURES AND OUTCOME IN CHILDREN WITH IMMUNE THROMBOCYTOPENIA

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Background: Immune thrombocytopenia (ITP) is the most common cause of abrupt onset thrombocytopenia in an otherwise healthy child. Many efforts have been made to identify patients at risk of developing persistent or chronic ITP.

Aims: The aim of this research was to investigate the relationship of clinical features and outcome of patients receiving intravenous immunoglobulin (IVIG), with the intent of identifying prognostic factors.

Methods: During the period between January 2009 and July 2014, we retrospectively analyzed 94 children newly diagnosed with ITP who received IVIG treatment. Medical records of all patients were reviewed retrospectively. CBCs with differential counts were obtained at diagnosis, 1, 3, 6, and 12 months.

Results: Ninety four patients aged between 1.3 months and 15 years (median 27.3 months) were enrolled. Multivariate analysis showed that patient gender, age at diagnosis, history of viral infection or vaccination prior to disease onset and IVIG dosage was not statistically correlated with platelet recovery at 6 and 12 months. Also, hemoglobin count, total leukocyte count (TLC), absolute neutrophil count (ANC), and absolute lymphocyte count (ALC) at diagnosis was not correlated with patient outcome. However, early platelet count recovery of ≥ 100,000/μl at 1 and 3 months after IVIG treatment was significantly correlated with platelet recovery at 6 (P<0.001 and P<0.001, respectively) and 12 months (P=0.014 and P=0.019, respectively).

Summary and Conclusions: To date, there is no consensus regarding the management of acute ITP, however IVIG use to expedite the recovery of a platelet count adequate for hemostasis is commonly practiced for newly diagnosed ITP children. The results of this study indicate that early platelet count recovery was the only significant prognostic factor associated with a short disease duration and favorable outcome. Further investigation of a larger pool of patients is warranted to validate these findings and an understanding of the pathophysiological mechanism underlying this association is needed to properly identify prognostic factors of patients at risk of developing persistent or chronic ITP.

Key words: immune thrombocytopenia (ITP); children, prognostic factor, outcome.

PB1960

THERAPY WITH RITUXIMAB AND TPO-RA IN THE REAL-LIFE CLINICAL PRACTICE OF ITP

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Background: The classical therapeutic approach for Immune Thrombocytopenic Purpura (ITP), provides the use of corticosteroids and intravenous immunoglobulins (IVIg) as first line treatment and, in case of refractoriness, the splenectomy as second line therapy. Since more than ten years other two drug regimens with Rituximab and TPO-ra, are available for the ITP treatment but their precise location in the stages of the disease is still debated.
Aims: Goal of this study was to evaluate, retrospectively, the use and efficacy of Rituximab and TPO-ra in the real-life practice of ITP to define the best time for their use.
Methods: The response to Rituximab and TPO-ra was investigated in patients treated (21 and 15 ITP patients respevtively) between May 2004 and February 2015 at our ward. The response was evaluated in terms of efficacy (CR*= Complete Response >100 pltx 10^9L, PR= Partial Response >50 pltx 10^9L, MR= Minimal Response >30 pltx 10^9L, NR= None Response <30 pltx 10^9L or no change from the baseline platelet count) and duration (SR**= Sustained Response >6 months, PeR= Persistent Response >3 months, TR= Transitory Response <3 months).
Results: The results are summarized in Table 1 and Table 2. Overall response to Rituximab was 62% and to TPO-ra was 67%. The response rate in Rituximab group decreases significantly if it was used as second line of treatment (69%) rather than as third (23%) or fourth (8%) line. Our results don't show the same trend for the TPO-ra group where the response seems to be independent from the time of use and from the previous treatments (40% of rat response in second line; 40% in the third line; 20% in the fourth line).

Table 1. Efficacy and duration of the response

	Rituximab	TPO-ra
Total treated patients	21	15
Complete Response* (CR) n (%)	11 (52%)	10 (67%)
Partial Response (PR) n (%)	1 (5%)	0
Minimal Response (MR) n (%)	1 (5%)	0
Sustained Response (SR)** n (%)	6 (47% of responders)	6 (60% of responders)
Persistent Response (PeR) n (%)	5 (38% of responders)	3 (30% of responders)
Transitory Response (TR) n (%)	2 (15% of responders)	1 (10 % of responders)
None Response (NR) n (%)	8 (38%)	5 (33%)
Response Onset-time (weeks) Median (min; max)	2 (1; 8)	1 (1; 2)

Table 2. Relationship between response and previous treatments

	Rituximab		TPO-ra	
	Responders	Non-responders	Responders	Non-responders
Total patients n (%)	13 (100%)	8 (100%)	10 (100%)	50 (100%)
Second line n (%)	9 (69%)	5 (63%)	4 (40%)	1 (20%)
Third line n (%)	3 (23%)	3 (37%)	4 (40%)	1 (20%)
Fourth line n (%)	1 (8%)	0	2 (20%)	3 (60%)
Splenectomy n (%)	3 (23%)	4 (50%)	3 (30%)	2 (40%)
Plts (n x 10.9L) before treatment Median (min; max)	5 (1; 74)	12 (3; 50)	12 (1; 30)	4 (2; 5)

Summary and Conclusions: The overall response is than clearly higher when Rituximab was used in an early stage of ITP showing a marked trend to inefficacy in the chronic and multi-refractory phases. Actually we can't say the same thing for TPO-ra. Prospective and multicenter studies about the use of these drugs in acute ITP should be performed to validate these results.

PB1961

INCREASED PRODUCTION OF ROS BY PLATELETS IN EXPERIMENTAL CIRRHOSIS

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Background: Despite the endogenous coagulopathy of cirrhosis, some patients experience venous thromboembolism. Previous studies in experimental cirrhosis by bile duct ligation (BDL) have shown a platelet hyperactivity, similar to humans with biliary cirrhosis disease (Clin.Sci., 2007,112(3):167-74). In these experiments we have found abnormal intracellular Ca^{2+} homeostasis and alterations in the metabolism of homocysteine (Hcy) that may contribute to those platelet alterations (J. Hepatology 2009, S280). The role of ROS in this platelet hyperactivity is not known.
Aims: Investigate agonist-induced production of ROS in platelets of bile duct ligated rats and the effects of chronic folic acid treatment (an essential cofactor in the metabolism of homocysteine (Hcy)).
Methods: Cirrhosis was induced in rats by bile duct ligation (BDL) and sham-operated rats were used as control. The experiments were performed after 21 days (without ascites) and 28 days (BDL group with ascites) after surgery. Platelet rich plasma aggregation in response to ADP (5μM) was analyzed using a lumiaggregometer. To analyze ROS, by fluorescence spectroscopy, platelet suspensions were washed and incubated with CM-H_2DCFDA acetyl ester and stimulated with thrombin (0.3U/mL), ADP (5μM) and homocysteine (Hcy, 50-200μM). In some experiments, sham and BDL rats were treated with folic acid (8 mg/Kg/day in drinking water).
Results: Platelet stimulation with thrombin and Hcy induced an increase in ROS production that was of greater magnitude in platelet from BDL rats than in control. Ascites reduced aggregation response and increased ROS production. Chronic treatment with folic acid decreased more platelet aggregation and ROS production in BDL than in control group.
Summary and Conclusions: Folic acid show antiaggregant actions in cirrhotic rats due in part to attenuation of a high ROS production and might be used for prevention of venous thromboembolism in liver cirrhosis. But, with proper control, due to the folic acid reduces platelet aggregation excessively in cirrhosis with ascites and can produce increased maintenance of gastrointestinal bleeding due to portal hypertension characteristic of this disease.

PB1962

STUDY OF IMMATURE PLATELET FRACTION AND POTENTIAL ROLE IN DIFFERENTIATING VARIOUS CAUSES OF THROMBOCYTOPENIA

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Background: Immature platelets are newly released reticulated platelets into circulation. They can be measured using a specifically designed automated hematology analyzer as immature platelet fraction (IPF). IPF was shown to be high in immune thrombocytopenic purpura (ITP) due to rapid clearance and enhanced production of platelet. In aplastic anemia (AA), IPF was lower because of low platelet production rate. Utilization of this parameter to differentiate causes of thrombocytopenia between underproduction and peripheral destruction has been proposed. There is no IPF data on other diseases with similar mechanisms such as hypersplenism, Evans syndrome (ES), thrombotic thrombocytopenic purpura (TTP), or chemotherapy-induced bone marrow suppression (CIBMS).
Aims: To measure IPF in healthy Thai volunteers, and patients with thrombocytopenia from various causes and to compare IPF from different thrombocytopenic mechanisms *i.e.* underproduction (AA, CIBMS) and peripheral destruction/ sequestration (ITP, TTP, ES, hypersplenism).
Methods: This was a prospective, cross-sectional, observational study. Each EDTA-anticoagulated blood from Thai healthy volunteers (NL; 51) and thrombocytopenic patients (67) (platelet count<150x 10^9/L) with known diagnosis of AA (14), CIBMS (22), ITP (20), TTP (2), ES (3) and hypersplenism (6) was analyzed by Sysmex XE-5000 for complete blood count, reticulocyte parameters (percentage, absolute count and fluorescent ratio), IPF, and mean platelet volume (MPV). Peripheral blood smears were simultaneously examined for RBC, WBC and platelet morphology. Clinical parameters were collected on fever, anemia, jaundice, bleeding status with/ without ecchymosis, and concurrent therapy for thrombocytopenia.
Results: Mean platelet number (x 10^9/L) for NL, AA, CIBMS, ITP, TTP, ES and hypersplenism were 277.25, 27.21, 35.55, 50.20, 54.50, 22.67, and 63, respectively. Negative correlation between platelet number and IPF value was shown in which the lowest median IPF (%) was found in NL (1.50; 0.6-4.5) compared among underproduction (2.95; 0.7-24.8) and peripheral destruction group (4.80; 0.2-25.7). In each underproduction subgroup, median IPFs (%) for AA and CIBMS were 4.25 (1.8-24.8) and 2.50 (0.7-15.5). In each peripheral destruction/ sequestration subgroup, median IPFs (%) were 5.45 (0.2-25.7), 4.90 (2.4-7.4), 8.90 (4.8-11.4), and 3.95 (1.3-17.2) for ITP, TTP, ES and hypersplenism, respectively. A cut-off absolute immature platelet count value of more than 1,000x 10^9/L was proposed to differentiate peripheral destruction from underproduction mechanism with sensitivity and specificity of 75.2% and 63.9%, respectively.
Summary and Conclusions: IPF is a simple, reliable parameter and can potentially be used to differentiate mechanisms causing thrombocytopenia. More beneficial roles of IPF are expected.

PB1963

PROPHYLAXIS OF FETO-MATERNAL ALLOIMMUNE THROMBOCYTOPENIA (FMAIT) WITH ENRICHED INTRAVENOUS IMMUNOGLOBULINS (IVIG) AND CORTICOTHERAPY

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Background: FMAIT can lead to serious complications such as intracranial hemorrhage. The current consensus is to initiate first line prophylactic treatment with IVIg and corticotherapy. We describe 2 cases of successful prophylactic treatment of FMAIT with enriched IVIg and corticotherapy. Infusion of enriched

IVIg is interesting to the quality of life in outpatient setting. Time of infusion is faster than standard IVIg.
Aims: Ms VH born in 1982 gave birth normally to a 2.130 g girl in 2005. In 2008 a second pregnancy was medically terminated during week 30 because of fetal hydrocephalus. The post expulsion evaluation identified FMAIT caused by incompatibility of parental HPA1 and HPA3 platelet groups and anti-platelet alloantibodies against HPA1a/a and HPA3a/a. In 2009 this woman begin a third pregnancy and was treated prophylatically with lyophilized IVIg (Tegeline 5%, 1 g/kg bodyweight) from the 16th week combined with Prednisolone (60 mg/day) from week 30. A healthy 2.070 g girl was delivered by caesarean section during week 33. This woman initiated 2 new pregnancies in 2011 and 2013 treated with prophylactic infusion of enriched IVIg (Privigen 10%, 1 g/kg bodyweight) from week 19 combined with Prednisolone (60 mg/day) started during week 32. In 2011 she gave birth by caesarean section at 37 weeks to a 3.370 g girl. In 2013 a scheduled caesarean section was performed on week 37 and a 2.460 g girl was born.
Methods: Ms SA born in 1973 is mother of 1 child with no reported clinical issues. During her second pregnancy in 2014, thrombocytopenia was observed at the 5th month with a maternal platelet count of 35 000/mm3. FMAIT is diagnosed with parental HPA5 incompatibility and anti-platelet alloantibodies against HPAa/b. Prophylactic treatment with enriched IVIg (Privigen 10%, 1 g/kg bodyweight) was started during week 18. Platelet count of 190 000/mm3 was observed 2 weeks after. Prednisolone (40 mg/day) was started during week 32. A caesarean section was performed on week 38 with birth of a 2. 800 g boy.
Results: Owing to the morbidity of FMAIT IVIg prophylactic treatment with corticotherapy is nowadays considered a standard approach. IVIg are generally intiated during weeks 16 – 18 of pregnancy and continued until planned caesarean section 2 – 4 weeks before the due date. The current consensus on the IVIg dose is 1 g/kg bodyweight per week. Corticotherapy is regularly combined with IVIg from week 30. Interest of enriched IVIg concerns particularly the quality of life of these patients.
Summary and Conclusions: To our knowledge this is the first report of FMAIT treatment with enriched IVIg preparation which was well tolerated and effective consistent with the profile of standard IVIg. Prophylactic treatment of FMAIT depends on identification of risk factors from previous pregnancies with diagnosis by laboratories experienced in biological platelet testing. Management must involve close multidisciplinary collaboration between obstetrics team and hematologists experienced in IVIg use. Surveillance mainly involves fetal echographic monitoring and maternal biological parameters follow-up. Based on our experience use of enriched IVIg in women at high risk of FMAIT is efficient and safe.

PB1964

BORTEZOMIB IN THE MANAGEMENT OF ACUTE TTP – DIFFICULTY IN MONITORING PROGRESS

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Background: Thrombotic Thrombocytopenic Purpura (TTP) is a rare but life threatening condition that has an untreated mortality of 90%. It is characterised by the presence of autoantibodies against the von Willebrand factor cleaving enzyme ADAMTS13.(1) Therapeutic plasma exchange (PLEX) remains the mainstay treatment – adjuvant immunosuppressant therapy with rituximab is also required in some patients.(2)
Aims: We report the case of an acute acquired TTP that did not respond to PLEX or rituximab however rapidly responded to the proteasome inhibitor, commonly used in multiple myeloma, bortezomib.
Methods: The case is of a 59 year old gentleman who presented with vomiting, progressive dizziness and double vision. Acute TTP was confirmed and the patient was commenced on once daily PLEX to which he had an inadequate response. He was subsequently initiated on twice daily PLEX and prescribed four doses of rituximab. Again only a partial response was observed, so based on case report data, a course of bortezomib was trialed alongside the twice daily PLEX.
Results: A relatively quick recovery in platelet count was observed, and although difficult to interpret the ADAMTS13 inhibitor titre and ADAMTS13 activity normalised. The patient was discharged 57 days post diagnosis and has remained stable since.
Summary and Conclusions: This case supports the use of bortezomib as an adjuvant therapy in the management of acute TTP where PLEX and rituximab have been ineffective. Flow cytometry conducted on this patient's bone marrow post-rituximab revealed a normal CD19 count supporting the failure of rituximab in this case. One potential reason that is important to consider, is that effect of twice daily plasma exchange on the pharmacokinetics of rituximab, evidence suggests peak plasma concentrations are difficult to achieve during PLEX.(3) The PLEX also made it difficult to monitor progress with bortezomib, as both ADAMST13 and its inhibitor titre were unreliable representations of disease activity.

PB1965

IMMUNE THROMBOCYTOPENIA: AN EGYPTIAN EXPERIENCE IN MANAGING ADULT PATIENTS WITH ITP

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Background: Idiopathic thrombocytopenic purpura (ITP) is a heterogeneous clinical disorder characterized by immune-mediated platelet destruction, it may present both as an acute, self-limiting condition and as a recurrent (chronic) form. The clinical differences between newly diagnosed and chronic ITP suggest the existence of different pathophysiological mechanisms in the two forms.
Aims: We aimed to study the clinical, laboratory parameters as well as response to therapy in Egyptian adults with ITP.
Methods: We investigated 108 Egyptian patients with immune thrombocytopenia who were registered in clinical hematology unit, internal medicine department, Cairo university, Egypt during period between 2013 and early 2015 through history, physical examination, laboratory tests including CBC, reticulocyte ounts, ESR, PTT, PT, virology markers; CMV IgM, EBV IgM, HCVAb, HBsAg and HBcAb, ANA, Lupus anticoagulant, anticardiolipine, H pylori antigen in stool and TSH and response to therapy including response to rituximab and thrompopoietin agents recently introduced as line of therapy in our center.
Results: We had investigated 108 ITP patients, Female (*n*;89) were 82% while male were 18%, The median age at the time of diagnosis was 30 years and its range was (14–70) years, Duration of disease ranged between (3 months-21 years) where the median duration was 2.5 years, 45% were newly diagnosed, 44% had chronic ITP and 10% had persistent ITP. Bleeding symptoms were present in 88% (the frequency of various bleeding symptoms were as follows: cutaneous bleeding 79%; gingival hemorrhage 33%; epistaxis 30.5%; vaginal bleeding 27.7%; melena 3.7%; hematuria 4.6%; 1.8% fresh bleeding per rectum and post-partum hemorrhage 0.9%), The median platelet count at the time of diagnosis was 15,000/ mm3 where 38.8% patients had a platelet count<10,000/mm3, ANA was positive in 13.8%, and anti-DNA was positive in 1.8% of ITP patients who had symptoms and signs fulfilling criteria to diagnose SLE. APL antibodies were positive in 4.8% who also had history either of thrombosis or abortion. HBsAg was negative in all studied patients where anti-HCV antibody was positive in 13.8% of patients, also 15.7% of our patients had positive H pylori antigen in stool with silent gastritis, 2.7% had positive anti EBV IgM with high titer and none of studied patients had positive anti CMV IgM. Regarding the thyroid functions 6.4% had abnormal functions where 3.7% of ITP patients had overt hypothyroidism. Also the onset of disease was related to pregnancy in 12% of ITP patients. Regarding Treatment and follow-up; There was an indication for treatment in 96% of patients, Of the 104 ITP patients who were given first-line therapy (corticosteroid 1mg/kg/day PO), there was complete response (CR) in 40.3% and 59.7% patients were nonresponsive to therapy. Patients who had failure of response to 1st line of therapy were given a 2nd line of therapy and the details of it were as follow (splenectomy was done in 16,1% and CR was 3%, 14% received rituximab and CR was 60%, 3% received (TPO) agonist; Eltombopag and CR was 100%, 45% received combined azathioprine and steroid therapy and CR was 64%, 4.8% received triple therapy in form of steroid, azathioprine and danazole where CR was 66%, 8.1% received vincristine and CR was 20% and 5 patients received anti H pylori triple therapy and CR was 20%)
Summary and Conclusions: Most ITP patients were females and investigating 2ry causes of ITP cases even when there is no clear symptoms of the 2ry cause is very important and may be a rising issues in future. Using another agents as 2nd line therapy rather than splenectomy they proved its value although it still need more time and number of ITP patients to prove its efficacy that's why our study still ongoing recruiting more chronic ITP patients to represent our single center Egyptian experience.

PB1966

MUTATION ANALYSIS AND CLINICAL SPECTRUM IN PATIENTS WITH BERNARD SOULIER SYNDROME: A SINGLE CENTER EXPERIENCE

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Background: Bernard Soulier Syndrome (BSS) is a rare inherited disorder of platelet function due to absence of obviously decreased expression of the platelet Gp Ib-V-IX receptor. This complex plays a critical role in hemostasis by initiating platelet adhesion and subsequent activation at the site of injured vessel. Due to rarity of disease, there are reports only a few cases about the relationship between phenotype and genotype.
Aims: We aimed to evaluate clinical and genetic characteristics of the patients with BSS diagnosed at our department.
Methods: Five patients with BSS diagnosed at department of Pediatric Hematology, Necmettin Erbakan University, Meram Medical Faculty, were enrolled the study. The diagnosis of patients was based on low platelet count, presence of giant platelets and aggregometric studies. Flow cytometry to assess the surface Gp Ib-V-IX complex showed reduced expression. Clinical manifestations and lab-

oratory investigations of the patients were evaluated. We also analyzed the coding regions of genes GpIBA (NM-ooo173,4), GpIBB (NM-000407.4_ and GpIX (NM-000174.3) using DNA samples of affected patients and their family members.
Results: Four of the patients have mutation in GpIBB gene. Two patients have c.233T>G (p.Leu78Arg) in GpIBB gene. Two patients have c.[470T>A(+)472_473del(CT)] (p.Leu157GlnfsX151) in GpIBB gene. One patient has c.1A>C in GpIA gene. Clinical and laboratory findings of the patients are summarized in table 1.

Table 1.

Table 1. Clinical and laboratory Characteristics of the patients with Bernard Soulier Syndrome

Patient no	1	2	3	4	5
Age (year)	29	28	8,5	11,5	24
Gender	female	female	female	female	female
Age at diagnosis (year)	2,5	2	0,5	8	2
Platelet count(x10^9/L)	44000	42000	40000	35000	45000
Mean platelet volume (fl)	16	15,2	11,8	11,2	12,1
Aggregation with Ristocetin	%0	%0	NA	Anormal	%2
CD42a	%0	%0	%0,03	%89 (CD42b %75)	NA
Mutation type	c.[470T>A(+)472_473del(CT)] (p.Leu157GlnfsX151)	c.[470T>A(+)472_473del(CT)] (p.Leu157GlnfsX151)	c.233T>G (p.Leu78Arg)	c.1A>C	c.233T>G (p.Leu78Arg)
Screened area	GpIBB	GpIBB	GpIBB	GpIBA	GpIBB
Bleeding phenotype	Menorhagia, epistaxis, gum bleeding, gastrointestinal system bleeding, splenic rupture	Menorhagia, epistaxis, gum bleeding	Epistaxis, gum bleeding, gastrointestinal system bleeding	Epistaxis, gastrointestinal system bleeding, ecchimousis	Menorrhagia, gum bleeding
History of surgery	Uterin polipectomy, tooth extraction	Uterin polipectomy	-	-	-

Summary and Conclusions: Patients with BSS often suffer from mucocutaneous bleedings. All of our cases are female. Menorrhagia is the major problem for girls at the age of menstruation. One patient, who carrying the mutation of c.[470T>A(+)472_473del(CT)] (p.Leu157GlnfsX151) at GpIBB gene, had experienced spontaneous splenic rupture and successfully treated with conservative approaches such as transfusion of erythrocyte and thrombocyte suspensions. Three different mutations were identified in 5 patients. All of them are previously described mutations. Larger collaborative studies are needed to describe relationship between clinical phenotype and genotype.

PB1967

THE EVALUATION OF OXIDATIVE STRESS IN CHRONIC IDIOPATHIC THROMBOCYTOPENIC PURPURA (ITP)

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Background: Chronic Idiopathic Thrombocytopenic Purpura (ITP) is an autoimmune disease characterised by a low platelet count determined by an increased platelet destruction or a decreased platelet production. Oxidative stress, defined as an imbalance between the reactive oxygen species and the antioxidant defence system, plays an important role in the pathophysiology of autoimmune diseases determined by DNA, protein and lipid oxidation.
Aims: To evaluate if oxidative stress is involved in the pathogenesis of chronic ITP.
Methods: We studied 28 patients with chronic ITP hospitalized in the Clinic of Hematology of Craiova (Romania) between 2012 and 2014 (group A). The A group was compared to 30 healthy people who represented the control group (group B). Both free oxygen radicals and total antioxidant capacity were evaluated by FORT (Free Oxygen Radicals testing) and FORD (Free Oxygen Radical Defence) tests from a single drop of capillary blood, at the time of diagnosis, before the administration of any drug. The normal value of FORT was less than 2.3 mmol/L H_2O_2 and the normal value of FORD was in between 1.07-1.53 mmol/L. The statistical analysis was performed and a p value ≤0.001 was considered significant.
Results: A statistically significant difference was determined for both FORT and FORD levels between the two groups. The FORT and FORD levels were higher in group A compared with group B.
Summary and Conclusions: We believe that oxidative stress is involved in the pathophysiology of the chronic ITP. When free oxygen radicals become excessive and surpass the total antioxidant defence capacity, they are destructive and attack the fundamental cellular components such as proteins that may be highly immunogenic and induce autoantibody production which is involved in platelet destruction.

PB1968

EVALUATION OF A FLOW CYTOMETRIC PLATELET COUNT METHOD

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Background: The platelet count is an important orienting test for clinicians to diagnose various pathologies and in the therapeutic monitoring of patients, this is why the accuracy of the results is essential and any measurement faults may leads the clinician to errors in the diagnostic and the treatment.
Aims: We tried to use the high precision of flow cytometry in the platelet count. In this respect we adapted a simple method and tried it.
Methods: We compared the platelet count obtained by flow cytometry based on red blood cells/platelet ratio with 2 automated hematology analyzers. : one with impedance principle and another with optical principle. The flow cytometric count is performed after labeling platelets with specific monoclonal antibody (CD61), then the red cells/platelet events ratio is determined on a forward scatter/Fl1 cytogram and the platelet count is calculated from the number of red blood cells obtained precisely by an hematology analyzer. A total of 105 blood samples were analyzed including 3 levels quality control: 45 samples with a normal platelet count, 29 samples with a low platelet count and 31 samples with a high platelet count.
Results: The correlation of the results of the platelet count obtained by the 3 methods is excellent for samples with normal and low platelet count. However the impedance hematology analyzer shows less accuracy in the high platelet count samples. The stability of the flow cytometric platelet count over the time is good. The mean of the CV over the time is 08%, which is accepted in biological analysis.
Summary and Conclusions: Finally, we suggest using the flow cytometric platelet count as a gold standard since the other methods have a large margin of error and we need exactly results to manage thrombopenic patients.

PB1969

THE BURDEN OF GAUCHER DISEASE: A REVIEW OF THE LITERATURE

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Background: Gaucher disease (GD) is a rare, genetic lysosomal storage disorder caused by a deficiency in the enzyme β-glucocerebrosidase. This enzymatic deficiency leads to progressive accumulation of the substrate glucosylceramide in organ tissues resulting in debilitating hematologic, visceral, and skeletal manifestations that span a wide spectrum of disease severity and adversely impact patients' quality of life. Glucosylceramide accumulation in macrophage lysosomes of the liver, spleen, and bone marrow leads to anemia, thrombocytopenia, hepatosplenomegaly, and bone disease as defining clinical signs. Three subtypes of GD are recognized: type 1 (GD1, non-neuropathic), type 2 (GD2, acute neuropathic), and type 3 (GD3, sub-acute/chronic neuropathic), with GD1 being most prevalent. Diagnosis of GD relies on clinical presentation, heightened diagnostic suspicion and exclusion of other hematologic diseases. Enzyme assay to measure deficiency of β-glucocerebrosidase in leukocytes is the definitive diagnostic test. However, owing to low disease awareness and symptoms that overlap with other diseases, diagnostic delay is common, which substantially impairs patient care.
Aims: To better understand the burden of GD, a comprehensive review of the published literature was conducted.
Methods: MEDLINE, EMBASE, CENTRAL, and conference proceedings literature sources published in English between January 1990 and February 2013 were searched for relevant publications. Eligible studies were primarily of observational design and examined the disease epidemiology, clinical and socioeconomic disease burden of GD and the impact of current treatment options.
Results: Overall, 2034 citations were identified through a systematic search of databases, conference abstracts, and manual searches of published reviews. Full text was reviewed in-depth for 177 relevant studies and a total of 97 publications were summarized. In the reviewed studies, the standardized incidence and prevalence of GD in the general population varies from 0.30 to 5.80 per 100,000 and 0.33 to 1.75 per 100,000, respectively, and GD1 is the predominant type in most regions. The risk of mortality is highest in GD patients who present younger than age 5 years and generally increases after age 55; the life expectancy is lower than the general population. Prior to treatment, anemia was present in 11–75% and thrombocytopenia in 20–62% of GD patients in the reviewed studies. Prevalence of splenomegaly and hepatomegaly varied widely among small samples of patients with untreated GD (15–96% and 10–86%), respectively. Bone pain and bone crises were reported frequently in studies of patients with untreated GD (8.0–64.2% and 3.4–24.2%), respectively. Parkinson's disease and cancer were frequently reported medically important comorbidities among patients with GD.
Summary and Conclusions: GD is a rare, chronic disease associated with significant disease burden to patients and their families. Signs and symptoms of Gaucher disease can be nonspecific and are often suggestive of hematologic abnormalities, including neoplasms. Disease awareness is of critical importance in facilitating clinical diagnosis and optimal management. Current treatment options for GD include enzyme replacement therapy or substrate reduction therapy. However, there are still medical unmet needs and further research is needed in this area.

PB1970

IMPACT OF THE ABNORMALITIES IN IMMUNOGLOBULIN LEVELS IN THE EGYPTIAN PATIENTS WITH ITP

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Background: Immune thrombocytopenic purpura (ITP) is an autoimmune disorder that characterized mainly by a low platelet count and absence of any apparent initiating and/or underlying cause of the thrombocytopenia. ITP is associated with a loss of tolerance to platelet antigens and a phenotype of accelerated platelet destruction with impaired platelet production. Common variable immunodeficiency (CVID) is a mixed group of disorders characterized by decreasing the Immunoglobulins (Igs) and an increased susceptibility to repeated infections, autoimmunity and malignancies. Both ITP and autoimmune hemolytic anemia (AIHA) are autoimmune disorders which can be seen together with CVID.
Aims:. This study aimed to assess the immune system deregulation in the form of measuring the immunoglobulins (Igs) levels in patients diagnosed as ITP and correlates the relation of these levels to the initial platelet counts, severity of the disease, treatment response and clinical outcome.
Methods: Across-section study was done on 105 patients' age range from 10-61 years, with newly diagnosed ITP at the Oncology Center Mansoura University over the past 2 years.
Results: The patients with IgA level below or equal to median had good response to stander treatment (steroids, intravenous Ig (IVIG), or intravenous anti-D), while the patients with a higher IgA level than median showed excellent response with stander treatment (P=0.02). Subjects with High IgA levels associated with increased major bleeding but not platelet count. Low IgM levels associated with failure to response to stander treatment and platelet count but not major bleeding. Changes in IgG levels wasn't associated with either response to treatment nor bleeding tendency or platelet count.
Summary and Conclusions:. We recommend initial measure of immunoglobulin level in patients with ITP to help us in the treatment protocol for each patient.

Quality of life, palliative care, ethics and health economics

PB1971

UNMET NEED FOR TREATMENT RELAPSES IN MANTLE CELL LYMPHOMA: DECREASING INTERVALS BETWEEN SEQUENTIAL TREATMENT LINES IN THE US

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Background: No large scale description of sequential treatment (tx) lines and time to the next treatment (TTNT) exists for mantle cell lymphoma (MCL).
Aims: This study evaluated first, second, and third tx lines (1L/2L/3L) and TTNT for US patients in the usual care setting.
Methods: MCL patients (pts) were identified from the IMS PharMetrics Plus database (>100 million US pts) having ≥2 medical claims (ICD-9-CM 200.4x) at least 30 days apart in 2008-2012 (the first diagnosis date as the index), no MCL claims in the 6 months pre-index and continuous enrollment in the health plans for ≥3 months post-index. The 1L therapy was defined as all MCL tx administered within 90 days of tx start. A new tx line was defined as addition of a new agent ≥90 days from tx start, or when tx was restarted following a 90 day gap in therapy. For ASCT pts, TTNT to 2L was the duration from ASCT procedure date to the start of subsequent tx; for non-ASCT patients and ASCT pts post-2L, TTNT was the time between the initiation of current line tx to the beginning of next line tx. Rituximab (R) monotherapy was not considered a new tx line when initiated within 12 months post any tx line.
Results: Of the 872 pts in the study, 172 (19.7%) received ASCT, 628 (72.0%) were males and mean age was 63.3 years. Comparison between transplanted and non-transplanted pts revealed ASCT pts were younger (median: 56.8 vs 64.9 years). Compared to non-transplanted pts, ASCT pts had also fewer comorbidities calculated by Charlson Comorbidity Index (mean: 1.63 vs 2.44). Within 1L, the majority (52.9%) of ASCT pts received tx regimens containing anthracycline + R, followed by regimens containing cyclophosphamide or vincristine +/- R (18.6%). The most frequent 1L tx regimens for non-ASCT pts were anthracycline +/- R (30.7%), R monotherapy (19.9%) and bendamustine +/- R (13.9%). The most common tx regimens in 2L contained R monotherapy (32.3%), bendamustine +/- R (21.9%), bortezomib +/- R (16.8%) and other chemotherapy +/- R (24.3%), and the most common regimens in 3L contained bendamustine +/- R (29.4%), R monotherapy (20.2%), bortezomib +/- R (14.3%), and other chemotherapy +/- R (23.5%). Of the pts not followed to TTNT (n=538), median follow-up time in days was 517 from the start of 1L tx, 304 from 2L tx, and 243 from 3L tx. The median TTNT to 2L was 349.5 days for pts who relapsed after ASCT (n=54) and 421.5 days for relapsed non-ASCT pts (n=280). For all relapsed/refractory pts, median TTNT was 403.5 days from 1L to 2L (334 pts); 272 days from 2L to 3L (119 pts), and 213 days from 3L to 4L (41 pts).
Summary and Conclusions: Within each tx line, there was substantial variation in treatment regimens. The decreasing interval from one line of therapy to the next line of therapy suggests that once patients relapse, they deteriorate at an increasing pace. Taken together, the current results suggest an unmet need exists for effective treatment of patients that relapse.

PB1972

A TRANSITION FROM USING "OFF-LINE" TO INTEGRATED PROCEDURES FOR PERFORMING EXTRACORPOREAL PHOTOPHERESIS: A COMPARISON OF COSTS AND EFFICIENCIES

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Background: The Hemobiotherapy Department at Pitié Salpêtrière Hospital has been providing extracorporeal photopheresis (ECP) procedures for patients presenting with graft *versus* host disease (GvHD) and cutaneous T-cell lymphoma (CTCL) since November 2011 and for lung transplant rejection patients since June 2013. Initially, an "off-line" system was used for all procedures, and in the first 12 months we carried out a total of 225 treatments. At the end of 2012 we commenced a transition from the off-line system to a Therakos integrated photopheresis system. In 2013 we carried out a total of 527 ECP procedures with 397 using the integrated system. At the start of 2014, the unit was thought to be close to capacity so we participated in a ProcEx Solutions workflow assessment where we successfully implemented improved ways of managing the increasing demand with the same number of nurses in the unit. Then, in 2014 we transitioned to performing all procedures using the integrated system and a total of 797 treatments were performed.
Aims: The aim of this study was to compare the cost and efficiency of performing ECP using the off-line system *versus* the Therakos integrated system.

Methods: An activity-based costing method was applied to provide an accurate and meaningful cost comparison between administration of ECP using the off-line method or the integrated system. This was important so as to not be misled by comparing the costs of off-line and integrated system procedural kits alone.
Results: The cost of performing ECP using the off-line system was more expensive than the Therakos integrated system (€1,429 vs €1,265) resulting in a cost avoidance of €131,242 for 2014 by performing all ECP treatments on the integrated system. Of note, the lymphocyte PHA and OKT3 inhibition tests mean scores were 99.2% (Integrated system) and 97.4% (off-line system), respectively. Off-line (Macopharma) Integrated (Therakos) Collection of leukocyte concentrate (PTC) €173.75 €1,009.20 Biologic analysis (patient) €59.00 €59.00 Biologic analysis (cell collection) €31.05 — Transportation of cells to CT area €7.76 — Irradiation of leukocyte concentrate €616.55 — Biologic analysis (irradiated cells) €76.00 — Transportation of cells to ward €7.76 — Injection (triple access) €12.00 — Personnel costs €108.00 €40.50 Bed retention cost/treatment (per hours used) €337.50 €156.00 Total cost €1,429.37 €1,264.70Number of treatments in 2014 797 Cost per treatment €1,429.37 €1,264.70 Total cost in 2014 €1,139,208 €1,007,966 Difference in cost (2014) €131,242.
Summary and Conclusions: The transition from off-line to the Therakos integrated system proved beneficial in terms of cost and ease of implementation. The integrated system has resulted in efficiency gains in terms of the number of patients who can be treated in a day with the same number of nurses being employed. For hospitals considering a transition from off-line methods where cost is a barrier, activity-based costing should be applied to gain an accurate understanding of the true situation. Also, reimbursement factors should be considered when making a judgement; the off-line system allows significantly fewer treatments, so reimbursement levels received by the treatment center will be substantially reduced.

PB1973

INNOVATION IN ONCOLOGY: WHY FOCUSING ONLY ON BREAKTHROUGH INNOVATION MAY BE COUNTER-PRODUCTIVE

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Background: Innovative oncology products are routinely perceived to be those that offer a substantial increase in overall survival (OS), usually above a certain threshold. However, this growing emphasis on significant OS gain (breakthrough innovation), to the exclusion of other clinical and non-clinical gains, undervalues new products and may not capture aspects of treatment that are important to patients. Clinicians, patient groups, and manufacturers argue that progressive innovation in other domains needs to be considered and valued in regulatory and health technology assessment (HTA) decisions.
Aims: The aim of this paper is to bridge the perspectives on progressive innovation and the approaches taken to measure and value innovation among different stakeholders at all levels of drug approval, appraisal and access.
Methods: We conducted a targeted literature search for definitions of innovation in academic and grey literature, covering regulatory, HTA, and industry bodies. Current/proposed OS thresholds were applied to standard of care (SOC) therapies in colorectal cancer (CRC) and non-small cell lung cancer (NSCLC).
Results: The magnitude of OS benefit consistently emerges as key in the definition of innovation from policy (*e.g.*, England's Cancer Drugs Fund) and clinical (*e.g.*, American Society of Clinical Oncology [ASCO]) perspectives. Regulators and HTA agencies do not provide clear consistent definitions, however. Emphasis is increasingly on 'clinically meaningful' change in survival, expressed as minimum thresholds: OS gain >2.5months, HR>0.8; progression-free survival gain >3months, HR>0.5. Only one of six CRC products approved since 2000 met the OS threshold published by ASCO of three to five months (Ellis *et al*. 2014) whereas survival has doubled in that time. None of the products approved for NSCLC since 2005 met the OS threshold, for 3.25 to 4 months, while survival in patients receiving first-line treatment for advanced NSCLC has also doubled.
Summary and Conclusions: Innovation should not be defined solely on one-time large survival gains but should be evaluated according to the value provided to patients and health systems. Smaller sequential gains in clinical benefit and improvements in quality of life, safety, convenience, and system efficiency should be considered in assessing value and innovation. Products that have smaller outcome gains can be combined to provide an amplification of effectiveness. Similarly, small incremental advances can lead to significant improvemments over time. If the focus moves too far towards breakthrough innovation, these treatment opportunities will be lost. Progressive innovation in these aspects provides opportunities for immediate benefit, including survival until the next therapy is available, and may uncover new clinical pathways with significant cumulative benefit over time. Recognition of this 'option value' for future health and research advances is needed. Focussing only on breakthrough OS gains Regulatory and HTA agencies should balance clinical and economic gains and societal and patient preferences when evaluating innovation in a new therapy.

PB1974

IMPACT OF THE ECONOMIC CRISIS IN GREECE ON THE MANAGEMENT OF THALASSEMIA PATIENTS

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Background: Economic crisis has had a major impact on public health in Greece during the last 5 years, negatively affecting both ability for health insurance coverage and availability of medical and laboratory services. As all patients suffering from chronic illnesses, thalassemia patients have had to live with and try to overcome relevant difficulties.
Aims: Purpose of the present retrospective study was to evaluate the impact of economic crisis on management of thalassemia patients for the 5 year crisis period (January 2010-December 2014) in a single pediatric institution.
Methods: A total of 17 multi-transfused pediatric patients participated in the study. Patient data assessed included annual heart and liver MRI T2* measurements, transfusion frequency, chelation therapy, as well as mean annual pre-transfusional hemoglobin and ferritin level. Changes in family working status of the patients as well as in insurance coverage ability were recorded.
Results: Mean study population age was 10.5 years (range 6.5-18 years), with a baseline mean pre-transfusional hemoglobin of 9.5g/dl, a mean ferritn value of 1380ng/mL, a mean heart T2* of 32m (20-58ms) and a mean liver T2* of 7.4ms (3.1-26ms). No significant change in pre-transfusional hemoglobin was recorded during the study period, as all patients continued their regular transfusion program. All patients continued chelation therapy during follow-up. Changes in chelation therapy during study period were only based on medical criteria and were not related to economic issues. With regards to ferritin levels, 13/17 patients showed an improvement, with an overall statistically significant improvement in the mean value at the end of study. As to iron concentration, 15/17 patients improved their liver measurements and 2/17 remained stable. Out of 17 patients, 8 improved their heart measurements, 2 remained stable and 8 showed decrease in heart values – however, in only one case did the measurement reach an abnormal value, consistent with mild heart siderosis. With regards to insurance ability, 3/17 patients lost coverage due to parents' unemployment, but Social Security provided free services, enabling patients to continue regular transfusions and chelation treatment.
Summary and Conclusions: Although Greece has seriously suffered during the last years because of the economic crisis, provision for health has managed to survive-at least for patients suffering from chronic illnesses, such as thalassemia.

PB1975

PREVALENCE OF TRANSFUSION TRANSMITTED INFECTIONS IN INDIVIDUALS WITH THALASSEMIA IN PAKISTANI POPULATION

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Background: Individuals suffering from thalassemia requires regular transfusion in absence of expensive stem cell transplant treatment. However, repeated or frequent blood transfusion along with post transfusion hepatitis intensifies the severity of disease and it continues to be a problem in these high risked individuals.
Aims: Present study undertaken at multicenter of Pakistan aims to estimate the prevalence of blood transfusion transmitted infections (TTI) in individuals suffering from beta thalassemia.
Methods: In a cross-sectional study, after consents 350 individuals suffering from beta thalassemia excluding patients of any other blood disease were interviewed for different epidemiological parameters *e.g.* Gender, age, transfusion history, family and personal history from October 2012-December 2013. Haematological parameters such as RBC indices and quantification of haemoglobin were recorder retrospectively. Individuals were screened for transfusion transmitted infections (TTI) mainly anti-HIV, HBsAg and anti-HCV.
Results: Out of 300 thalassemic individuals 47% (142/350) were infected with TTI including 78 males and 64 females. The seroactivity for HCV was highest 93% (133/142) followed by for HBV 6.3% (9/142) and no seroactivity for HIV. Beta thalassemia major (>95%) was most common followed by thalassemia intermedia (<5%) and few structural variants (<1%) *e.g.* HbS and HbH. When compared to the normal age span, it was observed that only 3.14% (11/350) of the patients crossed the second decade of their life. Early onset disease (before 6 months) was more common (24%; 124/350) than the late onset *e.g.* above 36 months (06%; 21/350).
Summary and Conclusions: Short life span and high number of HCV/ HbBAg status depicts that in a country like Pakistan insufficient facilities, poor management and compromised socioeconomic status are deteriorating the disease

status. More multicenter studies covering cities from different regions of country are needed in developing preventive measurements at regional and national level.

PB1976

THE QUALITY OF LIFE OF ANEMIC PATIENTS WITH LYMPHOPROLIFERATIVE DISORDERS TREATED WITH RED BLOOD CELL TRANSFUSIONS AND ERYTHROPOIESIS-STIMULATING AGENTS

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Background: Although haematological toxicities, such as anemia, are common in lymphoproliferative disorders (LPD), no clear consensus exists on the use and impact of Erythropoiesis-stimulating agents (ESA) and Red blood cells transfusions (RBCsT) on quality of life (QoL).
Aims: To assess alteration of QoL in anemic patients with LPD treated with RBCsT and ESA.
Methods: There were included 131 anemic patients with LPD (multiple myeloma, low-grade non-Hodgkin's lymphoma, chronic lymphocytic leukemia). Median age of patients was 67 years (range 24-85). All persons were divided into two groups: 1st group included patients (n=54) treated with RBCsT, 2nd (n=77) – treated with ESA. Posetive response for ESA considered as achievement of hemoglobine concentration 120 g/L or increase Hb ≥ 20 g/L. QoL was studied with Functional Assessment of Cancer Therapy Anemia subscale (FACT-An) and Fatigue subscale (FACT-F).
Results: In the 1st group of patients treated with RBCsT (in average 3.7±0.3 Units) concentration of Hb increased from 70.0±1.6 g/L to 93.1±1.2 g/L. Statistically significant improvement of their QoL was found in FACT-An subscale – from 41.1±2.0 (95% CI=37.1-45.0) to 34.2±2.1 (95% CI=30.0-38.3) points (P<0.001; n=54). Improvement of QoL was also found in FACT-F subscale – from 30.2±1.4 (95% CI=27.8-32.9) to 23.2±1.5 (95% CI=19.8-26.9) points (P<0.0001; n=54). In the 2nd group positive response for ESA-therapy was found in 52 (67.5%) out of 77 patients: 39 (50.6%) patients achieved the Hb concentration 120 g/L and 13 (16.9%) ones increased Hb more than 20 g/L. The patients with positive response increased the Hb concentration significantly from 88.4±1.4 g/L to 123.1±2.4 g/L (P<0.0001; n=52). We found out improvement of QoL in both subscale: in FACT-An – from 34.5±1.7 (95% CI=31.1-37.9) to 30.1±1.6 (95% CI=26.9-33.2) points (P<0.001; n=52) and in FACT-F – from 22.8±1.3 (95% CI=20.3-25.4) to 19.7±1.2 (95% CI=17.3-22.1) points (P=0.013; n=52).
Summary and Conclusions: Both RBCsT and ESA-therapy are able to increase Hb concentration considerably and improve QoL. However the RBCsT patients had more severe anemia and over a period of its correction the RBCsT patients's planned level of Hb was less than ESA-patietnts's one therefore their final QoL was less. So using the ESA-therapy can increase higher Hb concentration and improve QoL more than RBCsT. It's indicating importance of ESA-therapy not as alternative RBCsT method of anemia's correction but also as a way to prevent decreasing of the Hb induced by toxic effect of chemotherapy and as a method of anemia treatment with which a normal Hb level can be achieved.

PB1977

VITAMIN D DEFICIENCY IN SURVIVORS OF CHILDHOOD ACUTE LEUKEMIA

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Background: Vitamin D plays an important role in calcium and bone metabolism. It also exerts some biological effects in regulatory systems including host defense, inflammation and immunity. Vitamin D deficiency is to be a worldwide problem, especially in developing countries. Low serum levels of 25-hydroxy-vitamin D (25(OH)D) have been associated with a number of diseases like cardiovascular diseases, diabetes, cancer, and infections. However, recent studies demonstrated evidence for vitamin D in preventing the development of many diseases including cancer. 25(OH)D is formed by hydroxylation of cholecalciferol in microsomes of hepatocytes. The serum concentration of 25(OH)D is used to determine vitamin D status in the body.
Aims: The prevalence of subclinical vitamin D deficiency has been reported to be higher in adult survivors of childhood cancer (1). However, the data on this subject are scarce.In this study, we aimed to determine the frequency of vitamin D deficiency/insufficiency in acute leukemia patients who completed treatment.
Methods: Serum 25(OH)D levels of leukemia survivors were measured using liquid chromatography-mass spectrometry (LC-MS) technique who were followed-up at Late Effect Clinic in Lösante Hospital between January 2013 and December 2013. According to criteria published by Mısra M *et al.* the serum 25(OH)D levels<15-20 ng/mL is considered insufficiency, or deficiency if<15 ng/mL or severe deficiency if ≤10 ng/mL for children and adolescents (2,3). According to criteria published by Kuchuk NO *et al.* the serum level of 25(OH)D was considered insufficiency if the level was ≤30 ng/mL and deficiency<10 ng/mL for young adults. (3,4)
Results: Two hundred and forty-eight patients (113 female and 135 male) aged between 3 and 31 were recruited. Of these, 233 patients had acute lymphoblastic leukemia (ALL), 12 acute myeloid leukemia (AML), and 3 myelodysplastic syndrome (MDS).Treatment completion times were between 2 months and 17 years. The results of serum 25(OH)D levels in survivors of childhood leukemia are shown in Table 1. Overall, 95 children and adolescents (38.1%) had normal levels of 25(OH)D whereas only two young adults older than 21 years (10.5%) had normal levels.

Table 1. Distribution of 25-hydroxy-vitamin D Levels after Chemotherapy According to Age.

	<5age (n:4)	6-9age (n:82)	10-18age (n:113)	19-21age (n:30)	>21age (n:19)	Total (n:248)
Children and adolescents						
Normal (%)	4 (100)	34 (41.5)	51 (45.1)	6 (20.0)		95 (38.3)
Insufficiency (%)	None	22 (26.9)	28 (24.8)	7 (23.4)		57 (23.0)
Deficiency (%)	None	14 (17.0)	20 (17.7)	13 (43.3)		47 (19.0)
Severe deficiency (%)	None	12 (14.6)	14 (12.4)	4 (13.3)		30 (12.1)
Young adults						
Normal (%)					2 (10.5)	2 (0.81)
Insufficiency (%)					14 (73.7)	14 (5.64)
Deficiency (%)					3 (15.8)	3 (1.21)

Summary and Conclusions: The results of the current study showed that vitamin D insufficiency/deficiency is an important problem in survivors of leukemia even in a country which the rate of sunlight exposure is high. Studies in recent years, which show that vitamin D deficiency plays an important role in many diseases, including cancer, emphasize the importance of detection of the insufficiency/deficiency of Vitamin D and the necessity of the planning the corrective treatment. Vitamin D deficiency is a health problem that concerns all age groups. Vitamin D inadequacy is now an internationally recognized health problem and survivors of neoplastic diseases may be at even higher risk than healthy children, adolescents, and adults. Thus, the measurement of vitamin D level in survivors of neoplastic diseases seems mandatory during their follow-up.(3,5).

PB1978

THE RULE OF QUALITY OF LIFE QUESTIONNAIRE IN OLDER ADULTS PATIENTS WITH ACUTE MYELOID LEUKEMIA IN A MONOCENTRIC EXPERIENCE

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Background: In older patients the concept of frailty is changing, infact actually it's necessary to analize not only the age and the comorbidities but also how the tumoral pathology involve and modify the performance status of the patient s(generally calculated by karnosky, IADL and ADL scale)
Aims: We investigated the value and utility of a simple and fast questionnaire about physical and mental quality of life (SF12 Standard V1) administered to a group of elderly consecutive patients with acute myeloid leukemia in our single center.
Methods: During the years 2013-2015 twenty consecutive patients with acute myeloid leukemia (AML) and age >65 years treated with hypomethylating agents (azacitidine 75mg/m2 for seven consecutive days each twenty-eight days) compiled a simple questionnaire(SF12 Standard V1) with twelve questions to determine and quantify the degree of physical and mental fitness/frailty at the onset of AML and during the treatment(each six months with a monitoring an year.
Results: We observed that the patients with higher score at the questionnaire (major impairments in physical activity and mental discomfort) had a poor outcome, while patients with lower score at AML onset or score improvement of 6th months questionnaire(during treatment) predicted longer overall survival (median 200 days *versus* 600 days;p0.03) and better response to therapy (overall response vs non response p0.04).
Summary and Conclusions: The quality of life questionnaire could help to have a more complete vision of the pathology in those vulnerable patients and to take therapeutic decision (non intensive care *versus* intensive care)considering the reversibility of frailty caused in part of the acute leukemia.Clinical trials are necessary to confirm the rule predictor of this quality of life questionnaire for overall survival and response to therapy in a large population of older AML patients.

PB1979

HALOPERIDOL CURES COGNITIVE DYSFUNCTION; THE MECHANISM OF ACTION IS EXPLAINED.

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Background: Cognitive disorders occur in the course of life threatening situations such as deportation and diseases such as cancer and are often associated with acute stress and post traumatic stress disorders induced by the treatment received. A battery of function tests have been used to assess cognitive disorders but cure for these disorders is not known.
Aims: Cognitive dysfunction has been reported after cancer treatment and after hematopoietic stem cell transplantation. The observations in this case lecture how to proceed in patients with similar conditions in the treatment of cancer.
Methods: The subject experienced a life changing situation of major impact that induced a cognitive disorder, which expressed itselves in difficulty writing letters, documents and composing presentations; moreover she reported her capacity after deportation to be 10% of her former capacity (1). At a conference in 2013 a video was presented of a patient who had been treated for breast cancer who had the same cognitive disorder, being difficulty writing.
Results: Treatments that impact life drastically induce stress that may result in acute stress disorders, posttraumatic stress disorders and cognitive impairment. The subject was initially not treated and noticed slow improvement; however the old level of functioning was not reached and work finally resulted in burnout. Although time off work improved the status it was not until haloperidol was instated that she noticed improvement of writing skills after about three weeks of treatment. A few years later upon new exposure to stress she noticed a decrease in capacity that could not be overcome with rest and requested reinstatement of haloperidol. She reported recovery in about three weeks after start of therapy. The subject first received haloperidol at a dose of 5 mg/day which was too high and induced extrapyramidal side effects. The dose was reduced to 2 mg/day, which was well tolerated. Although the subject had been advised to take the drug life long she stopped after nine to ten months and remained well. The second time haloperidol was prescribed at 2 mg/day and well tolerated and the function recovered in about three weeks. The subject took the drug four months and alternated 2 mg and 1 mg every other day. Since stopping the subject's status has remained well.
Summary and Conclusions: Biochemical studies have demonstrated that stress increases dopamine turn over in the prefrontal cortex, which is associated with cognitive deficits (2). Haloperidol is a dopamine receptor antagonist and administration reverses cognitive impairment.Van Hoef MEHM. Successful treatment of stress induced cognitive impairment with haloperidol; evaluation also warranted in hematopoietic stem cell transplantation. The observations described hereabove warrant evaluation of haloperidol as treatment for cognitive disorders that may develop in the course of treatment for cancer; although these are generally attributed to chemotherapy, the mechanism of action may rather be attributable to stress induced biochemical changes in the brain. In prescription of the drug, doctors should inform the recipient what to expect from the drug, that treatment is temporary, that they can relapse and that at a dose of 2 mg/day driving capacity is affected.

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PB1980

DEVELOPMENT OF A PROGRESS TEST BASED ON THE EHA CURRICULUM AND EHA CV PASSPORT, USED FOR YEARLY EVALUATION OF HEMATOLOGY RESIDENCY IN SWEDEN

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Background: In Sweden, after 5.5 years of medical school and 18-21 months of general internship, you can pursue a residency in internal medicine and hematology, which takes an additional 6.5-7 years. The Swedish Hematology Association (SHA) [AE1] appointed a Work Unit (WU) of resident hematologists lead by two senior colleagues with a vast teaching experience, to develop a yearly progress test for evaluation of hematology residents in Sweden. The framework for the progress test was the EHA curriculum and EHA CV Passport, which divides the Hematology Specialty into eight large parts, with subunits to systematically cover the whole field of Hematology.
Aims: The aim for the WU was to develop a yearly MCQ based progress test, based on the eight parts of the EHA CV Passport, and to launch it among Swedish resident hematologists, with a questionnaire to fill out along with the assessment.
Methods: Individuals or groups of Swedish experts, *e.g.* the National Diagnostic Groups, were asked to produce a number of multiple choice (MCQ) questions. The questions were read, revised and sorted by the WU, after which 75 MCQ questions, covering the eight major fields of the EHA CV Passport, were selected. The test was available via the SHA homepage, www.sfhem.se for one week in October 2014, whereafter the test was removed and the answers posted online. Instructions were to complete the test within 2 hours, and when answers were available, sit down and discuss with a clinical mentor, and fill out an internet based questionnaire on your impressions of the MCQ test.
Results: Among 77 listed resident physicians in the SHA registry, 54 (70%) answered the questionnaire. Out of these, 83% had completed the test. All except one valued the subjective usefulness of the test to high/very high, and interestingly, 37 of 54 reported that their test result would contribute to them actively re-planning their education in some way, mostly by adding courses in specific subjects, or by increasing literature studies. 67% had not yet gone through the test with mentor, but almost all claimed that they would do so in the near future. There was an even spread in years of education, with 21 in the beginning, 10 in the middle, and 23 physicians at the end of their residency. All but two residents acknowledged that they would do the test again next time it is given, to assess progress on an individual level. Comments from residents were that the test was well received, the questions adequate to assess skills, and helpful to point out weaknesses to work on.
Summary and Conclusions: There is a need for continuous evaluation of acquired skills during residency. The Swedish MCQ progress test reflecting the EHA CV Passport was well received by the resident doctors and may be an appropriate tool for yearly progress evaluation.

PB1981

ANDRAL NETWORK: OPEN ACCESS SOLUTION FOR BEST CYTOLOGICAL EXPERTISE

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Background: In hematology, the microscopic analysis of cellular morphology of blood, bone marrow, lymph node… samples remains the first important stage for diagnosis and patient follow-up. In our field, the application of information technology has allowed remarkable developments: iconographic databases, e-learning systems, automated systems of classification of blood cells, and cytological web-review of clinical protocols. However, the cytological diagnosis itself has yet to really benefit from the great possibilities offered by modern technology.
Aims: In this aim, the French speaking Group of Cellular Hematology in partnership with the College of Hematology established the open access solution of "tele-expertise" in cytology: the ANDRAL network.
ANDRAL proposes remote expert decision-making support in cytology: for any transmitted request for classification, an image file with a clinical/biological form is submitted. The network then allows remote classification and diagnosis to be obtained by opinion of two expert reviewers within 24h.
Methods: The ANDRAL network is accessible on www.gfhc-reseau-andral.fr. It is free and accessible to both hospital and the private biologists. ANDRAL is linked to a group of 45 international expert reviewers, who assure by paired review, continuous cytological care service. Globally, the system satisfies the strictest requirements regarding medical data exchange in terms of security, confidentiality, and sample/patient traceability.
Results: From October 2012 through August 2014, the network recorded more than 300 registrations with approximately 40% from private biologists, 60% from biologists practicing in a local or university hospital. 15% of the subscribers (n=42) practiced outside of France. Over the same period, more than 200 dossiers were submitted. 70% were de novo diagnoses; 15% were urgent requests. Image files were selections of images and rarely wide field images. The requests were variable ranging from benign to malignant pathologies and common occurrences to rare hemopathies. The average time of request management was 1h50min, and the time to obtain a classification was on average 6h00min. The ANDRAL network also features specific evaluation tools which allow for follow up activity and also allow the measure of the quality of service provided.
Summary and Conclusions: Today, the assessments and the perspectives offered by ANDRAL network are real and very encouraging. In term of assessments, the constant and rapid increase of membership, the incredible motivation of all the expert reviewers and the high quality of exchanges are highlights. Several developments are further committed: collaboration with other networks and other countries and the development of a permanent economically feasible medical model. For the hematologists involved in morphological diagnoses, the ANDRAL network is a working example of cooperative function of which the first results are very encouraging.

PB1982

PREVALENCE AND TREATMENT OF MANTLE CELL LYMPHOMA (MCL) IN GERMANY: AN ANALYSIS OF SICKNESS FUNDS

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Background: No central registries for patients with mantle cell lymphoma (MCL) exist in Germany.
Aims: The objective of this analysis was to determine the number of patients with MCL diagnosed (with or without other diagnoses of cancer) and to characterize the types of treatment being utilized and care settings using sickness funds claim data.
Methods: This analysis evaluates data from 1,771,225 beneficiaries in 2012 from different statutory sick funds (SHI). MCL patients were identified by ICD-10 C83.1, oncological co-diagnoses by ICD-10 C00-79; D37-48 and chemotherapy by Anatomical Therapeutic Chemical (ATC) Code L01*, pharmacy number (PZN) 9999092 and/or operating and procedure code (OPS) 854*.
Results: 78 patients with a diagnosis of MCL (C83.1) could be identified (4/100.000). Overall gender ratio was 76.9% male and 23.1% female. Additional oncological diagnoses were found in 43.6% of patients. 20.7% had malignant neoplasms of ill-defined, other secondary and unspecified sites (C76-80), 17.2% had malignant neoplasms of digestive organs (C15-26), 13.8% had melanoma (C43-44), 10.3% had neoplasms of uncertain behavior, polycythemia vera and myelodysplastic syndromes (D37-48), 8.6% had malignant neoplasms of urinary tract (C64-68) and malignant neoplasms of lip, oral cavity and pharynx (C00-14) (multiple diagnoses possible). The outpatient diagnosis rate was 64.1%, inpatient rate 15.4% and in- and outpatient 20.5%. From a total of 78 patients 44 (56.4%) patients received chemotherapy (40 men [90.9%] and 4 women [9.1%]) in 2012. 25.0% of patients received both out- and in-patient treatment, 65.9% received out-patient treatment and 9.1% in-patient treatment. Identification of the administered substances was possible when ATC codes were reported. The most commonly used treatments were rituximab (34.7%), bendamustine (14.9%), cyclophosphamide (11.9%), vincristine (8.9%), doxorubicine (21.8%) and other treatments (21.8%) (multiple treatments possible).
Summary and Conclusions: Prevalence and gender ratio 3:1 was consistent with previously reported[1,2]. Most patients were diagnosed and treated as outpatients. Other oncological disorders are higher than in chronic lymphocytic leukemia[3]. About half of the patients were treated with chemotherapy within a year.

PB1983

THE SOUTHERN TRANSYLVANIA HEMATOLOGICAL PATIENTS' OPINION ON THE QUALITY OF MEDICAL CARE

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Background: The interest about the opinion of our patients on various aspects of health management has become a constant in our work, helping us to improve our activity.
Aims: To find the opinion of our patients on the quality of medical care.
Methods: A transversal study was performed on a sample of 214 consecutive hospitalized patients in our Hematology department in January 2015 who responded to a questionnaire on the quality of medical care. The results were analyzed and they have allowed conclusions that we hope to have implications for clinical practice.
Results: The mean age of surveyed patients was 61,79+/-16,05 years. Distribution by gender: women 51,19%, men 48,81%. Most surveyed patients (92.8%) believe that regular consultations in hematology service is a useful opportunity for drug doses adjustment and early detection and treatment of possible recurrences. Most of them (84.14%) believe that the time interval between two successive control consultations is suitable and only 8.33% think it is too short. It is gratifying that all surveyed patients believe that the attitude of physicians towards them is polite and 94.04% of them think that nurses are polite, too. However, the lack of courtesy of nurses was observed by 5.95% of respondents. Most patients (83.33%) think that the time established by the doctor for patient consultation is long enough, 3.57% of them consider that it is too short. All surveyed patients said they had been informed by their doctor on the diagnosis of hematological disease,the therapeutic plan has been exposed to them, and they understand its necessity. Most patients (94.04%) are satisfied with the results of treatment, and the rest do not know. The possible adverse effects of transfusions were explained to 82.82% of them. A percentage of 13.64% of them said they did not receive such information, and 4.55%-did not remember about this. While 82.82% of them admited to having read and signed a transfusion consent before they were transfused, 15.91% of them said they did not remember hereof. A proportion of 83.33% of surveyed patients is satisfied with the conditions of hospitalization and 14.29% of them are not. A proportion of 11.9% of them would like the medical staff to offer more attention to them. If they had to decide again in what hematology department in the country to be hospitalized and treated, 100% of respondents would still come in Sibiu.
Summary and Conclusions: Although most patients are satisfied with the medical care they have expressed several opinions that may contribute to increase their quality: a more polite attitude of the nurses, more time dedicated by physicians for consultation, explaining the possible adverse effects of transfusion to all patients, additional efforts to improve the conditions of hospitalization, more attention offered to the patient and their needs, greater concern for the introduction of innovative therapies. The fact that, despite the reported imperfections, patients prefer to be hospitalized again in our service, means that the general opinion is favorable and that they trust the provided medical services, which are perfectible.

PB1984

LIPOSOMIAL IRON IMPROVES FATIGUE IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES AS REFRACTORY ANEMIA. MULTICENTRIC STUDY.

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Background: Fatigue is the most invalidating symptom in neoplastic disease. Fatigue frequently is linked to an iron deficiency. In inflammatory diseases as myelodysplastic syndromes fatigue might be linked to a functional iron deficiency with elevated ferritin level and a saturation of total iron binding capacity<20%.
Aims: Aim of this study is to verify if liposomial iron support in myelodysplastic syndromes as refractory anemia improves fatigue perception in patients with a saturation of total iron binding capacity<20%.
Methods: Between june 2011and december2014,20patients affected by refractory anemia were studied. Median follow-up was12 months(R10-24).Patients were randomized1:1 to receive in groupA alpha erythropoietin 40000IUsc/week+calcium levofolinate7.5mg/day orally+Vitamin B12:400mg/day orally.In group B patient received liposomial iron14mg1 tablet orally/day+alpha erythropoietin 40000IUsc/week+calcium levofolinate7.5mg/day orally+Vitamin B12:400 mg/day orally.In group A median age was60 years(R65-70),M/F:8/2.In groupB median age was66 years(R60-75),M/F:6/4.Caryotype was normal in groupA andB patients.Median level of haemoglobin was 9 g/dl in group A(R8.5-11) and8.8g/dl(R8.5-11.5)in group B.Fatigue was measured with Modified Fatigue Impact Scale (FISC-Fisk 1994).
Results: Patients in group A reached a median hemoglobin level of11.5g/dl after 3 month of therapy and referred a median FISC score of74 (R65-80). Patients in group B reached a median hemoglobin level of12.5g/dl after 3 month of therapy and referred a median FISC score of54 (R42-68).
Summary and Conclusions: Liposomial iron support improves fatigue perception in patients with refractory anemia. This study needs confirmation on a lager cohort of patients.

Red blood cells and iron - Biology

PB1985

MOLECULAR CHARACTERIZATION OF BETA GLOBIN GENE OF THALASSEMIA REVEALS NEW AND RARE MUTATIONS IN PAKISTANI POPULATION

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Background: Imbalance in synthesis of α- or β- chains of hemoglobin results in thalassemia that has clinically diverse manifestations ranging from asymptomatic to regularly transfusion dependent individuals. More than 200 deletions or point mutations have been identified that impair transcription, processing, or translation of α- or β-globin mRNA. In Pakistan, a carrier rate of 5 to 7% demands communal preventive measures such as carrier identification, genetic counseling and prenatal diagnosis.
Aims: Present multicentre study undertaken in four cities of Pakistan examines the frequency and spectrum of alpha and beta thalassemia in Pakistani population.
Methods: 161 seronegative beta thalassemia patients, identified on the ground of haematological parameters, were screened for mutations of the Alpha (HBA2 and HBA1) and Beta (HBB) genes and G Gamma (HBG2) Globin gene XmnI polymorphism using a combination of Multiplex Gap PCR, Sanger sequencing and RFLP PCR.
Results: Among 16 mutations identified in the beta gene, HBB:c.27_28insG (p.Ser10Val) was the most prevalent. α-3.7 and α-4.2 deletions were also found in coinheritance with beta thalassemia mutations. Rare mutations such as HBB:c.-138C>T and HBB:c.315+1G>A were also identified. Interestingly, one novel mutation (HBB:c.-148T>A), two rare variants (HBB:c.332T>C (p.Leu111Pro) and HBB:c.92G>C (p.Arg31Thr) and a novel association (HBB:c.92G>C (p.Arg31Thr)/HBB:c.-92C>G) were also reported for the first time in our study in the Pakistani population. HBG2:c.-211C>T base-pair substitution (historically described as -158 Gγ XmnI polymorphism) was present in 36% of the patients analysed.
Summary and Conclusions: Such comprehensive studies revealing some rare mutations are essential for prenatal screening and control of this highly prevalent disease in Pakistani population along with nationwide awareness campaign.

PB1986

Abstract withdrawn

PB1987

HEME EXPORT AND HEME DEGRADATION DURING MURINE ERYTHROPIESIS

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Background: Erythropoiesis is the biological process that consumes the highest amount of body iron for heme synthesis. Heme accumulation within differentiating erythroid cells is crucial for globin genes expression and hemoglobin production. Recently, a role for heme oxygenase (HO)-1and Feline Leukemia Virus subgroup C Receptor (Flvcr)-1 genes in the control of cytosolic heme during erythropoiesis has been reported. HO-1 is the inducible enzyme responsible for heme degradation. Flvcr1 gene encodes for two heme exporters, Flvcr1a and Flvcr1b, localized at the plasma and mitochondrial membrane, respectively. Flvcr1 plays a crucial role in erythropoiesis since loss of both *Flvcr1a* and *Flvcr1b* in mice and zebrafish results in anaemia. Moreover, we demonstrated that *Flvcr1b* is required along all phases of erythropoiesis while *Flvcr1a* is important for the expansion of committed erythroid precursors but dispensable for their differentiation.
Aims: The aim of this work was the elucidation of the interplay between heme export and heme degradation during erythropoiesis.
Methods: We isolated erythroid sub-population from murine bone marrow and analyse the expression of *Flvcr1* isoforms and HO-1. Moreover, we compared HO-1 level observed in wild-type mice to that of conditional knock-out mice that do not express *Flvcr1a* and *Flvcr1b* in bone marrow. The same analysis was performed on the fetal liver of mouse embryos carrying alleles that differentially affect *Flvcr1a* and *Flvcr1b* expression.
Results: Our data indicated different profiles of expression for HO-1, *Flvcr1a* and *Flvcr1b* during erythropoiesis. Moreover, when *Flvcr1a* or *Flvcr1b* were lost, HO-1 resulted up-regulated.
Summary and Conclusions: These results suggest that during erythroid differentiation, heme export and heme degradation control different pools of heme.

PB1988

HEME, INFLAMMATION AND PARKINSON'S DISEASE

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Background: Heme is a metallo-compound, essential for the survival of most organisms. However, the ability of the central iron (Fe) atom, contained within its protoporphyrin ring, to participate in the Fenton chemistry and generate highly reactive hydroxyl radicals renders heme potentially toxic. Under inflammatory conditions, the release of heme from hemoproteins leads to unfettered oxidative stress, a deleterious effect allowing this molecule to sensitize parenchyma cells to undergo programmed cell death. We previously demonstrated that heme critically contributes to the pathogenesis of immune-mediated inflammatory diseases, by promoting the activation of the c-Jun N-terminal kinase signaling transduction pathway in response to pro-inflammatory agonists released in the course of the infection. The deleterious effect of heme is associated to tissue damage and disease severity, as upon hemolysis its release into circulation dictates the outcome of infectious diseases.
Aims: As infectious and inflammatory diseases are often associated to a certain degree of vascular leakage, it is possible that under these conditions the cytotoxicity of heme may affect also organs of restricted accessibility, such as the brain, thus increasing the risk and severity of neurodegenerative diseases.
Methods: This hypothesis has been investigated in mice by assessing whether the peripheral exogenous administration of heme or the release of heme upon exposure to immune-mediated inflammatory diseases enhances the severity of Parkinson's disease.
Results: Preliminary data demonstrates that heme triggers neurodegeneration and enhances the susceptibility to Parkinson's disease.
Summary and Conclusions: The protective effect of heme scavengers and proteins counteracting the pro-oxidant reactivity of Fe in preventing the development of Parkinson's disease suggests that heme cytotoxicity may critically contribute to this disorder.

PB1989

GHRELIN GENE POLYMORPHISM IN IRON DEFICIENCY ANEMIA

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Background: Iron deficiency anemia (IDA) is the most common in infancy and childhood hematologic diseases. It is seen in all age groups. Nutrition plays an important role in the development of IDA which is the most common cause of nutritional anemia. In our country, IDA has been determined in 12.7% of children between 4-months and 18-years old. One of the clinical features of IDA is loss of appetite and nutrition plays a major rol in IDA. Ghrelin stimulates appetite and food intake. It is considered that appetite and eating behavior are controlled with complex mechanisms by certain areas located at hypothalamus in central nervous system (CNS). The neurons containing ghrelin involved in appetite were found at arcuate nucleus of hypothalamus. Ghrelin at this localization controlles food intake. In addition to becoming hunger hormone, ghrelin regulates eating behavior and weight balance. Also ghrelin, before and after fasting, controlles behavioral, metabolic and gastrointestinal adaptations. The significant positive correlation was found between body iron and ghrelin levels. Ghrelin levels progresively decrease from iron decrease to development of IDA.
Aims: The frequency of ghrelin gene polymorphism was aimed to be investigated in children diagnosed as IDA and healthy control group. In this way, we aimed to determine ghrelin gene polymorphism in healthy children of our society. It was aimed which determined polymorphisms are seen more frequently. Thus, we aimed to determine difference between the ratios of healthy children and children with IDA. Among children with the same nutrition manner, common living conditions, we aimed to answer question that the real reason of IDA development may be occurance of a polymorphism at ghrelin gene.
Methods: In our study, total 57 children with IDA, 27 female (47.4%) and 30 male (52.6%), and 57 healthy control group were assessed. -501 promoter, arg51Gln, Leu72Met, and Gln90Leu polymorphisms at ghrelin gene were studied in patients and contol group.
Results: In terms of -501 A/C polymorphism at ghrelin gene, A allele was found statistically significant in patients group (P<0.05). For promoter -501 A/C polymorphism, the frequencies of genotype was not different from the control group (P<0.05). When genotype and allele frequencies were compared with control group for Arg51Gln, Leu72Met and Gln90Leu polymorphisms, there is no statistically significant difference (P<0.05).
Summary and Conclusions: We suggest that ghrelin gene may play an important role in IDA immunogeneticy. Also we think that studies are needed to be

done in both Turkish population and other populations for the determiation of other polymorphisms in this gene which may contribute to this disease. To verify our data, the same polymorphisms should be futher investigated in patients with IDA in different populations by more futher studies. We suggest that increased promoter -501 variant frequency in ghrelin gene may be one of he mechanisms involved in etiopathogenesis of IDA.

PB1990

RESPONSIVENESS TO PARENTERAL IRON THERAPY IN THE DIFFERENTIAL DIAGNOSIS FROM UNEXPLAINED REFRACTORY IRON DEFICIENCY ANEMIA TO IRON-REFRACTORY IRON DEFICIENCY ANEMIA

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Background: We evaluated responsiveness to parenteral Iron therapy in the differential diagnosis from unexplained refractory iron deficiency anemia to iron-refractory iron deficiency anemia.
Aims: We evaluated responsiveness to parenteral Iron therapy in the differential diagnosis from unexplained refractory iron deficiency anemia to iron-refractory iron deficiency anemia.
Methods: This study analyzed responses to IV iron sucrose therapy given to 15 children with unexplained refractory IDA who were unresponsive to oral iron therapy.
Results: Ferritin, MCV, MCH and Hb values were normal range in 10 patients at 6 weeks following the first therapy. The increase in Hb, MCH, MCV, and ferritin values were ranging from 2.6 to 3.5g/dL, 1.7 to 4.2 pg, 2 to 9 fL and 13 to 25 ng/mL in table 1, respectively. In five patients, Hb, MCH and MCV mean (range) values [11.2 g/dL (11-12.2), 24.5 pg (24-25.6) and 67fL (65-70)] were nearly normal but ferritin mean (range) values [9.8 ng/mL (8-11)] were below normal. The increase in Hb, MCH, MCV and ferritin were ranging from 2.6 to 3.5g/dL,7 to 4.2 pg, 2 to 9 fL and 5 to 12 ng/mL, respectively. Children with IRIDA in previous our study, Hb, MCH, MCV and ferritin values increased 6 weeks after the first therapy. The increase in Hb was 0.8–2.7g/dL and that of MCH, MCV, and ferritin values were 1.7–4.2 pg, 2–9 fL, and 13–25 ng/mL, respectively.
Summary and Conclusions: Intravenous iron therapy increased rapidly blood parameters in children with unexplained refractory IDA. The hematologic response to IV iron therapy should be used differentiation from unexplained refractory IDA to IRIDA.

PB1991

PHENOTYPIC PROFILE OF PAROXYSMAL NOCTURNAL HÉMOGLOBINURIA CLONES BY FLOW CYTOMETRY IN BLIDA, ALGERIA.

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Background: Paroxysmal nocturnal hemoglobinuria (PNH) is a rare, genetically acquired, life-threatening disease. We report 24 cases of PNH diagnosed and followed by flow cytometry (FC).
Aims: To evaluate the size of PNH clones and their clinical impact
Methods: The PNH clone was required in the event of bone marrow aplasia with or without hemolysis, of regenerative haemolytic anemia with negative direct Coombs test, in case of Myélodysplastic syndrome, in case of unexplained cytopenia and in thrombosis. The research of the clone by FC is carried out by analysis of the following monoclonal antibodies: Flear, CD59, CD14 with gating on the CD45, CD64 and on Glycophorin A; the clone was required on the red cells, neutrophil graulocytes (NG) and monocytes. We judged that the patients (pts) has a HPN clone when the deficit is >5% on at least two markers highlighted on two different lines. PNH clone was diagnosed among 151 pts since August 2009. A monitoring by CMF is ensured in the event of absence of deficit or a very moderate deficit or concerning only one line.
Results: On 151 analyzed cases, absence of HPN clone in 127 cases (84,2%); In 24 cases (15,8%), a deficit ≥ two markers in two lines or more was present. They are 10 women and 14 men; sex ratio=1,4, median age=41 years (21-58). PNH clone was found in bone marrow aplasia: 15/67 cases (22, 4%), hemolytic anemia with negative direct coombs: 2 /28 case (7,1%), other hemolysis : 2/11 cases (18%), thrombosis: 2 /21 (9,5%), bone marrow aplasia in pregnancy: 1/3 cases (33%), myelodysplastic syndrome : 02/14 cases (14.3%) and cytopénia: 0/6 cases. Types of HPN: type II: 2 cases (8,4%), type III: 16 (66%), mixed type: 6 cases (25%). The median rate of deficit of the CD59 on red cells=31.7% (5-72), of the CD59 on neutrophil granulocytes = 58,7% (7-99) and of Flear carried out in 11 cases=61.42% (6-98) and the deficit on the monocytes was required in 8 cases, the median rate of the deficit of CD14=77.7% (22-97) and of Flear (4 cases)=72,2% (19,4-92,1). In monitoring: appearance of the PNH clone in 1 case, increase in the size of clone in 4 cases, initially lower than 2%, and in a case the clone has doubled of size from 54% to 98% after 6 months and the patient developed a wide thrombosis. In 13 cases, a moderate deficit of a marker was noted on only one line.
Summary and Conclusions: In our study HPN clone was noted in 14% of the cases of MDS which joined the literature.
In the group where moderate deficit was observed, biological signs of hemolysis were absent; the indication of a follow-up of the size of HPN clone is then necessary and must include an assessment of hemolysis repeated with evaluation of the LDH; that is checked in our study since in 04 cases of increase size of the clone, a thrombosis occurred in one case.. B. Höchsmann showed after follow-up of 155 cases a significant increase of the clone in 28% of cases.
The application of FC, enabled us to make the diagnosis of PNH. Moreover it determined the phenotypic profile of PNH clones in our area and specified their size; it is also very useful for the follow-up of the patients. It remains a key examination in the diagnosis of the PNH and in some cases it will be necessary to repeat research of the clone after a first negative analysis in particular in the event of bone marrow aplasia and of hemolysis associated, and also in the event of small clone to follow its evolution.

PB1992

IRON AND TRANSFERRIN IN CHLDREN WITH SIDEROPENIC ANEMIA

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Background: Introduction: Sideropenia means lack of iron within the organism. As the result of the absence of the iron the synthesis of hemi decreases in hemoglobin. Sideropenic anemia is the most common anemia to children because of the specific metabolic needs associated with growth and development. Anemia as a lack of iron is charachteriszed by a defect in the synthesis of hemoglobin and as a result of that we end up to a reduction of the red blood cells (micro cells), and to a reduction of amount of hemoglobin (hypochromic).
Aims: The purpose of the paper: was the presentation of cases with sideropenic anemia to our patients.
Methods: Materials and methods: Within the study we had 200 children consisting of all age groups, hospitalized in the Pediatric Clinic, in hemato-oncology department. The diagnosis is done based on the past history, physical examination, and laboratory analysis. The test of iron and transferrin is done using the electronic modern method.
Results: Results: Regarding the age of children diagnosed with sideropenic anemia, the most endangered age group was 0-1 years where 113 cases or (56.1%) were diagnosed, whereas the ages with lower risk were ages 7-15 years which consisted of 18 cases or (5.6%). Further on, according to settlements, we did not have many differences. From villages we have had 109 cases (54.5%), while from the cities 91 cases (45.5%). Male children dominated by 107 cases (53.5%), whereas females consisted of 93 cases (46.5%). In terms of nutritional status, eutrophic children dominated by 131 cases (65.6%). Moreover, according to iron values of 1-2.9 micromoles/L have been presented 14 cases (70%), and of 6-10 micromoles/L 108 cases (54.0%) have been presented. By nominal values of UIBC transferrin have been 187 cases (93.5%), while those with higher values of TIBC consisted of 128 cases (64.8%).
Summary and Conclusions: Conclusion: Based on our data, it is obvious that sideropenic anemia is not rare to our children, however it tends to be quite frequent. A very important part to this disease we consider some factors such as: socio-economic low status, the low level of parental education, and the nutritional manner.

Red blood cells and iron - Clinical

PB1993

MULTI-PARAMETRIC CARDIAC MAGNETIC RESONANCE FOR PREDICTION OF CARDIAC COMPLICATIONS IN THALASSEMIA INTERMEDIA: A PROSPECTIVE MULTICENTER STUDY

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Background: Cardiovascular Magnetic Resonance (CMR) has an established role in managing and predicting prognosis of patients with Thalassemia Major (TM). Thalassemia Intermedia (TI) is a milder variant of beta-thalassemia showing a different clinical and prognostic profile; pulmonary hypertension (PH) is a more common complication in TI patients.
Aims: We prospectively determined the predictive value of CMR parameters, including measurement of right ventricular mass, for cardiac complications in TI.
Methods: We considered 342 TI patients enrolled in the Myocardial Iron Overload in Thalassemia network; about half of them (178/302, 58.9%) became transfusion-dependent in the adult age. Myocardial and liver iron overload were measured by T2* multiecho technique. Atrial dimensions, left and right ventricular mass and systolic function were quantified by cine SSFP images. Late gadolinium enhancement (LGE) images were acquired to detect myocardial fibrosis.
Results: Twenty-three patients were excluded because a cardiac complication was present at the time of first CMR, so we prospectively followed 319 patients. All 319 patients were white, with a mean age at time of their first scan of 38.02±11.69 years and 165 (51.7%) of them were females. Mean follow-up time was 52.24±24.87 months (median 54.64 months). Cardiac events were recorded in 22 patients (6.9%): heart failure (HF) in 1 patient, arrhythmias in 12 patients, pulmonary hypertension (PH) in 7 patients and myocardial infarction (MI) in 2 patients. The mean time from the first MR scan to the development of a cardiac complication was 30.77±23.48 months and 8 (36.4%) cardiac events occurred within the first year of follow-up. Significant myocardial iron overload (global heart T2*<20 ms), ventricular dilation, right ventricular (RV) hypertrophy, myocardial fibrosis and atrial dilation were identified as univariate prognosticators. In the multivariate analysis the independent predictive factors were RV hypertrophy (HR=24.12, 95% CI=5.09-114.12, P<0.0001) and myocardial fibrosis by LGE (HR=6.59, 95% CI=1.33-32.67, P=0.021). The Figure displays the Kaplan–Meier curves showing the impact of each predictive factor on the development of cardiac complications. The log-rank test revealed a significant difference in the curves for each predictor factor (RV hypertrophy: P<0.0001 and myocardial fibrosis: P<0.0001).

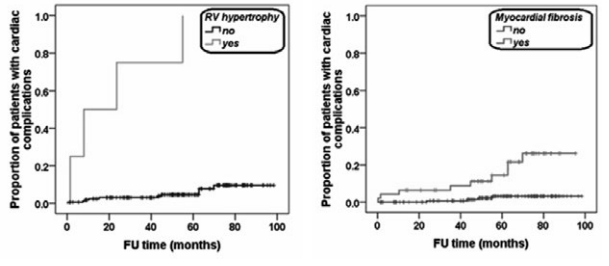

Figure 1.

Summary and Conclusions: For the first time we studied the prognostic value of right ventricular mass as part of multiparametric CMR imaging in a population of TI patients. Both RV hypertrophy and fibrosis detected by LGE were independent predictive factor for cardiac complications. Measurement of RV mass should be part of the multi-parametric CMR study of patient with thalassemia intermedia.

PB1994

A PROSPECTIVE MRI STUDY OF CARDIAC AND HEPATIC IRON AND CARDIAC FUNCTION IN NON-TRASFUSION-DEPENDENT THALASSEMIA INTERMEDIA PATIENTS TREATED WITH DESFERRIOXAMINE

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Background: In thalassemia intermedia (TI) patients no observational study prospectively evaluated in the real life the efficacy of the desferrioxamine (DFO) therapy in removing or preventing iron overload from the heart and the liver by T2* Magnetic Resonance Imaging (MRI).
Aims: The efficacy endpoint of this study is represented by the changes in cardiac T2* values, MRI LIC (liver iron concentration) values and biventricular function parameters in non-transfusion dependent (NTD) TI patients after 18 months of desferrioxamine therapy.
Methods: Among the 325 TI patients enrolled in the MIOT (Myocardial Iron Overload in Thalassemia) network, we selected 129 TI patients NTD. We considered 29 patients who had been received DFO alone between the two MRI scans. Cardiac iron overload was assessed by the multislice multiecho T2* technique. Hepatic T2* values were assessed in a homogeneous tissue area and converted into LIC. Biventricular function parameters were quantified by cine SSFP sequences. Myocardial fibrosis was evaluated by late gadolinium enhancement (LGE) acquisitions.
Results: Mean age was 39.69±8.12 years and 14 (48.3%) patients were females. Patients started regular chelation therapy at a mean age of 21.92±15.89 years. The mean administered dosage of DFO via subcutaneous route was 38.46±10.27 mg/kg body weight on 3.32±1.54 days/week. The percentage of patients with excellent/good levels of compliance to the chelation treatment was 82.1%. At baseline only one patient showed cardiac iron overload (global heart T2*=15.23 ms) but he recovered at the FU (global heart T2*=26.93 ms). All patients without cardiac iron maintained the same status at the follow-up (FU). Eighteen patients (62.1%) had hepatic iron overload (MRI LIC ≥3 mg/g/dw) at the baseline. For this subgroup, the baseline and the FU LIC values were, respectively, 9.15±7.97 mg/g/dw and 7.41±6.28 mg/g/dw. The reduction in MRI LIC values was not significant (P=0.102). Out of the 11 patients with a baseline MRI LIC<3 mg/g/dw, only one (9.1%) showed hepatic iron at the FU. The Figure shows the evolution of different hepatic iron overload risk classes between the baseline and the FU. Due mainly to technical reasons, cardiac function was assessed at both baseline and FU MRIs in 24 patients. At baseline all patients had a normal LV ejection fraction (EF) and 4 of them showed a reduced LV ejection fraction (LVEF) at the FU. No patient had a pathological RV EF. No significant change between the two MRIs was detected in biventricular volume indexes, biventricular EFs and LV mass index. For 21 patients the presence of myocardial fibrosis was investigated at both baseline and FU MRIs, and this subgroup was considered. Three (14.3%) patients had myocardial fibrosis at the baseline, all with a non ischemic pattern. At the FU two new occurrences of non-ischemic myocardial fibrosis were detected.

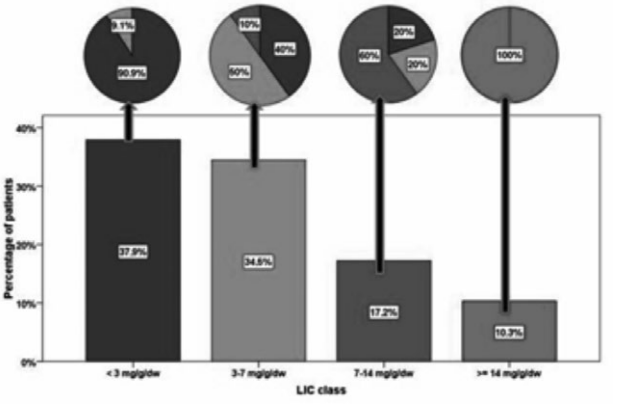

Figure 1.

Summary and Conclusions: In this small population of sporadically or non transfused TI patients, the DFO therapy showed 100% efficacy in maintaining a normal global heart T2* value. As regards as the hepatic iron overload, the DFO therapy did not prevent the transition to a worst class in 2 patients. Moreover, the DFO therapy did not prevent the worsening of the LV function and the occurrence of new myocardial fibrosis.

PB1995

THE ROLE OF NITRIC OXIDE IN SLEEP OF ADULTS PATIENTS WITH SICKLE CELL DISEASE

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Background: Nitric oxide (NO) is widely implicated in the modification of the sleep-wake states and, in obstructive sleep apnea, decreased NO levels have been implicated as possible mechanisms for development of cardiovascular diseases. Previous studies have shown that sleep disorders are prevalent in sickle cell disease (SCD) patients, and it is well known that NO plays a significant role in the pathophysiology of this disease.
Aims: The aim of the present study was to analyze the relationship between NO and sleep disorders in a group of SCD patients.
Methods: Steady-state adult patients with SCD followed at Ambulatory of Hereditary Anemias from Escola Paulista de Medicina/UNIFESP were invited to this study. Exclusion criteria were: chronic blood transfusion, pregnancy, and vaso-occlusive episodes and transfusion in the last three months. Sleep disorders were evaluated by standard overnight multichannel polysomnography (Somnologica, Embla™). NO plasma samples were determined by chemiluminescence using Model 280 Nitric Oxide Analyzer (NOA™) from Sievers Instruments, Inc. (Boulder, CO, USA). This study was approved by Ethical Committee, and all patients agreed to participate.
Results: 64 consecutive patients were enrolled, 33 (51.6%) males, with median (range) age of 28 (19–60) years. 42 (65.6%) had sickle cell anemia in use of hydroxyurea (SS-HU), 13 (20.3%) SS without HU, and 9 (14.1%) SC disease. The NO median concentration was 71.75 uM (40.5–243.5), with no difference among SS-HU, SS and SC patients (P=0.40). According with median level of NO, the patients were divided in two groups: G1 ≤71.75 and G2 >71.75uM. Among the sleep parameters analyzed, only two showed significant difference when compared G1 with G2: AHI – apnea-hypopnea index: 3.1(0–43.2) *vs.* 0.8 (0–10.4) (P=0.008), and DI – desaturation index: 5.1 (0–106.3) *vs.* 3.3 (0–29.9) (P=0.011). Considering the normal values of AHI and DI (≤5 and ≤10, respectively), we found association between lower levels of NO and AHI (OR=5.05, 95%CI: 1.25-20.43, P=0.03), and between lower levels of NO and DI (OR=3.81, 95% CI: 1.04–13.95, P=0.07).
Summary and Conclusions: Obstructive sleep apnea is, probably, the most known sleep-disordered breathing (SDB). It is a multifactorial process that leads not only to chronic intermittent hypoxia and sleep fragmentation, but also to increased cardiovascular morbidity. NO plays a contributory role in the pathophysiology of this process. Interestingly, SDB and SCD present many aspects in common, like chronic inflammatory status, endothelial dysfunction, and lower levels of NO. Moreover, SDB is prevalent in SCD and there is a relationship between NO level and SCD manifestations. The AHI and DI are important indexes related to SDB and risk of cardiovascular morbidity. The fact that we found both indexes altered in SCD, especially in patients with lower levels of NO, confirm that SCD patients suffered from sleep disturbances, and implies that NO is probably associated with it. This is one of the first studies about sleep disorders in adult patients with SCD, reinforcing the need of more studies in this field.
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PB1996

Abstract withdrawn

PB1997

Abstract withdrawn

PB1998

LESSONS FROM VERY SEVERE, REFRACTORY AND FATAL PRIMARY AUTOIMMUNE HAEMOLYTIC ANAEMIAS

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Background: Autoimmune haemolytic anaemia (AIHA), usually classified as warm (WAIHA), cold (CAD), mixed, and atypical forms, is a greatly heterogeneous condition both in terms of clinical presentation and response to treatment, varying from mild/fully compensated to very severe, life-threatening and fatal.
Aims: To describe acute and very severe cases of AIHA who displayed multidrug refractoriness and related complications.
Methods: 13 consecutive patients were identified among 157 primary AIHA followed from 1996 to 2014 at our institution. Patients were selected on the basis of very severe clinical presentation (Hb<6 g/dL, 51 cases) and requirement of at least 3 lines of therapy [steroids +/- IVIG, splenectomy, various immunesuppressants, plasma-exchange (PEX) and erythropoietin, 13/51].
Results: Table 1 shows the clinical characteristics of patients: 7 cases were direct antiglobulin test (DAT) positive with IgG+C antisera, 4 with IgG, and 1 with C; 1 case was DAT negative. All displayed marked intravascular haemolysis, 8 inadequate reticulocytosis, and 6 immune thrombocytopenia. Thrombotic events occurred in 8 patients (of whom 4 pulmonary embolism and 3 disseminated intravascular haemolysis) and acute renal failure in 3. All patients (but a Jehovah witness) received transfusions and steroids +/- intravenous immunoglobulins, 11 rituximab, 9 immunosuppressants, 5 PEX, 3 erythropoietin and 5 underwent splenectomy. Seven cases (54%) were fatal. In most cases death was due to infections and multiple organ failure, and was related with multi-treatment, particularly splenectomy, and with concomitant thrombocytopenia. Thrombotic complications were not fatal, and occurred more frequently in splenectomised cases.

Table 1.

	Date of diagnosis	DAT positivity	Hb g/dL	Ret 10^3 /mmc	LDH U/L	Treatment	Complications	Outcome (date last f-u or death)
Case 1 (F/ 31yrs)	1986	IgG	3.5	139	7665	Azathioprine, Splenectomy Vincristine, Cyclophosphamide	ITP, Gastro-intestinal bleeding Deep venous thrombosis Pulmonary embolism Acute renal failure, MOF	Death (May, 2014)
Case 2 (F/ 61 yrs)	May 2001	IgG+C	6.0	297	10404	Cyclophosphamide, Splenectomy Cyclosporine, Plasma exchange Rituximab, Vincristine	Pulmonary embolism Pneumonia	In follow-up, off therapy (Sept 2014)
Case 3 (M/ 50 yrs)	Nov 2004	IgG	5.2	500	12787	Cyclophosphamide, Azathioprine Rituximab, Splenectomy	ITP, Pneumonia Peritonitis Acute renal failure Sepsis/ARDS, MOF	Death (March 2006)
Case 4 (M/ 37 yrs)	Oct 2006	DAT-	5.4	200	839	Plasma-exchange, Cyclophosphamide, Rituximab	ITP Deep venous thrombosis	In follow-up off therapy (July 2014)
Case 5 (F/ 33 yrs)	Dec 2009	IgG+C	4.3	429	955	LD-Rituximab, Azathioprine Splenectomy	Sepsis,MOF	Death (April 2012)
Case 6 (F/ 46 yrs)	Sept 2009	IgG+C	4.9	157	1074	LD-Rituximab Cyclophosphamide Splenectomy	Pulmonary embolism Pneumonia	In follow up off therapy (Sept 2014)
Case 7 (F/ 46 yrs)	Dec 2010	IgG+C	5.9	114	835	Rituximab	ITP, Pneumonia HCV/toxic hepatitis MOF	Death (March 2013)
Case 8 (F/ 67 yrs)	Jan 2008	C3d	6	271	468	LD-Rituximab Cyclophosphamide, EPO	Pneumonia Pulmonary embolism	In follow-up off therapy (June 2014)
Case 9 (M/ 40 yrs)	Sept 2011	IgG+C	5	90	960	LD-Rituximab	ITP, Pneumonia Respiratory insufficiency	Death (April 2013)
Case 10 (F/ 73 yrs)	June 2011	IgG+C	2	58	703	Plasma-exchange, EPO	ITP, Pneumonia /Respiratory insufficiency Acute renal failure, DIC	Death (July 2014)*
Case 11 (F/ 18yrs)	April 2012	IgG	2.1	50	4000	Plasma-exchange, Rituximab Cyclophosphamide, EPO	Pneumonia, DIC	In follow-up off therapy (Sept 2014)
Case 12 (M/ 40 yrs)	Feb 2009	IgG	5.9	397	579	Cyclophosphamide, Rituximab	Osteonecrosis Septic arthritis Mastoiditis	In follow-up off therapy (Sept 2014)
Case 13 (M/ 66 yrs)	April 16, 2014	IgG+C	1.8	48	2200	Plasma-exchange, Rituximab	DIC, MOF	Death (April 23, 2014)

All patients were treated with steroids +/- IVIG. Bone Marrow evaluation and imaging studies excluded AIHA secondary to lymphoproliferative diseases in all patients. Autoimmunity profile was negative in cases 1, 2, 4, 6, 7, 9, 10, 11, 12, 13. In case 10 death was not related to AIHA

Summary and Conclusions: The challenging AIHA cases described showed a greatly unpredictable clinical course. Evans syndrome and previous splenectomy, particularly when performed after multiple immunosuppressive therapies, were associated with poor prognosis. Rituximab, when promptly administered, was effective in solving the clinical emergency in most cases unresponsive to steroids and transfusions. Plasma exchange and erythropoietin were valuable options. Above all, in very severe AIHAs a scrupulous and continuous attention by experienced clinicians is required in order to avoid a delay in potentially life-saving strategies.

PB1999

THE CORRELATION BETWEEN CAROTID ARTERY INTIMA MEDIA THICKNESS AND ANTIOXIDATIVE ENZYMES ACTIVITIES IN CHILDREN WITH IRON DEFICIENCY AND IRON DEFICIENCY ANEMIA

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Background: Lipid peroxidation increased in iron defiency anemia (IDA) as a result of increase capasity of oxydant and also decreases of antioxydant capasity. Though there are many studies that damaged antioxydant activity affects atherogenonosus in IDA; there are not any studies over intima-media thickness of carotid artery (CIMT).
Aims: The aim of this study is to assess the antioxydant enzym activity in iron defiency (ID) without anemiea and thickness of carotid intima media as an early indicator of premature of atherosclerosis.
Methods: In this study, we searched for the relationship between carotid intima-media thickness and antioxidant enzyme activities in adolescents with iron deficiency or IDA. The study was performed on 71 children aged 11 to 17 years old including 25 children with ID, 25 children with IDA and 21 healthy children. Complete blood count, the serum levels of iron and iron binding capacity, ferritin, lipid profile, glutathione peroxidase, glutathione reductase, paraoxonase activity were carried out. The CIMT were assessed by using echocardiography. All blood tests and CIMT were reevaluated after three months oral ferric iron treatment in the group with iron deficiency anemia.
Results: We founded significantly lower paraoxonase activity in groups of iron deficiency and iron deficiency anemia (P<0.05), but there was not a difference between this two groups. In addition, glutathione peroxidase and reductase activities were similar (p>0.05). CIMT in ID and IDA were higher than the control group (P<0.05), but there was not a difference between this two groups. After the iron treatment, significantly increased PON, GSH-Px, GSH-R activities (P<0.05) and CIMT were decreased. It has been determined that a significant correlation between PON enzyme activity and levels of hemoglobin, serum

iron, ferritin, serum transferrin saturation. Moreover, there was an inverse correlation between carotid intima thickness and levels of hemoglobin, ferritin, and serum transferrin saturation. It was also revealed PON was negatively correlated with CIMT in IDA group (P<0.05, r: -0.410).

Table 1.

Table I. Corelation between PON and CIMT in ID, IDA, and control groups.

	ID	IDA	Control	*p* value
PON (U/L)	132±60[a]	105± 29[b]	209±38	p<0.05
CIMT(mm)	0.44±0.01[a]	0.44±0.01[b]	0.41±0.02	p<0.05

(a) There was a signicifant between ID and Control groups.
(b) There was a signicifant between IDA and control groups.

Table 2.

Table II. Corelation between PON and CIMT in pre and post treatment groups.

	Pre-treatment	Post-treatment	*p* value
PON(U/L)	105± 29	242± 77	*p*<0.05
CIMT(mm)	0.44±0.01	0.42±0.02	*p*<0.05

Summary and Conclusions: We think that impaired antioxydant capasity exist during iron defiency without anemia and this lead up to atherosclerosis. In addition, iron defiency even if not anemia should be treated to prevent prematüre aterosclerosis

PB2000

A COMMON GENETIC VARIANT OF THE TMPRSS6 GENE INFLUENCES THE SUSCEPTIBILITY TO IRON DEFICIENCY ANAEMIA IN PORTUGUESE WOMEN

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Background: Anaemia is a worldwide blood disorder affecting about one-quarter of the world's population. Iron-deficiency anaemia (IDA) is the most common type of anaemia and is caused by inadequate iron availability for haemoglobin synthesis. The disorder development can be attributed to nutritional factors, namely to an iron deficiency diet. However, genetic variants may also be involved. Genome-wide association studies have suggested that common variants in the liver-expressed *TMPRSS6* gene might modulate haematological phenotype and iron status. This gene encodes for a membrane-bound serine protease, matriptase-2, that plays an essential role in down regulation hepcidin, the key regulator of iron homeostasis. One of the suggested genetic variant is the SNP rs855791 c.2207 C>T, p.Ala736Val.
Aims: The objective of this work was to evaluate whether the SNP rs855791 in *TMPRSS6* gene may influence IDA susceptibility in Portuguese women.
Methods: The SNP rs855791 (c.2207 C>T, p.V736A) was characterized at DNA level, using an *allele specific amplification* approach, in 25 Portuguese women presenting IDA (mean age=38 years) and in another 89 women without IDA, corresponding to the normal control group (mean age=39 years).
Results: We have found that the frequency of the three genotypes at SNP rs855791 is different between the two groups of women. The CC genotype frequency (736A) is lower in the IDA group than in controls and, inversely, the TT genotype frequency (736V) is higher; (P=0.037). Also, the TT genotype is associated with low haemoglobin level (P=0.036), serum iron (P=0.009) and transferrin saturation (P=0.015).
Summary and Conclusions: The genotype TT at SNP rs855791 within the gene *TMPRSS6* is associated with the lowest haemoglobin levels and serum iron parameters of the studied women. Moreover, it is more frequent in the IDA group. Therefore, we can conclude that this variant is a genetic risk factor to develop IDA, which corroborates results obtained by previous studies in other populations. So, this study has revealed that the *TMPRSS6* gene, besides its association with the rare iron deficiency iron refractory anaemia (IRIDA), has also an important role as genetic modifier of common iron-related disorders. The polymorphism p.Ala736Val effect is probably due to a partial impairment of matriptase-2 and, consequently, to an increased expression of hepcidin which perturbs iron absorption in the duodenum as well as iron recycling by macrophages. *Partially funded by FCT: PEst-OE/SAU/UI0009/2013 and Pest-OE/SAU/UI4013/2011.*

PB2001

LONG TERM FOLLOW-UP OF HYDROXYUREA IN SICKLE CELL DISEASE

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Background: Hydroxyurea (HU) is considered to be the most successful drug therapy for severe sickle cell disease (SCD) and has many characteristics of an ideal drug for sickle cell anemia (SCA) and provides therapeutic benefit through multiple mechanisms of action. HU emerged as an important therapeutic option for children and adolescents with recurrent vaso-occlusive events and sustained long-term benefits were documented
Aims: To assess long term effects of hydroxyurea therapy in infants and adults with sickle cell disease (all types)
Methods: Since 2000, there were 54 SCD patients treated with HU, 39 SS 14S/βthal and 1 SC: Mean age at inclusion was 12+/-4.4 years (4 – 22 y), just 3 patients was aged above 40y at the inclusion, sex ratio: 29F/25M. Thirty night included for standard criteria (more than 3 severe crisis pain/year and/or recurrent acute chest syndrome >1 ACS/y) and 7 because of severe chronic anemia (baseline of hemoglobin lower than 6g/dL), 2 for complex red cell alloimmunization, 1 for delayed posttransfusional hemolysis, 1 invalidant priapism, 2 association with auto-immun disease(sarcoidosis, Systemic lupus erythematosus), 1 history of stroke with transfusional difficulties, 1 for nephropathy and renal failure (creatinine 18 mg/mL). 13 patients have been splenectomized before the inclusion; Avascular Necrosis of hips was noted in 5 patients. Ferritine in 27 patients polytransfused (more 20 blood units) was 3700 ng/mL. The median daily dose of HU was 18 mg/kg (14 to 22 mg/kg/d)
Results: After a follow-up of 9 years(5 to 14 years), we observed a significant decreased (P<0,0001) of severe and recurrent acute pain crisis (5,5→0,7/y) and Acute chest syndrome (1,5→0,3/y), need of blood transfusions (8→2/year), days of hospitalization (17→3/y). Sustained clinical benefits for the most patients received long-term HU treatment. Total hemoglobin increase was 1,1g/dl (0.5 à 2.5). In the subgroup of 7 patients treated for severe anemia, the mean hemoglobin level was 6.6±0.5 g/dL prior to HU and increased to 8.2±1.5 g/dL after 1 year of therapy. The iron chelation Deferroxamine (30mg/kg/j, 3j/7) and phlebotomy, performed in 11 patients when Hb level was upper 9g/dl). We noted some severe events: acute priapism in 4 patients (1 shunt cavernospongieux),4 hip osteonecrosis**.** We deplored 7 deaths: recidive of stroke (1), sepsis (1), ACS (2), progressive renal failure (1), acute hepatic sequestration (1), undetermined (1). Five were lost to follow-up after a median of 48 months. Very young patients treated have been associated with excellent growth and development. Sexual maturation, including menarche, has occurred without apparent delay. No severe side effect was related to HU treatment, which was discontinued in children mainly for transient neutropenia or thrombopenia, hypersplenism or acute splenic sequestration, followed by splenectomy(9 cases) or non-compliance (2 cases). Four women were pregnant after 8 years of HU. Long-term clinical benefits have also been observed wth improved quality of life. Ferritin levels were decreased with significant decline in group of patients treated with Deferroxamine and/or phlebotomy (3700→950 ng/mL).
Summary and Conclusions: Long-term exposure to HU seems associated with significantly reduced mortality, perhaps by reducing long-term end organ damage and does not appear to be associated with increased risk of secondary malignancies nor myelodysplasic syndrome. Our experience, mainly in children allows us to consider that this drug is a very potent therapeutic option for the management of severe forms of the disease, particularly in countries with limited blood supply. An updated national registry is highly needed to further assessment.

PB2002

GEOGRAPHICAL DIFFERENCES IN IRON OVERLOAD AND IRON CHELATION PRACTICES IN ANEMIA PATIENTS: BASELINE RESULTS FROM THE TRANSFUSIONAL HEMOSIDEROSIS REGISTRY STUDY (TORS)

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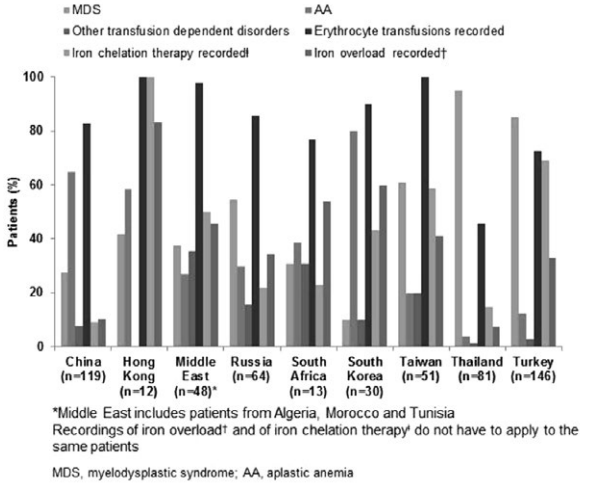
Background: Data describing transfusion practices and diagnoses of iron overload as well as iron chelation in patients with anemia across geographical regions are limited. A prospective epidemiological study may provide further insights into clinical practices for these conditions.
Aims: This prospective, multinational, non-interventional study, aimed to (1)

evaluate the differences in baseline characteristics among early diagnosed patients with anemia requiring chronic transfusion therapy; (2) assess the extent of iron overload and patterns of care surrounding the use of iron chelation therapy in different geographical regions.
Methods: Patients aged >2 years requiring chronic transfusion therapy with newly diagnosed anemias (<12 months from diagnosis), including thalassemia, low and intermediate-1 myelodysplastic syndromes (MDS), aplastic anemia (AA), Diamond-Blackfan anemia (DBA) and other transfusion-dependent anemias were enrolled. Patients were recruited from Turkey, Russia and South Africa as well as from countries within the Asia-Pacific and Middle East regions. Patients with secondary or therapy-related MDS; intermediate-2 or high-risk MDS; or acute leukemia were excluded. Patients were evaluated at baseline and at follow-up visits according to the standard practice for up to 3 years or until death. At baseline, patient demographics and details of iron overload and iron chelation therapy use were assessed. Transfusional hemosiderosis was defined as ≥1 serum ferritin measurement >1000 ng/mL after onset of transfusion therapy; or a liver iron concentration (LIC) >2 mg Fe/g dry weight; or if serum ferritin or LIC measurements were not available, evidence of ≥20 units of red blood cell transfusions.
Results: Of the 564 patients (including patients aged ≤18 years, n=57) recruited, majority had a diagnosis of MDS (58.5%, n=330), followed by AA (31.2%, n=176), other transfusion-dependent disorders (6.2%, n=35), β-thalassemia major (2.0%, n=11), β-thalassemia intermedia (1.1%, n=6), sickle cell anemia (0.5%, n=3) and DBA (0.4%, n=2). The mean age (±SD) of the patients was 51.9±23.87 years (range, 2-92); 49.5% (n=279) were male. At study entry, >70% of patients in all countries, except Thailand (45.7%), were receiving transfusion therapy. Overall, 29.4% (166/564) patients and 36.8% (21/57) pediatric patients were assessed as having iron overload during the study; extent of iron overload across countries varied and ranged from 7.4% (6/81) in Thailand to 83.3% (10/12) in Hong Kong. Patients receiving drug therapy only were less likely to be assessed for iron overload (1.2% [1/86]) than patients receiving erythrocyte transfusion (33.0% [33/100]) or erythrocyte transfusion and drug therapy (38.1% [131/344]). Overall, 39% of the population received iron chelation therapy and ranged from 9.2% (11/119) in China to 100.0% (12/12) in Hong Kong. Despite receiving iron chelation therapy, 56.4% (124/220 patients) were documented as having iron overload (Figure 1).

Figure 1- Geographical Differences in Diagnoses, Iron Overload, Erythrocyte Transfusions and Iron Chelation Use in Patients Included into the TORS



*Middle East includes patients from Algeria, Morocco and Tunisia
Recordings of iron overload† and of iron chelation therapy‡ do not have to apply to the same patients

MDS, myelodysplastic syndrome; AA, aplastic anemia

Figure 1.

Summary and Conclusions: In this large observational study, MDS was the most common disease type enrolled. Majority of patients received transfusion therapy across countries; consequently there was a proportion of patients that had iron overload. Despite a large percent of patients receiving transfusion therapy and having developed iron overload, there was a broad spectrum of iron chelation use. Notably, over half of patients still had iron overload despite receiving chelation therapy. Overall, these results suggest that iron overload management practices may still be suboptimal in many parts of the world. Further studies are required to clearly understand these geographical differences in management of transfusion–dependent anemias.

PB2003

CHRONIC HCV INFECTION AND HEMOSIDEROSIS AMONG EGYPTIAN THALASSEMIA PATIENTS: THE ROLE OF HOMA INDEX

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Background: Hepatitis C virus (HCV) is a major cause of chronic hepatitis C (CHC) which increases the risk for insulin resistance, glucose intolerance and type 2 diabetes. Egypt has the highest reported rates of HCV infection especially among chronically transfused patients. Abnormal glucose tolerance is common in multitransfused thalassemia and is attributed to early impaired beta cells function with increasing insulin resistance (IR). The HOMA index (homeostasis model assessment) has been extensively used to investigate insulin resistance in CHC
Aims: to detect insulin resistance in transfusion dependent thalassemia major patients and to evaluate its possible link to chronic hepatitis and iron overload
Methods: : Fifty nine Egyptian thalassemia major patients were divided into hepatitis C (HCV) positive (group A; n= 39) and HCV negative thalassemics (group B; n=20) by HCV antibody and RNA. Thalassemia patients were compared to 20 age and sex matched HCV positive (non thalassemic) patients (group C). Blood samples were withdrawn from all patients for assessment of HOMA index, serum ferritin, AST and ALT
Results: : Abnormal HOMA test was evident in 30.8% (n=12) of group A and 40% (n=8) of the control group while none of group B had abnormal test. Significant results of HOMA-IR was observed between the three groups (P=0.029) and between group B and each of groups A and C (P= 0.012, 0.043 respectively) while no difference was observed between HCV positive thalassemics and control group (P=0.99) Positive correlation (P=0.038) was observed between HOMA- IR and AST but no correlation to age or serum ferritin level
Summary and Conclusions: Chronic Hepatitis in transfusion dependent thalassemia major patients is a major risk factor for insulin resistance in these patients

PB2004

MRI BRAIN IN YOUNG EGYPTIAN SICKLE CELL DISEASE PATIENTS: RELATION TO CEREBRAL BLOOD FLOW AND NEUROPSYCHOMETRIC EVALUATION

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Background: Stroke is an important and common complication of sickle cell disease (SCD), affecting children as well as adults.
Aims: To evaluate the prevalence of abnormal MRI brain in sickle cell disease (SCD) patients and its relation to transcranial Doppler findings and the results of neurosychometric evaluation
Methods: A cohort of 30 SCD patients aged less than 18 years were recruited from the Children's Hospital, Faculty of Medicine, Ain Shams University, Cairo, Egypt, examined in the steady state.Patients were subjected to full clinical and neurological examination and psychometric evaluation using Wechsler intelligence scale and Benton visual retention test, laboratory investigations including CBC,Hb electrophoresis,serum ferritin, MRI brain,transcranial Doppler ultrasonography for MCA,ACA,PCA,ICA,vertebral artery and basilar artery.
Results: The cohort included 12 males and 18 females, mean age 11+/-3.5 years., including 14 splenectomized patients. Abnormal MRI findings were present in 7/30 patients(23.3%), as tiny cortical infarctions in 5 patients (all were asymptomatic) and large infarctions in two patients (both had previous history of stroke). The presence of abnormal MRI was not related to age,sex,family history of SCD or stroke,neurological symptomatology,abnormal findings on neurological examination,or frequency of blood transfusion. Abnormal brain MRI was significantly related to frequency of sickling crises (P=0.002), surgical splenectomy (P=0.002), HDU therapy (P=0001), scholastic retardation (P=0.0001), impaired Penton visual perception (P=0.0001), impaired attention (P=0.001), impaired comprehension (P=0001), hematocrit value(P=0.001), HbS%(P=0.0001), HbF%(P=0.001), Total WBCs count(P=0.0001), absolute neutrophil count(P=0.0001), serum ferritin(P=0.0001).Abnormal MRI was related to cerebral blood flow in MCA (P=0001)and ICA (P=0001) but not related to cerebral blood flow in ACA,PCA, vertebral artery and basilar artery.
Summary and Conclusions: Conclusion: In patients with SCD,the neurologist should not only rely on standard neurological examination,but should add cerebral doppler and neuropsychometric assessment tools as soft signs to identify even minor MRI lesions and to identify the children at risk of stroke

PB2005

THE PREVALENCE OF ANEMIA, IRON DEFICIENCY AND IRON DEFICIENCY ANEMIA, AND THEIR ASSOCIATION WITH HEAVY METALS IN BLOOD AND NUTRITIONAL INTAKES IN KOREAN WOMEN AGED 10 YEARS OR OLDER

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Background: Anemia, iron deficiency (ID) and iron deficiency anemia (IDA) continue to be common disorders in the world.
Aims: This study was aimed at assessing the prevalence of anemia, ID and

IDA in apparently healthy Korean women aged 10 years or more. We also aimed at verifying an association of heavy metals in blood or nutritional intakes with ID and IDA.
Methods: We used the fifth Korea National Health and Nutrition Examination Survey (KNHANES V; 2010~2012) data by the Korea Centers for Disease Control and Prevention. This study was performed on 10,169 Korean women including 1,232 anemia, 2,030 ID and 690 IDA subjects. Prevalence and 95% confidence intervals (CI) were calculated using the SAS Survey Procedures. Logistic regression analyses were performed to identify significant factors that affect the risks of ID and IDA.
Results: The overall prevalence rate of anemia, ID and IDA was 12.4%, 23.11% and 7.7%, respectively, between 2010 and 2012 in Korean women. The most prevalent age groups for anemia were ≥ 70 (17.8%) and 19-49 years (15.0%). The women of 15-18 (36.5% for ID; 10.7% for IDA) and 19-49 years (32.7% for ID; 11.3% for IDA) were most prevalent for ID and IDA. The risks of ID and IDA conferred by the blood cadmium level were significant (OR=3.03, 95% CI=2.45-3.75, P<0.0001 for ID; OR=3.14, 95% CI=2.41-4.10, P<0.0001 for IDA). Vitamin D intake showed significant protective effects of ID and IDA (OR=0.96, 95% CI=0.94-0.98, P=0.0004 for ID; OR=0.94, 95% CI=0.91-0.98, P=0.0015 for IDA).
Summary and Conclusions: This study shows that the prevalence of anemia, ID and IDA was relatively high in late adolescents and reproductive ages, and there has been minimal improvement in the burden of anemia and IDA compared with the previous data. The cadmium in blood was found to increase the risk for ID and IDA, however, vitamin D intake contributed to prevent the risk for ID and IDA. Our findings indicate that systematic health systems between policymakers and specialists are needed to control anemia, ID and IDA.

PB2006

EFFICACY OF DEFERIPRONE IN THALASSEMIA: POSSIBLE ROLE OF UGT1A6 GENETIC POLYMORPHISM AND NON-GENETIC FACTORS
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Background: Deferiprone(DFP) is an oral iron chelator widely used in thalassemia(thal) patients(pts). It is mostly glucuronidated by liver UGT1A6 to an inactive metabolite before renally excreted. UGT1A6 polymorphism has been studied as a potential cause for variability in DFP's efficacy. No significant(sig) effect of UGT1A6 polymorphism was shown on the single-dose pharmacokinetics of DFP in healthy volunteers, while another study on β thal major pts treated with DFP showed an association of UGT1A6 single nucleotide polymorphism(SNP) 541A>G with lower ferritin. There are no studies on UGT1A6 polymorphism's association with MRI-T2*, which allows more specific measurement of DFP response by non-invasive liver and heart iron quantification.
Aims: Determine associations of UGT1A6 genetic polymorphism and other non-genetic factors with MRI-T2* responses to DFP in thal.
Methods: Thal pts(aged ≥18 years) who had ≥1 year of DFP and at least two MRI-T2* of ≥1 year apart for assessment of DFP response were enrolled after consenting for UGT1A6 DNA analysis. 3 common non-synonymous SNPs(19T>G, 541A>G, 552A>C) and 1 synonymous SNP(105C>T) of UGT1A6 were detected by sequencing PCR-amplified target DNA segments. Data on MRI-T2* response to DFP was retrospectively collected and analysed for associations with UGT1A6 SNPs/haplotypes and other non-genetic factors(gender, race, splenectomy, baseline cardiac/liver T2*, type of thal, transfusional iron load, DFP and concomitant deferoxamine(DFO) doses). Liver T2* was converted to liver iron concentration(LIC). Improvement in cardiac T2*(if baseline cardiac T2*<20ms) or LIC without discrepant changes in corresponding LIC or cardiac T2* were considered to have responded to DFP.

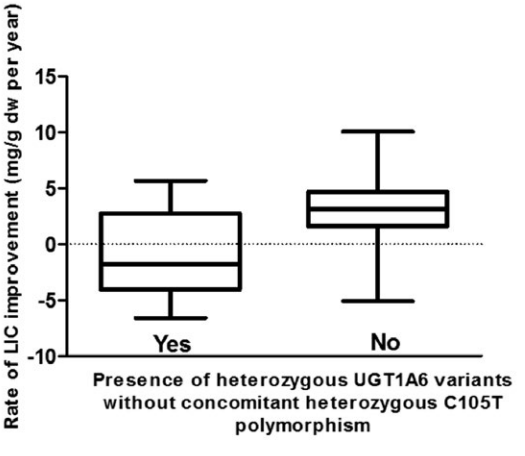

Figure 1.

Results: 32 pts(Chinese 56%, Malay 35%, Indian 3%, others 6%) were treated with median 6(1-11) years of DFP at median average dose of 68.2(24.4-93.3) mg/kg/day. 53% received concomitant DFO at median average dose of 23.1(11.4-40.6) mg/kg/day; 84% was regularly transfused, with median transfusional iron load of 0.4(0.1-0.6) mg/kg/day. Genotypic frequency was UGT1A6*1/*1: 75%, UGT*1/*2: 18.8%, UGT*1/*4: 6.2% (UGT1A6*1=wild type haplotype; UGT1A6*2=variant haplotype with SNPs 19T>G, 541A>G, 552A>C; UGT1A6*4=variant haplotype with SNPs 19T>G and 552A>C). In addition, 15.6% were heterozygous for 105C>T SNP. On multivariate analysis, splenectomy(OR 0.002, 95% CI[0.000, 0.697], P=0.04) and UGT1A6 variant haplotypes(*2 or *4) without concurrent 105C>T(OR 0.016, 95% CI[0.000, 0.766], P=0.03) independently associated with absent DFP response. The median rate of LIC improvement was 2.7(-12.0 to 10.1) mg/g dw per year after excluding 2 patients with discordant liver and cardiac T2* changes. On multivariate analysis, thal major(-2.8, 95% CI[-5.5, -0.04] mg/g dw per year, P<0.05) and UGT1A6 variant haplotypes without concurrent 105C>T (-4.8, 95% CI[-7.8, -1.7] mg/g dw per year, P<0.01) independently associated with poorer LIC improvement rate (see Figure). Other analysed factors did not have sig independent associations with DFP response and LIC improvement rate(P>0.15).
ISummary and Conclusions: Splenectomy decreases iron storage capacity, might lead to iron redistribution to other organs and poorer MRI-T2* responses to DFP. Thal majors had poorer DFP response than other types of thal likely due to higher body iron load. The poorer DFP response observed in our pts with UGT1A6 variant haplotypes (and no concurrent 105C>T) was contrary to expectations since recombinant UGT1A6 variants have slower *in vitro* DFP glucuronidation rates than wild type. One possibility is UGT1A6 variants have different in vivo activities on DFP since conflicting activities of recombinant and liver UGT1A6 allozymes are reported for other substrates. Concomitant 105C>T appeared to negate the adverse impact of UGT1A6 variants on DFP response in our pts. *in vitro*, this SNP increased mRNA stability despite no amino acid changes. This might influence mRNA or enzyme protein stability in vivo for heterozygotes of UGT1A6 variants, leading to changes in DFP metabolism and efficacy. More studies on UGT1A6 SNPs' clinical impact and interactions with other factors on DFP's efficacy are needed for better insights into individualisation of DFP therapy for thal.

PB2007

CHARACTERISTICS OF FEBRILE CONVULSIONS OF PEDIATRICS RELATED TO IRON DEFICIENCY IN EAST DELTA OF EGYPT
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Background: Iron is clearly important for brain development as well as for prevention of anemia, and more study is warranted to understand its role in these common neurodevelopmental disorders.Iron deficiency has also been associated with alterations in synaptic neurotransmitter systems including norepinephrine, dopamine, serotonin, glutamate and gamma-aminobutyric acid (GABA). Febrile seizures may reflect different facets of altered brain excitability that is enhanced by iron deficiency and also influenced by genetic factors.
Aims: To find the association between iron deficiency anemia and febrile convulsions among children aged 6 to 36 months presenting at Pediatric Hematology and Oncology out patients clinic of Zagazig university hospital- Zagazig city – Egypt, from January 20011 to December 20014..As well as to find characteristics of febrile convulsion with iron deficiency in Pediatrics.
Methods: A total of 200 patients fulfilled the study criteria of febrile seizure and 100 patients with any febrile illness without convulsion. These were divided into two groups with children having febrile seizures comprised the cases (Group I) while those having only febrile illness with no seizures comprised the controls (Group II). Both matched for age and gender. Workup for seizures [History and complete neurological and developmental examination.EEG and MRI according the need for each case] and iron deficiency anemia [CBC-Serum iron – Serum Ferritin- Total Protein Iron Binding Capacity – Serum Calcium ionized and total] were done and data was analyzed using SPSS version 10. According to iron deficiency, Group I had been subclassified into Group IA with iron deficiency and Group IB without iron deficiency. Any case with abnormal calcium level and those with MRI findings were excluded.
Results: A total of 24% of group I had iron deficiency (ID) and 16% had iron deficiency anemia (IDA), compared to 17% and 12% of controls respectively. There was significant decrease of Hb level, serum iron, serum ferritin and total iron binding capacity in children with febrile convulsion than that of controls. There was significant decrease age, HB conc, serum iron, ferritin and total protein iron binding capacity in group IA than group IB. One attack of febrile convulsion at presentation was significant higher in group IA than group IB. Duration of convulsion was significantly lower in group IA than group IB.
Summary and Conclusions: Serum Iron, Total Protein Iron binding capacity and Plasma ferritin level were significantly lower in cases as compared to controls suggesting that iron deficient children are more prone to febrile seizures. The characteristics of iron deficiency related febrile convulsions were young age [9-21 month], short duration [not exceed 3 minutes],usually first attack,high with non-breast fed baby.

PB2008

PREVALENCE AND CLINICAL SIGNIFICANCE OF XMNI ΓG-158 (C/T) POLYMORPHISM IN AN EGYPTIAN COHORT OF SICKLE CELL DISEASE PATIENTS

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Background: Sickle cell disease (SCD), well-known for its clinical and hematologic variability, comprises a heterogeneous group of hemoglobin genotypes where affected individuals have sickle hemoglobin (HbS). This considerable clinical heterogeneity among SCD patients is still not well understood. Genetic variants that modulate fetal hemoglobin (HbF) level have a strong impact on ameliorating the clinical phenotype.
Aims: The current study aimed to evaluate the prevalence of *Xmn*1 γ^G-158 (C/T) gene polymorphism in an Egyptian cohort of SCD patients and to investigate the possible association between this polymorphism and HbF level.
Methods: This case control study included 111 Egyptian SCD patients aged 1-21 years (mean age 11.08±5.5 years; 77 HbSS and 34 S/β thalassemia) and 100 age and gender matched unrelated healthy controls. All subjects were recruited only after informed consents were freely obtained from their guardians. Genotyping of *Xmn*1 γ^G-158 (C/T) polymorphism was performed by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) method.
Results: In our cohort, wild genotype *Xmn1* (-/-) was the most prevalent (91.9%), while polymorphic genotypes whether heterozygous or homozygous (-/+ and +/+) were detected in 8.1% of SCD patients; six (6/77-7.8%) were HbSS and three (3/34-8.8%) were S/β thalassemia. No statistically significant differences were detected between SCD patients and controls or between HbSS and S/ β-thalassemia patients regarding prevalence of Xmn1 polymorphic genotypes (P=0.50 and 0.73 respectively). Baseline hemoglobin, HbF level and mean corpuscular volume were significantly higher in patients harboring polymorphic genotypes (P=0.01, 0.001 and 0.04 respectively). We found no significant effect of γ^G-158 (C/T) polymorphism on age of first transfusion, vasoocclusive crisis frequency or transfusion frequency (P=0.067, 0.778 and 0.053 respectively). Although HbF level was significantly higher in patients with polymorphic genotypes (19.24±7.0 vs. 9.06±7.45, P<0.001), Xmn1 γ^G-158 (C/T) polymorphism did not seem to influence HbF levels in patients receiving hydroxycarbamide (P=0.68, 95% Confidence interval= 5.08-7.68). By multiple regression analysis, only baseline HbF correlated independently with elevation of HbF in response to hydroxycarbamide therapy (P<0.001, 95% Confidence interval=0.75-1.31).
Summary and Conclusions: Egyptian sickle patients have low frequency of *Xmn1* γ^G-158 (C/T) polymorphism. The presence of *Xmn1* γ^G-158 (C/T) polymorphism had positive influence on HbF level.

PB2009

ELECTIVE CHOLECYSTECTOMY IS ASSOCIATED WITH INCREASED MORBIDITY AND MORTALITY IN PATIENTS WITH SEVERE THALASSEMIA: A RETROSPECTIVE CASE CONTROL STUDY

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Background: Haemoglobin disorders including thalassemia and sickle cell disease are often complicated with gall stone formation. The co-existence of Gilbert's syndrome together with these diseases further increases the risk of gall bladder disease. Some of these patients develop symptomatic disease which necessitates surgical intervention. At present the timing of cholecystectomy for thalassemia is no different from that of the general population with the exception of removal of the gall bladder at the time of splenectomy. This is no longer the case in sickle cell disease where, laparoscopic cholecystectomy is recommended even in asymptomatic patients. This practice however has not been extended to other types of haemoglobin disorders.
Aims: 1.To assess the perioperative complications of patients with thalassaemia during cholecytetomy and to compare it with non thalassaemics who undergo the procedure. 2. To see if there is enough evidence to recommend elective cholecystectomy for thalassaemics.
Methods: We retrospectively studied case notes of thalassemia patients who had cholecystectomy (cases) in two of the biggest thalassaemia centres in Sri Lanka and also of 62 non-thalassaemics (controls) with gall bladder disease who had been scheduled to have gall bladder surgery in the same hospitals and looked at their peri-operative complications.
Results: 98 out of 540 (18%) thalassaemics in the two centres had gall stones. Mean age of cases was 26.8 (SD 10.9) years and of controls 47.5 (SD 19.7) years. 19 (19%) thalassaemics with gall stones had undergone cholecystectomy. Ten patients had cholecystectomy simultaneously with splenectomy. The majority of non-thalassaemic "controls" had laparoscopic cholecystectomy 53/55 (96.3%) whilst the patients with thalassaemia were mostly operated with laparotomy 13/19 (68%). There was a significant excess complications occurring in both early (42.11 vs. 18.1%) and late (31.5 vs. 12.7%) phases in the thalassaemic patients compared with the controls.
Among the early complications, sepsis (10.5% vs. 1.8%) and liver abscess formation (5.2 vs. 0%) was significantly different in the groups, adversely affecting the thalassaemics. Recurrent abdominal pain was more common among the thalassaemics as a late complication (P<0.05). Six thalassaemic patients with gall stone disease died during this study, 5(5%) while awaiting surgery and 1(1%) after surgery. There were no deaths among the controls. Out of the deaths, 3 (50%) were directly attributable to gallstone disease. In all three septicemia precipitated heart failure. We found a significant increase of both early and late post-surgical complications in the thalassemia group and also increased mortality most of which was related to severe sepsis. Higher perioperative mortality and morbidity were seen among symptomatic thalassaemic patients with gall stone disease undergoing cholecystectomy. This seems to suggest a strong case for supporting elective cholecystectomy in thalassemics before they develop symptoms.
Summary and Conclusions: We suggest that laparoscopic elective cholecystectomy be considered for non-sickle, thalassemia patients too who have asymptomatic gall bladder disease, in an attempt to reduce this morbidity and mortality.

PB2010

EFFICACY AND SAFETY OF DEFERASIROX IN SINGLE VS DIVIDED DOSAGE IN THALASSEMIC CHILDREN.

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Background: Iron chelation has improved the prognosis in thalassemia major. DFX had been the recent oral iron chelator in use. Dose-dependent effect of DFX had been observed in many studies. Initially it was used at 20 – 30 mg/kg/day but efficacy proved to be better when used at higher doses of 30 – 40 mg/kg/day with good tolerability. Ferritin levels tended to be high even on DFX (30 – 40 mg/kg/day) with good compliance, so safety and efficacy of higher doses (40 – 50 mg/kg/day) in once daily vs two daily doses needed to be compared.
Aims: To study the efficacy and safety of DFX (40-50 mg/kg/day) in single vs divided doses in thalassemic children
Methods: The prospective study was conducted in thalassemia ward of Department of Pediatrics, Dayanand Medical College and Hospital, Ludhiana from Jan 2013 to June 2014. Patients were randomly allocated in two groups, Group A & Group B. Both took DFX in doses of 40 – 50 mg/kg/day, group A once daily doses and group B in two divided doses. Efficacy was studied in terms of change in ferritin levels, cardiac & liver MRI T2 * values and LVEF. Safety profile was studied in terms of gastrointestinal side effects, rash and change in serum creatinine values, SGPT and GFR.
Results: Serum ferritin levels reduced in both the groups viz. Group A (2692.5±1232.35 ng/mL to 1959.5±696.19 ng/mL p value=0. 07), Group B (2766.2±897.51 to 2569.9±762.6 ng/mL ;p value=0.38) with significant difference between the two groups at the end of study (P=0.05).Liver MRI T2* values improved in group A (2.1±1.50 to 3.1±1.84 ms; P= 0.096)as compared to group B (2.8±1.84 to 2.2±2.71 ms; P=0.70). Mean LIC decreased in group A (17.4±7.83 mg/g dw to 12.4±8.05 mg/g/dw; P= 0.197) as compared to group B (15.3±10.42 mg/g dw to 22.1±11.93 mg/g/dw; P= 0.340).Overall mean cardiac MRI T2 * values decreased (30.5±10.30 ms to 24.5±4.94 ms; P=0.202) in group A and (31.7±14.78 ms to 20.5±11.78 ms; P=0.167) in group B but remained within normal limits. No change in LVEF was observed. No significant adverse effects were noted with 40–50 mg/kg/day dose of DFX. There was increase in liver transaminases (SGPT) levels (64.4±54.0 to 34.5±14.1 mg/dl in group A; P= 0.05) and (97.9±79.3 to 61.9±39.2 mg/dl ;P= 0.134) in group B
Summary and Conclusions: DFX is safe in higher dosages (40 – 50 mg/kg/day) and once daily dosage is more efficacious as compared to twice a day dosage schedule.

PB2011

MICROPARTICLE PROFILE DURING PAINFUL CRISIS AND NON-CRISIS PERIOD IN SICKLE CELL ANEMIA

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Background: Sickle cell anemia is a disease which is characterized with hemolytic anemia, hypercoagulopathy and painful crisis. Microparticles are 0,1-1 µm sized little membrane particles which are derived during activation or apoptotic phase of cell cycle. It is reported that microparticles are increased in many systemic disease including sickle cell anemia.
Aims: In this study we aimed to investigate the role of microparticles on clinical state and prognosis during crisis and non-crisis periods in sickle cell anemia patients.
Methods: Twenty nine patients, following by Çukurova University Department of Pediatric Hematology, are included in this study. Blood samples were collected in 26 of these patients in non-crisis period. Control group was formed

with 18 healthy children without any systemic disease. Complete blood count, hemoglobin electrophoresis and biochemical parameters were studied in both groups. Also patients' total microparticle levels, erythrocyte (CD235a), endothelia (CD106), monocyte (CD14) particle levels and tissue factor expressing (CD142) microparticle levels were studied by flow cytometry and whole data was statistically analyzed.
Results: Hemoglobin and hematocrit levels were significantly low in sickle cell anemia patients (P<0,001). Levels of HbS were significantly high during crisis period comparing with mean HbS levels during non-crisis period (P<0,001). Total microparticle levels were significantly high in sickle cell anemia patients with painful crisis comparing with control group (P<0,05). Erythrocyte and monocyte microparticle levels were significantly high in patients with painful crisis comparing with non-crisis periods (P<0,05). Endothelial and tissue factor expressing microparticle levels were high in patiens with crisis comparing to non-crisis period but this was not statistically significant (p>0,05). There was not any significant relation with microparticle levels and femur or humerus head avascular necrosis (p>0,05). There was not any significant relation with frequency of crisis and microparticle levels (p>0,05). Microparticle levels were low in patients whose were taking hydroxiurea treatment comparing with not taking hydroxiurea treatment but this was not statistically significant (p>0,05).
Summary and Conclusions: As a result we found high levels of total microparticle, erythrocyte and monocyte microparticles in sickle cell anemia patients during painful crisis period. These microparticles may be role in pathophysiological process of crises in SCA patient. We need further studies in order to understand the effect of microparticles on prognosis of sickle cell anemia.

PB2012

TRANSIENT RED CELL APLASIA "THE GIFT OF PROGRESS"
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Background: "What we call progress is the replacement of one trouble to another",H.Ellis. Modern technologies that enabling blood transfusion even in utero, possibilities to identify new viruses and so on that is a background why doctors have to solve previously never-existing problems.Transient aplasia crisis is an often situation for the pediatric hematologist in Russia. It would seems strange, but we have decided to unite under this name two quite different nosological forms:hemolytic disease of the newborn,complicated with an absence of reticulocytosis, and transient erythroblastopenia of childhood.
Aims: To analyze the clinical features and management of aplasia crisis in children with hemolytic disease of newborns, complicated with an absence of reticulocytosis and children with transient erythroblastopenia.
Methods: There were reviewed medical histories of 62 patients (16 babies with hemolytic disease of the newborn, complicated absence of reticulocytosis, 46 children with transient erythroblastopenia of childhood). We have evaluated duration of hospitalization, number of transfusions,the connection of the disease with infection, therapeutic options. Period of evaluation was since 2009 to 2014 years.
Results: Children with hemolytic disease of the newborn were aged from 0 to 3. Children with transient erythroblastopenia of childhood were aged from 12 months to 5 years.The length of hospitalization of children ranged from 16 days to 35 days, the average was 24 days, and from 9 to 25 days on average 16 days, respectively.Children with hemolytic disease of the newborn were closely observed immediately after birth, and were admitted to hospital in adequate time. Only 12 of the 46 children with transient erythroblastopenia of childhood were applied to hospital immediately after infection. During the observation period the number of patients with transient erythroblastopenia of childhood increased from 7 (2009) to 15 (2014).Children with hemolytic disease of the newborn has increased from 3 (2009) to 5 (2014).6 children with hemolytic disease of the newborn required intrauterine transfusions, 9 children required exchange transfusion immediately after birth, 12 babies needed transfusion during the first month of life.There were 21 child with transient erythroblastopenia of childhood that required blood transfusions because of the hemic hypoxia signs.In 18 children with transient erythroblastopenia of childhood was verified acute viral infection (6 EBV,2 Parvovirus 19,4 CMV, 5 herpes virus type 6,1 herpes type 1).In children with hemolytic disease of the newborn, complicated by aplastic crisis, acute viral infections has not been found. Two children with severe long regenerating form of hemolytic disease of the newborn held a bone marrow biopsy, to 44 patients with erythroblastopenia was also made this study.According to the results of myelogram in all patients there was excluded systemic blood diseases. All patients with hemolytic disease of the newborn received drugs of erythropoietin from 5 to 15 injections depending on the severity of the condition. Reticulocytosis crisis was reached in an average of 4-5 injections. All patients with erythroblastopenia received B group vitamins, 16 children received a course of prednisolone. Children with erythroblastopenia had normalization of blood count at 12-25 day.
Summary and Conclusions: Transient aplasia crisis, that presented by normochromic aregenerating anemia, is severe, but in the modern world, treatable condition. Duration and severity of hemolytic disease of the newborn regenerating status is determined by prenatal transfusion therapy. Severity and length of transient erythroblastopenia of childhood is determined by the etiology of the infectious agent and the time of anemia status. Parvovirus B19 is not the only reason for the erythroblastopenia of childhood.Both of these diseases are relatively benign conditions, it can pass without trace, but in view of the high transfusion dependence it requires further study and observation. Regularly blood tests after birth and diseases can help to find this conditions earlier and to reduce transfusion rate.

PB2013

IL28B GENE POLYMORPHISMS IN EGYPTIAN PEDIATRIC PATIENTS WITH SICKLE CELL DISEASE: PREVALENCE AND RELATION TO SPONTANEOUS CLEARANCE OF HCV
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Background: Egypt has the highest prevalence of hepatitis C virus (HCV) worldwide. Patients with sickle cell disease (SCD) are exposed to the risk of HCV infection. Genetic polymorphisms in IL28B gene have been associated with spontaneous HCV clearance.
Aims: To determine the prevalence of both HCV infection and IL28B gene polymorphisms among pediatric patients with SCD and to explore the relation between IL28B gene polymorphisms and spontaneous HCV clearance.
Methods: Seventy SCD patients were screened for HCV antibody. All patients were recruited only after informed consents were obtained freely from their guardians. Detection of IL28B polymorphisms (rs 12979860 SNP and rs 12980275 SNP) was done using Taqman QRT-PCR and sequence specific primers PCR respectively. HCV RNA was measured in sera of HCV positive patients using quantitative real time PCR.
Results: Sixteen patients (23%) were positive for HCV antibodies. Nine patients (56.3%) had undetectable HCV RNA in serum (spontaneously cleared) and 7 patients (43.8%) were not cleared. Genotypes CC/CT/TT of rs12979860 were found in 30 (42.9%), 29 (41.4%) and 11 (15.7%) while rs12980275 AA/AG/GG were found in 8 (11.4%), 59 (84.3%) and 3 (4.3%). There was no significant difference in the frequency of IL28B (rs 12979860) genotypes between HCV patients who cleared the virus and those with persistent viremia (P=0.388). In addition, frequency of IL28B (rs12980275) genotypes did not differ between the two groups (P=0.438).
Summary and Conclusions: Egyptian sickle cell disease patients have high prevalence of HCV. IL28B gene polymorphisms are not associated with spontaneous clearance of HCV in this cohort of Egyptian children with sickle cell disease.

PB2014

GLUCOSE PHOSPHATE ISOMERASE DEFICIENCY: CLINICAL AND MOLECULAR CHARACTERIZATION OF 10 CASES
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Background: Glucose-6-phosphate isomerase (GPI, EC 5.3.1.9) is a dimeric enzyme that catalyses the reversible isomerisation of glucose-6-phosphate (G6P) to fructose-6-phosphate (F6P), the second reaction step of glycolysis. GPI deficiency, transmitted as an autosomal recessive trait, is the second most common erythro-enzymopathy of anaerobic glycolysis, after pyruvate kinase. GPI deficient patients are affected by chronic non-spherocytic hemolytic anemia of variable severity; in rare cases mental retardation or neuromuscular symptoms have also been reported. The gene locus encoding GPI is located on chromosome 19q13.1 and contains 18 exons. So far about 60 cases of GPI deficiency have been described, and 35 mutations have been reported at the nucleotide level.
Aims: We report the clinical, haematological and molecular characteristics of 10 GPI deficient cases
Methods: 10 patients (6 males, 4 females) from 9 families, with a median age at admission of 7 yrs (range 1-51) were studied. Eight patients were of Italian origin and 2 were Turkish. Haematological parameters and red cell enzyme activity were determined according to standard methods. The entire coding region of the *GPI* gene was amplified by PCR and automatically sequenced.
Results: Main clinical and haematologic parameters and results of the molecular characterization are reported in Table 1. All patient displayed anaemia at birth or in the early infancy; neonatal jaundice was observed in 4 patients, 3 of whom required exchange transfusion. All patients needed blood transfusion in childhood: 3 regularly (every 4-8 weeks), the remaining occasionally in concomitance of haemolytic crises due to infections. Splenectomy, performed in 5 patients, did not lead to normalization of anaemia, only resulting in a slightly increase of Hb levels (0.5-1 g/dL) but it eliminated transfusion requirement;

bilirubin levels increased after splenectomy in 3 cases. No patient needed transfusions in adulthood; however, two underwent chelation therapy for iron overload. None showed neurological signs. Eleven different mutations were found in *GPI* gene, 4 of them never described before (c.311 G>A, c.307C>G, c.269T>C, c.1066G>A); all the new mutations affect highly conserved residues, were not detected in Ensembl and 1000 genomes database, and were predicted to have pathogenic effects by Polyphen, SIFT analysis. In particular, mutation Asp356Asn falls in a region involved in the sugar isomerase domain and dimer-dimer interface, and it is part of active site, further suggesting its drastic effect. In one patient we were unable to find the second mutation; however, the loss of heterozygosity at the cDNA level suggested the presence of a null mutation of the paternal GPI allele

Table 1.

Pt	Neon. Jaund.	Tx	Splenect. (age)	Hb g/dL	Retics 10^9/L	Hb g/dL	Retics 10^9/L	SF ng/ml	Mutation	Effect
				Pre splenectomy		Post splenectomy				
1	yes	1	no	6,1*-10,2	231	-	-	n.a.	C.145G>C/c.921C>A	Gly49Arg/Phe307Leu
2	no	1	no	6,2*-11,6	166	-	-	n.a.	**c.311 G>A/c.584C>T**	**p.Arg104Gln/p.Thr195Ile**
3	yes	1	yes (9)	n.a.	n.a.	11,5	445	2356	**c.307C>G / c.307C>G**	**p.Leu103Val/ p.Leu103Val**
4	no	1	yes (7)	8,4-10	113	9,4-10,5	364	488	c.301G>A/c.1009G>A	p.Val101Met/ p.Ala337Thr
5	yes	1	no	10	n.a.	-	-	202	c.1009G>A/c.1009G>A	p.Ala337Thr/ p.Ala337Thr
6	no	1	yes (17)	n.a.	n.a.	10,5	170	353	c.584C>T/c.584C>T	p.Thr195Ile/p.Thr195Ile
7	no	1	no	11,7	347	-	-	210	?/c.1415G>A	LOH/p.Arg472His
8	yes	3	no	8,5	410	-	-	n.a.	**c.269T>C/ c.1066G>A**	**p.Ile90Thr / p.Asp356Asn**
9	no	2	yes (6)	5,4*-8,9	210	9,4	1420	1123	c.1040G>A/c.1040G>A	p.Arg347His/p.Arg347His
10	no	2	yes (3)	2,7*-8.4	660	9,2	1740	185	c.1040G>A/c.1040G>A	p.Arg347His/p.Arg347His

Tx: transfusions: 1 = occasional, 2= regular until splenectromy, 3= regular; SF: Serum Ferritin, n.a.: not available;
*= during hemolytic crises. New mutations are reported in bold.

Summary and Conclusions: We characterized the largest series of GPI deficient patients so far reported. Patients present with moderate/severe anaemia that improve with aging. The study confirms the great heterogeneity of the molecular defect. Although neurological impairment is sometime reported in literature in GPI deficiency, none of the cases here described displayed extra-haematological symptoms.

PB2015

ASEMPTOMATIC NEPHROTOXICITY IN PATIENTS WITH TRANSFUSION DEPENDENT BETA THALASSEMIA

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Background: There is few information about nephrotoxicity than other organ damages in patients with transfusion-dependent Beta thalassemia(TDT). Creatinine levels are not sensitive indicators and do not change unless renal injury is significant. Cystatin C(Cys-C) is a marker to predict glomerular dysfunction with higher sensitivity and specificity than serum creatinine and creatinine clearance.
Aims: The aim of this study was to evaluate renal tubular and glomeruler functions with Glomerular Filtration Rate(GFR), Beta 2 microglobuline(B2 MG), mean fractional Na excretion (FENe) and cystatin C in patients with TDT.
Methods: Fifty patients with TDT who were being followed up at Istanbul University, Istanbul Medical Faculty, Thalassemia Center between January 2014 and May 2014, were included in this study after the approval of the ethical committee and informed consents were signed by patients. The mean age was 18.4±11.8 years (28 female and 22 male, age range:2-45 years). Thirty healthy volunteers with matched age and sex were also evaluated as the control group. Patients with known renal disease were excluded from our study. The patients were evaluated using GFR, FENe for glomerulopathy and serum Cys-C and urine β-2 MG for tubulopathy. These results were compared with the controls. Serum Cys-C and urine β-2 MG were measured by a latex particle-enhanced nephelometric immunoassay (DadeBehring, Liederbach, Germany).
Results: We studied 50 patients, all of them have normal GFR according to age.Thirty patients (60%) had at least one sign of glomerulopathy and tubulopaty. Twenty-seven of 50 patients (55%) had higher serum Cys-C values (mean : 0,75+0,12) compared with controls (mean:0,66+0,09), P<0.001) while no patient had increased levels of serum Cr according to age. There was a linear relationship between Cystatin C and GFR. When we evaluated the etiology of glomerulopathy and tubulopathy, we found that; there were no statistically significant differences between pretransfusion Hb, ferritin, liver iron overload, cardiac iron overload, and cystatin C, β-2 MG, FENe and GFR. Cystatin C increases with the age, the beta-2 MG and FENe does not change with age. 27 of 30 patients with renal injury (90%) had an increased Cys-C levels compared to the control group. Forty-two of 50 patients (84%) used Deferasirox (DFX), 8 (16%) used Deferipron (DFP). When patients were evaluated with respect to chelation they received, nephrotoxicity was determined in 22 of 42 patients using DFX (52%). Only FENe level in 2 of these patients, only Cys-C level in 11, both FENe and Cys-C levels in 2, both B2 MG and Cys-C levels in 4, all three parameters had increased in 3 of these patients. Nephrotoxicity was detected in 7 of 8 patients (85%) using DFP. Only Cys-C level increased in 4 of these patients (57%), only FENe level increased in 1, both B2 MG and Cys-C levels had increased in 2 of these patients. Also, Cys-C level increase was detected in one TDT patient who didn't receive any chelator yet. In patients receiving DFX, nephrotoxicity was more obvious and related with dosage.

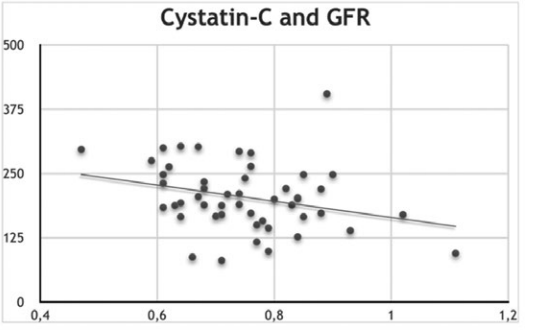

Figure 1.

Table 1.

Findings of patients with nephrotoxicity

Age	Drug	Duration of Drug (year)	Dose mg/kg/d	Ferritin ng/ml	Cardiac T2*	LIC	Hb	FENa	B-2 MG mg/L	Cys-C mg/L	TSH mIU/L	Ft4 mol/L	GFR ml/dk/(1.73m²)	Splenectomy
15	DFX	5	34.8	8482	**39.8**	**18**	8.3	0.8	<0.21	0.89	2.6	15.7	405	No
23	DFX	4	20	248	**19**	**3.2**	9.07	0.73	0.22	0.84	2.7	18.8	203	Yes
36	DFP	10	70	640	**23**	**19**	10.5	0.45	<0.21	0.88	2.25	13.4	173	Yes
6	DFX	1	25	815	///	///	8.03	0.42	<0.21	0.79	2.4	15.7	144	No
15	DFP	3	45	401	**32**	**18.1**	9.5	0.23	0.29	0.75	2.3	16.7	241	Yes
25	DFX	5	20	778	**20**	**35.80**	9.1	1.15	0.93	0.78	4.05	13	158	Yes
16	DFX	4	18.8	1300	**40.8**	**4.6**	8.7	0.55	<0.21	0.85	4.6	13.7	248	No
33	DFP	2	50	1992	**6.7**	**4.8**	8.6	0.56	<0.21	0.76	3.2	12.5	173	Yes
24	DFX	8	37	1151	**40.7**	**7.5**	7.9	0.7	<0.21	0.76	3.3	16.3	174	No
34	DFP	9	65	978	**25.9**	**18.3**	7.4	0.69	<0.21	0.84	1.31	11.6	127	Yes
23	DFX	9	39	1327	**8.7**	**11**	8.1	0.42	0.74	0.79	2.1	16.9	99	No
23	DFX	5	33	3224	**19.9**	**8.36**	8.79	1.06	<0.21	0.93	0.79	13.0	139	No
34	DFX	7	24.5	812	**33**	**1.53**	7.9	0.51	<0.21	0.77	4.0	16.3	150	Yes
17	DFX	2	36	2074	**45.4**	**17.9**	8.9	0.7	0.75	0.88	2.6	17.8	220	Yes
7	DFX	1	37.5	1741	**32.4**	**11.2**	8.6	0.42	<0.21	0.82	4.9	14.6	221	No
30	DFX	4	15	3000	**34.5**	**36.3**	8.16	1.28	<0.21	0.90	3.4	17.2	248	Yes
13	DFP	12	73	1500	**31,4**	**10.5**	8.9	0.36	<0.21	0.84	2.1	13.1	201	No
11	DFP	3	32	430	**40.7**	**2.31**	9.3	0.53	1.72	0.76	0.77	13.1	290	No
21	DFX	5	35	10119	**14**	**42**	9.0	0.8	<0.21	0.77	2.3	11.3	117	No
49	DFX	5	27	418	**76**	**5.5**	8.8	1.54	0.31	1.11	2.9	17.4	95	Yes
2	None			780	///	///	9.2	/////	/////	0.83	////	////	189	No
34	DFX	9	23.5	800	**27.2**	**4.46**	9.26	0.49	<0.21	1.02	1.17	17.1	170	Yes
15	DFX	9	18	609	**24.2**	**5.5**	9.3	0.36	<0.21	0.76	3.1	18.2	264	Yes
29	DFX	9	38	4600	**50**	**40**	8,7	0.65	<0.21	0.8	2.2	17.1	200	No
2	DFX	8	20	1223	**40.8**	**8.4**	7.7	1.3	0.58	0.72	2.6	16.2	210	No
27	DFX	3	33	1386	**40.8**	**6.4**	9.1	0.31	0.56	0.71	3.4	14.3	188	No
8	DFX	2	34	1263	**50**	**11.7**	10.4	0.39	2.6	0.68	4.1	16.2	221	No
10	DFX	6	20	735	**50**	**2.8**	8.9	1.23	**<0.21**	0.62	3.6	14.1	263	No
24	DFX	10	26.9	1596	**28.6**	**17**	8.3	1.2	**<.21**	0.47	3.8	13.6	297	Yes
11	DFP	7	65	3712	**27.6**	**25.6**	7.5	1.26	**<0.21**	0.64	2.9	17.0	166	No

Summary and Conclusions: Nephrotoxicity in thalassemia patients is reported with rate of 4-80% and is multifactorial, even if DFX receiving patients have more risk, all TDT patients should be evaluated with Cys-C for early identification of asymptomatic nephrotoxicity. We suggest intermitten evaluations (at least every 6 months) with Cys-C in addition to routine renal function tests to diagnose and prevent major renal injury in patients with TDT.

PB2016

SPURIOUSLY LOW PULSE OXIMETRY SATURATION ASSOCIATED WITH HEMOGLOBIN SYDNEY IN A CHILD AND RELATIVES: IDENTIFICATION OF THIS UNSTABLE HEMOGLOBIN MAY AVOID UNNECESSARY TESTING AND HOSPITAL ADMISSIONS

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Background: Pulse oximetry is a noninvasive method to measure hemoglobin oxygen saturation and is widely used in different medical settings. Very few variant hemoglobins have been associated with unexpectedly low SpO2. We describe a new family with the unstable hemoglobin Sydney associated to artifactually low SpO2, which, to our knowledge, has not been reported as a cause of this phenomenon. Identification of these rare hemoglobinopathies may avoid cardiac and pulmonary testing, unnecessary treatments and even hospital admissions.
Aims: To describe the association of Hemoglobin Sydney with artifactually low SpO2.
Methods: HPLC analysis with the VARIANT II equipment and CDM software (Bio-Rad) and acid and alcaline electrophoresis (HYDRASIS, SEBIA HISPANIA) were performed on the hemoglobin of the index case and his father. PCR amplification of the exons 1, 2, 3 and adjacent intronic regions was performed on the HBB gene (betha-hemoglobin gene) and sequence analysis of the amplified region.
Results: A 19-month-old male and his father had low pulse oxygen saturation measured by pulse oximetry (SpO2), which was not confirmed by arterial blood oxigen measurement (SaO2) in the child (index case). The child was referred by his primary care pediatrician to the Emergency ward because of bronchospasm that, in spite of salbutamol nebulizations and oxygen did not improve SpO2 (87%). He was admitted to the hospial ward and treated with salbutamol, corticosteroids and oxygen. A chest XR and cardiac ultrasound were performed which were normal. He had mild compensated hemolysis (hemoglobin 125g/L, VCM 94 fL, reticulocytes 4.5%, haptoglobin<40 mg/L. SaO2 was 96% (FiO2 21%) when SpO2 was 85%. The patient's father, grand-father and aunt had history of chronic hemolysis due to an unstable hemoglobin, not further characterized. The grand-father had a history of splenectomy. A hemoglobinopathy causing this falsely low SpO2 was suspected and confirmed. Both the patient and his father had anomalous hemoglobin peaks on HPLC that did not separate at all from HbA1 and DNA sequence analysis exhibited a mutation in heterozigosity in the HBB gene (Beta 87 (E11) Val>Ala) consistent with Sydney hemoglobin. The artifactuosly low SpO2 may be due to abnormal absorption peak measured by pulse oximeter.
Summary and Conclusions: Hemoglobin Sydney is a known unstable hemoglobin which can result in an artifactually low oxygen saturation measurement with pulse oximetry, previously not reported. To describe this association is important in order to avoid unnecessary tests in patients with this rare hemoglobinopathy.

PB2017

FREQUENCY OF MUSCULOSKELETAL AND OTORHINOLARYNGOLOGIC MANIFESTATIONS IN CHILDREN WITH B- THALASSEMIA

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Background: Beta-thalassaemias have a wide variety of musculoskeletal system manifestations. Spinal involvement related to disease course and treatment is common in patients with thalassemia syndromes. Also, a high frequency sensorineural hearing loss is observed in a large percentage of patients during intensive Desferrioxamine therapy.
Aims: To study the clinical and radiographic skeletal changes in transfusion-dependent beta-thalassaemia patients and to present a comprehensive overview of spinal involvement in those patients.
Methods: this cross sectional study was carried out on 240 patients with b-thalassemia major who came weekly for blood transfusion at the hematology outpatient clinic of Pediatric Department at Zagazig University hospital during a period from 2012-2013. In all patients studied, detailed history regarding musculoskeletal involvement was taken and locomotor examinations were performed. Also, preliminary auditory perceptual assessment of patient's voice and careful laryngeal examination. All patients underwent all routine laboratory investigations (CBC, Serum iron and ferritin, Liver and renal functions), Imaging study with standing anteroposterior and lateral X-rays of the spine,Ultra sonography on affected knee (s) joint, augmentation and documentation of the glottic picture and high-fidelity voice recording and acoustic analysis was done.
Results: locomotor system involvement was found in 56 patients (23.3%). Most frequent complaints were arthralgia and low back pain in 21.3% and 6.3% of patients respectively. Scoliosis was detected radiologically in 8 patients (3.3%). The most common curve pattern in thalassemia was the left lumbar (62.5%) followed by the right lumbar (37.5). Ultrasonography of affected knee (s) joint showed synovitis, effusions, signs of metaphysis dysplasia and increased power Doppler signals in (23.8%,14.3%,85.7%,19% respectively). There was mild to moderat laryngeal congestion nearly in all cases but the vocal fold mobility (crico-arytenoid joint) is not affected.
Summary and Conclusions: Patients with beta thalassemia have a variety of musculoskeletal problems and spinal involvement is common. Further studies will be needed to detect the risk factors involved in the development of these musculoskeletal problems.

PB2018

STUDY OF GROWTH DIFFERENTIATION FACTOR 15 IN EGYPTIAN PATIENTS WITH BETA THALASSEMIA

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Background: The thalassemia syndromes represent the most common causes of ineffective erythropoiesis. The increased but ineffective erythropoiesis resulting in tissue iron overload induces numerous endocrine diseases, hepatic cirrhosis, cardiac failure and even death. Growth Differentiation factor 15 (GDF-15) is a member of the transforming growth factor β super family. It was recently studied as a marker of ineffective erythropoiesis in different types of anemia.
Aims: Our objective was to study the serum level of GDF- 15 in β thalassemia major (TM) and thalassemia intermedia patients (TI) and to correlate it to the iron status and different clinical and laboratory parameters in these patients
Methods: twenty five thalassemia major patients (mean age of 12.08± 4.6 years) and 25 thalassemia intermedia patients (mean age of 9.3± 4.7 years) were randomly selected from the hematology clinic of the children hospital at Cairo University together with 30 age and sex matched healthy controls. All patients were subjected to thorough history taking and clinical exam. Blood samples were withdrawn for the assessment of complete blood count, liver function tests and serum ferritin. Estimation of GDF-15 level was done using ELISA technique.
Results: On comparing the level of GDF-15 among the 3 groups, it was significantly elevated in the TM group (8769.6±3560 pg/mL) *versus* the TI group (7090±3656 pg/mL); P< 0.05 and *versus* the control group (385.1±244); P< 0.05. GDF-15 showed positive correlation to the age of starting chelation among TM patients; P= 0.04 and negative correlation to the frequency of transfusion and hemoglobin level in the TI group; P= 0.001.
Summary and Conclusions: GDF-15 in our study represent a first step for its use as a biomarker in thalassemia patients. Future studies with large number of patients are needed to confirm this hypothesis.

PB2019

EFFECT OF IRON FORTIFIED FORMULA FEEDING IN THE SECOND 6 MONTHS OF LIFE ON IRON STATUS AND ZINC LEVELS

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Background: Malnutrition, iron deficiency anemia, zinc deficiency and associated conditions are among the significant health problems of infancy especially in developing countries. The influence of nutrition types on iron deficiency has not been adequately studied.
Aims: The aim of this sudy is to investigate the relationship between iron status and zinc levels in infancy and type of milk consumed.
Methods: All infants were exclusively breatfed in the first 6 months life. All mothers were recommended to start complementary foods at 6 months of age. Infants, without a nutritional problem, weighing over 10th percentile and continue to be breastfed in addition to adequate complemantary foods were enrolled in the control group (n: 45), babies weighting over 3rd percentile, did not get adequate complementary foods and an iron fortifed formula was added to the diet were included in the study group (n: 39). All infants were followed-up monthly for 3 months. Hemoglobin, ferritin, iron, iron binding capacity and zinc levels were assessed in relation to the type of milk.
Results: Mean hemoglobin values were similiar in the two groups at the end of 3 months of follow-up. Mean ferritin values increased from 53,2±41,8 mg/L to 64,7±89,7 mg/L in the study group, while decreased from 42,8±34,0 mg/L to 34,6±29,3 mg/L in the control group. In the study group, significantly higher levels of ferritin were measured in the last assessment, compared to the control group. There was no difference in the zinc levels between breastfed and formulafed babies.
Summary and Conclusions: An increase in the iron stores of iron fortifed formula fed infants was observed during the follow-up period

PB2020

DO PANCREATIC FUNCTIONS PREDICT CARDIAC AND LIVER IRON LOADING IN TRANSFUSION-DEPENDENT BETA THALASSEMIA MAJOR PATIENTS USING CARDIOVASCULAR AND LIVER T2-STAR (T2*)MAGNETIC RESONANCE ?

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Background: In Egypt, b-thalassemia is the most common genetically determined chronic hemolytic anemia. Regular and frequent red blood cell transfusions have significantly increased the life expectancy of patients with b-TM.

The transfusions reduce some of the consequences of anemia, such as growth deficit. However, when no appropriate chelation therapy is available, patients accumulate iron in the heart, liver, spleen, pancreas, and endocrine glands, leading to progressive organ dysfunction.
Aims: To assess the correlation between cardiac and hepatic T2*MRI findings with the endocrine and exocrine pancreatic functions in known β-TM patients.
Methods: A total of 44 children and adolescents β-TM patients and 44 healthy controls were investigated via: serum amylase, lipase, triglyceride index, oral glucose tolerance test, and T2* MRI to assess iron content in the heart and liver.
Results: Overt diabetes was found in 9.4% and 45.5% of patients had impaired fasting glucose. Median cardiac T2* was 22ms (12-31 ms) and LIC was 6ms (4-9 ms). CardiacT2* was less than 10ms in 21.4% indicating heavy load with iron in cardiac tissues. There is a significant decrease in serum amylase (87.5 vs. 63.5 IU/L, p =0.003) and lipase (94 vs.70 IU/L, P=0.056) among enrolled patients in comparison to control group. Thalassemic diabetic showed low serum amylase (32.5 vs. 59.5,P=0.0005),serum lipase (39.5 vs. 68, P=0.0007), low cardiac T2* was found (7 vs. 22 ms,p =0.0006) and low LIC (2 vs. 6ms,P=0.0006) than other β-TM patients without diabetes. Inverse correlation was found between triglyceride index with cardiac T2*(r= -0.376, P=0.014) and low LIC (r=-0.376, P=0.014 respectively) but not with serum lipase (r= -0.099,P=0.533), (r= -0.222,P=0.1570) and serum amylase (r =-0.191P=0.225), (r=-0.053 ,P= 0.738) respectively. In Egypt, β-thalassemia is the most common genetically determined chronic hemolytic anemia.
Summary and Conclusions: Follow up of thalassemic patients with impaired fasting glucose together with intensive chelation therapy may help to prevent the development of cardiac and hepatic siderosis.

PB2021

USEFULNESS OF DUAL-ENERGY CT FOR DETECTION OF LIVER IRON DEPOSITION IN TRANSFUSION-DEPENDENT PATIENTS

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Background: Chronic red blood cell transfusions lead to iron overload, which has been implicated in the development of serious complications. Liver iron concentration (LIC) is considered an indicator of total body iron stores, and the measurement of T2* values by MRI represents a standard noninvasive technique to evaluate LIC. However, some limitations are associated with MRI, such as its high cost and the overestimation of LIC. Although the application of CT, which is easy to use and widely applied to measure LIC, needs to be considered, conventional single energy CT (SECT) also has limitations for the detection of LIC due to normal variations in CT attenuation, predominantly in patients with mild iron overload. Moreover, SECT fails to detect iron in fatty livers, which has an inverse effect on attenuation by lowering CT numbers. Dual-energy CT (DECT) is used to obtain additional information on tissue composition over that provided by SECT. This technique is based on substances showing different densities with two different energies, with each substance displaying its own energy-dependent change in CT attenuation. However, the role of DECT in the detection of LIC remains to be clarified.
Aims: The aim of this study was to examine whether DECT represented a novel and useful technique for the detection of LIC.
Methods: Eight transfusion-dependent patients underwent liver DECT using a dual-source 128-slice CT system, serum ferritin levels were measured, and liver MRI was performed. DECT images were acquired using a tube voltage pair of 140 kV and 80 kV or 140 kV and 100 kV, and the three-material decompositions of fat, soft tissue and iron. All patients provided informed consent. Patient 1 was a 54-year-old male with MDS (RCMD-RS). Patient 2 was a 66-year-old female with AML with MRC in 1st CR. Patient 3 was a 47-year-old female who had undergone renal transplantation for chronic renal failure. Patient 4 was a 65-year-old male with AML with MRC receiving iron chelation therapy. Patient 5 was a 47-year-old male with AML in 3rd CR. Patient 6 was a 52-year-old female with AA. Patient 7 was a 63-year-old male with AIHA. Patient 8 was a 56-year-old female with AML in 2nd CR. All patients had long transfusion histories and received red blood cell transfusions of at least more than 12 U.
Results: Serum ferritin levels in patients 1 to 8 were 961, 2168, 7875, 795, 1921, 5104, 2401, and 2916 ng/mL, respectively. Although patient 8 with fatty liver, which was confirmed by abdominal ultrasonography, showed hypodensity on SECT images, liver iron deposition was detected on DECT images. All patients showed diffuse severe hypointensity on MRI T2*-weighted images. Patients 1, 2, and 3 also showed severe diffuse iron deposition on DECT images. However, patients 4, 5, 6, and 8 showed mild focal iron deposition, while patient 7 did not show iron deposition on DECT images.
Summary and Conclusions: Our results suggest that DECT has the ability to detect liver iron deposition in transfusion-dependent patients more precisely than MRI. DECT may be a new tool that can overcome the limitations of MRI.

PB2022

INCIDENTAL DETECTION OF HAEMOGLOBIN VARIANTS DURING GLYCATED HEAMOGLOBIN ANALYSIS

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Background: The presence of a haemoglobin variant (Hb variant) influences the glycated haemoglobin (HbA1c) analysis in a method-depending manner and may erroneously lead to underestimated HbA1c measurements by high performance liquid chromatography (HPLC).
Aims: The aim of this study is the retrospective registration of the cases of Hb variant detected during HbA1c analysis by HPLC, in order to reevaluate the use of this specific method.
Methods: All patients who had their HbA1c levels determined in our laboratory during the period of time from 01-01-2004 to 31-03-2013 were included in the study. The measurements of HbA1c were performed by automated reversed phase cation exchange HPLC on an ADAMS™ A1c HA-8160, ARKRAY® analyzer. All Hb variant cases found during routine HbA1c analysis and further identified with HPLC analyzer (HLC-723 G7 β-Thalassemia Mode, TOSOH®) were recorded. Only one HbA1c result per patient was included in the study.
Results: Out of a total of 12617 patients, 122 (54 men & 68 women) was found to have Hb variant. In particular, 83 patients (68,03%) had Hb-O Arab (1 homogyzous & 82 heterozygous), 36 patients (29,5%) had HbS (heterozygous), two patients (1,64) had HbJ (heterozygous) and one patient (0,82%) had HbD (heterozygous). The determination of HbA1c was impossible in the case of homozygous O-Arab (no s-A1c peak detected). In all cases, the presence of Hb variant was first diagnosed during the routine HbA1c analysis.
Summary and Conclusions: The analysis of HbA1c using the HPLC method is a simple and reliable screening test for Hb variant. The incidental finding of haemoglobinopathy is very important, because old people that usually suffer from diabetes mellitus, probably did not have previous haemoglobinopathy investigation. Furthermore, homozygous O-Arab and heterozygous HbS present a significant clinical importance. As the measurement of HbA1c can potentially be affected by the presence of a Hb variant, and in order to avoid mismanagement of diabetes mellitus (pre-diabetes, diagnosis, monitoring) in individuals with Hb variants, the visual inspection of chromatograms obtained from HPLC analysis for the detection of additional peaks is recommended. The laboratory should report the presence of Hb variant with the HbA1c result.

Stem cell transplantation - Clinical

PB2023

CHARACTERIZATION OF CHRONIC GRAFT-VERSUS-HOST-DISEASE IN MALIGNANT HEMATOLOGICAL DISORDERS AFTER USING G-CSF-PRIMED BONE MARROW AS A SOURCE OF HEMATOPOIETIC STEM CELLS FOR ALLOGENEIC TRANSPLANTATION

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Background: A major complication of allogeneic stem cell transplantation (allo-SCT) is chronic graft-*versus*-host disease (cGVHD). Risk factors for the development of cGVHD include prior acute GVHD, increasing patient age, and use of a parous female donor for a male recipient. GVHD is also associated with significant morbidity and mortality, commonly requiring long-term immunosuppressive therapy after allo-SCT. It has been documented that the use of G-CSF-primed bone marrow as a source of hematopoietic stem cells decreases the frequency and severity of cGVHD.
Aims: To describe the frequency and characteristics of cGVHD in 35 patients who underwent allogeneic, G-CSF-primed bone marrow transplantation, at INCMNSZ, from November 1998 to December 2014.
Methods: A retrospective analysis was performed in 35 patients who underwent allogeneic, G-CSF-primed bone marrow transplantation, describing the characteristics and outcome of 12 of them who developed cGVHD, using the Statistical Software Package SPSS v21.0.
Results: Twelve out of 35 patients who underwent allogeneic, G-CSF-primed bone marrow transplantation, from November 1998 to December 2014, developed cGVHD (34.2%). Median age was 33 years (range 16-49), and 60% were male. The patients had a following range of underlying diseases: acute lymphoblastic leukemia (LLA, n=4, 33.3%), myelodysplastic syndrome (n=5, 41.6%), chronic myeloid leukemia (CML, n=2, 16.6%), and acute myeloid leukemia (AML, n=1, 8.3%). Median infused CD34+ stem cell dose was 2.04x 10^6/kg. All patients received conditioning with busulphan and cyclophosphamide, and prophylactic immunosuppression with methotrexate (10 mg/m2 days 3, 6, and 11) and cyclosporine A (CsA 5mg/kg) for the first 4 months. The median interval from transplantation to occurrence of cGVHD was 275.3 (range, 101-1359) days. 83.3% of the patients had limited cGVHD (10/12). Among the risk factors associated with cGVHD we identified the following: five male patients were given grafts from female donors, 2 patients with CML developed cGVHD after donor lymphocyte infusion, three patients previously experienced acute GVHD, and only one patient had an antigen mismatch (9/10). In 11 patients (31.4%), hepatic dysfunction was consistent with elevation of transaminases and alkaline phosphatase. Nine patients developed skin involvement characterized by exanthema; 6 had mucous lesions: 2 patients lichen reticularis, 2 developed ulcers, and one leukoplakia. None of the patients had pulmonary or ophthalmic lesions. Initial treatment included steroids in all patients, without complete response and additional Immunosupressive therapy was prescribed including: CsA, sirolimus, mycophenolate mophetyl, cyclophosphamide, ruxolitinib, and imatinib. Median time of duration of immunosuppression therapy was 12.9 months (range, 3-41) with an overall response of 66% (8/12 patients). GVHD associated mortality occurred in one patient, and one patient was lost during follow up. At last follow up, the remaining 10 patients were alive and 8 were out of immunosuppressive therapy without evidence of GVHD.
Summary and Conclusions: Using G-CSF-primed bone marrow as a source of hematopoietic stem cells for allo-SCT is associated with a different pattern of cGVHD tissue impairment, characterized by skin and liver involvement, in most cases mild and limited. At least two immunosuppressive therapy approaches were needed to induce clinical remission. Further studies including a larger number of patients are required in order to validate this strategy.

PB2024

IMPACT OF PLATELETS ALLOIMMUNIZATION IN ALLOGRAFT

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Background: The prolonged thrombocytopenia after hematopoietic stem cell transplantation (HSCT) present factor of poor prognosis. There causes are varied and complex; whose platelet alloimmunization, which is responsible for refractoriness platelet transfusions.
Aims: The objective is to demonstrate the impact of mismatches between donor and recipient for human platelet antigens (HPA) on recovery of platelet counts after transplantation.
Methods: A retrospective analysis of 96 patients transplanted at the Department Hematology at Hospital Henri Mondor, Creteil, between January 2011 and December 2013 was performed. Only geno and pheno-identical HSCT are studies.
Results: We tested each of the four HPA systems (HPA1, HPA3, HPA5 and HPA15), the platelet recovery in recipients "aa" as they receive HSC donor "aa" or "ab or bb". We demonstrated no significant differences between groups with or no mismatch HPA, compared at 1, 3, 6, and 12 months after graft. While there was a trend (P=0.07) in HPA3 system at 24 months to transplant. In HPA5 system, the differences were in the expected direction without statistically significant.
Summary and Conclusions: It would be important to demonstrate the impact of mismatch HPA on platelet recovery in a most important sample of patients to prevent the onset of refractoriness to platelet transfusions in the course of allogeneic haematopoietic stem cell.

PB2025

CLINICAL IMPACT OF HEPATITIS B SURFACE ANTIGEN POSITIVITY IN PATIENTS UNDERGONE ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Hepatitis B virus (HBV)-related events could increase in patients who have HBV before or after allogeneic hematopoietic stem cell transplantation (HSCT) due to their immunosuppressive status because of their primary diseases, conditioning regimens, immunosuppressive agents, and immature immune reconstruction. However, the consensus of the risk of HBV status and prophylactic or therapeutic strategies for these patients were not settled yet.
Aims: We intented to search the clinical impact of HBV surface antigen (HBS Ag) positivity in patients undergone allogeneic hematopoietic stem cell transplantation.
Methods: We reviewed clinical information of HBS Ag-positive patients who had been treated with allogeneic HSCT for various hematologic diseases to evaluate the influence of HBsAg positivity to the clinical outcomes. A total of 398 patients who had been treated with allogeneic HSCT between January 1998 and November 2014 in three domestic institutes of Korea were enrolled in this study.
Results: Eleven patients (2.8%) were HBsAg-positive among 398 patients treated with allogeneic HSCT. The diagnosis were acute myeloid leukemia (N=3), acute lymphoblastic leukemia (N=1), non-Hodgkin lymphoma (N=1), myelodysplastic syndrome (N=2), severe aplastic anemia (N=3), and acute biphenotypical leukemia (N=1). Ten patients were HBsAg-positive before HSCT, and one patient became seroreversion after HSCT due to HBsAg-positive donor. Seven patients (63.6%) were given prophylactic antiviral agents (lamivudine, N=4; entecarvir, N=3). Three patients (27.3%) experienced chronic active hepatitis (CAH) after HSCT, which was controlled well with antiviral agents. Of three CAH patients, two had not been give any HBV prophylaxis during HSCT. Sinusosidal obstruction syndrome (SOS) occurred in 3 patients (27.3%), and one died of SOS. Acute liver graft-*versus* host disease (GVHD) occurred in 4 patients (36.4%); and chronic liver GVHD did in 2 (18.2%). Interestingly, one patient whose donor had HBs antibody (HBsAb) lost HBsAg after HSCT.
Summary and Conclusions: HBsAg positivity could increase the risk of the incidence of chronic active hepatitis after allogeneic HSCT. However, it seems not to influence markedly to the overall clinical outcomes of HSCT including survival, SOS or liver GVHD. Loss of HBsAg might be expected if the donor has HBsAb. Further large-scaled studies designed to identify the risk of HBsAg positivity and the optimal strategy for HBsAg-positive patients is required.

PB2026

THE ROLE OF HLA HIGH RESOLUTION TYPING IN UMBILICAL CORD BLOOD TRANSPLANTATION

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Background: 1s smdy was pur10sed to investigate me clillical value of HLA mtchjn2(10w and tligh I esolution)aJld its effect on outcome of the patients received umbilical cord blood_ransplantation(UCBT). This study was purposed to investigate the clillical value of HLA matching(low and high resolution) and its effect on outcome of the patients received umbilical cord blood transplantation(UCBT)
Aims: To analyze HLA matching(low or high resolution) on outcomes of umbilical cord blood transplantation(UCB) and the value of clinical application.
Methods: From October 2010 to July 2012,26 patients were selected umbilical cord blood transplantation in our hospital,in the absence of a suitable HLA-identical sibling donor or alternative donors.We examined the effects of HLA matching (low or high resolution) on engraftment of leading, engraftment,graft-*versus*-host disease (GVHD), infection and mortality.Performed mainly by

sequence-specific oligonucleotide probe (SSOP) methods, sequence-based typing (SBT) methods and high resolution sequence-specific primers(SSP) methods,retrospective HLA matching of cord blood,typed at high resolution for HLA-A,-B, -C, -DRB1, -DQ, and -DP and low resolution for HLA-A,B,DRB1.
Results: Compared single UBC units transplantation with double UBC units,the hematopoietic reconstitution rate was 88% vs 56%.The median total nucleated cells(TNC) of single and double cord blood was 5.5×107/kg, when TNC≥5×107/kg could promote neutrophil recoveries (P<0.05). The UBC units were a 6/10 HLA high resolution match or better with each other could promote UCB implantation advantaged P<0.05, accelerate platelet recovery P<0.05 and reduce more risk of acute GVHD (grade III -IV) P<0.05. There are different between the group 5-6/6 HLA low resolution and 3-4/6.but no statistical difference P>0.05.
Summary and Conclusions: The HLA High resolution typing can select the better UCB than HLA low-resoulution. It has important clinical value on promoting hematopoietic reconstitution and reducing complications after UCB transplation.

PB2027

WHICH IS THE MOST ADOPTABLE PROGNOSTIC MODEL FOR DIFFUSE LARGE B CELL LYMPHOMA TREATED WITH R-CHOP FOLLOWED BY AUTOLOGOUS TRANSPLANTATION?

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Background: The ideal prognostic model has not been developed for patients with diffuse large B cell lymphoma (DLBCL) treated with rituximab combined with the CHOP regimen (R-CHOP) followed by upfront autologous stem cell transplantation (Auto-SCT).
Aims: Among the currently adoptable prognostic models for DLBCL, we investigated to find out which one is the most adoptable one for DLBCL treated with R-CHOP followed by upfront Auto-SCT.
Methods: We retrospectively evaluated survival differences between the risk groups based on the International Prognostic Index (IPI), the age-adjusted IPI (aaIPI), the revised IPI (R-IPI) and the National Comprehensive Cancer Network IPI (NCCN-IPI) at diagnosis in 63 CD20-positive DLBCL patients treated with R-CHOP followed by upfront Auto-SCT.
Results: Patients had either stage I/II bulky disease (6.3%) or stage III/IV disease (93.7%). The median age at diagnosis was 50 years (range, 22-66 years). 37 of 63 patients (58.7%) belonged to the IPI high-intermediate or high risk group. 48 of 63 (76.2%) received 6 or more cycles of R-CHOP. The median time to transplantation was 7.2 months (range, 3.4-40.3 months). At the time of Auto-SCT, 74.6% and 25.4% of patients achieved complete (CR) and partial remission (PR) after R-CHOP, respectively. As a whole, the 5-year overall (OS) and progression-free survival (PFS) were 78.8% and 74.2%, respectively. The 5-year OS and PFS rates according to IPI, aaIPI, R-IPI, and NCCN-IPI did not differ in statistical significance between the risk groups of each prognostic model (*P* values for OS: 0.255, 0.185, 0.881, and 0.803, respectively; *P* values for PFS: 0.177, 0.832, 0.295, and 0.609, respectively).
Summary and Conclusions: There is no ideal prognostic model among the currently adoptable ones for CD20-positive DLBCL treated with R-CHOP followed by upfront Auto-SCT. A new prognostic model is necessary to identify those who will gain the maximum benefit from upfront Auto-SCT in the rituximab era.

PB2028

EAM (ETOPOSIDE, CYTARABINE, MELPHALAN) BEFORE AUTOLOGOUS STEM CELL TRANSPLANTATION IS SAFE AND EFFECTIVE FOR RESISTANT/RELAPSED HODGKIN LYMPHOMA PATIENTS

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Background: High-dose chemotherapy (HDT) followed by autologous stem cell rescue has become the standard of care for patients with relapsed, chemosensitive Hodgkin lymphoma (HL). A BEAM regimen is widely used conditioning regimen for autologous stem cells transplant (ASCT) in patients with lymphoma because of its acceptable toxicity and high effectiveness. Adverse events associated with BEAM are related in part to BICNU.
Aims: On 2011-2012, we started a pilot study designed as follows: Etoposide 800 mg/m^2 J-5 to J-2, Cytarabine 1000 mg/m^2/12h, J-5 to J-2 and Melphalan 140 mg/m^2 J-1 as the conditioning regimen to ASCT for resistant/relapsed HL patientsto evaluate the safety and the efficacy of this regimen with high dose Cytarabine.
Methods: Seventeen patients, seven males and ten females, 17 to 51 years old (median, 28 years), with HL were consecutively treated in our center. Regarding the stage of disease, four patients were in early stage (I-II), thirteen in advanced stage (III-IV) and concerning their status at ASCT, thirteen were in complete remission, three in partial response and one presented a refractory disease. A median number of 3,73x10^6 CD34$^+$ cells/kg (range, 2, 97-6, 15) were re-infused.
Results: All patients had a full hematopoietic reconstitution. Median time to achieve neutrophils >500 /µl was 13 days (range: 9-19) and median time to achieve an unsupported platelet count >20,000/µl was16 days (range: 14-25). Toxicities included grade 4 hematologic in all patients, grade 3 mucositis in 4, grade 3 infectious in 2. Among 16 patients evaluable, fifteen patients (94%) achieved CR; one patient had progressive disease post-ASCT.Fivepatients died: one within the first 100 days that followed transplantation, one after progression of disease and three after relapse. After a median follow up of 37 months (2-45) (End point: 31/12/2014), the overall survival was 70% at 45 months, the DFS was 75% at 45 months and 12 patients are alive in continuous CR (80%).
Summary and Conclusions: In conclusion, this pilot studyof EAMas conditioning regimen seems to be safeand effective for heavily pre-treated Hodgkin lymphoma patients. Although these outcomes are encouraging, our results need to be confirmed by more other studies.

PB2029

CLINICAL IMPLICATION OF ΓΔ+ T CELL RECOVERY AFTER AB+ T CELL DEPLETED HAPLOIDENTICAL HEMATOPOIETIC CELL TRANSPLANTATION IN CHILDREN WITH HEMATOLOGIC MALIGNANCY

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Background: Human γ$^+$ T cells exhibit an ability to kill tumors but do not recognize major histocompatibility complex, thus have shown limited risk of graft-*versus*-host disease (GVHD).
Aims: The purpose of this study were to determine antitumor efficacy of γ$^+$ T cells in children with hematologic malignancy after αβ$^+$ T cell depleted haploidentical hematopoietic stem cell transplantation (haplo-HSCT).
Methods: Ten children (6 males, 4 females, median age 12.3 years) with hematologic malignancy received haplo-HSCT after *ex vivo* depletion of αβ$^+$ T cells between May 2012 and December 2013 at AMCCH. Of 10 patients, 1 had ETP-ALL in CR2 who relapsed after MSD-HCT, 1had MLL in relapse after 2 rounds of URD-HCT, 5 had AML [2 CR1, 2 CR2, 1 non-remission (NR)], 2 had JMML (1 CR1, 1 NR), and 2 had NHL (1 CR2 and 1 CR3) who relapsed after AUTO-HCT. The median number of CD34$^+$, αβ$^+$, γ$^+$ and CD3$^+$ cells infused was 10.6x 10^6/Kg, 6.3x 10^5/Kg, 2.1x 10^7/Kg and 22.9x 10^6/Kg, respectively. Proportion of TCR γ$^+$ T cells in T lymphocyte were analyzed by using Gallios flow cytometer™ (Beckman Coulter, Fullerton, CA, USA) after HSCT.
Results: All 10 patients engrafted and 1 patient experienced grade IV acute GVHD without treatment related mortality. At a median follow-up period of 387 days (range, 208-766), 5 patients (1 ETP-ALL, 1 MLL, 2 AML, 1 NHL) relapsed at the median 131 days (range, 42-234) after haplo-HSCT. The median dose of infused γδ$^+$ T cell for 5 patients without relapse and 5 with relapse were 2.2x 10^7/Kg and 1.8x 10^7/Kg. The median proportion of γ$^+$ T cells after HSCT was higher in non-relapsed patients than in relapsed patients. (69.4% vs 29.3% at 1 month, 44.2% vs 6.2% at 2 months and 36.8% vs 9.1% at 3 months post-transplant) At a median follow-up of 12 months probability of 1 year relapse-free survival was 48.0%, and probability of 1 year overall survival was 78.8%.
Summary and Conclusions: These findings suggest a correlation between an increase in the proportion of γ$^+$ T cells and improved relapse-free survival after αβ$^+$ T cell depleted haplo-HSCT. However, further studies including prospective large numbered trial are needed to confirm our results.

PB2030

EXTRACORPOREAL PHOTOPHERESIS AS A TREATMENT FOR ACUTE AND CHRONIC GRAFT VERSUS HOST DISEASE. A SINGLE CENTER EXPERIENCE IN SPAIN

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Background: Graft *versus* host disease (GvHD) is the most important cause

of mortality and morbidity in patients who have undergone allogeneic stem cell transplantation. Currently, high-dose steroid is the first line of therapy in this disease. Nevertheless, there are no guidelines or agreement about the second and subsequent lines of treatment, so this still remains a matter of discussion. Extracorporeal photopheresis (ECP) has shown to be an effective and safe option to treat GvHD.

Aims: To evaluate the efficacy and safety of ECP in GvHD treatment in our center.

Methods: Since 2011 we have treated with ECP (Therakos System) a total of 25 patients with acute GvHD (aGvHD) (group 1) (n=12) and chronic GvHD (cGvHD) (group 2) (n=13) with refractory disease to two or more previous lines of therapy. Patients in group 1 were treated with weekly cycles and response was evaluated at week 6-8 after treatment was started. Patients in group 2 were treated every 2 weeks and response was evaluated at month 3-4. In group 1 Complete Response (CR) was defined as resolution of all symptoms, Partial Response (PR) as improvement of at least one grade compared to baseline and, Non Response (NR) when patients did not accomplish any of the other definitions. In group 2 CR was defined as symptom resolution with immunosuppressant treatment discontinuation, PR as improvement of more than 50% over baseline and/or reduction of more than 50% in immunosuppressant treatment, Minimum Response (MR) as improvement less than 50% over baseline and/or immunosuppressant reduction between 25-50% and Progression (PG) as clinical worsening or new organ involvement. The Modified Glucksberg classification was used to stage aGvHD and the NIH criteria to stage cGvHD.

Results: GvHD staging was 16%, 42% and 42% for grade II, III and IV respectively in group 1 and 8%, 38% and 54% for mild, moderate and severe in group 2. Group 1 was treated with a median of 6 cycles (1-12) and group 2 with a median of 9 cycles (3-22). The responses in group 1 was CR= 25%, PR= 33% and NR= 42% and in group 2 was CR= 15%, PR= 54%, MR= 8% and PG= 23%. The mortality rate was 67% for group 1 and 54% for group 2. The main causes of mortality were infections (88% and 57% for group 1 and 2) and GvHD progression (12% and 29% for group 1 and 2). The only complication associated to ECP was catheter infection (2 cases).

Summary and Conclusions: In our experience, ECP has proven to be a safe and effective treatment for patients with aGvHD and cGvHD. Prospective studies are required to establish indications and treatment guidelines.

PB2031

EFFECT OF COMORBIDITIES ON HEMATOPOIETIC STEM CELL TRANSPLANTATION OUTCOME IN ADULT PATIENTS WITH DIFFERENT HEMATOLOGIC DIEASES :SINGLE CENTER EXPERIENCE IN EGYPT

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Background: Background Heamtopoeitic Stem cell transplantation (HSCT) is a procedure that can restore marrow function for patients who have had severe marrow injury, replacement of marrow by disease, or abnormalities of the immune system..It is either autologous or allogenic. It has become increasingly important to optimize pre-transplant risk assessment in order to improve HCT decision making and clinical trial assignments. Single-organ comorbidity involving liver, lung, heart, or kidney before HCT has been traditionally found to cause organ toxicity after HCT. The HCT-comorbidity index (HCT-CI) has provided better prediction of HCT-related morbidity and mortality than other non-HCT-specific indices

Aims: The aim of this work is to study the impact of various pretransplant comorbidities on outcome of patients who had undergone either-allogenic or autologous HSCT -in relation to treatment related mortality and disease related mortaliy with specific emphasis on overall survival.

Methods: A retrospective study was conducted at the bone marrow Transplantation Unit, of internal medicine department Ain Shams University on 119 patients who were transplanted either by autologous or allogenic HSCT (matched or mismatched related donor). All of them were aged >18ys.and had different types of hematologic diseases; the most frequent being AML(34.4%) followed by multiple myeloma(17.6%) then ALL(16.8%) and lymphoma (10.9%) whereas,aplastic anemia,MDS,CML and biphenotypic leukemia.collectively were20.1%.They were either in complete or partial remission according to the type of disease. They were categorized according to HCT-CI into mild score (0) 43 patients (36.2%), moderate score (1-2) 60 patients (50.4%) and severe score (≥3) 16 patients (13.4%). The study data were collected from medical notes, pathology reports and laboratory data.

Table 1.

Score	Median	SE	95% Confidence Interval	Log Rank	P-value
Mild	67.85	8.18	(51.82-83.88)	8.790	0.012 S
Moderate	62.06	7.14	(48.07-76.05)		
Severe	5.00	2.66	(0.00-10.21)		

Results: There was statistically significant relation between HCT-CI and overall survival (p 0.012), disease free survival (p 0.007), mortality(p 0.047). and the incidence of graft failure.(p 0.034). Comparison between different HCT-CI scores as regard overall survival (Kaplan- Maier curve): P<0.05S-

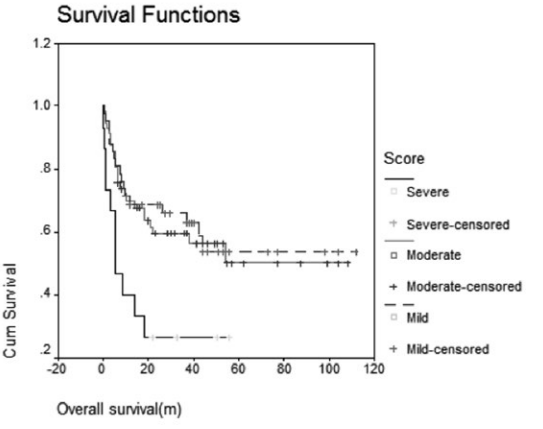

Figure 1.

Summary and Conclusions: In our study, we could conclude that the HCT-CI is a better predictor to detect the influence of comorbidity in patients with hematologic disorders on mortality, overall survival and disease free survival post HCT.

PB2032

ALLOGENEIC STEM CELL TRANSPLANTATION FOR MYELODYSPLASTIC SYNDROME

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Background: Myelodysplastic syndromes (MDS) are clonal hematopoietic disorders characterized by ineffective hematopoiesis, marrow dysplasia with cytopenias, abnormal blasts and variable rates of transformation to acute myeloid leukemia (AML). Allogeneic hematopoietic stem cell transplantation (HSCT) currently is the only potentially curative therapy for MDS. However, since most patients with MDS are older than 60 years, few are candidates for myeloablative transplantation. We present in this study our experience in HSCT for MDS.

Aims: Between May 2001 to June 2014 (13 years period), twenty two pts with MDS underwent allogeneic HSCT with HLA- identical sibling donors. The median age of the pts is 35,5 years (11-58), the Sex ratio is 1,44 (13M/9F), according to WHO classification 2008 (RA : 01, RA with del 5q : 01, RA with ring sideroblasts: 01, refractory cytopenia with multilineage dysplasia: 07, RA with excess blasts-1: 07, RA with excess blasts-2 : 05), 20pts were transfusion-dependent before transplantation. Median time from diagnosis to allogeneic HSCT was 17, 2 months (7-52).

Methods: Myeloablative conditioning (MAC) was employed in 08pts and the reduced intensity conditioning (RIC) in 14pts. GVHD prophylaxis consisted of association of Cyclosporin and Methotrexate according to Seattle protocol. All pts received G-CSF mobilised peripheral blood stem cells, with a median CD34+ cell count : 5 ,8.10^6 /Kg (3,18-19,9). At 31 December 2014, a median time of follow-up is 41, 5 months (6-135).

Results: Neutropenia occured in all pts and the median duration of aplasia was 14 (7-23) days. Median time to achieve neutrophils count >0,5.10^9/l : 13,5days (11-23) and platelets >20.10⁹/l : 13 days (9-35). Twenty pts (90%) required red blood cells transfusions (9, 9 units/pt) and 20 pts (90%) needed platelet transfusions (6 units/pt). Acute GVHD was observed in 05 cases (22,7%) grade II-IV and chronic GVHD in 07 pts (31,8%) of whom 5 with an extensive form.One pt (4,5%) relapsed at 4 months. 12 pts (54,5%) are still alive in complete remission, 10 pts (45,5%) died (4 : early severe infection, 1 : VOD, 1 : MAT, 3 : acute GVHD, 1 after relapse). The overall survival (OS) and the Disease free survival (DFS) are respectively 52, 4% in all pts. The OS and DFS for RIC group are 53, 9%.

Summary and Conclusions: This study, after a long follow-up, suggests that allogeneic HSCT is the treatment of choice for pts with advanced stage MDS and pts with dependent transfusion. Relapse seems low (4,5%) in our study and no difference in term of OS between MAC and RIC group. The advantage of RIC is the possibility of HSCT for pts up to 60 years of age and older.

PB2033

HAPLOIDENTICAL PERIPHERAL BLOOD STEM CELL TRANSPLANTATION WITH POST-TRANSPLANTATION CYCLOPHOSPHAMIDE FOR HIGH RISK PEDIATRIC LEUKEMIA. EXCELLENT ENGRAFTMENT AND ENCOURAGING LEUKEMIA FREE SURVIVAL

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Background: T cell replete haploidentical stem cell transplantation with post-transplantation cyclophosphamide (PTCy) has shown encouraging results for the treatment of hematologic malignancies; its main advantage is that almost every patient will have a donor. But this technique has been explored mainly in adults and using bone marrow as a cellular source
Aims: To present our experience using T cell replete haploidentical peripheral blood stem cell transplantation (TCR-Haplo-PBSCT) with PTCy for high risk pediatric acute leukemia
Methods: Donors were mobilized with filgrastim 5 ucg/kg/BID for five days, the PBSC were collected with one large volume apheresis procedure. The conditioning consisted of fludarabine 150 mgs/m2, busulfan 4-8 mgs/kg and Total Body Irradiation 400 cGy (Flu Bu TBI) or fludarabine 150 mgs/m2, melfalan 100-140 mgs/m2 and TBI 200 cGy(Flu Mel TBI); on days +3 and +4 PTCy 50 mgs/kg/day was given followed by cyclosporin and mycophenolate starting day + 5. All patients received post transplant filgrastim beginning on d + 6.
Results: After a signed informed consent, 18 patients, were transplanted; median age was 11.5 years (range 6-16), 8 were girls, the diagnosis were: acute lymphoblastic leukemia 8 patients, acute myeloid leukemia 9 and blastic phase of chronic myeloid leukemia one. 22% were in CR1, 39% in CR2 and 39% in CR3 or with refractory disease. Flu Bu TBI conditioning was given in 14 cases while Flu Mel TBI in 4. A median of 10 million/kg of CD34+ cells were infused. The engraftment rate was 100%, median time to achieve 500 neutrophil or more was 15 days (range 14-20), 1 patient out of 18 died without platelet recovery, the remaining had a self- sustained platelet count of 20.000 or more at a median of 14 days (range 10-21). Chimerism at day + 100 was available in 14 cases, all of them had full donor hematopoiesis. The cumulative incidence of aGVHD II-III and extensive cGVHD was 37.5% and 27% respectively. The median follow-up is 13.5 months (range 1-24), 4 patients have died, the causes were; pneumonia (n:1) and relapse of leukemia(n:3), other 2 have relapsed but are alive receiving low intensity chemotherapy. The cumulative incidence of transplantation related mortality is 5.6% and the 2 years actuarial overall survival and event free survival are 79.8% and 58.7% respectively
Summary and Conclusions: The use of TCR-Haplo-PBSCT with PTCy for treating pediatric acute leukemia is promising; it is associated with low transplantation related mortality, very good engraftment rate, acceptable incidence of GVHD, despite the use of peripheral blood, and encouraging leukemia free survival. It deserve more studies

PB2034

HIGHER DOSE CD34+ CELLS AFFECT ABSOLUTE IMMATURE PLATELET COUNT BEFORE ENGRAFTMENT AND PLATELET COUNT AFTER ENGRAFTMENT IN CORD BLOOD TRANSPLANTATION: A SINGLE-INSTITUTIONAL STUDY

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Background: Platelet recover is important to successful allogeneic stem cell transplantation and platelet is the last to be regenerated in bone marrow after stem cell transplantation. Especially, platelet increase is far slower in cord blood transplant(CBT). Recent retrospective studies suggested CD34+ cell and total nucleated cell affect blood cell recover. However, the impact of CD34+ cells dose on each blood cell lineages is not be sufficiently investigated. In addition, these studies mainly focus on engraftment.
Aims: Therefore, we analyzed cases in our database to evaluate the impact of CD34+ cell and total nucleated cell on each blood cell lineages in consecutive patients who were treated with CBT and achieved platelet engraftment over the past 6 years at our institution in Tokyo, Japan.
Methods: We included 95 consecutive patients treated at our institution between April 2008 and May 2014. The study population consisted of 58 male and 37 female patients with a median age of 53 years old (range: 16-69). Patient in this cohort underwent CBT as a part of therapy for myeloid malignancies(N=52) and lymphoid malignancies (N=43). 38 patients were treated with Flu+Cy+TBI(2gy), and 29 patients were treated with Flu+Bu+TBI(2Gy), and 29 patients were treated with AraC+CY+TBI(12gy). All patients received calcineurin inhibitor plus MMF for GVHD prophylaxis. Due to the influence of blood transfusion on blood cell counts and overall estimation of each blood cell movement, each blood cell lineages, and absolute Immature platelet count(ABPC) were assessed by the 5-day moving average,and then we checked movement of the 5-day moving average between patients receiving higher doses of CD34+ cells and patients receiving lower. The 5-day moving average was calculated from the time of day4 after transplantation until day 100 or death from any cause or the date on which the patient was received next chemotherapy.
Results: Among 95 patient, 69 patieunt achieved platelet engraftment. The median CD34+ cells doses and total nucleated cells doses were 0.8*10^5/kg and 2.0*10^7/kg. Among patients' age, total cell count and CD34 cell count in donor cord blood and disease status and conditioning regimen, disease status and myeloablative regimen impacted on pletelet engraftment on multivariate analysis. But, as shown figure 1, the curve of ABPC in patients receiving higher doses of CD34+ cells is highter than the other from day +30 to day +50, and the curve of platlet counts in platelet patients receiving higher doses of CD34+ cells were raising after day +40. In patient who attained platelet engraftment, only age and CD34 cells affect over all survival rate.

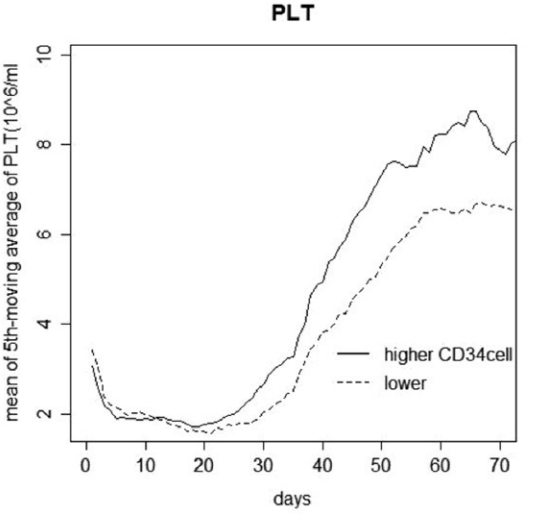

Figure 1.

Summary and Conclusions: The results of the present study highlighted the importance of CD34 cells on platelet recover and CD 34+ cell can be prognostic factor after engraftment in RIC-CBT.

PB2035

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PB2037

COMPARATIVE ANALYSIS OF FILGRASTIM, BIOSIMILAR FILGRASTIM AND LENOGRASTIM AS PERIPHERAL BLOOD CD34+ MOBILIZATION DRUG FOR AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: There are different strategies to mobilize peripheral blood CD34+ cells in oncohematologic patients that will receive an autologous stem cell transplantation (ASCT). As mobilizers drugs (G-CSF), in addition to the original filgrastim (Neupogen®) and lenograstim (Granocyte®), since 2012 we have a biosimilar filgrastim (Nivestim®).
Aims: Our objective was to compare the effectiveness and cost of these drugs in our hospital during the past six years.
Methods: We retrospectively analyzed patients undergoing peripheral blood CD34+ apheresis between January 2009 and December 2014. The collected data included: age, sex, disease (acute leukemia, plasma cell neoplasm, NHL, Hodgkin lymphoma and solid tumor), number of lines of prior chemotherapy, prior use of purine analogs or alkylating agents or radiotherapy, type of mobilization (G-CSF alone, associated with cyclophosphamide or mega-CHOP, or associated with disease-specific chemotherapy) days of administration and daily dose of G-CSF. As for the apheresis procedure: leukocytes, hematocrit,

platelets, percentage of mononucleated cells, percentage of CD34+ cells and CD34+ cells/μL in blood just before apheresis; total number of volemias and processed volume, total number of apheresis per mobilization, process time and obtained CD34+ cells (x10^6/Kg, target >2), and cost per total mobilization procedure. If ASCT was done, we also collected infused CD34+ cells, day to achieve a neutrophil count >500/μL and platelets >20,000/μL. For statistical analysis, SPSS was used.
Results: We studied 201 patients, mean age 50 years (range 2-71) and 64% males, and we used filgrastim in 85 cases, biosimilar 66 and lenograstim 50. There were no significant differences among the three groups for any of the analyzed variables, except for the total number of apheresis per mobilization (the lowest with biosimilar, 1.7, and the highest with filgrastim, 2.1; P=0.009), the number of lines of prior chemotherapy (biosimilar 1.4, lenograstim 1.5 and filgrastim 1.9; P=0.006) and the cost in euros per total mobilization procedure (biosimilar 200, lenograstim 531, and filgrastim 602; P<0.001). Average CD34+ cells in the final product was 6.7x 10^6/Kg (range 0.2 to 40.2) for mobilization and similar with all three drugs (P=0.38). In 25 cases (12%), CD34+<2x 10^6/Kg were obtained. ASCT was performed in 103 patients (51%) and no differences in infused CD34+ cells, days to achieve neutrophils >500/μL or platelets >20,000/μL (for the two last, average 11 and 17, respectively), were observed.
Summary and Conclusions: In our experience we found no differences between the three analyzed drugs in terms of number of CD34+ cells obtained at the final product of apheresis, or hematopoietic recovery after ASCT. However, for the biosimilar drug fewer apheresis for mobilization procedure were needed, and a significantly lower overall cost was observed.

PB2038

FROM HLA TYPING TO ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION: BARRIERS AND OUTCOME

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Background: Despite the advent of newer targeted and immune therapies, allogeneic hematopoietic cell transplantation (HCT) still offers an advantage in terms of disease free survival, overall survival and quality of life in appropriate settings.
Aims: Several studies have elucidated the heterogeneity in HCT practice among individual physicians, transplant centers and different countries. In our institution, we conducted a retrospective analysis describing and evaluating the outcome of patients for whom an allogeneic HCT was discussed or planned.
Methods: From 2007 till the end of 2014, an HLA typing was done for 156 patients treated for different hematological malignancies at Centre Rene Huguenin/ Institut Curie, France. We reviewed the files of these patients for the type of malignancy, the reason why HLA typing was done, the major barriers to allogeneic HCT and the different outcomes.

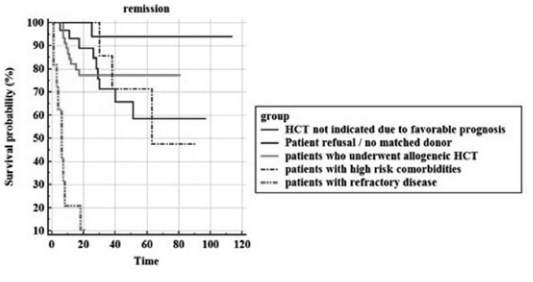

Figure 1.

Results: Among the 156 patients, 27.5% had acute myeloid leukemia, 5.8% acute lymphoblastic leukemia, 0.6% biphenotypic leukemia, 1.9% plasmacytic leukemia, 15.4% multiple myeloma, 7.1% hodgkin lymphoma, 16.1% non hodgkin lymphoma, 7.7% chronic lymphocytic leukemia, 12.8% myelodysplastic syndrome, 5.1% myeloproliferative neoplasms. Of these, 14.1% were typed before complete risk stratification of the disease, 37.2% for the presence of high risk factors (e.g karyotype, molecular markers, ...), 17.3% because their disease was refractory to primary treatment or had an early relapse, 30.8% for late and multiple relapses, and 0.6% upon patient's request. Fifty nine patients or 37.8% underwent allogeneic hematopoietic cell transplantation and 7.1% are currently being evaluated by the committee. The median age of the patients undergoing allogeneic HCT was 53 years (19-67years) and those for whom an HLA typing was done was 57 years (19-72years). Median time from HLA typing to HCT was 7 months ranging from 1 to 14 months. For the remaining patients, in 31.4% of the cases, allogeneic HCT was finally not indicated due to favorable prognosis, 25.6% didn't have a matched donor, 15.1% were ineligible because of refractory/resistant disease and 15.1% were also ineligible because of high risk comorbidities. In 10.5% of cases, the patients refused to undergo HCT and 2.3% were lost to follow up. In terms of outcome, the patients with no indication to HCT due to a favorable prognosis had a significantly better survival than all others. Patients ineligible to HCT with a refractory/resistant disease had the poorer outcome. The group of patients undergoing allogeneic HCT had a lower relapse rates (10.1%) compared to those who didn't have a matched donor or who refused transplantation (22.5%) but there was no significant difference in survival between these groups possibly due to a transplant related mortality of 20.4%.
Summary and Conclusions: In patients for whom HCT was discussed in our institution, a favorable prognosis was the main cause for not undergoing HCT. The major barriers were lack of matched donors, patient's refusal and significant comorbidities. Our results show that current risk stratification of the hematological malignancies correlates well with the patients outcome. Clear criteria for allogeneic HCT are still lacking especially with the advent of new targeted therapies and the persistence of a significant transplant related mortality and morbidity.

PB2039

HYPERBARIC CHAMBERS APPLICABLE IN THE TREATMENT OF HEMORRHAGIC CYSTITIS AFTER ALLOGENEIC STEM CELLS FROM UNRELATED DONORS.

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Background: Hemorrhagic cystitis (HC) is a diffuse inflammation of the bladder of an infectious or non-infectious etiology, causing bleeding of the bladder mucosa. Medications: (busulfan, endoxan, idarubicin, carboplatin), radiation, viruses, chemicals may be the cause of HC. HC occurs after approximately 35 days after stem cell/bone marrow transplantation and continues for approximately 23 days. Symptoms of cystitis may be caused by defect in the inflammatory response and increase the permeability of glycosaminoglycan layer, which covers the bladder mucosa, form a physiological barrier. There are no explicit guidelines defining appropriate treatment. Hyperbaric therapy (HBO) has been recognized clinically in the treatment of HC in the past 15 years. It is a non-invasive method involving the operation of 100% oxygen under increased pressure, which penetrates poorly perfused areas, patient exposure to 100% oxygen-saturated tissue oxygen efficiently.
Aims: We reviewed the records of 5 patients with HC who received hyperbaric oxygen as an adjunctive treatment.
Methods: We retrospectively analyzed the effectiveness of HOT in 5 patients in the years from 2012 to 2014. Among patients there were 3 men, aged from 31 to 41 years and 2 women aged from 40 to 43 years. Patients characteristics is shown in Table 1.

Table 1.

Patient No	Age yr	Sex	Diagnosis	Clinical status	Condidtioning regimen	Source for alloHSCT	GvHD prophylaxis
1	40	F	MDS	CR1	BuCyTym	PBSC, MUD	C, MTX, Gluc
2	41	M	AML	CR1	BuCyTym	PBSC, MUD	C, MTX, Gluc
3	39	M	ALL	CR1	TBI, CyTym	PBSC, MUD	C, MTX, Gluc
4	43	F	AML	CR1	BuCyTym	PBSC, MUD	C, MTX, Gluc
5	31	M	AML	CR1	BuCyTym	PBSC, MUD	C, MTX, Gluc

Abbreviations: C= cyclosporine, Tym= tymoglobuline, Gluc=glucocorticoids, MUD=matched unlrelated donor, MTX=methotrexate

Table 2.

Patient No	Onset days post BMT	Hematuria (grade)	Ultrasound	GvHD (type)	GvHD (grade)	Viruria	Antiviral treatment before HBO
1	14	2	Bladder wall thickening	Cutaneous And Gastrointestinal	III	BKV	Cidofovir
2	24	2	Normal	Cutaneous	II	ADV	Cidofovir
3	15	2	Bladder wall thickening	Cutaneous	II	BKV+ADV	Cidofovir+Rybavirin
4	11	3	Bladder wall thickening	Cutaneous	II	BKV+ADV	Cidofovir+Rybavirin
5	55	2	Not performed	None		ADV	Cidofovir

Results: Clinical presentations of 5 patients with haemorrhagic cystitis. The median time to onset of HC after allogeneic stem cell transplantation was 22 days (range, 11-55 days). In one patient, the symptoms did not appear until the day 55 after transplantation. Despite immunosuppressive therapy, all patients had macroscopic hematuria and GvHD. The BKV and ADV DNA were detected in urine and plasma samples in 2 patients, ADV DNA was detected in urine and plasma in 2 other patients, and in the one patient, BKV was detected in both samples, respectively. The patients were treated with HOT (2.5 atmospheres for 60 minutes, 5 days per week) after treatment failure of bladder drainage flow, hyaluronic acid administered intravesically, as well as antiviral treatment. All patients showed complete resolution of hematuria and eradicaton of the virus after a median of 13 sessions (range, 11-30) of HOT.
Summary and Conclusions: Hyperbaric oxygen therapy may be an alternative and promising therapy in the treatment of severe hemorrhagic cystitis.

PB2040

THE IMPACT OF INDUCTION THERAPY AND AUTOLOGOUS STEM CELL TRANSPLANTATION IN MULTIPLE MYELOMA ON TREATMENT OUTCOMES: A SINGLE CENTER EXPERIENCE

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Background: Treatment of symptomatic multiple myeloma (MM) in patients (pts) eligible for ASCT is a main approach, which can lead to a complete eradication of the tumor, improving or deepening of response, as well as increased survival rates.
Aims: The primary aim was to assess whether induction therapy and ASCT was associated with improved results on anti-tumor response and overall survival (OS). We also evaluated the impact of renal impairment and advanced stage of MM on OS in pts that underwent of ASCT.
Methods: The study included a cohort of pts with MM who underwent of ASCT for the period from 2001 to 2014. Induction therapy prior to ASCT included VAD-regimen and bortezomib-containing regimens with subsequent high-dose cyclophosphamide therapy. OS and progression free survival (PFS) estimated by the Kaplan-Meier method.
Results: The analyzed group consisted of 71pts with primary diagnosed MM (48 males/23 females; mean age 53.1 yrs (range 34-69). IgG MM was diagnosed in 39pts (55%), IgA MM in 13 (18%), Bence Jones myeloma in 16 (23%), primary plasma cell leukemia in 3pts (4%). In accordance with clinical stage, (Durie B.G.M. and Salmon S.E.) pts were distributed as follows: IA-2 (2.8%), IIA-23 (32.4%), IIB-2 (2.8%), IIIA-21 (29.6%), IIIB-22 (31%). Induction therapy was VAD in 28pts (39.4%), bortezomib (VDD, VD) in 40 pts (56.4%), and VAD-like and VD in 3 pts (4.2%). The median of follow-up was 26.7m (3.1-126.2m). The cohort studied performed 83 ASCT. 12pts received 2 ASCT including planned tandem ASCT (n=6) and 2nd ASCT due to progression (n=6). Before the 1st ASCT complete response (CR) was observed in 21pts (29.6%) including 8 stringent CR, which was preserved when restaging after 3m. We identified improving the quality of response from a very good partial response (VGPR) and partial response (PR) to CR in 16 (37.2%) of 43pts after ASCT. 5pts with stable disease (SD) reached VGPR and PR. Of the 2pts transplanted in early biochemical relapse, 1 was reached CR, and the 2nd developed clinical relapse. Before the 2nd planned tandem ASCT performed with an interval of 3m (2-5m), in 3 of 6pts were reported CR, which hasn't changed during 3m. Of the remaining 3pts the achieved VGPR and CR preserved in 2pts. VGPR and PR was noted before the 2nd ASCT after relapse/progression in 5pts; 3m later 1 patient achieved CR, and 4pts showed no change. A patient with signs of disease progression after the 2nd ASCT achieved SD. The impact of induction therapy (with or without bortezomib) did not affect the results of survival (P=0.57). Renal impairment and advanced stage of MM as a poor prognostic factors also had no effect on the survival rate after ASCT (P=0.1). PFS and OS after ASCT (n=71) was 19.6m and 45.2m, respectively. OS from the diagnosis of MM was 58.5m. OS after 1st-line therapy (n=62) was 69m compared to 29m after 2nd-line (n=9), P=0.02. Median PFS after the 1st and 2nd line therapy did not differ (P=0.14). OS was significantly greater in the group of pts with CR after induction therapy (n=21) than with PR (n=43): 94 m vs 43 m, P=0.04. PFS for pts with CR before transplantation also exceeded PFS in pts with PR (34m vs 16m, P=0.05).
Summary and Conclusions: The depth (degree) of response to induction therapy was positively associated with survival rates. ASCT followed by high-dose chemotherapy demonstrated improving responses in a substantial proportion of patients not only after the first ASCT but also after the second ASCT (tandem or due to relapse of MM), which confirms the important role of ASCT. No survival benefit was seen for patients treated with ASCT in different regimens of induction chemotherapy and if they have renal impairment.

PB2041

THE INFLUENCE OF HAPLOTYPE HLA-BW4 IN EVOLUTION AFTER HSCT

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Background: Continuing to study haplotypes of patients like ligands for inhibitories and activatories donors KIR allele in HSCT,it is an evidence that haplotypes can be protective or not against postHSCT complication,like ligand or not.
Aims: Haplotype HLA-BW4 is described in literature like,,bad,, haplotype.We try to demonstrate the influence of this haplotype at patients with acute leukemia after HSCT.
Methods: Eighteen pairs patients-donors are evaluated :patients with acute leukemia,lymphoblastic and non-lymphoblastic and their genoidentics donors.Eleven patients have HLA-BW4 haplotype,seven HLA-BW6(absence of HLA-BW4) Following the impact of inhibitory KIR3DL1 and activatory KIR3DS1 on survival and complication development,we proved the protective effect of absence of HLA-BW4 haplotype,HLA-BW6,respectively.The source of HSCT was PBSC.The method used was PCR-SSP (Innotrain DIAGNOSTIK GMBH,Dynal BIOTECH PEL-FREEZE)The complications like graft *versus* host disease acute and chronic,relapse,TMA and the recovery with leucocytes and thrombocytes are followed
Results: Absence of HLA-BW4 haplotype(HLA-BW6,respectively) is protective for both types of leukemia,the patients survival is 100% in presence of KIR3DS1(activatory allele) and with statistical significance (sig<0,05) and 83% in presence of KIR3DL1(inhibitory allele) Is protective against relapse, TMA, aGVHD, leucocytes and thromboytes recovery,with statistical significance in presence of KIR3DS4,and also in presence of KIR3DL1 is protective against aGVHD,TMA and relapse,also thrombocytes recovery,without statistical significance.
Summary and Conclusions: Absence of HLA-BW4 improve survival and protect against most complication at patients with acute leukemia and related donors with 100% allele match,in presence of both types of KIR alleles and confirme the "missing " ligand theory.

PB2042

MATCHED UNRELATED DONOR HAEMATOPOIETIC STEM CELL TRANSPLANTATION OFFERS LONG-TERM SURVIVAL IN ABOUT HALF OF ADULTS WITH HAEMATOLOGICAL MALIGNANCIES. THE GREEK EXPERIENCE.

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Background: Allogeneic haematopoietic stem cell transplants (HSCT) are steadily increasing in number due to the enhanced availability of matched unrelated donors (MUD) over the last decade. The results of MUD-HSCT have improved considerably, mainly due to the introduction of high-resolution molecular HLA typing for donor selection. Currently, more than 50% of allogeneic HSCT are done with MUD worldwide.
Aims: The aim of this study is to retrospectively analyze the outcomes in a cohort of adult patients, who underwent MUD-HSCT for hematological malignancies at three major transplant centers in Greece.
Methods: From 01/2001 until 12/2013, 331 patients (M/F: 197/134) underwent MUD-HSCT for acute myeloid leukemia (n= 148), acute lymphoblastic leukemia (n= 74), non-Hodgkin's lymphoma/chronic lymphocytic leukemia (n=28), myelodysplastic syndrome (n=26), chronic myeloid leukemia (n=21), Hodgkin's lymphoma (n=20), myelofibrosis (n=7), or other haematological malignancies (n=7). The median age of patients was 40 (range, 14-66) years. Median time from diagnosis to transplantation was 13 (range, 2-235) months. Disease Related Index (DRI) was low, intermediate or high/very high in 11%, 66% or 23% of cases, respectively. EBMT risk score was ≤3 in 38% and ≥4 in 62% of patients. The comorbidity-age index was ≤1 in 58.4%, and ≥2 in 41.6% of recipients. The conditioning regimen was myeloablative in 252 (76%) or reduced intensity in 79 (24%) of procedures. Peripheral blood was the source of graft in 92% of cases. Donor/recipient HLA allele match at HLA-A, -B, -C and -DRB1 loci was 8/8, 7/8 or<7/8 in 51%, 47% and 2% of cases, respectively. *in vivo* T cell depletion with antithymocyte globulin or alemtuzumab was performed in 58% of cases. The combination of tacrolimus and methotrexate was used for GvHD prophylaxis in 234 (71%) patients.
Results: Engraftment was achieved in 97% of patients. The incidence of acute and chronic GvHD was 42.5% and 55.5%, respectively. The cumulative incidence (CI) of treatment related mortality (TRM) was 26% and 33% at 1 and 3 years post transplant, respectively. CI of relapse was 25%. With a median follow-up of 36.5 months, 3-year event free survival (EFS) and overall survival (OS) were 44% and 47%, respectively. The major prognostic factors for OS in multivariate analysis were EBMT score (≥4 vs. ≤3, 37.4% vs. 64.1%, HR: 2.32, P<0.001), comorbidity-age index (≥2 vs. ≤1, 32.6% vs. 59.7%, HR 1.81, P<0.001), DRI (high/very high vs. intermediate/low, 26.3% vs. 54.3%, HR: 1.79, P<0.004) and HLA match (≤7/8 vs. 8/8, OS 40% vs. 56.1%, HR: 1.68, P<0.002).
Summary and Conclusions: MUD-HSCT is an effective therapeutic option for adults with haematological malignancies, and offers long-term survival in about half of them. Apart from age and comorbidities, the outcome greatly depends on HLA match and disease stage at transplant. Therefore, timely referral of patients for MUD-HSCT early in the course of disease may further improve the results.

PB2043

COMPARISON OF ORAL BUSULFAN (BU) AND INTRAVENOUS BU THERAPY FOLLOWING HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR HEMATOLOGICAL MALIGNANCY IN CHILDREN AT A SINGLE INSTITUTION

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Background: Busulfan (BU) is a frontline agent in the hematopoietic stem cell transplantation (HSCT); however, severe complications including sinusoid obstructive syndrome (SOS) have been reported, particularly with oral administration. Since intravenous BU (i.v. BU) was introduced in 2006, some reports have demonstrated i.v. BU reduces hepatic complication such as SOS and treatment related mortality in adults. However, there have been few studies in pediatric populations comparing i.v. BU and oral BU (p.o. BU).
Aims: The purpose of this study was to determine the efficacy and safety of i.v. BU in children.
Methods: We retrospectively analyzed 38 cases of hematological malignancy in which HSCT was performed with myeloablative dose of BU between January 2000 and March 2014.
Results: Twenty-six cases received p.o. BU and 12 cases received i.v. BU. The median age at HSCT was 5 years (range, 3months-17 years). The median overall follow up was 777.5 days (range,7 -4893 days), 1400 days (range, 7-4893 days) in the p.o. BU group, and 459.5 days (range, 33-2360 days) in the i.v. BU group. Diagnosis of hematological malignancies were as follows: 11 AML, 10 ALL (5 infan) and 5 others in the p.o. BU group, and 10 ALL (4 infant) and 2 others in the i.v. BU group. Disease status at HSCT with BU were as follows: 14 cases (36.8%) were CR1 overall, 11 cases (42.3%) in the p.o. BU group, and 3 cases (25%) in the i.v. BU group (P=0.32). Overall survival (OS) at 1 year was 52.5% overall, 56% in the p.o. BU group, and 50% in the i.v. BU group (P=0.83). Event free survival (EFS) was 35% overall, 42.8% in the p.o. BU group, and 33.3% in the i.v. BU group (P=0.35). Relapse rates at 1 year were 27.5% overall, 21.4% in the p.o. BU group, and 41.7% in the i.v. BU group. Non relapse mortality at 1 year was 26.3% over all, 26.9% in the p.o. BU group, and 25.0% in the i.v. BU group. SOS occurred in 6 cases (15.8%) overall, 4 (15.4%) in the p.o. BU group, and 2 (16.7%) in the i.v. BU group (P=0.92). Adverse events above grade 3 included pulmonary complications such as interstitial pneumonia is 3.8% (2/26) in the p.o. BU group, while 50% (6/12) in the i.v. BU group. We also analyzed OS and EFS, cumulative incidence of relapse, non-relapse mortality of patiens with no prior HSCT (n=23) in this cohort. There were no statistically significant differences in OS, EFS, cumulative incidence of relapse or non-relapse mortality between p.o. BU (n=16) and i.v. BU (n=7) among patients with no prior HSCT.

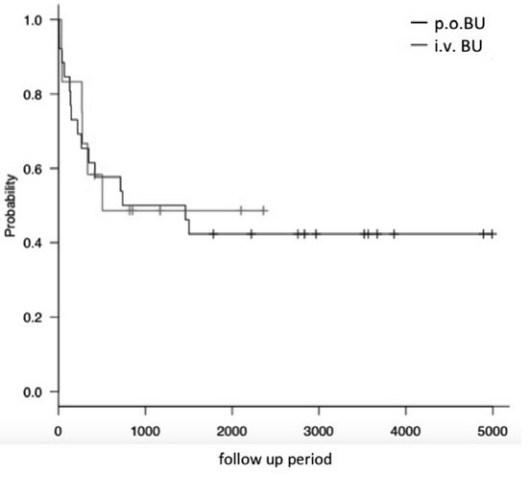

Figure 1.

Summary and Conclusions: In summary, our analysis demonstrated no difference in OS and EFS at 1 year or probability of SOS between myeloablative i.v. BU and p.o. BU doses for the treatment of hematological malignancies. Recent reports have demonstrated i.v. BU can improve the outcome following HSCT in adult. However, retrospective analysis of a large number of pediatric HSCT cases in Japan found no significant benefit of i.v. BU on the survival probability (M Kato *et al.*Biol Blood Marrow Transplant 2013; 19: 1690-1694). Our study corroborated these result; however, this was a retrospective study with different patients backgrounds. These are the major limitations of this study. In terms of adverse events, pulmonary complication was more frequent with i.v. BU than that with p.o. BU. This result may be due to the proportion of infantile cases at a high risk of pulmonaly complications with BU included in this study. Although i.v. BU is considered the gold-standard, i.v. BU does not confer benefit on survival probability and is still associated with severe complications including SOS and interstitial pneumonia. It is important that complications arising from the use of i.v. BU are fully elucidated by long term observational studies and the accumulation of evidence regarding the outcomes following i.v. BU use.

PB2044

THE NUMBER OF ANTI-MYELOMA DRUGS IN PRIOR TREATMENT REGIMENS DOES NOT NEGATIVELY AFFECT THE SUCCESS OF AUTOLOGOUS CD34+ HEMATOPOIETIC STEM CELL MOBILIZATION

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Background: Post-induction Autologous Hematopoietic Stem Cell (HSC) Transplantation (aHSCT) remains the standard of care for multiple myeloma pts (MM) who are fit enough to tolerate it. Although treatment guidelines favour transplantation after induction, recent drug developments have lead to prolonged survivals and the ability to salvage patients who were not transplanted at diagnosis, and who now present at aHSCT Centers, referred from hospitals with differing practices regarding the timing for autologous HST and the protocols used. While it is known that prior treatment can adversely affect the success of HSC collection, consensus is lacking on whether this is only due to the myelotoxicity of the drugs used, or also to the type of regimen chosen.
Aims: We aim to clarify the influence of the type of prior antimyeloma regimens on the success of HSC collection in a real world sample of newly-diagnosed and previously treated patients (pts).
Methods: We analysed 96 MM pts undergoing their first aHCT. HSC collection success was evaluated by the number of peripheral blood autologous CD34+ cells ("PB CD34"), per kilogram, available for infusion at the first transplant ("Number of Cells").
Results: The median overall survival was 104.5 m after a median follow-up of 59.1 m. Overall, 68.8% of pts underwent their first aHSCT after 1st line therapy, 23.9% after 2nd line and the remaining 7.3% after 3rd line or greater. The mean Number of Cells infused was 3.4±1.9x10^6, 3.3±1.7, and 2.6±0.7, respectively, P=0.05. The regimens received prior to apheresis ranged from doublets up to seven-drug associations (VBMCP/VBAD), and included alkylator-, bortezomib, IMID- and anthracycline-based therapies. Considering pts undergoing aHSCT after 1st line, 40.9% were treated with doublets, 47.0% with triplets and 12.1% with associations of ≥4 antimyeloma agents. Pts receiving doublets were transplanted with a larger number of PB CD34 cells (4.1±2.6 vs 3.0±1.1x10^6, P<0.001), compared to pts receiving ≥3 drug-associations. Within the same cohort, the 57.6% who were treated with alkylator-based regimens were transplanted with a lower Number of Cells than pts treated with alkylator-free protocols (3.0±1.0 vs 4.1±2.6x10^6, P<0.001). In contrast, neither the presence of anthracyclines nor of bortezomib in the 1st line regimen influenced the success of apheresis; the 21.1% of pts treated with anthracycline-based regimens were infused with 4.1±1.7x10^6 cells vs 3.3±1.9 (P=NS) in pts without anthracyclines; the 74.2% of pts treated with bortezomib-based regimens received 3.4±2.0x10^6 cells vs 3.8±1.7 (P=NS) in pts without bortezomib. Only 7.6% of pts received one of the IMIDs at 1st line in our cohort, limiting our analysis. We found that 0% of doublet-regimens included alkylators, compared to 96.8% of triplets and 100% of ≥4 drugs (P<0.001). Taking this into account, after multivariate analysis the number of drugs used lost its predictive value, while only the presence of an alkylator maintained its effect over the success of HSC collection.
Summary and Conclusions: We found that the presence of an alkylator in the 1st line regimen negatively impacted HSC collection success, as expected from its myelotoxic effects. Neither the anthracyclines nor proteasome-inhibitors influenced the success of HSC collection. While there was a significant difference favouring the use of doublets prior to apheresis, this was due to the higher likelihood of the inclusion of an alkylator in higher-number drug-associations. Our results support the option of saving alkylators for advanced-lines of treatment, in pts who have not yet undergone HSC collection.

PB2045

HIGH INCIDENCE OF NEUROLOGICAL COMPLICATIONS IN SICKLE CELL DISEASE UNDERGOING ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION: EARLY DIAGNOSIS CAN CHANGE THE PROGNOSIS

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Background: Hematopoietic stem cell transplantation (HSCT) was offered primarily as a therapeutic option for severe sickle cell disease in the context of myeloablative matched sibling donor transplants over the last two decades and helped to establish the benefits of transplantation for this disorder. In recent years, the transplant community has set out to explore ways to make stem-cell transplantation more available to patients with the disease, define indications and better timing, and offset toxicities with novel approaches to conditioning and better supportive care. In this context, neurological complication such as stroke and blood flow alterations in cerebral artery constitute the main indications of HSCT but neurological complications are the main causes of TRM in D+100 and is important to find ways to prevent this problem.
Aims: Describe early neurological complications in sickle cell patients undergoing related HSCT on single center and its following after the early diagnosis.
Methods: Seven patient undergone related HSCT for sickle disease from 2011 to 2013. All patients filled inclusion criteria in the study and signed agreement term. Seven HSCT were developed in the period, being four males. The aver-

age age was 13 years old (7-24). The HSCT indications were previous stroke, cerebral flow alteration on Doppler, acute chest syndrome and alloimunization. All patient were on blood transfusion therapy. The conditioning regimen was BuCy + ATG and the GVHD prophylaxis was MTX and CSA. Related donors were chosen with 10/10 HLA match and graft source marrow

Results: The median of the neutrophil engraftment was D+ 25 and the platelets engraftment was in D+60. Two patient died, one by intestinal and liver GVHD on D+120, and another with sagittal sinus thrombosis and hemorrhagic stroke on D+3. Other two patients showed PRES syndrome related to cyclosporine use. The patients showed generalized seizures with tomographic neurological alteration. After the imunossupressor change to tacrolimus and the change of Phenytoin to Lamotrigine, the patient had total resolution of neurological complications without development of neurological sequelae. Patients who used Lamotrigine since the beginning of the conditioning have not shown neurological alterations.

Summary and Conclusions: The PRES Syndrome and Stroke are two of the main causes of mortality related to the use of calcineurin inhibitors. Patients with sickle disease have shown endothelial and cerebral microcirculation changes, which made them highly susceptible to neurological complication. The control of blood pressure, maintenance of 50.000 platelets level and the use of Lamotrigine as prophylaxis of seizures seems to decrease the risk of neurological complications. Prospective studies with lamotrigine as primary prevention of PRES Syndrome must be performed. Patients with severe neurological alteration as vessels stenosis more than 90% and Moya-Moya Syndrome must be better evaluated before the conditioning because of high TRM risk.

PB2046

THE COMPARISON OF LENOGRASTIM AND FILGRASTIM IN ALLOGENEİC STEM CELL TRANSPLANTATİON: EFFECTS ON BONE MARROW STEM CELL PRİMİNG

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Background: Hematopoietic stem cell transplantation from HLA-matched siblings improved long-term survival of the children with hematologic malignancies and bone marrow failure syndromes.Recombinant human granulocyte colony stimulating factor (G-CSF) is used to mobilize hematopoietic stem cells for allogeneic and autologous hematopoieitc stem cell transplantation.

Aims: In this study, we aimed to compare the efficacy profile of different G-CSF agents including biosimilar filgrastim (Leucostim®) and lenograstim (Granocyte®) on bone marrow stem cell mobilization in healthy donors

Methods: A total of 29 healthy donors were enrolled in the study and analysed retrospectively. Fifteen donors received biosimilar filgrastim and fourteen donors received lenograstim. The dose and duration of G-CSF treatment was 10 μcgr/kg/dose for 3 days and similar between two groups. Post-G-CSF white blood cells, harvested stem cell volume, number of total nucleated cells (TNC) and CD34+ cells, CFU-GM colony number has been compared between biosimilar filgrastim and lenograstim groups.

Results: There were 16 (55.2%) male and 13 (44.8%) female donors. The median age and body weight of donors was 12.5±10.2 years and 37.1±19.3 kg respectively. The median number of post G-CSF white blood cells ($x10^3$/μL) [32.6 (28-41.2) vs. 37.3 (25.7-40), P=0.663], harvested total stem cell volume (cc) [437 (385-850) vs. 643 (324-1018), P=0.694], number of total nucleated cells($x10^8$/recipient kg) [6.4 (5.1-8.7) vs. 7.1 (5.1-8.6), P=0.861], CD34+ cells (μL) in the harvested stem cell [183 (104-272) vs. 160 (93-259), P=0.801], number of CD34+ cells ($x10^6$/recipient kg) [3.1 (2.1-5.2) vs. 2.6 (2.3-5.5), P=0.930], CFU-GM colony number ($x10^4$/recipient kg) [13.2 (8-15.4) vs. 14.1 (9.8-25.6), P=0.538] were similar between filgrastim and lenograstim groups.

Summary and Conclusions: In conclusion, the current retrospective study shows that biosimilar filgrastim and lenograstim are similar in terms of efficacy for the mobilization of bone marrow hematopoietic stem cells in healthy donors.

PB2047

FACTORS RELATED TO THE FINAL NUMBER OF CD34+ CELLS OBTAINED BY APHERESIS FOR AUTOLOGOUS STEM CELL TRANSPLANTATION: EXPERIENCE IN 181 CONSECUTIVE PROCEDURES IN A SINGLE CENTER

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Background: The successful mobilization of hematopoietic progenitors (CD34+) for autologous stem cell transplantation has been associated with the number of cycles of previous chemotherapy (CT), recent administration of CT (<3 months), prior use of purine analogs or alkylating agents, radiotherapy, age, marrow involvement by the disease, and leukopenia or thrombocytopenia before mobilization.

Aims: Our objective was to analyze our experience on CD34+ cell collection by apheresis in the past 5 years, focusing in what factors are implicated in a better or worse final product.

Methods: We retrospectively analyzed all consecutive mobilization procedures performed in our department between 2009 and 2014. Analyzed data included: age, sex, underlying desease (acute leukemia, plasma cell neoplasm, small cell NHL, aggressive NHL, Hodgkin's lymphoma or solid neoplasm), type of used G-CSF (filgrastim, biosimilar filgrastim or lenograstim), type of mobilization (G-CSF alone, associated with cyclophosphamide or mega-CHOP CT, or disease-specific CT), number of previous CT lines, prior radiotherapy or purine analogs or alkylating agents, marrow involvement at diagnosis, percentage of CD34+ cells and CD34+ cells/μL in blood just before apheresis, and CD34+ cellsx 10^6/kg at the final product. For statistical analysis (SPSS adapted to Windows) we used the Pearson correlation coefficient, ANOVA and Tukey HSD test for multiple comparisons.

Results: We studied 181 patients, mean age 51 years (range 3-71) and 64% males. We found that the type of G-CSF, the number of previous lines, the use of purine analogs or alkylating drugs, and marrow involvement at diagnosis did not influence significantly in the final number of CD34+ cells obtained. In the other hand, we observed a tendency to a poorer final product in older patients (P=0.09) or when there was a history of radiotherapy (P=0.062). Significantly, the richest final product was achieved in patients with Hodgkin's disease compared with poorer in those affected of acute leukemia, plasma cell neoplasm or small cell NHL (P=0.012). Similarly, a higher number of CD34+ cells were collected in patients mobilized with disease-specific CT, compared with the other types of mobilizations (P=0.006). Finally, as expected, the pre-apheresis mononuclear cells and CD34+ cell percentages, and circulating CD34+ cells/μL, were directly related to the achieved final product (P=0.004, P<0.001 and P<0.001, respectively).

Summary and Conclusions: In our experience, the factors that most influenced the amount of CD34+ cells harvested by mobilization and apheresis, are the type of hematologic disease (from most to least favorable: Hodgkin's disease, solid tumors, aggressive NHL, small cell NHL, plasma cell neoplasms and acute leukemia) and type of mobilization scheme used (the best product was obtained with disease-specific CT, and worst with G-CSF alone). To a lesser extent, the patient's age and history of radiotherapy may also influence the final product.

PB2048

ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION USING FLUDARABINE, MELPHALAN, TOTAL BODY IRRADIATION AS REDUCED-INTENSITY CONDITIONING REGIMEN FOR MYELOID MALIGNANCIES IN A SINGLE INSTITUTION.

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Background: Traditional transplant conditioning regimens have a limiting factor regarding co-morbidities or old age. Therefore, reduced intensity conditionig (RIC) regimens have been increasingly used since RIC regimens have lower rates of severe toxicity. Although RIC regimen for allogeneic hematopoietic transplants (allo-SCT) have become common usuful strategy for high risk myeloid malignancy, optimal conditioning regimen has not been established.

Aims: To define the role of RIC and MAC condition for patients with myeloid malignancy.

Methods: We retrospectively compared long-term outcomes of 38 consecutive transplants for adult patients with high-risk myeloid malignancy. Fifteen out of 38 patients who were older age (□55 year-old) or unfit received RIC regimen consisting of fludarabine (125mg/m2), melphalan (80mg/m2) and total body irradiation (TBI) (4Gy). Twenty-three patients received MAC regimens containing TBI (12Gy). All transplants received uniform strategy of graft *versus* host disease (GVHD) prophylaxis.

Results: All patients achieved engraftment in RIC comparing with 2 patients were engraftment failure in MAC. High level of treatment related toxicities was observed in patients with MAC rather than RIC. Compared with MAC, relapse rate was higher in RIC. Overall 2-year survival rate and relapse free survival were 42.3%, 36.7% in RIC and 43.5%, 39.1% in MAC respectively. Grade III to IV of acute GVHD were occured 6.6% in RIC and 30.4% in MAC, chronic GVHD were appeared 46.6% in RIC and 34.7% in MAC. In unitivariate analysis, good performance status (PS) and disease status CR, In multivariate analysis, disease status CR was the only prognostic variable for prolonged OS in high risk myeloid malignancy.

Summary and Conclusions: Our retrospective study, RIC was feasible and survival benefit was obtained in same level MAC. Lower tumor burden before transplantation but not conditioning intensity nor patient age intensity influenced longer survival. Although our study was small size of patient population, larger prospective randomized study should be required.

PB2049

EFFICACY OF COMPLEMENT MODULATING THERAPIES IN ALLOGENEIC STEM CELL TRANSPLANTATION ASSOCIATED THROMBOTIC MICROANGIOPATHY

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Background: Thrombotic microangiopathy (TMA) is a rare but potentially devastating complication of allogeneic stem cell transplantation (allo-SCT). Its pathophysiology remains poorly understood. Mechanisms associated with TMA include loss of endothelial cell integrity induced by intensive conditioning regimens, immunosuppressive therapy, irradiation, infections or graft-*versus*-host-disease (GVHD). Management and treatment strategies of MAT remain challenging. Strategies as the discontinuation of calcineurin inhibitors or plasma exchange have limited efficacy. Eculizumab could be a promising complement targeting therapy for allo-SCT associated TMA.
Aims: To describe our experience with eculizumab in two cases of TA-TMA.
Methods: Post-allo-SCT TMA was diagnosed according to diagnostic criteria (International Working Group Definition for TMA (2007)). All patients received Tacrolimus (T) and Sirolimus (S) as immunosuppressive therapy. ADAMTS13 and serum C3 complement were analysed in all patients.
Results: 2 patients with TA-MAT are described: Case 1: 53 year old woman diagnosed with refractory Hodgkin Lymphoma who underwent allo-SCT from a sibling donor. TA-TMA was diagnosed on day +34 post-allo-SCT. This patient had a severe kidney injury and neurologic disfunction and the blood tests confirmed an overdose of inmunosupressives. TMA treatment consisted in S and T withdrawal and plasma exchange with no response. ADAMTS13 was normal and serum C3 level was low. Induction therapy with 4 doses of Eculizumab was initiated with resolution of renal function and improvement of hemolysis parameters. Due to the excellent response we did not complete the maintenance treatment. Case 2: 52 year old woman diagnosed with AREB-II who underwent allo-SCT from a sibling donor. TMA was diagnosed on day +26 post-allo-SCT. This patient had a severe kidney injury, alveolar hemorrhage and the blood tests confirmed an overdose of inmunosupressives. ADAMTS13 and C3 were normal. The TMA treatment consisted in S and T withdrawal and plasma exchange with no response. Finally, induction therapy with Eculizumab was initiated. The patient received only 1 dose when normal C3 was confirmed and also because of the good evolution of renal function and hemolytic parameters.
Summary and Conclusions: Allo-SCT associated TMA is an uncommon but devastating complication of HCT. The pathogenesis is unclear. Although T and S have been implicated as risk factors for TA-TMA, there is no solid evidence supporting the discontinuation or dose reduction of these medications. Recently, it has been observed that both the classical and alternative complement pathways may be involved in TA-TMA, supporting the potential use of complement modulating therapies as Eculizumab in patients at highest risk for the worse outcomes. It poses certain challenges such as the reported difficulty of achieving therapeutic levels in critically ill HSCT patients, limited availability in certain countries and significant cost associated with this therapy. We achieved good response with the use of Eculizumab in one patient who had serum C3 low levels. Novel TMA biomarkers, reflecting predisposition for injury to specific organs, need to be identified in order to aid earlier TA-TMA.

PB2050

PULMONARY FUNCTION TESTS (PFTS) PRIOR TO AUTOLOGOUS STEM CELL TRANSPLANT AS PREDICTOR OF PULMONARY COMPLICATIONS AND SURVIVAL IN ADULTS WITH HEMATOLOGICAL MALIGNANCIES

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Background: Autologous Stem Cell Transplantation (ASCT) represents a standard-of-care for Multiple Myeloma patients eligible to receive high-dose chemotherapy, Lymphoma patients undergoing second-line treatments and for a small proportion of Acute Leukemia patients. Although all canditates to an ASCT are carefully evaluated for their eligibility with a complete screening of clinical, laboratory, imaging and functional tests to check comorbidities, global organ function and infections, pulmonary and infective complications are a significant cause of morbidity and mortality after ASCT. However, the relationship between pre-transplant Pulmonary Function Tests (PFTs), development of post-ASCT complications and mortality is unknown.
Aims: The aim of this study was to evaluate the role of pre ASCT PFTs on post-ASCT complications and mortality
Methods: We collected data for 88 patients undergoing ASCT between March 2008 and February 2015 in our Institution. Complete PFTs were obtained in 62 patients (74% males; median age 57 yrs, range, 18-69): Multiple Myeloma n=44, Non-Hodgkin Lymphoma n=18, Hodgkin Lymphoma n=4, Acute Myeloid Leukemia n=1). ASCT was performed as first line treatment in 42 (67%) patients, after first relapse in 17 (28%) and as salvage treatment after ≥2 relapse in 3 (5%). Previous regimens including drugs known to induce pulmonary toxicity, such as bortezomib and bleomycin had been administered to 34/62 (55%) patients
Results: Of the 62 transplanted patients, 9 (13.4%) had abnormal PFTs at baseline (5 obstructive and 4 restrictive PFTs) and 19 (28.4%) had two or more major chronic comorbidities (metabolic and cardiovascular disease). Infective complications occurred in 40/62 (64.5%) and respiratory complications in 9/62 (14.5%) cases. After a median follow-up of 25 months (range, 4-111), 48 out of 62 patients (77,4%) are alive. Post-ASCT respiratory complications were significantly higher (97% vs 83%; P=0.05) in patients with reduced pre-ASCT FEV1.
Summary and Conclusions: To reduce the risk of respiratory complications after ASCT, these patients might benefit from the use of a reduced intensity conditioning.

PB2051

COMPLETE RESOLUTION OF EXTENSIVE CHRONIC GRAFT-VERSUS-HOST DISEASE WITH IBRUTINIB

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Background: Chronic graft *versus* host disease (cGvHD) is mediated donor T cells. The role of B cells in the pathogenesis of cGvHD is increasingly recognized. Two murine studies have explored the role of ibrutinib in cGVHD-like syndromes, one in which there is T cell driven sclerodermatous cGvHD and a second in which there is Ab driven multiorgan system cGvHD that includes bronchiolitis obliterans.
Aims: We herein document a complete response of extensive chronic graft-*versus*-host disease (cGvHD) to a Bruton's tyrosine kinase (BTK) inhibitor, ibrutinib.
Methods: Following approval from Institute Review Board, patient disease and transplant related variables were studies in a single patient who received Ibrutinib for post- allogeneic transplant relapse of mantle cell lymphoma (MCL). NIH criteria for cGvHD diagnosis and staging were followed for disease assessment.
Results: A 41 years old female with primary refractory MCL underwent mismatched unrelated donor allogeneic hematopoietic stem cell transplantation in December 2011 (ablative conditioning with CY/TBI and alemtuzumab, graft=6.6x 106/kg CD-34+ cells, tacrolimus alone for GVHD prevention). Following engraftment on day 11, she developed grade III acute GvHD involving the skin and gut on day 17 of transplantation that persisted beyond 100 days post-transplant. Her cGvHD was treated with steroids, but remained active and extensive. Despite persistent cGvHD and 100% donor chimerism she relapsed in July 2012. Treatment with radiation, bendamustine and rituximab failed. By December 2013, the patient had extensive cGvHD manifesting as scleromatous skin thickening, oral ulcers and sclerosis of the buccal mucosa, ocular dryness and diarrhea, and was started on ibrutinib[1] 560 mg once daily for relapsed MCL. After 8 weeks of therapy, cGvHD had begun to improve. Oral steroids were reduced and ultimately stopped after 26 weeks of ibrutinib; after 30 weeks treatment all cGvHD manifestations resolved completely. A complete remission for MCL was documented at 8 weeks of ibrutinib initiation. Currently she continues to be on 560 mg daily ibrutinib without cGvHD exacerbation or MCL relapse for 22 weeks and 52 weeks, respectively.
Summary and Conclusions: Our report provides the evidence for BTK inhibition led complete cGvHD resolution and supports exploration of its role in future clinical trials.

Stem cell transplantation - Experimental

PB2052

RIC WITH BUSILVEX 4 DAYS OF ONCE-DAILY 100MG/M² WITH FLUDARABINE AND ANTITHYMOCYTE GLOBULINS PRIOR TO ALLOGENEIC STEM CELL TRANSPLANTATION IN PATIENTS WITH HIGH RISK HEMATOLOGICAL MALIGNANCIES.

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Background: The optimal intensity of myeloablation delivered as part of a reduced-intensity/toxicity conditioning (RIC/RTC) regimen to decrease the recurrence rate, without increasing non relapse mortality (NRM), remains to be established and the disease control remains a major challenge. The introduction of RTC regimens has allowed allogeneic hematopoietic cell transplantation to be performed in patients who were previously considered too old or otherwise unfit.
Aims: When busulfan Pharmacokinetic is not available the optimal dose is difficult to determine. In this perspective we made the hypothesis that decreasing the daily dose can be safer and efficient in high risk patients.
Methods: We studied the outcome of 27 patients (median age, 50 years; range, 21-65 years) with hematological malignancies were included. The conditioning regimen based on busulfan at a dose of 100 mg/m^2 /day intravenously for 4 days, fludarabine at a dose of 30 mg/m^2 /day for 5 days, and antithymocyte globulins at a dose of 2.5 mg/kg/day for 2 days. Patient, disease and transplant characteristics are shown in Table 1.

Table 1.

	N=27
Sex ratio M/F	15/12
Median age (years)	50
Age < 60years	25 (93%)
Donor	
Sibling	10(37%)
MUD	15(56%)
MMUD	2(7%)
Graft source	
PBSC	23(85%)
Bone marrow	3(11%)
PBSC+BM	1(4%)
Diagnostic	
AML	13(48%)
ALLB (Phi+/Phi-)	8 (30%) (6/2)
ALLT	2(7%)
CML	1(4%)
MDS	2(7%)

Results: No patients experienced graft rejection. The median HCT comorbidity index score was 2 (range, 0 to 5). With a median follow-up of 13 months (range, 3-16months), the cumulative incidences of grade 2 to 4 acute graft-*versus*-host disease (GVHD) and chronic GVHD (all grades) were 43% (95% CI, 26%>60%) and 44% (95% CI, 20%>68%), respectively. The Kaplan-Meier estimates of overall and disease-free survival at 1 year were 63% (95% confidence interval [95% CI], 42%>84%) and 49% (95% CI, 27%>71%), respectively. At 1 year, the cumulative incidence of recurrence/disease progression was 32% (95% CI, 12%>52%). Non relapse mortality (NRM) was 4% and 19% at day 100 and at 1 year respectively. Patient age, diagnosis, donor type, sex, presence of comorbidities, and the Hematopoietic cell transplantation-specific comorbidities index did not appear to have any statistically significant impact on NRM, recurrence/disease progression, disease-free survival, or overall survival.
Summary and Conclusions: This well-tolerated conditioning platform can lead to long-term disease control The RTC regimen used in the current study appeared to be safe, with a low NRM rate at 1 year noted among high-risk patients, and efficient disease control, warranting prospective phase 3 trials.

Thrombosis and vascular biology

PB2053

VENOUS THROMBOEMBOLISM (VTE) IN CHILDREN WITH CANCER AND HEMATOLOGICAL DISORDERS; 16 YEARS OF EXPERIENCE IN A GREEK CENTER

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Background: Guidelines addressing the management of venous thromboembolism (VTE) in pediatric cancer and hematological patients remain heterogeneous while the identification of independent risk factors for VTE, represents a major challenge that may help in refining prevention and treatment strategies.
Aims: Our aim is to define characteristics, treatment and outcome of pediatric hematology-oncology pts with proven VTE and illustrate possible risk factors and genetic predisposition.
Methods: Data from all pts with benign or malignant hematological disorders and solid tumors consecutively diagnosed and treated at the Department of Pediatric Hematology-Oncology (N=532,1999-2014) have been retrospectively recorded and analysed. Pts with superficial thrombosis were excluded from the study.
Results: In our cohort, a total of 32 pts (median age 5.7 yrs, range 1.1-18.6) developed VTE with the following disease background: acute lymphoblastic leukemia(14), T-lymphoblastic lymphoma(2), RAEB-T/acute myeloid leukemia(1), nephroblastoma(3), neuroblastoma(3), hemophagocytic lymphohistiocytosis(1), autoimmune hemolytic anemia(2) and other rare solid tumors(6). In our cohort, 21 pts had high risk disease features and 6 pts presented with bulky mediastinum involvement. Median time of VTE occurrence from disease diagnosis was 129 days (range 0-4244). 25/32 pts developed DVT during the intensive chemotherapy regimen (16 not in CR). Most commonly used drugs within the last 30 days from DVT diagnosis were Asparaginase and steroids, followed by VCR, MTX, CPM and cisplatin. 13/32 pts had undergone major surgery and no pt received hormone therapy. In 26/32 pts CVL was placed; median time between CVL placement and VTE occurrence was 60 days (range 0-1352). VTE diagnosis was symptom-driven (n=16) or incidental finding (n=16); in 15 pts VTE was CVL-related (4 with infection) and 6 cases presented in tumor adjacent areas. The most common VTE sites were right atrial and jugular veins (16), subclavian veins and superior vena (2), kidney and inferior vena cava (6), pulmonary embolism (2), femoral and iliac vein (3), cerebral sinovenous thrombosis (5) [ischemic cerebral infracts, cavernous sinus (after XRT), upper sagittal and transverse sinus], with some patients presenting with multiple VTE sites. Additionally, 2 pts presented with VOD, as well. WBC or PLTs count at diagnosis did not have a statistically significant impact on the probability of VTE. Thrombophilia evaluation that was performed in 16/32 pts did not reveal an increased incidence of predisposing factors [factor G20210A variant homozygous or heterozygous (1/1), heterozygous MTHFR variant (8), elevated FVIII (7)]. Treatment consisted mainly of sc LMWHs as anticoagulation therapy in 28/32 pts. Median time of treatment duration was 6 months (range 3-12) and was guided by the continuing presence of CVL, CR status and until establishing vascular patency and stable imaging findings. Within a median follow-up time of 33.8 months (range 2.1-195.1) no VTE recurrence occurred while overall survival was 78.12% with morbidity not related to VTE.
Summary and Conclusions: In our study, VTE was not very common among our pediatric haematology-oncology pts and continuation of chemotherapy was feasible in all cases, with no VTE recurrence. Furthermore, thrombophilia evaluation neither revealed an increased incidence of predisposing factors nor altered our therapeutic decisions; thus, extensive baseline screening or DVT prophylaxis are not supported in pediatric pts who can be successfully diagnosed and treated with current recommendations.

PB2054

ARTERIAL ISCHEMIC DISEASE AND CONGENITAL THROMBOPHILIA TESTING IN YOUNG PATIENTS: DO OR NOT DO?

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Background: Cardiovascular disease (CVD) includes coronary, renal, cerebral and peripheral artery atherosclerosis and ischemia. It may be caused by several cardiovascular risk factors (CVRF), the most are acquired and modifiable like high blood pressure, smoking, diabetes, obesity or high blood cholesterol, and others unchangeable factors like age or family history of premature illness. Many researchs which have investigated association between congenital thrombophilia (CT) and CVD have obtained positive and negative results. In spite of the doubtful utility of CT analysis in these cases, it is not infrequent its request for young patients with CVD.
Aims: Our aim in this research is to assess CT prevalence in young patients (less or equal than 55 years old) with CVD and comparing to observed in patients with venous thrombotic disease with same age and that has been described in general population.
Methods: Sequential and retrospective analytical study of 149 patients (68 men and 81 women) ≤55 years old with arterial isqhemic event in different vascular areas whom were made CT analysis that included: Antithrombin (AT) and functional chromogenic Protein C (PrC), Functional coagulative Protein S (PrS) and immunological PrS; resistance to Activated Protein C and molecular analysis of factor V Leiden (FVL) and Prothrombin G20210A (FIIG20210A) by PCR. Data were taken from database of our Unit.
Results: Mean age at presentation was 42.9 years (18-55). Ischemic location was cerebral in 122 cases, 16 cardiac and 11 peripheral cases. Twelve patients (8%) presented postivie CT: six of them were heterozygous FIIG20210A mutation (4.02%), four heterozygous FVL mutation (2.6%) and one case was positive for type I PrS deficiency (0.67%); a patient presented double thrombophilia (FIIG20210a and PrS deficiency). Eight of twelve patients with TC (66,6%) presented some CVRF and the patient with double thrombophilia was taking oral contraceptives and she was obese, smoker, diabetic and hypertensive. Comparatively, CT prevalence in other previous research of ours about 253 young patients with venous thrombotic disease was 26.8%.
Summary and Conclusions: Congenital thrombophilia prevalence in young patients with CVD is not higher than in general population. Congenital thrombophilia positive result did not trigger any change in therapeutic attitude and it is the reason why its routing testing in arterial thrombosis cases presents little or no use.

PB2055

EFFICACY OF TREATMENT WITH ASPIRIN IN THE PREVENTION OF RECURRENT THROMBOTIC EVENTS

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Background: The risk of recurrent venous thromboembolism persists for several years after the interruption of anticoagulant treatment. The role of aspirin in the primary prevention of venous thromboembolism has been evaluated by different groups. In these studies, treatment with low-dose aspirin (100 mg / day) was associated with a reduced risk from 20-50%. The benefit of antiplatelet therapy for secondary prevention of VTE was evidenced by the results of WARFASA study.
Aims: To evaluate the efficacy of aspirin for the prevention of recurrent venous thromboembolism after treatment with vitamin K antagonists in an unselected group of patients with VTE.
Methods: 105 patients with VTE referred to the Hematology consultation during last year were included, the mean age of the group was 52.83±18.40 (63.8% were women, n=67). From each patient cardiovascular risk factors and the location of thrombosis were collected. Aspirin administration was decided depending on the presence of cardiovascular risk factors. Aspirin was prescribed in patients with 2 or more risk factors.

Figure 1: Distribution of patients depending on the location of thrombosis.

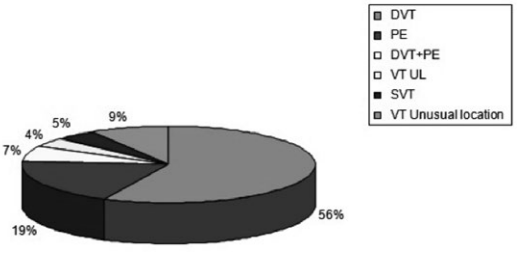

Figure 1.

Results: The characteristics of the population and the result of the comparison of both groups are shown in Table 1. The mean follow-up period after discontinuation of OAT was 35.96±19.49 months, after which 15 cases of recurrent thrombosis (14.3%) and 31 cases of post-thrombotic syndrome (29.5%) were diagnosed. Figure 1 shows the distribution of patients according to the location of the thrombosis. The mean duration of OAT was 12.63±9.79. 87.6% of patients (n=92) were treated with acenocoumarol and 12.4% were treated with LMWH (n=13). Finally, 49 patients were treated with low dose aspirin (46,7%). In this group 20.4% (n=10) patients had recurrent VTE, compared to the patients not prescribed with aspirine 8,9% (n=5) (P=ns). The number of PTS diagnosed was similar in both groups (26.8% (n=15) vs 32.7% (n=16); P=0.6577). VTE Aspirin No aspirin p Age 52,83±18,40 55,45 ±16,06

50,54±20,10 0,1735 Dyslipemia 32,7 (33) 41,7 (20) 24,5 (13) 0,1049 Smoke 24,8 (26) 18,4 (9) 30,4 (17) 0,2327 Obesity 15,2 (16) 16,3 (8) 14,3 (8) 0,9855 Hypertension 26,7 (28) 30,6(15) 23,2 (13) 0,5261 DM 3,8 (4) 6,1 (3) 1,8 (1) 0,3370 CVD 5,0 (5) 6,1 (3) 3,6 (2) 0,0491.

Summary and Conclusions: The results we obtained are consistent with those published in the WARFASA study, although we found no statistically significant difference in the recurrence rate. It would be interesting to extend this study including a larger population and review the selection criteria to choose patients who may benefit with aspirin treatment; and also a prospective analysis of the evolution of the patients treated with low-dose aspirin.

PB2056

RESISTANCE TO ACTIVATED PROTEIN C AS PATHOGENIC MECHANISM UNDERLYING THROMBOSIS ASSOCIATED WITH LUPUS ANTICOAGULANTS

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Background: Resistance to activated protein C (APCR) is associated with high risk of venous thromboembolism (VTE). After the factor V Leiden (FVL) mutation was found as the main cause of APCR little attention is paid to the role of acquired APCR. An acquired resistance to activated protein C has been previously shown in patients with antiphospholipid antibodies. The Calibrated Automated Thrombography (CAT) is now considered to reflect patients phenotype better than traditional coagulation tests. When thrombomodulin (TM) is used thrombin generation (TG) becomes sensitive to all disorders of the PC system.
Aims: Aim of our study was to evaluate the prevalence of acquired APCR and its reasons in patients with VTE manifestation under the age of 45 years.
Methods: The study involved 35 patients (M/F 15/20, mean age 37,0±9,0 yr) with early manifestation of VTE and 30 controls. All patients received vitamin K antagonists (VKA) treatment at least for 6 months, 18 of them were still on VKA. TG was measured according to Hemker *et al*. with fluorogenic assay, at 5 pM TF and 4 µM phospholipids in platelet poor plasma (PPP) with PPP plasma+/-TM reagent (Thrombinoscope BV, Maastricht, The Netherlands).
Results: Endogenous thrombin potential (ETP) and peak thrombin (PT) were markedly reduced in the presence of TM. None of the patients had increased ETP measured as values above 95th percentile in controls (*i.e.* >2114 nMmin in the absence of TM and >1433 nMmin in the presence of TM). Lupus anticoagulants (LA) were found in 5 patients. From parameters derived from the thrombin generation curves measured both with and without TM significant correlation with LA (P<0,05) was found for lag-time (R=0,45), ETP (R=-0,45) and PT (R=-0,44) both in presence and absence of TM. Normal ranges of ETP and PT inhibition calculated in controls were 21-62% and 14-51% respectively. Values below 21% for ↓ETP and/or 14% for ↓PT (*i.e*. APRC) were found in 20 (57%) of samples. In the studied group 100% of patients with FVL demonstrated APCR phenotype. So, in 50% of cases APCR was due to FVL mutation (10 patients, among them 2 patients with acquired protein C deficiency on VKA treatment). As for the rest 50% of detected APCR cases 25% (5 patients) were associated with LA, 10% (2 patients) with isolated increased FVIII activity, 10% (2 patients) with prothrombin G20210A mutation and increased FVIII activity. The reasons of APCR in the rest 5% of cases need further elucidation. APCR was found in 100% of LA samples. Importantly all these patients had protein C activity within the normal range. Abnormal PT inhibition seemed the most sensitive to detect APCR. Abnormal PT inhibition was found in all patients, contrary to ETP inhibition-50% cases with FVL and 75% with LA.
Summary and Conclusions: In conclusion LA was the most prevalent cause of acquired APCR in our patients. Beta2 glycoprotein I, anti-prothrombin antibodies and lupus anticoagulants previously demonstrated association with APCR, determined by classic activated partial thromboplastin time-based tests. We used a novel integrated approach with calibrated automated thrombography made in parallel with and without TM. This modification of the thrombin generation test allows effectively detecting APCR and LA even in patients on vitamin K antagonists treatment, that is very important for clinical practice. APCR in patients with lupus anticoagulants is strongly associated with thrombotic complications; it was demonstrated by CAT in all patients with VTE manifestation under the age of 45 years and LA laboratory phenotype. Acquired APCR could be the main pathogenic mechanism underlying thrombosis associated with LA.

PB2057

RELATIONSHIP BETWEEN ANTIPHOSPHOLIPID SYNDROME CLINICAL MANIFESTATIONS AND MULTIPLE POSITIVE ANTIPOSPHOLIPID ANTIBODIES

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Background: The Antiphospholipid syndrome (APS) is characterized by the development of thrombosis and/or pregnancy complications in addition to the persistent presence of antiphospholipid antibodies (APLS): Lupus Anticoagulant, Anticardiolipin antibodies, Anti-beta 2 GP1 antibodies. At this moment, is not well known that risk factors could be associated to any clinical manifestations of APS.
Aims: We investigated if there are relationship between the different clinical manifestations of APS and the presence of cardiovascular risk factors, hereditary and acquired thrombophilia or multiple positive APLS.
Methods: Retrospective we investigated the relationship between clinical manifestations and the presence of cardiovascular risk factors, hereditary and acquired thrombophilia or the existence of multiple positive APLS in 65 patients consecutive diagnosed of APS in our hospital from 1 February 2000 to 31 January of 2015. The patients ages were between 21 to 88, gender 53.8% male and 46.2% females. 43 patients present cardiovascular risk factors (26 smokers 24 hypertension, 22 dyslipidemia, 3 Diabetes Mellitus), 21 patients with thrombophilia (8 hyperhomocysteinemie, 2 antithrombine deficiency, 3 protein S deficiency, 1 protein C deficiency, 4 FV Leiden mutations and 3 prothrombin G20210A mutation), 17 patients with triple APLS (TP) and 48 with one/double APLS (ODP). 37 patients presented venous thrombosis (22 deep vein thrombosis, 6 pulmonay embolism, 9 both), 15 patients with arterial thrombosis manifestations (2 arterial thrombosis, 3 myocardial infarction and arterial thrombosis, 10 cerebrovascular accident), 4 with pregnancy complications, and 9 with combined complications (arterial/venous thrombosis/pregnancy complications). The statistical analysis was performed with Chi2 test realized by JMP 9, being statistically significant P<0.05.
Results: We found statistically significant difference between the presence of TP and the development of arterial thrombosis (OR 0.16 y IC 95% 0.03 – 0.76 P=0.01).
Summary and Conclusions: Our data show an association between TP and development of arterial thrombosis in APS patients. We did not found association between one or multiple positivity on APLS and the other clinical manifestations of APS, and the presence or not of cardiovascular risk factor or thrombophilia with clinical manifestations of APS.

PB2058

THE INFLUENCE OF CYP2C9 AND VKORC1 GENE POLYMORPHISMS ON THE RESPONSE TO WARFARIN IN EGYPTIANS

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Background: warfarin is the most commonly used drug for chronic prevention of thromboembolic events and stroke, it also ranks high among drugs that cause serious adverse events. The narrow therapeutic index coupled with substantial inter-individual variability in warfarin dose requirement puts patients at increased risk for hemorrhagic events, and the variability in dose requirements has been attributed to inter-individual differences in medical, personal, and genetic factor. Cytochrome P-450 2C9 is the principle enzyme that terminates the anticoagulant effect of warfarin by catalyzing the conversion of the pharmacologically more potent S-enantiomer to its inactive metabolites. Warfarin exerts its anticoagulant effect by reducing the regeneration of vitamin K from vitamin K epoxide in the vitamin K cycle, through inhibition of vitamin K epoxide reductase. This protein is encoded by the recently identified vitamin K epoxide reductase complex subunit 1 gene (*VKORC1*).
Aims: The current study aimed to investigate the pharmacogenetic effect of CYP2C9 and VKORC1 gene polymorphisms on the patients response to warfarin.
Methods: This study was carried out on 100 cases starting warfarin treatment. Informed consent was obtained from each subject or patient prior to participating in this work. The mean daily dose of warfarin (mg) was calculated from patient's medical records. For each patient,<10% variability in warfarin dose and a target international normalized ratio (INR) within the therapeutic target range (2–3) and for patients having valve replacement and recurrent pulmonary embolism target was (2.5-3.5), were required for at least 3 months for one of the following indications (Deep vein thrombosis, Pulmonary embolism, cerberovascular stroke and Myocardial infarction) prior to inclusion in the study. Molecular genetic study was performed to determine *CYP2C9**2, *CYP2C9* *3, and the *VKORC1* 1639G >A genetic polymorphisms. The genotypes were determined with the tetraprimer amplification refractory mutation system (T-ARMS) using specific outer and inner primers. Plasma warfarin determination was performed using rapid flouremetric assay.
Results: The median age of the studied cases as shown in table 1 was 49 years (range, 33–68).The males represented 56% of the cases while the females represented 44%. The body mass index of the patients ranged from 19.81 to 42.44. The plasma warfarin concentration ranged from 2.19-10.98 µg/mL with a median 3.52 µg/mL. The patients were stratified into three different groups based on their maintenance dose (>2.5 mg is the low dose group, ≤2.5 mg to 7 mg is the standard dose group and >7 is the high dose group). The INR ranged from 2 to 4 wth a Mean of 2.58±0.62(SD).The incidence of supratherpeutic INR was presented in 11% of the cases. Thromboembolic complications occurred to 7% of the cases. While 8% of the cases experienced

major bleeding. The majority of the patients receiving high maintenance dose (>7 mg/day) had combined *non VKORC1*2 and* homozygous wild-type *CYP2C9* (*CYP2C9*1*1*) alleles, representing 52.2% of the high dose group while the majority of the patients recieving low maintenance dose are carriers of the Variant (AG + AA)/ Wild (*1/*1) representing 55.6% of the low dose group.These results were statistically significant with p value<0.001. With respect to Incidence of supratherapeutic INR, a significantly increased risk occurred in patients carrying the CYP2C9 variant in the multivariate logistic regression model. Also with respect to thromboembolic complications and incidence of supratherapeutic INR, higher risk was observed in patients carrying combined VKORC1 Variant (AG + AA) and CYP2C9 Variant (*2/*3).
Summary and Conclusions: Data from our study suggest that together with clinical factors, VKORC1 and CYP2C9 polymorphisms are important contributors to warfarin dosing and may help predict adverse effects in Egyptian patients.

PB2059

ASSOCIATION OF FACTOR V LEIDEN G1691A AND PROTHROMBIN GENE G20210A MUTATION WITH ADVERSE PREGNANCY OUTCOMES
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Background: Familial defects and polymorphisms of clotting cascade proteins protein S, protein C, factor V Leiden G1691A and factor II G20210A are linked with increased risk of thromboembolism which is better known as *inherited thrombophilia*. Thrombophilia causes deep venous thrombosis, pulmonary embolism and is strongly associated with poor pregnancy outcomes. Pathophysiology of these outcomes is thought to involve thrombosis in uteroplacental blood flow hence, anticoagulation therapy can potentially improve obstetric outcome in females with thrombophilias. To date, there is limited data from this region on the role of these genetic abnormalities causing adverse pregnancy outcomes.
Aims: Determine the association of factor V Leiden G1691A and prothrombin gene G20210A mutation with adverse pregnancy outcomes.
Methods: It was a case control study, conducted at clinical laboratory, section of haematology, and PCR-RFLP technique is used at multi-disciplinary laboratory, Aga Khan University Hospital. Females with adverse pregnancy outcomes who came to obstetrical clinic were included in the study as cases. Adverse pregnancy outcomes included recurrent pregnancy loss (defined as >2 first trimester miscarriages or one or more second trimester miscarriage), severe pre-eclampsia, placental abruption, intrauterine growth restriction and still birth. Control samples are selected from females with ≥ 2 consecutive normal pregnancies. Calculated sample size is 172 which comprise of 86 cases and 86 controls.
Results: Overall mean age of all subjects was 28.5 years (±4.9). Mean age of cases was 29.3 (±5.17) years and of controls was 27.6 years (±4.5). 73 (84.8%) cases had recurrent pregnancy loss, 12 (13.9%) had pre-eclampsia, 8 (9.3%) had IUGR while placental abruption and still birth was present in 2 (2.3%) cases each. 10 (11.6%) cases had more than one adverse pregnancy outcomes. 19 (22.09%) cases had >4 pregnancy losses. Among cases, 40 (46.5%) females had previous live births and 9 (10.4%) were pregnant at the time of sample collection. Two cases with recurrent pregnancy loss (P=0.155 OR=0.49) showed heterozygous mutation of factor V Leiden G1691A and while no mutation identified in the control arm. Heterozygous prothrombin gene mutation was identified in one case with recurrent pregnancy loss (P= 0.316 OR=0.497) while none of the control exhibited this mutation.
Summary and Conclusions: This is a small sample sized study which does not support a significant association between inherited thrombophilia mutations and adverse pregnancy outcomes. The apparent lack of association may be reconciled by the low numbers of subjects recruited.

PB2060

LONG-TERM ANTICOAGULATION WITH DABIGATRAN IN PATIENTS WITH SEVERE LACTOSE INTOLERANCE
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Background: Chronic anticoagulation is realized mainly with vitamin K antagonists (VKA), but the use of VKA is problematic in patients with severe lactose intolerance. Dabigatran is currently the only available lactose-free oral anticoagulant, however its use in patients with lactose intolerance has not been described.
Aims: Evaluate long-term anticoagulation with dabigatran in patients suffering lactose intolerance.
Methods: We present two patients with severe lactose intolerance on long-term anticoagulation with VKA, that were transitioned from VKA to dabigatran because the treatment with VKA worsened symptoms of their lactose intolerance. Case 1. A 75-year-old female was diagnosed with nonvalvular atrial fibrillation. Long-term anticoagulation with VKA was started for stroke prevention. Two weeks later, the patient was admitted by recurrence of acute diarrhea in the setting of her lactose intolerance, well controlled so far. VKA was replaced by dabigatran and fifteen months later, she remains asymptomatic. Case 2. A 40-year-old female was diagnosed with antiphospholipid syndrome (APS) when she experienced a non provocated acute left upper extremity venous thrombosis. She was started on anticoagulation with VKA with an INR target of 2.5 and had no recurrence of thrombosis. However, VKA therapy was complicated by frequent episodes of diarrhea that the patient suffered from several years. She was evaluated by a gastroenterologist and was diagnosed with lactose intolerance. Due to progressive worsening gastrointestinal clinical despite a lactose free diet, three years later she was transitioned from VKA to dabigatran. One year later, she is asymptomatic and has not suffered thromboembolic recurrence. We present two patients with severe lactose intolerance on long-term anticoagulation with VKA, that were transitioned from VKA to dabigatran because the treatment with VKA worsened symptoms of their lactose intolerance. Case 1. A 75-year-old female was diagnosed with nonvalvular atrial fibrillation. Long-term anticoagulation with VKA was started for stroke prevention. Two weeks later, the patient was admitted by recurrence of acute diarrhea in the setting of her lactose intolerance, well controlled so far. VKA was replaced by dabigatran and fifteen months later, she remains asymptomatic. Case 2. A 40-year-old female was diagnosed with antiphospholipid syndrome (APS) when she experienced an unprovoked acute left upper extremity venous thrombosis. She was started on anticoagulation with VKA with an INR target of 2.5 and had no recurrence of thrombosis. However, VKA therapy was complicated by frequent episodes of diarrhea that the patient suffered from several years earlier. She was evaluated by a gastroenterologist and was diagnosed with lactose intolerance. Due to progressive worsening gastrointestinal clinical despite a lactose free diet, three years later she was transitioned from VKA to dabigatran. One year later, she is asymptomatic and has not suffered thromboembolic recurrence.
Results: Our patients with lactose intolerance on long-term anticoagulation with dabigatran have not experienced gastrointestinal symptoms or thromboembolic complications months after transitioning to dabigatran.
Summary and Conclusions: Switching from VKA to dabigatran for patients with lactose intolerance seemed clinically reasonable, but until results of prospective randomised trials are available, we recommend caution in using dabigatran in patients with APS.

PB2061

THE PENETRANT FEATURE OF VENOUS THROMBOEMBOLISM IN FAMILIES WITH INHERITED THROMBOPHILIA FROM NATURAL ANTICOAGULANT DEFICIENCY IN KOREA
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Background: Inherited thrombophilia is a genetic predisposition to develop venous thromboembolism (VTE) from either deficiency of natural anticoagulants (NA) or increased procoagulant activity. Deficiencies of NA such as protein C (PC), protein S (PS) and antithrombin (AT) are relatively common in Asian populations. It has been acknowledged that the development of VTE is affected by various factors other than genetic predisposition such as environmental influences and other modifiers.
Aims: The purpose of this study was to investigate the penetrance of VTE in families with inherited thrombophilia from NA deficiency in Korea.
Methods: The authors reviewed the family history and family study results in a cohort of consecutive patients with VTE from genetically confirmed NA deficiency between January 2005 and December 2014 at a single tertiary institution in Seoul, Korea.
Results: A total of 87 probands were VTE patients with PC (N=44), PS (N=21), or AT (N=22) deficiency confirmed by molecular genetic tests (mean age: 40 years; 62 men and 25 women). The family history of the parents was available in 78 probands, and a positive VTE history was ascertained in 7 (9%). Family study was performed in both or either one of the parents in 10 probands, and 11 parents of either side (mean age: 55 years; 4 fathers and 7 mothers) were confirmed to have the same mutation as in the probands. Among them, 2 had a history of VTE (18%).
Summary and Conclusions: To our knowledge, this is the first study to investigate the family history of VTE in a large cohort of probands with genetically confirmed NA deficiency. The results demonstrated a low penetrance of VTE in the parents of the probands, even in genetically confirmed cases. The data suggested that the environmental factors (possibly generation-dependent) have implications for the index of suspicion in inherited thrombophilia and genetic counseling in VTE patients. A further study involving siblings of the probands is believed to better delineate the penetrance feature.

PB2062

A LOCAL PROCESS AND MANAGEMENT FOR HOSPITAL ASSOCIATED VENOUS THROMBOEMBOLISM EVENTS
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Background: In 2005 a House of Commons Health Committee in England reported an estimated 25000 avoidable deaths occur annually in the UK from hospital associated venous thromboembolism (VTE)[1]. There is ongoing work in England to develop up-to-date and accurate statistics about incidence and death from hospital associated VTE although this is difficult due to often clinically silent nature of VTE and a decrease in post-mortems in recent years. Root cause analysis (RCA) is required for all confirmed cases of pulmonary embolism and deep vein thrombosis associated by patients in hospital, with an aim to reduce avoidable death, disability and chronic ill heath from VTE[2].
Aims: To implement a process for identification and management of hospital associated VTE events (HATs). To determine incidence of HATs and potentially preventable HATs. To identify contributory factors for potentially preventable HATs
Methods: Support from senior Executives were essential in prioritising VTE as a patient safety quality improvement objective. RCA tools were developed to explore the *how*, the *what*, and most importantly the *why* of HAT. RCA will identify contributory factors and inform an action plan developing solutions with monitoring to prevent reoccurrence. The VTE RCA team is multidisciplinary with Consultant Haematologist, Anticoagulation Pharmacist, lead clinician, clinical governance and radiology departments. Weekly imaging reports are sent by the radiology team. Reports are screened to identify new VTE diagnosis. Patient records are reviewed to establish whether the VTE event is hospital associated. Lead clinicians are requested to complete the RCA tool to determine whether the HAT was potentially preventable. An action plan is implemented to address contributory factors.
Results: From April 2014-January 2015, 6 potentially preventable HATs identified. Interventions to address contributory factors for HATs were: A 'Preventing Harm' group was introduced to investigate omitted doses of thromboprophylaxis, without clinical omission. An electronic report was created for 'prescribed but not given' medications, by ward. Agency nursing staff were given access to the electronic prescribing system to allow for documentation of medications given. VTE ward rounds introduced on medical, surgical and obstetric wards to review VTE risk assessment completion and accuracy, and whether thromboprophylaxis is appropriate. A summary report of findings/learning points is circulated to division. Risk assessment and guidance for patients with lower limb immobilisation was updated with appropriate management. Patient agreement to investigation or treatment consent form updated to include VTE as a significant, unavoidable or frequently occurring risk related to surgical procedures. WHO checklist updated to define if thromboprophylaxis is required and if prescribed. Educational support for accurate VTE risk assessments at booking appointments for antenatal women, to identify those at risk requiring antenatal thromboprophylaxis. Audits performed to assess whether patients receive verbal/written information on VTE prevention.
Summary and Conclusions: The VTE team implemented a robust and sustainable system for identifying and analysing HATs with feedback to departments. Successful interventions are embedded into clinical practice to reduce HATs. *Key messages:* Continuous VTE awareness, education and stewardship, Robust action plans to drive and excel performance, Real-time reporting, Maintain VTE momentum – cause some noise about VTE prevention.

PB2063

INHERITED AND AQUIRED THROMBOPHILLIAS IN WOMEN WITH RECCURENT PREGNANCY LOSS- OUR EXPERIENCE

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Background: Inherited thrombophilias are the leading cause of maternal thromboembolism and are associated with an increased risk of certain adversy pregnancy outcomes including second and third trimester fetall loss, abruptions, severe intrauterine growth restriction and early onset severe preeclampsia. The antiphospholipid antibody syndrome (APS) is an acquired autoimmune thrombophyllia in which vascular thrombosis and/or reccurent pregnancy losses occur in patients having laboratory evidence for antibodies againts phospholipids or phospholipid-binding protein cofactors in their blood. Pregnant women with these highly thrombogenic conditions are at very high risk for both thromboembolism and adverse pregnancy outcomes.
Aims: Aim was to determine the presense of inherited or acquired thrombophillias in women with reccurent pregnancy losses (RPL).
Methods: Women with RPL were tested for heterozygosity for the factor V Leiden and prothrombin G 20210A mutations, homo and heterozygosity in the type 1 plasminogen activator inhibitor gene (PAI-1) and the thermolabile variant of the methylentetrahydrofolate reductase gene (MTHFR). They were also tested for deficiencies of protein C, protein S and antithrombin, as well as for antiphospholipid antibodies-anti beta 2 glycoprotein I antibodies, anticardiolipin antibodies and lupus anticoagulant assays.
Results: From January 2011 till January 2015 260 women were tested for the presense of inherited or acquired thrombophillias because of reccurent pregnancy loss or treatment of infertility. Median age of patients was 33.4 (19-45). Six women (2.3%) was negative for both inherited or APS and others (97.7%) were positive. Criteria for APS fullfilled 57 patinets (21.9%), 8 patients (3%) had only APS and 49 patients (97%) had APS with some of inherited thrombophillias. 246 patients (94.6%) were positive for one or more inherited thrombophillias. 99 patients (38.1%) were positive for one inherited thrombophillia: 4 (1.5%) for Factor V Leiden, 2 (0.7%) for prothrombin G 20210A, 47 (18%) for MTHFR and 46 (17.7%) for PAI-1. 133 patients (41.1%) were positive for 2, 13 patients (5%) for 3 inherited thrombophillias. 105 patients (40.3%) with low molecular weight heparin plus aspirin (LMWH/ASA) or ASA alone had successfully pregnancy outcome-live birth.
Summary and Conclusions: Some form of thrombophillia-inherited or acquired was found in most of tested women with reccurent pregnancy loss (97.6%) and 163 patients (62.7%) had more then one thrombophillia. With adequate anticoagulant therapy patients with these conditions had chance for successfully pregnancy outcome.

PB2064

INHERITED PROTHROMBOTIC RISK FACTORS IN CHILDREN WITH INTRACRANIAL VENOUS THROMBOSIS: SINGLE CENTER EXPERIENCE IN TURKEY

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Background: Intracranial venous thrombosis (IVT) is a rare condition in childhood, with a wide variety of clinical features and etiologies.
Aims: We aimed to describe the inherited prothrombotic risk factors in children with IVT, confirmed by neuroimaging.
Methods: The retrospective study involved reviewing the records of patients who were admitted to our hospital during the years of 2010-2014. The patients diagnosed as having IVT, confirmed by neuroimaging were investigated for the common thrombophilia markers, such as protein C (PC), protein S (PS), antithrombin III (AT III), factor V G1691A and prothrombin 620210A mutations, methylenetetrahyrafolate reductase (MTHRF) C677T, and MTHFR A1298C genotypes.
Results: The clinical manifestations in 18 patients with IVT included headache, seizures, cranial nerve palsy, and hemiparesis. Transfer sinus thrombosis was the commonest site (50%) followed by diffuse sinus thrombosis (31.25%). Two patients had PC deficiency. Furthermore, one of them had homozygous MTHFR C677T genotype. No patient had PS and ATIII deficiencies, and prothrombin 620210A mutations. Eleven patients showed the MTHFR genotype (homozygous C677T, n=4, heterozygous C677T, n=5, heterozygous A1298 C, n=2); and 1 patient was carrier of heterozygous factor V mutation.
Summary and Conclusions: Early diagnosis by fast and safe radiological methods (neuroimaging of the brain), investigation of thrombophilia markers and the appropriate anti-clotting therapy in acute phase may prevent death due to IVT at the pediatric age.

PB2065

PAIN AS UNIQUE MANIFESTATION OF HIGH TITER ANTI-PF4/HEPARIN ANTIBODIES.

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Background: A 68 years old male patient was addressed for hematological care in the context of polycythemia vera, a recent proximal deep vein thrombosis, and a newly diagnosed rectal tumor requiring resection. The patient had been known for polycythemia vera for 10 years, was treated by hydroxycarbamid and aspirin, and presented stable blood counts. A recently diagnosed left femoro-popliteal thrombosis had been treated by dalteparin. One week after the initiation of anticoagulation he signaled the apparition of extreme, burning pain in the distal region of the thrombosed leg and in the right hand, always starting 1 hour after each injection of dalteparin. The pain persisted for a couple of hours and was not responsive to anti-inflammatory and analgesic treatment. The switch of the low molecular weight heparin (LMWH) to nadroparin did not affect the pain. Clinical examination showed discrete lower limb edema predominantly in the affected side, without sign of ischemia. Imaging did not show an extension of the thrombosis. Discrete thrombocytopenia (137 G/l) was the only anomaly of blood count. Coagulation tests were normal.
Aims: To describe laboratory findings and clinical course of this unsusual case.
Methods: Because of the consistent temporal relation of the pain to the administration of LMWH and the slight degree of thrombocytopenia, anti-PF4/heparin antibodies were assessed. The IgG-monospecific ELISA was highly positive (OD 1.86; cut-off : 0.32). The HIPA test performed in a reference laboratory was negative even if the local ELISA was also highly positive.
Results: Nadroparin was immediately changed to fondaparinux, which allowed prompt and complete resolution of the pain. After one month of fondaparinux treatment a Doppler control of the thrombosis showed thrombus persistence without complete canalization. Blood counts showed discrete anemia and thrombocytosis, and the anti-PF4/heparin antibody level decreased to OD 1.45.

The rectal surgery was performed with no complication 36 hours after the last dose of fondaparinux and anticoagulation was resumed in the post-operative phase with argatroban followed by fondaparinux 3 days later. At last follow-up, 1 month after surgery the patient was still on fondaparinux, free of pain and the anti-PF4/antibody level had further decreased (OD 0.98).
Summary and Conclusions: Because of the temporal relation of the patient's symptoms with the administration of LMWH and the steady decline of anti-PF4/heparin antibodies after switch to fondaparinux, we consider this the first case report of high titer anti-PF4/heparin antibodies uniquely presenting with post-injection pain. We propose that clinicians should be aware that the presence of high titer anti-PF4/heparin antibodies might be related to otherwise unexplained pain after injection of low-molecular weight heparins. In our case, switch to a non-heparin based anticoagulation lead to an immediate disappearance of the post-injection pain.

Transfusion medicine

PB2066

PREVENTING UNNECESSARY TRANSFUSIONS DURING ELECTIVE PROCEDURES

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Background: In 2013 the UK chief medical officers iniative Patient Blood Management superseded Better Blood Transfusion. Patient Blood Management is a multidisciplinary, evidence-based approach to optimising the care of patients who might need blood transfusion. Patient Blood Management puts the patient at the heart of decisions made about blood transfusion to ensure they receive the best treatment and avoidable, inappropriate use of blood and blood components is reduced. Reduced blood usage will reduce demand, therefore less pressure on Northern Ireland Blood Transfusion Service. In turn will mean less shortages and more blood available for emergencies. If a transfusion is avoided then there is no risk of a transfusion reaction. Safest blood transfusion is the one not given.
Aims: Can blood transfusions be prevented during elective procedures?
Methods: A surgical list of all elective hip replacements was obtained between 01/09/2013 and 31/12/2013. Preoperative assessment including baseline haemoglobin was recorded. Post operative complications including haemoglobin drop and blood transfusions were looked at.
Results: 68 patients underwent elective hip replacements during this time. 5 patients underwent post operative blood transfusions 12/68 had pre operative haemoglobins<120g/l 56/68 had pre operative hb >120g/l 0% of patients with a pre operative hb >120g/l required a blood transfusion. 5/12 patients with a pre operative hb<120g/l required at least 1 unit of packed red cells post operatively.
Summary and Conclusions: All patients with a hb<120g/l at present are not being added to the elective hip replacement list until their anaemia is investigated and managed appropriately. General Practitioners received a letter indicating that the pre operative assessment has detected anaemia, also enclosed is guidelines regarding the investigation of anaemia. Once the patients anaemia has been treated or hb optimised the patient is returned to the waiting list.
The safest blood transfusion is the one not received.

PB2067

PROGRESSIVE DECLINE OF HBSAG SEROPREVALANCE IN BLOOD DONORS IN NORTHERN TURKEY: THE TWENTY ONE-YEAR EXPERIENCE

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Background: The high prevalence of HBV, HCV, HIV-1/2 and treponema pallidum carriers among blood donors has the unpleasant results for cost- effective, transfusion security and contagious disease.
Aims: The aim of this study was to determine the seroprevalence of HBV, HCV, HIV-1/2 and treponema pallidum in blood donors who applied to our Blood Bank in an twenty one –year period retrospectively.
Methods: The single donations and first time donation of repeat donors entered statistical analysis. HBsAg, anti-HCV treponema pallidum and anti-HIV-1/2 screening tests were performed using ELISA kits.
Results: Between 1994 and 2015, total 267,242 blood donation records at the Blood Banks of University were retrieved. Of them 206,027 were first time donors. HBV, HCV, HIV-1/2 and treponema pallidum seroprevalences among these first time donors were analyzed. The mean seroprevalence of HBsAg was 3.6% between 1994 and 2000 years. The rate of HBsAg positivity was unchanged in these seven years. But prevalence began to decrease in 2001. The positivity rates were 3.7% in 2000 and 3.1% in 2001. This difference was significant statistically. This declining continued progressively in later years and the rate dropped to 0.5% in 2014.
Summary and Conclusions: This retrospective study showed a significant and progressive decline in the HBsAg seropositivity while no apparent change were those of HCV, HIV and T. Pallidum among blood donors in Northern Turkey at last thirteen years. The drop in HBsAg seroprevalence is probably multifactorial such as public education activities and use of mandatory disposable injectors.

Index of authors

B

C

D

E

F

G

H

I

J

K

L

M

N

O

P

Q

R

S

T

U

V

W

X

Y

Z

Late breaking authors

W

X

Y

Z