EUROPEAN HEMATOLOGY ASSOCIATION

haematologica

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Florence, Italy, October 4-7, 2015

ABSTRACT BOOK

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

Ancient Greek

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

Scientific Latin

haematologicus (adjective) = related to blood

Scientific Latin

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

Modern English

The oldest hematology journal, publishing the newest research results.
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BEST ABSTRACTS

BEST1 - B001

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

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Background: Prognosis for older patients with relapsed or refractory (R/R) acute myeloid leukemia (AML) is poor and treatment options are limited. Vosaroxin is a first-in-class anticancer quinolone derivative that is active in AML. VALOR, a phase 3, randomized, double-blind, placebo-controlled trial, evaluated vosaroxin plus cytarabine (vos/cyt) *vs* placebo plus cytarabine (pla/cyt) in patients with R/R AML (NCT01191801). *Aims:* To evaluate the efficacy and safety of vos/cyt *vs* pla/cyt in patients ≥60 y of age enrolled in the VALOR trial. *Methods:* Patients were randomized 1:1 to receive cytarabine (1 g/m^2 IV over 2 h, d 1-5) plus either vosaroxin (90 mg/m^2 IV over 10 min d 1 and 4; 70 mg/m^2 in subsequent cycles) or placebo. Eligible patients had refractory disease or were in first relapse after 1-2 cycles of prior induction chemotherapy. Primary endpoints were overall survival (OS) and 30- and 60-day mortality. Here we report results of predefined subgroup analyses in patients ≥60 y. *Results:* Of the 711 patients randomized, 63% (n=451) were ≥60 y (n=226 randomized to vos/cyt and n=225 to pla/cyt). At final analysis, OS was improved with vos/cyt (7.1 mo *vs* 5.0 mo with pla/cyt; HR=0.75; P=0.003) (Figure 1). Event-free survival was also improved (2.1 mo *vs* 1.3 mo with pla/cyt; HR=0.61; P<0.0001). Complete remission (CR) was achieved in 31.9% of patients treated with vos/cyt *vs* 13.8% treated with pla/cyt (P<0.0001). Responses were durable; median leukemia-free survival among patients who achieved CR was 10.3 mo with vos/cyt *vs* 6.5 mo with pla/cyt (P=0.20). Thirty- and 60-day all-cause mortality was similar between treatment arms (30-day: 10.2% *vs* 9.0%; 60-day: 20.4% *vs* 22.6% with vos/cyt *vs* pla/cyt, respectively). Serious AEs were more common with vos/cyt treatment (57% *vs* 33% with pla/cyt); most common serious AEs were febrile neutropenia (9.3% with vos/cyt *vs* 5.9% with pla/cyt), sepsis (9.3% *vs* 5.0%), pneumonia (7.5% *vs* 4.5%), bacteremia (7.5% *vs* 2.3%), and stomatitis (4.4% *vs* 1.8%). Serious and non-serious cardiac, renal, neurologic, and hepatic AEs were comparable between treatment groups. *Conclusions:* Addition of vosaroxin to cytarabine improved OS and increased CR rates without increasing early mortality in patients ≥60 y with R/R AML. The additional toxicity observed in the vosaroxin arm was acceptable in light of the benefit received. VALOR results support the use of vos/cyt as a standard of care in patients ≥60 y of age with R/R AML.

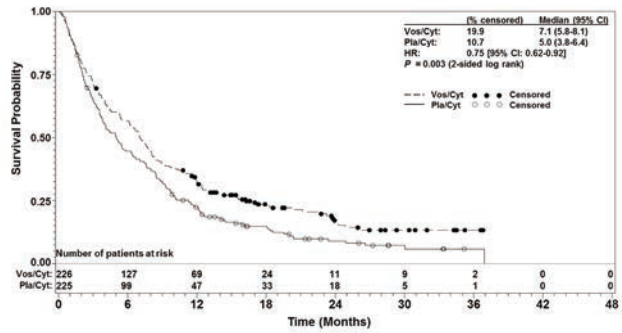

Figure 1. OS in patients age ≥60 y, by treatment arm (n=451).

BEST1 - B002

THE BRAF INHIBITOR VEMURAFENIB IS SAFE AND HIGHLY EFFECTIVE IN REFRACTORY AND RELAPSED HAIRY CELL LEUKEMIA: RESULTS OF THE HCL-PG01 PHASE-2 ITALIAN CLINICAL TRIAL

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Background: Hairy cell leukemia (HCL) responds well to purine analogues, but about 40% of patients relapse. Because BRAF-V600E is the genetic lesion underlying HCL, refractory/relapsed HCL patients may benefit from targeted therapy with the oral BRAF inhibitor vemu-

rafenib. *Methods:* We conducted an academic phase-2, single-arm, multicenter study to assess vemurafenib safety and activity in refractory/relapsed HCL. Twenty-eight patients (see Table 1) were enrolled in 11 months. Patients received vemurafenib 960 mg twice daily for a median of 16 weeks. We report here the mature results of this trial with almost 2 years of follow-up. *Results:* Vemurafenib was generally well tolerated. Drug-related adverse events were mostly of grade 1-2, including arthralgias, skin toxicities and pancreatitis. No myelotoxicity nor cutaneous squamous cell carcinomas/keratoachantomas were observed; however, 2 patients developed skin basaliomas and 1 patient a cutaneous superficial melanoma, all treated with simple excision. *In vivo* exposure to vemurafenib caused smoothening of the hairy surface and decrease of CD25 expression in leukemic cells, indicating a key role of mutant BRAF in determining the hairy morphology and suggesting that the HCL marker CD25 may not be ideal for monitoring the leukemic burden during vemurafenib treatment. In 26 evaluable patients, the overall response rate was 96%: 9/26 (34.6%) complete remission (CRs) and 16/26 (61.4%) partial remissions (PRs), obtained after a median of 8 weeks. At a median follow-up of 23 months, the median relapse-free and treatment-free survivals were respectively 19 and 25 months in CR patients, and 6.5 and 18 months in PR patients (Table 1). In all patients, immunohistochemistry showed residual HCL cells at the end of treatment, that in 6/13 evaluable cases exhibited persistent phospho-ERK expression, suggesting by-pass MEK-ERK reactivation as a potential resistance mechanism. Four CR patients and 6 PR patients, who respectively relapsed 9-18 months and 5-14 months post-treatment, received a second short course of vemurafenib for 4-12 weeks. The 3/4 evaluable CR patients obtained a second CR (n=2) and a PR (n=1). In the 5/6 evaluable PR patients, 4 minor responses and only one second PR were observed. *Conclusions:* A short oral course of vemurafenib proved safe and highly active in heavily pre-treated HCL patients. Retreatment was effective in patients relapsing after CR, but less so in patients relapsing after PR.

Table 1.

Pt.	Sex	Age	Previous lines of therapy	Blood counts and spleen size: Neut. /mm³	PLT x10³/mm³	Hb g/dl	Spleen[a] cm	Response to Vemurafenib	Weeks of treatment until best response	total	Relapse-free survival (in months[b])	Treatment-free survival (in months[b])
1	M	44	DCF, IFN, CDA, IFN, RTX, IFN	596	39	13.1	18	PR	8	20	9	9
2	M	76	DCF, DCF, CDA	399	97	9.8	13	CR	8	20	12	18
3	M	56	IFN, RTX, CDA	480	62	11	18	PR	12	20	3	8
4	F	52	Spl., 2CDA*, DCF+RTX	1443	139	10	-	PR	12	16	7	8
5	F	45	CDA*	1050	140	9.8	≤12	PR	16	16	24+	24+
6	M	81	DCF*	560	76	12.6	16	PR	12	16	15	21+
7	M	27	CDA*, Spl., RTX	1363	177	14.5	-	PR	8	16	9	11
8	M	77	Spl., IFN, CDA, CDA, IFN, CDA, RTX, RTX	179	36	8.9	-	CR	8	8	5	9
9	M	57	IFN, IFN, DCF, CDA, CDA, IFN, DCF+RTX	480	73	8.6	25	MR	16	16	/	4
10	M	68	Spl., CDA, DCF, CDA, IFN, CDA	290	6	8.2	-	PR[§]	12	12	14	18
11	M	49	DCF, CDA, CDA, RTX, IFN, CDA	861	66	15.3	25	CR	12	12	9	25
12	M	57	CDA, CDA, CDA, DCF	844	70	12.7	25	PR	4	16	3	18+
13	M	71	DCF, 2CDA, RTX, DCF, RTX, CDA	830	51	11.9	≤12	CR	8	8	24+	24+
14	M	70	Spl., IFN, CDA, RTX	710	47	9.2	-	PR	8	16	5	5
15	M	80	CDA+RTX, RTX, Spl., CDA	5710	85	15.4	-	not evaluable[¶]				
16	M	84	IFN, DCF, CDA, CDA, IFN	1123	79	11.2	≤12	CR	4	8	23+	23+
17	M	50	CDA, CDA	966	94	13.4	13.5	PR	16	16	3	18+
18	M	43	CDA, RTX	985	30	15.2	18	PR	4	16	1	13
19	M	52	CDA*, IFN	1160	66	13.9	≤12	PR	4	16	3	21+
20	M	51	CDA, CDA	1336	71	13.5	16	PR	8	16	9	14
21	M	36	CDA, IFN, CDA	628	48	17	13.5	CR	4	8	21+	21+
22	M	67	IFN, CDA, CDA+RTX	749	56	15.3	17	CR	8	8	24+	24+
23	F	39	DCF*	213	23	9.2	17.5	CR	4	8	12	18
24	M	39	CDA	1363	113	14.8	17	CR	5	8	19	23+
25	M	57	IFN, CDA, CDA, CDA, IFN	2310	49	10.5	≤12	PR[§]	8	16	20+	20+
26	M	68	Spl., IFN, IFN, IFN, DCF, DCF, IFN, CDA, RTX, CDA, CDA, CDA	1300	120	10	-	PR	10	14	8	18
27	M	72	CDA, CDA+RTX	1664	306	9.7	≤12	not evaluable[¶]				
28	M	56	IFN, CDA*, DCF, RTX, Spl.	425	304	14.5	-	PR	8	14	3	18+
								Median	8	16	9	25

Neut.: neutrophils; PLT: platelets; Hb: hemoglobin; DCF: pentostatin; IFN: interferon; CDA: cladribine; RTX: Rituximab; S
*Primary refractory
[a]Severe septic arthritis after DCF
[b]Longest diameter
[§]With delayed platelet recovery
[¶]Off study after ≤1 week of therapy for drug-unrelated acute myocardial infarction (pt. 15) or for consent withdrawal due to drug-related, reversible, grade-3 pancreatitis (pt. 27)
[¶]From the end of treatment

BEST1 - B003

MESENCHYMAL STEM CELLSAND AML CELLS DO NOT SHARE IDENTICAL CHROMOSOMAL DEFECTS: A CYTOGENETICS, FISH AND aCGH/SNPa STUDY

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Concerns have been raised on the possibility that MSCs may undergo malignant transformation and various studies have discovered cytogenetic defects in MSCs from AML patients (pts) suggesting that they may represent a mechanism of leukemogenesis.We tested whether leukemic cells and MSCs from 18 AML pts, at clinical diagnosis, share the same alterations on three technologies: conventional cytogenetics (CC), FISH and aCGH/SNPa. BM cells were submitted to routine CC and FISH. MSCs were isolated from BM cell suspension (10-15 ml) using Lympholyte®-H and cultured in flasks at a cell density of 106 cells/cm^2 at 37°C, 5% CO2 in MEM-α medium. After 48h, non-adherent cells were removed and culture medium replaced (Achille, 2011). Mesenchymal phenotype was evaluated by flow cytometry. with the fluorochrome-conjugated antibodies anti-CD90-FITC, anti-CD105-PE, anti-CD14-FITC, anti-CD73-PE, anti-CD34-FITC, anti-CD80-PE, anti-CD133-APC, anti-CD31-PE and anti-CD45-APC-Alexa750. The FISH commercial probes were applied according to manufacturer's guidelines: LSI D7S486/CEP7, LSI AMLETO (Abbot Molecular Inc. Chicago, Il, USA) and ON c-Myc/SE8, SE10(D10Z1) (Kreatech, Amsterdam, NL). aCGH/SNPa was carried out with the SureScan Microarray Scanner G4900DA (Agilent Technologies Inc. Santa Clara, CA). CC on AML cells revealed normal cariotype in 10 pts, -7 in 2 pts, del(7)(q31) in 1 pt, +8 in 2 pts, +10 in 1 pt, t(8;21)(q24,q22) in 1 pt and complex karyotype in the last pt. All these defects were confirmed by FISH. Flow-cytometry revealed a MSCs purity of 50-87%. On FISH the MSCs from all the 18 pts showed a normal pattern. In contrast, on aCGH/SNPa the MSCs from 6 pts presented chromosomal alterations. An amplification of the chr.5 was discovered in 1 pt (FISH showed a true +5), a LOH of a 3.8 Mb sized region located on 13q31.1 in one, a LOH of a 4.3 Mb region mapped on chrs. 6 and 18 in 2 pts, an amplification of three 71Kb, 322Kb and 47Kb sized regions of chr 5, 18 and 20 in 1 pt5 in another pt who presented an amplification of six genes including JAK1, ELN, FGFR2. In conclusion i) MSCs from chromosomally abnormal AMLs may have a normal FISH pattern, but on aCGH/SNPa may contain LOH or amplifications different from those of leukemic cells; ii) the MSCs defects may flag a leukemogenic-induced genomic instability which affects not only the hematopoietic tissue but also the niche; iii) aCGH/SNPa is a useful technique to identify potential clonal markers.

BEST1 -B004

THE ITALIAN FANCONI ANEMIA REGISTRY: A TWENTY-YEAR EXPERIENCE

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Fanconi anemia (FA) is a rare inherited hematological disorder biologically characterized by hypersensitivity to DNA cross-linking agents. The phenotype of FA patients is largely heterogeneous, since the natural history of the disease entails different clinical manifestations which may be present at birth, or rather develop later during the disease course. To accomplish the need of a National Database, in 1994 the "Registro Italiano Anemia di Fanconi" (RIAF) was established at the local health unit "ASL Napoli 1", as a non-interventional observational study; here we report on a 20-year experience of this Registry. Between 1994 and 2014 a total of 180 patients were included in the RIAF, belonging to 151 distinct families; all diagnoses were based on standard chromosome (DEB or MMC) breakage test. The median age at diagnosis was 3170 days, and it was lower in patients born in more recent periods. In the majority of patients the diagnosis was suspected based on typical morphological abnormalities and/or growth retardation; congenital abnormalities (mostly skin pigmentation and skeletal abnormalities) were demonstrated in 90% of patients. The majority of patients (77%) exhibited some hematological abnormalities at diagnosis, which in most cases was a mild-to-moderate cytopenia. Looking at the subsequent disease course, a total of 172 (96%) of FA patients developed some hematological manifestations, typically progressive cytopenia due to bone marrow failure; the cumulative incidences (CI) of any hematological disorder were 62%, 88% and 94% at 10, 20 and 30 years, respectively, whereas those of a hematological malignancy were 5%, 8% and 22% at 10, 20 and 30 years. The CI of BMT in our patient cohort was 33%, 64% and 72%% at 10, 20 and 30 years, with patients born in the most recent years transplanted earlier. The CI of solid tumor was 1%, 15% and 32% at 10, 20 and 30 years respectively, with head and neck as the most common site of cancer. Eighty-eight of the 180 FA patients enrolled in the RIAF died during their follow up; in non-transplanted patients, the main causes of death were related to the underlying disease (infections, bleeding, and solid tumors); in transplanted patients graft *versus* host disease and other transplant-related complications contributed to death. With a median follow up of 15.6 years, median survival was 22,5 years; probability of survival at 10, 20 and 30 years were 88%, 56% and 37%, respectively, with no improvement in most recent years.

BEST1 - B005

PHASE 1-2 CLINICAL STUDY OF WEEKLY CARFILZOMIB IN COMBINATION WITH CYCLOPHOSPHAMIDE AND DEXAMETHASONE AT DIAGNOSIS

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In this Phase I/II study we determined the maximum tolerated dose (MTD) of weekly carfilzomib in combination with cyclophosphamide-dexamethasone (wCCd) and assessed safety and efficacy of this combination. Preliminary results from the Phase I portion of the study are reported, the phase II portion is still ongoing. Elderly patients (≥65 years) with newly diagnosed multiple myeloma (NDMM) could be enrolled. In the Phase I, we used the 3+3 dose-escalation scheme. IV carfilzomib was given on days 1, 8, 15 in 28-day cycles at the starting dose of 45 mg/m^2 (level 0), and it could be escalated to 70 mg/m^2 (level 2). Carfilzomib dose was escalated based on dose-limiting toxicities (DLTs) occurring in cycle 1. 30 NDMM patients were enrolled. Median age was 74 years, 30% were ≥75 years, 37% had ISS stage III, 33% had t(4;14) or t (14;16) or del17p by FISH. In the Phase I, 12 patients were enrolled. At dose level 0, no DLT was reported; at dose level 1, 1 of 6 patients had a DLT (grade 3 creatinine increase); at dose level 2 no DLT occurred. The MTD of weekly carfilzomib was established as 70 mg/m^2. Toxicity and response data are available for 28 patients who completed at least the first cycle. Grade 3-4 adverse events occurred in <15% of patients and included neutropenia (10%), acute pulmonary edema (8%), pulmonary embolism (3%), fatigue (3%), and nausea (3%). No peripheral neuropathy was reported. Overall, wCCd was well tolerated, carfilzomib was reduced in 3 patients (10%) due to grade 3 creatinine increase, grade 3 transaminase increase and grade 2 fever (1 patient each), and it was interrupted in 3 patients (12%) due to acute pulmonary edemas (2 patients) and creatinine increase (1 patient). After 6 induction cycles, 91% achieved at least a partial response, 63% at least a very good partial response, and 27% complete response. Responses improved over the cycles (Table 1). Only 4 patients progressed and 1 died, due to acute pulmonary edema probably related to treatment. In conclusion, wCCd is safe and effective in NDMM patients. Responses improved over time and toxicities were manageable. The response rate observed with weekly carfilzomib compares favorably with similar studies with standard twice weekly carfilzomib infusion.

Table 1.

	2nd cycle	4th cycle	6th cycle
Complete Response	5%	22%	27%
At least Very Good Partial Response	9%	39%	63%
At least Partial Response	73%	83%	91%

BEST1 - B006

LONG-TERM RESPONDERS AFTER BRENTUXIMAB VEDOTIN: EXPERIENCE ON 57 PATIENTS WITH RELAPSED AND REFRACTORY HODGKIN AND ANAPLASTIC LARGE CELL LYMPHOMA

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Brentuximab vedotin (BV) is an antibody drug-conjugate targeting CD30 linked to monomethyl auristatin E. Several studies have shown the efficacy of BV in patients with refractory or relapsed Hodgkin lymphoma (HL) and systemic anaplastic large cell lymphoma (sALCL). We reviewed our clinical database to evaluate the long-term efficacy of this treatment. From July 2009 to February 2015, 57 patients were treated with BV in our Institute: 43 with a diagnosis of HL and 14 with sALCL. Thirty-six were males and twenty-one were females, with a median age of 33 years (range 16-77). All of them had been heavily pretreated before BV with a median number of previous therapies of 3 (range 2–10). Thirty-nine had refractory disease and eighteen were relapsed. Autologous stem cells transplantation had failed in 30 patients. BV was administered at a dosage of 1.8 mg/mq, every 21 days, for a maximum of 16 cycles. The median number of cycles was 8 (range 2-16); 13 patients completed the entire schedule. The best overall response rate was globally 57,8% (33 of 57 patients), including 25 (43.8%) complete responses (CR): 18 with HL and 7 with sALCL). At present, 20/25 (80%) patients are still in continuous CR (CCR) with a median follow up of 9 months (range 3–41): 10 of them have consolidated the response with a stem cell transplantation (SCT) (4 auto-SCT and 6 allo-SCT) and 10 patients have remained in CR without any other therapy after BV. Among these long-term responders without any consolidation (7 patients with HL and 3 with sALCL), the median follow-up is 12 months (range 3–37); in particular there are 3 patients in CCR after at least 24 months. The global overall survival rate at 68 months is 71% (no patients with sALCL dead) and the median overall survival has not been reached yet. The global progression-free survival rate at 48 months is 30%, the median is achieved at 11,7 months. Toxicity was primarily neurological with peripheral sensory symptoms (30%) and motor neuropathy (5%); the majority were Grade 3 in severity (8 patients). This study confirms the safety and the high efficacy of BV that can be considered an effective treatment in patients with relapsed or refractory HL or sALCL. This drug can induce a durable complete response representing a "bridge" to auto-SCT or allo-SCT. However our data show a subset of patients that can be considered "long-term responders", who have remained in CCR without any consolidation after BV.

BEST2 - B007

CLINICAL FACTORS PREDISPOSING TO ACHIEVEMENT OF RBC TRANSFUSION INDEPENDENCEIN LENALIDOMIDE-TREATED PATIENTS WITH LOW/INTERMEDIATE-1-RISK MYELODYSPLASTIC SYNDROMES) WITHOUT DEL(5q) IN MDS-005 STUDY,

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In the phase 3 MDS-005 study, lenalidomide treatment was associated with a significant achievement of RBC-TI ≥8 weeks in 26.9% of RBC transfusion-dependent patients with IPSS Low/Intermediate-1-risk MDS del(5q) negative who were unresponsive or refractory to ESAs. This analysis evaluated RBC-TI according to different clinical variables. Patients were randomized 2:1 to lenalidomide 10 mg once daily or placebo. Age, sex, transfusion burden, previous therapy WHO classification, IPSS classification, WHO 2008 classification, BM blasts, cytopenias, gene signature were evaluated in multivariate analysis. Rates of RBC-TI for ≥8 consecutive weeks were analyzed according to baseline EPO levels prior to randomization (≤500 mU/mL and >500 mU/mL) in patients randomized to lenalidomide. Of the 160 lenalidomide-treated patients, 5 were excluded from this analysis due to missing baseline EPO data. Factors predictive of response to LEN were previous treatments, treatment with ESAs, low transfusion burden (<4U/mos), female gender and levels of endogenous EPO. In a sub analysis conducted on 155 patients (EPO ≤500 mU/mL, n=97; EPO >500 mU/mL, n=58). Patients with EPO ≤500 mU/mL were older than those with EPO >500 mU/mL (median age 72 *vs* 68 years), had longer duration of MDS (median 3.7 *vs* 2.2 years), had lower RBC transfusion burden (median 3.0 *vs* 3.8 packed RBC units/28 days), and more received prior ESAs (96.9 *vs* 44.8%). There was a linear increasing trend in rates of RBC-TI ≥8 weeks across patients with decreasing EPO level from >500, 500–200, 200–100 to ≤100 mU/mL (15.5%, 23.3%, 33.3%, 42.5%, respectively;

Fisher exact P=0.023; linear trend test P=0.002). Similarly, RBC-TI rates were significantly higher in patients with EPO ≤500 mU/mL *versus* EPO >500 mU/mL (34.0% *vs* 15.5%; Fisher exact P=0.015).In lenalidomide-treated patients with IPSS lower-risk MDS without del(5q), response to LEN correlated with female gender, previous treatment with ESAs, and endogenous serum EPO levels in a linear fashion. Patients with baseline EPO ≤100 mU/mL achieved RBC-TI ≥8 weeks in >40% of the cases, while a baseline EPO <500mU/mL was anyhow accompanied by higher response rates compared with EPO >500 mU/mL (P=0.015). Patients with high EPO may be less responsive due to intrinsic defects in erythroid signaling pathways including STAT5. These results suggest it may be possible to identify a subset of patients with lower-risk MDS without del(5q) who have a higher sensitivity to LEN.

BEST2 - B008

RECURRENCES AFTER SPLANCHNIC VENOUS THROMBOSIS: RISK FACTORS AND EFFECT OF DIFFERENT TREATMENTS IN A RETROSPECTIVE MONOCENTER COHORT OF 154 PATIENTS

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Background: Chronic myeloproliferative neoplasms (MPN) and thrombophilia are the leading systemic causes of non-cirrhotic non-malignant splanchnic vein thrombosis (SVT). Long-term treatment is a clinical challenge and the optimal duration of anticoagulation is uncertain. *Patients and Methods:* We analysed a retrospective cohort of 154 pts. with a first SVT (M/F 68/86, median age at SVT 45 yrs, range 1-83), with thrombosis of hepatic veins in 12% and of one or more sites of the spleno-mesenteric-portal axis in 88%. SVT was unprovoked in 84 (54%); 56 (36%) had inherited or acquired thrombophilia and 47 (30%) overt MPN; 50/131 tested pts. (38%) had the JAK2V617F mutation. Anti-vitamin K agents (AVK) were given to 80 pts. (53%), aspirin (ASA) to 16 (10%), and AVK+ASA to 8 (5%); 50 (32%) discontinued prophylaxis after 6-12 months of AVK. The probability of recurrent thrombosis and the impact of different risk factors or treatments were estimated by a Cox regression model including as covariates gender, age >45 yrs at SVT, family history of venous thrombosis, absence of provoking causes of SVT, thrombophilia, overt MPN, cytoreduction, long-term AVK or ASA. *Results:* During a total time of 748 yrs, 25 pts.(16%) had a recurrence, 10 during AVK, with a cumulative probability of 6.1% at 1 yr, 16.2% at 5 yrs, and 25.7% at 10 yrs. Recurrence was SVT in 15 pts., and involved other venous or arterial sites in 7 and 3, respectively. The overall incidence of recurrences was 3.3% pts-yrs: 2.1% during AVK and 5.3% without AVK. In the multivariate model male gender and age >45 yrs were associated with a higher risk of recurrence (hazard ratio HR 2.61, 95%CI 1.04-6.53, and HR 3.26, 95%CI 1.18-8.97, respectively); AVK significantly prevented recurrences (HR 0.35, 95%CI 0.13-0.91). A modified model including JAK2V617F and without the redundant variable MPN applied on the 131 pts. checked for the mutation, retained only JAK2V617F as predictor of recurrence (HR 6.7, 95%CI 1.76-25.44) and confirmed the efficacy of AVK (HR 0.15, 95%CI 0.04-0.57). Efficacy of AVK was further confirmed in a sub-analysis of the pts. with overt MPN or carriers of JAK2V617F. *Conclusions:* Thrombophilia and MPN/JAK2V617F mutation were the systemic risk factors for SVT more represented. Male gender, age >45 years, and the JAK2 V617 mutation were independent risk factors for recurrence. Long-term treatment with AVK reduced recurrences in the overall cohort as well as in the MPN subgroup.

BEST2 - B009

RITUXIMAB, BENDAMUSTINE AND CYTARABINE AS INDUCTION THERAPY IN ELDERLY PATIENTS WITH MANTLE CELL LYMPHOMA: A PHASE 2 STUDY FROM THE FONDAZIONE ITALIANA LINFOMI

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The combination of rituximab (R, 375 mg/m^2 intravenously [IV], day 1), bendamustine (B, 70 mg/m^2 IV, days 2 and 3), and cytarabine (800 mg/m^2, IV on days 2 to 4) was highly active in patients with mantle-cell lymphoma (MCL) [Visco *et al.*, JCO 2013]. Overall, this regimen was well tolerated, but hematologic toxicity was relevant (grades 3 to 4 thrombocytopenia in 76% of cycles). Aiming at reducing the haematologic toxicity, the Fondazione Italiana Linfomi (FIL) designed a phase 2 trial lowering cytarabine dose to 500 mg/m^2 (RBAC500). Patients with newly diagnosed MCL, aged 61 to 80 years, not eligible for autologous transplant and fit according to the comprehensive geriatric assessment were enrolled. The primary endpoints were complete remission rate (CR) measured by FDG-PET according to Cheson criteria 2007, and safety. Secondary endpoints included rate of molecular response (MR), progression-free (PFS) and overall survival (OS). MR was assessed by nested-PCR using patient specific IGH or BCL1 based targets. Starting from May 2012 to February 2014, 57 patients were recruited. Central pathology revision was performed in 87% of cases. Median age was 71 years (range 61-79), 75% were males, and 91% had Ann Arbor stage III/IV disease. Mantle Cell International Prognostic Index (MIPI) was low in 15%, intermediate in 40%, high in 45%, and 9% had the blastoid variant. Overall, 53 patients (91%) received at least 4 cycles (median 5.3 cycles). Fifteen patients (26%) discontinued treatment because of toxicity/adverse events, mainly consisting of hemo-toxicity between cycles (11). Only one patient discontinued due to progressive disease. Grade 3 or 4 neutropenia and thrombocytopenia were observed in 49% and 52%, respectively. Febrile neutropenia occurred in 6%. Extra-hematologic toxicity was mainly cardiac (5%). Overall response rate was 96%, and CR was 93%. The MR rate at the end of treatment was 76% on peripheral blood and 55% on bone marrow (BM). With a median follow-up of 18 months (11-34), the projected 2-years PFS was 83%±5% and the OS 91%±4%. The MIPI score (high *vs* low/int), blastoid variant *vs* classical and the failure of achieving MR on BM samples were the only statistically significant adverse prognostic factors for PFS. Hematologic toxicity is substantially reduced compared to our previous experience. With 93% of FDG-PET negative CR, 55% MR on BM, and a projected 2-years PFS of 83% without maintenance therapy, the R-BAC500 regimen is a highly effective treatment for patients with MCL (Figure 1).

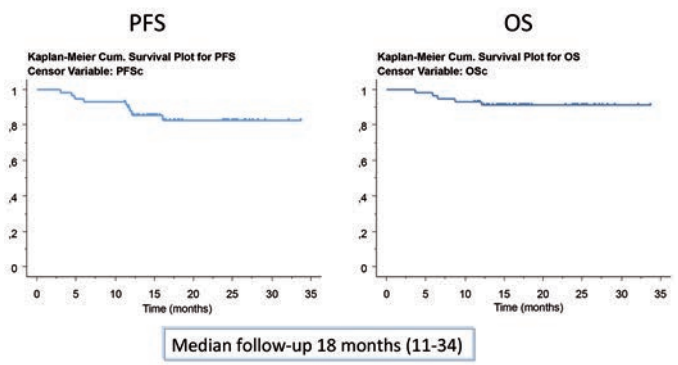

Figure 1.

BEST2 - B010

INTERVENTIONAL STRATEGIES TO CONTAIN COLONIZATION AND INFECTIONS CAUSED BY CARBAPENEMASE PRODUCING KLEBSIELLA PNEUMONIA IN HAEMATOLOGICAL MALIGNANCIES

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Background: The emergence of Carbapenemase producing Klebsiella pneumoniae (KPC) represent a serious threat to public health and is associated with high mortality.We carried out a prospective investigation to assess the prevalence of KPC in Hematology and the impact of a strategy control to limit the spread. *Methods:* Infections caused by KPC have been recorded in our Unit from January 2012 through March 2015. From January 2012 to July 2013, we developed an intensive control program, (Plan A): 1. Weekly colonization screening; 2. Educate healthcare personnel; 3. Promotion of hand hygiene 4. Physical separation of carriers from non-carriers 5. A double-carbapenem plus colistin therapeutic empiric regimen for infections. Since July 2013, we decided to implement additional measures (Plan B): 1. Drastic reduction of beds. 2. 2% chlorhexidine body washing for colonized pts. 3. Donning gown and gloves before entering the affected patients's room and removing prior to exiting the affected room. *Results:* Since January 2012 perianal swabs were detected weekly from 835 consecutive patients affected by hematologic malignancies, for a total of 1384 admissions and 16.328 days of hospital stay. KPC colonization was present in 51 out of 835 (6.1%) screened patients at some time during their hospitalizations; 12 bloodstream infections were reported in 11 patients (23,5%). Three deaths (3/12, 25%) due to KPC were reported before implementation screening (overall mortality rate was 5.8% of colonized patients). KPC-decolonisation was achieved in 13/51 pts (25%) after a median duration of 52 days (range 7-115). Most patients who recovered from KPC infection or were just colonized by KPC went on to receive additional chemotherapy without any life threatening KPC infection occurring. Since screening cultures or further clinical cultures identified a progressive increase KPC-colonized or -infected patients, we decided to implement additional measures (Plan B). From fourth trimester 2013 of study, we have observed a progressive decrease in rate of new colonization: 2 patients (4.17%) *vs* 15 patients (30%) in the third trimester, and a very low rate was maintained until to date. *Conclusions:* The prevalence of KPC colonization in our hospital is high. An implementation of additional measures was able to contain KPC colonization and infection. However, the success of our preliminary interventions should be monitored constantly and a coordinated regional control effort among healthcare facilities should be recommended.

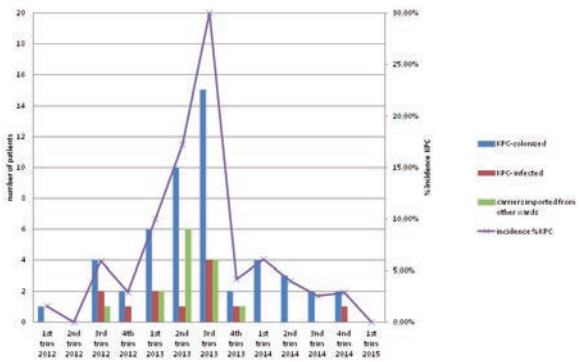

Figure 1. Prevalence of KPC infections 2012-1st trim 2015.

BEST2 - B011

LONG TERM FOLLOW-UP OF A MULTICENTER RETROSPECTIVE STUDY BASED ON DONOR AVAILABILITY IN MULTIPLE MYELOMA PATIENTS RELAPSED AFTER AUTOTRANSPLANT

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The role of allogeneic stem cell transplantation (allo-SCT) in patients with relapsed multiple (MM) is still a matter of debate and comparative studies are lacking. We had previously reported a significant progression free survival (PFS) benefit of salvage treatment with bortezomib or immunomodulatory agents followed by allo-SCT in patients with MM who had relapsed after autologous stem cell transplantation (auto-SCT) and had a suitable donor (BBMT 2012; 18: 617-626). Here, we report the long-term clinical outcome of this study with a median follow-up of 87 months. This study was structured similarly to an intention-to treat analysis and included only those patients who underwent HLA typing immediately after the relapse. Patients with a donor (donor group) and those without a suitable donor (no-donor group) were compared.. A total of 169 consecutive patients were evaluated retrospectively in a multicenter study. Of these, 75 patients found a donor and 72 (96%) underwent RIC allo-SCT, including 24 from an HLA-identical sibling (32%) and 48 from an unrelated donor (68%). The starting point of outcome analyses was the day of relapse after auto-SCT. The 2-year cumulative incidence of non relapse mortality was 22% in the donor group and 1% in the no-donor group (P<.0001). The 5-year PFS was 21% in the donor group and 3% in the no-donor group (P<.0001) (Figure 1 A). The 5-year OS was 40% in the donor group and 19% in the no-donor group (P=0.007) (Figure 1 B). In multivariate analysis, availability of a donor, chemo sensitive disease before transplant and a longer duration of salvage treatment were significant predictors for favourable PFS. Moreover, high-risk karyotype at diagnosis significantly shortened OS. Interval duration between auto-SCT and relapse had no significant impact on the outcome of the following allo-SCT. The long term follow-up of the study confirms the significant PFS benefit and provides evidence of an OS advantage of RIC allo-SCT in relapsed MM patients who have a suitable donor, suggesting that allo-SCT could be an option in young patients with high-risk relapse after first- line treatment.

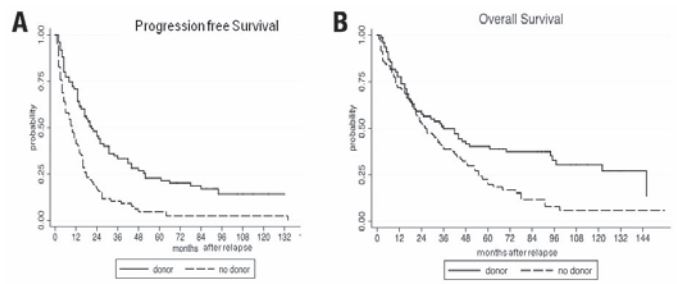

Figure 1.

BEST2 - B012

LONG TERM OUTCOME OF INTERMITTENT IMATINIB TREATMENT POLICY FOR MANAGING CHRONIC MYELOID LEUKEMIA IN THE ELDERLY

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Chronic myeloid leukemia (CML) patients treated with tyrosine kinase inhibitors (TKIs) have a life expectancy close to normal. Some of them can enter into a treatment-free remission status (TFR), but many of them are expected to continue to receive standard TKI treatment indefinitely. The main question is: is the standard dose of TKIs (any) required lifelong? To investigate an alternative policy to standard TKI treatment, we started in 2008 a phase II multicentric study of Intermittent imatinib (IM) treatment (INTERIM) in elderly patients (over 65 years) with Ph+ CML who achieved a stable complete cytogenetic response (CCyR) with at least 2 years of standard IM therapy. Seventy-six patients were treated with intermittent imatinib, one month on and one month off, and were monitored regularly for response. With a minimum follow-up of six years, 16/76 patients (21%) lost complete cytogenetic response (CCyR) and major molecular response (MMR or MR3.0), and 16 other patients (21%) lost MMR only. All these patients with the exception of one who was lost to follow-up, were given imatinib again, the same dose, on the standard, continuous, daily schedule, and achieved CCyR, MR3.0 or even better again. At last follow up, 4 patients are in CCyR, 4 are in MR3.0, 19 are in MR4.0 and 2 are in MR4.5. The probability of remaining in the intermittent imatinib schedule at 6 years was 48% (95% CI 35-59%). Nine patients, 72 to 80 years old (median age 75), died in remission. No loss of complete hematologic response (CHR) or progressions to accelerated/blastic phase (A/BP) was recorded. Side effects of continuous treatment were reduced by 50%. In optimal and stable (CCyR/MMR) responders, a policy of intermittent imatinib treatment is feasible: it is successful in about 50% of patients and is safe, as all the patients who relapsed could be brought back to optimal remission. Acknowledgments: This work was supported in part by EuropeanLeukemiaNet (ELN) - European Treatment and Outcome Study (EUTOS) and by Cofin 2009.

ORAL COMMUNICATIONS

Hodgkin Lymphoma

C001

THE SURVIVAL OF RELAPSED/REFRACTORY PATIENTS WITH HODGKIN'S LYMPHOMA TREATED WITH ALLOGENEIC TRANSPLANT IS NOT IMPACTED BY THE TIME OF RELAPSE AFTER AUTOLOGOUS TRANSPLANT OR REFRACTORY DISEASE

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Relapsed/refractory Hodgkin's lymphoma (RR-HL) has few options of cure. We analyzed our RR-HL patients to understand whether the timing of relapse after autologous transplant (autoSCT) or refractory disease may change the survival after allogeneic transplant (alloSCT). Of 221 patients referred to our center with HL, 105 were RR-HL. Sixty-three (60%) RR-HL patients received alloSCT, 41 patients (40%) did not for PD (29), advanced age (3) or CR after autoSCT after a 3rd-line chemotherapy (9 pts). Patients' donors were 38% HLA identical siblings, 35% matched unrelated and 27% haploidentical. Median age was 33 (range, 17-60), and 43% of patients were in CR, 30% in PR, 27% in SD/PD. 83% of patients relapsed <12 months from autoSCT or with primary refractory disease, 17% relapsed >12 months after autoSCT. We included in the multivariate analysis the disease pre-transplant status (CR, PR, SD/PD), donor (HLA identical sibling, unrelated, haploidentical), and time of relapse after autoSCT/refractoriness (relapse <12mts after autoSCT or primary refractory, relapse >12mts). Median follow-up was 5.0 years (range, 0.5-10.8). Overall survival (OS) was 60% at 3 years and 55% at 5 years of follow-up. In multivariate analysis, disease status before alloSCT significantly impacted OS (p=0.003, HR 1.6, CI95% 1.2-2.2), whereas donor and timing or relapse/refractoriness did not change OS (p=0.164, HR=1.3, CI95% 0.9-2.0, and p=0.587, HR=1.2, CI95% 0.6-2.5, respectively). Progression free survival (PFS) was 43% at 3 years and 43% at 5 years. Pre transplant disease status impacted PFS (p<0.001, HR=1.6, CI95% 1.2-2.1), which was not influenced by donor (p=0.349, HR=0.8, CI95% 0.6-1.2) and timing of relapse/refractoriness (p=0.912, HR=1.0, CI95% 0.5-1.9). Relapse incidence was 38% at 3 years and 38% at 5 years of follow-up. Relapse was impacted by donor (0.033, reduced risk for MUD donors, HR=0.5, CI95% 0.3-0.9), and pre-transplant disease status (p=0.003, HR=1.7, CI95% 1.2-2.4), whereas timing of relapse/refractoriness did not change relapse incidence (p=0.591, HR=0.8, CI95% 0.3-1.8). NRM was 10% at 100 days, 13% at 6 months, 18% at 1 year and reached a plateau of 20% at 2 years of follow-up. NRM was not impacted by the factors analyzed (donor p=0.158, pre-transplant status p=0.134, refractoriness p=0.327). In conclusion, PFS and OS for RR-HL after alloSCT are 42% and 55%. Disease pre-transplant status, and not the time of relapse after autoSCT or refractory disease impact OS and PFS.

C002

EARLY REDUCTION OF TARC LEVELS MAY PREDICT SUCCESSFUL TREATMENT IN NEWLY DIAGNOSED HODGKIN LYMPHOMA PATIENTS

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In this study we prospectively investigated the correlation between success of therapy and early reduction of Thymus and Activation-Regulated Chemokine (TARC) in newly diagnosed Hodgkin Lymphoma (HL) patients treated with ABVD. Serum samples of 115 HL patients (median age 32, range 26 – 44) were collected at baseline, after every cycle, at the end of therapy and during follow-up. TARC assessment was performed by ELISA, we estabilished 800 pg/ml as cut-off discriminating pathologic and non-pathologic values of TARC, as this value represented the 99th centile of TARC distribution in a group of 156 independent healthy subjects. Stage IIB, III and IV patients (n=76) received ABVD x 6-8 cycles +/- RT on bulky disease, stage I and IIA received ABVD x 4 cycles+IFRT (n=39). Bulky disease was present in 63% of patients, extranodal involvement in 26%, 55% of patients reported B symptoms. All patients underwent PET at baseline, after second cycle (PET-2) and at the end of therapy. PET-2 was negative in 83% and positive in 14% of patients, in 4 patients was not performed. In PET-2 negative subgroup 8% of patients progressed or relapsed, whereas among PET-2 positive progression or relapse occurred in 62% of patients. Median baseline TARC value was 22272 pg/ml (range, 125 – 191839), baseline levels were significantly higher in patients with B symptoms (29020 *vs* 15120), with bulky disease (29020 *vs* 8096) and with extranodal involvement (49770 *vs* 17590). No differences in baseline TARC were observed between patients achieving PET-2 negativity or positivity. TARC levels after the 1st ABVD cycle (TARC-1) were significantly reducted in comparison to baseline, TARC-1 median value was 528 pg/ml (range, 68 - 108185). Patients achieving a negative PET-2 had significantly lower levels of TARC-1 in comparison to patients with positive PET-2 (467 *vs* 2192, p <0.0001). The TARC-1 cut-off of 800 pg/ml had a sensibility of 75% and a specificity of 81% for PET-2 results. Moreover, 4-years PFS was better in patients with TARC-1 levels below the cut-off of 800 pg/ml than in patients with levels higher than 800 pg/ml, PFS was 91% (95%CI 85%-98%) *vs* 58% (95% CI 42% - 81%) (p=0.001). A multivariate analysis evaluating the risk of failure of treatment showed increased risk (expressed as log-relative hazard) at increasing of TARC-1 value. In conclusion, early reduction of TARC seems to be correlated to PET-2 negativity and success of ABVD treatment in HL patients.

C003

THE RISK OF PROGRESSION OF HODGKIN LYMPHOMA IN PATIENTS WITH NEGATIVE INTERIM PET:A ROLE FOR THE NUMBER OF TUMOR-INFILTRATING MACROPHAGES (CD68+ CELL COUNTS) AND B-SYMPTOMS

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Interim PET (iPET) according to the 5-point Deauville score (5p-DS), has been indicated as the strongest predictor for outcome in HL. However there is a small, but consistent proportion of iPET-negative patients who will progress or relapse. This leaves room for other potential prognosticators in addition to interim PET. Aim of this retrospective study was to evaluate if integration of the response evaluation with iPET with parameters available at diagnosis could add prognostic information, allowing a better risk-stratification of patients with HL. We studied 102 patients with classical HL (median age 38 years, range 15-74; 47 females, 55 males) diagnosed at our Institution between 2007 and 2014. Stage was limited in 53 patients, and advanced in 49 patients. IPS was higher than 2 in 25 patients. The number of tumor-infiltrating macrophages stained with the CD68 monoclonal antibody PGM1 was higher than 5% in 35/68 patients. B-symptoms were present in 38/102 patients All patients were treated with ABVD. iPET according DS performed after 2 cycles was negative (score 0-3) in 85 patients and positive (score 4-5) in 15 patients (2 patients technically not evaluable). Strong predictors for progression-free survival (PFS) were a negative interim PET (85% *vs* 28%, p<0.0001) and CD68+ cell counts <5% (89% *vs* 67%, p=0.006). In multivariate analysis, iPET, CD68+ cell counts and presence of B-symptoms were independently associated with PFS. We identified three risk groups. In the low risk group of patients with CD68+ counts below 5%, without B-symptoms probability of PFS was 92%, independent from interim PET scan result. The high-risk group of patients with B-symptoms and CD68+ cell counts over 5%, had a 45% probability of PFS. In this group PET strongly predicted prognosis, resulting in PFS of 72% and 0%, respectively (p=0.003). The 72% probability of PFS in the high-risk group despite a negative iPET indicates that these patients may require more attentive control of disease activity. We conclude that combination of clinical (B-symptoms), and pathological characteristics (CD68+ cell count) could add prognostic information to the early assessment of disease with iPet and identify patients still at increased risk for progression despite a negative iPET.

C004

A RANDOMIZED TRIAL OF ROUTINE SURVEILLANCE IMAGING PROCEDURES: ULTRASONOGRAPHY PLUS CHEST RADIOGRAPH *VS* FDG PET/CT FOR DETECTING RELAPSE IN PATIENTS WITH ADVANCED STAGE HODGKIN LYMPHOMA

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Background: Despite the high complete response (CR) rate to induction therapy with ABVD, about 1/3 of Hodgkin lymphoma (HL) patients with extensive disease at presentation are expected to relapse. The existing guidelines for post-treatment follow-up are not evidence-based. New imaging approaches are now available and require validation in a randomized fashion. *Aims:* This randomized study compared the use of [18F]FDG PET/CT *vs* ultrasonography (US) plus chest radiograph (CXR) to systematically follow-up patients with high risk HL. *Methods:* In this single centre equivalence trial, patients with advanced stage HL, in first CR, were randomly assigned (1:1) to either PET/CT-based or US+CXR-based follow-up. Imaging procedures in the US+CXR group comprised US scans for the evaluation of superficial-anterosuperior mediastinum-abdominal-pelvic (S-M-A-P) lymph nodes and frontal and lateral CXR for the evaluation of mediastinum compartments. In the PET/CT group, total-body PET/CT scans were carried out using a combined in-line system. The surveillance schedule for each arm implied 12 checkpoints at 4, 8, 12, 16, 20, 24, 30, 36, 48, 60, 84 and 108 months after treatment discontinuation. When imaging procedures were positive, recurrence was histologically confirmed. The primary end-point was to compare the sensitivity of the two follow-up imaging approaches. Secondary endpoints were their specificity, positive and negative predictive values, time to recurrence detection, radiation risks and costs. *Results:* Overall, 300 patients were randomized in the two arms. The study was closed after a median follow-up of 60 months, with a relapse rate of 27%. Sensitivity in detecting HL was similar for the two follow-up approaches: 40/40 relapses were identified with FDG PET/CT (100%) *vs* 39/40 relapses were identified with US plus CXR (97.5%; P equivalence=0.0001). US plus CXR showed significantly higher specificity and positive predictive value than PET/CT: 96% (106/110) *vs* 86% (95/110; p=0.02) and 91% (39/43) *vs* 73% (40/55; p=0.01), respectively. Exposure to ionizing radiation was estimated to be 14.5 mSv per one PET/CT *vs* 0.1 mSv per one CXR. Estimated cost per relapse diagnosed with routine PET/CT was 10-fold higher compared with routine US+CXR. *Conclusions:* US and CXR are diagnostic tools that enable effective, safe and low-cost routine surveillance imaging for patients at high risk of HL relapse. This study could have an important impact on practice for the follow-up of patients with advanced stage HL in CR.

C005

MOLECULAR DETERMINANTS OF BENDAMUSTINE ACTIVITY AS A SINGLE AGENT AND IN COMIBINATION WITH LENALIDOMIDE IN TUMOR CELLS OF HODGKIN LYMPHOMA

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Bendamustine (BDM) is a bi-functional alkylator with significant single-agent acitvity in recurrent HL. Despite the recent introduction of BDM in salvage and upfront regimens for HL, mechanisms underlying its effects on tumor cells of HL are still unkwnown. We evaluated the molecular determinants of BDM activity as a single agent and in combination with lenalidomide (LEN). To this end, a panel of HL-derived cell lines (L1236, L428, KMH2, HDLM2, L540) were exposed to increasing concetration of BDM (12.5 to 100 micromol/L) and LEN (0.1 to 50 micromol/L) alone and in combination. While BDM elicited a dose-dependent increase of apoptosis (30% to 50% at 72 hrs) in all cell lines (Annexin-V/PI), exposure to LEN did not cause any significant pro-apototic effect. Similarly, the significant cytotoxic activity of BDM towards HL cell lines, with IC50 at 48 hrs ranging from 10.7 to 16.2 micromol/L, was not paralleld by LEN that did not elicit any direct cytotoxicity. In contrast, exposure to BDM and LEN (50 micromol/L) resulted a signficant increase in the S-Phase population in all HL cells (45% to 200%, BDM; 25% to 35%, LEN). Q-PCR studies disclosed that BDM induces an early (8-24 hrs) and sustained (72 hrs) up-regulation of NOXA and p21, along with a delayed (72 hrs) induction of EXO1 and ATR, but not ATM, of the mitotic catastrophe (MC)-related genes (PLK1, CCNB1, AAK) and of the O6-methylguanine methyltransferase (MGMT). Confocal microscopy evidenced changes indicating early apotosis followed by MC upon exposure to BDM but not LEN. Intriguingly, a 24 hrs pre-exposure to LEN (10 micromol/L) resulted in a statistically significant increase in the cytotoxic effects of BDM towards all HL cell lines. Most notably, after a long-term exposure to BDM, surviving L1236 cells displayed a striking up-regulation of surface CD47 and its ligand SIRP. Concurrent exposure to LEN prevented such phenomenon. Overall, results indicate that: a) BDM is very active towards HL tumor cells and its cytotoxic activity is enanched by pre-exposure to LEN; b) overexpression of ATR, EXO-1 and MGMT represent different mechanism used by HL cells to survive cytotoxic effects of BDM; c) after exposure to BDM, HL cells upregulate CD47, which may prevent macrophage-mediated tumor clearance, but the phenomenon is inhibited by LEN. Our data provides a strong support to implement the BDM/LEN combination in progressive HL, as currently tested in a Phase 1/2 trial from our group (LEBEN; NTC01412307)

C006

RUOLO DELLA BIOPSIA OSTEOMIDOLLARE NEI LINFOMI DI HODGKIN IN ERA FDG-PET/CT: ESPERIENZA DEL MASTER "DIAGNOSIS AND TREATMENT OF PATIENTS WITH LYMPHOMA" DELLA FONDAZIONE ITALIANA LINFOMI

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Background: In recent years,, several studies were conducted to investigate the role of routine BMB in newly diagnosed cHL staged with PET/CT: recently, a meta-analysis [1] reported data of 955 patients in 9 different studies, to determine whether BMB is still necessary in patients staged at diagnosis with PET/CT. *Aims:* here we report data of patients (pts) with cHL assessed at diagnosis with both BMB and PET/CT, to evaluate their concordance in the detection of bone marrow lymphomatous involvement. *Methods:* we retrospectively analyzed data of consecutive pts since 2007 to 2013 referring to 16 Hematology departments of the Fondazione Italiana Linfomi (FIL). All pts underwent at baseline to both unilateral or bilateral BMB and PET/CT; stage assessment was performed with PET/CT according to the Ann Arbor classification, and it was compared to that resulting from PET/CT combined to BMB. The predictive significance of PET/CT was determined in terms of positive (PPV) and negative predictive value (NPV), sensitivity and specificity. *Results:* in this survey we included 1244 pts, 159 were excluded due to the lack of baseline BMB or PET/CT. Median age 32 (range, 14-80 years), 567 male (52%). Nodular sclerosis (70,9%) and mixed cellularity (19,3%) were the most common histotypes; bulky disease and B symptoms were present in 27% and 42% of pts, respectively. 169 pts (16%) presented one or more focal skeletal lesions at PET/CT and 55 (5%) had a positive BMB; other patients' characteristics are summarized in table 1. In 34/55 pts focal skeletal lesions evidenced by PET/CT revealed a positivity of BMB, while in 948/1030 pts the absence of skeletal lesions or a diffuse skeletal FDG uptake combined with a negative BMB. Based on these data, PPV and NPV resulted to be 20% and 98%, respectively; sensitivity and specificity were 62% and 87%, respectively. In 54/55 patients with positive BMB had PET/CT in stage III or IV, while 1/1043 (0.09%) would have been treated differently if he had not performed the BMB. *Conclusions:* NPV of PET/CT for bone marrow involvement was very high (98%). Moreover, the influence of BMB on the planning of treatment was minimal. BMB may be omitted in cHL patients staged with PET/CT.

Acute Leukemia 1

C007

TP53 MUTATIONS IN ADULT ACUTE MYELOID LEUKEMIA PATIENTS: STRONGLY ASSOCIATION WITH POOR PROGNOSIS, MUTUAL EXCLUSIVITY WITH FLT3 AND NPM MUTATIONS AND MORE SURVIVAL PREDICTABILITY THAN FLT3 MUTATIONAL STATUS OR COMPLEX KARYOTYPE

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Background: The reported TP53 mutation rate in AML is low (2.1%), but in AML with a complex karyotype (CK) is higher (69-78%), and generally these patients (pts) with complex karyotype (CK-AML) have a poor outcome. By contrast, the incidence of FLT3 disruption is frequent (20-30%) and is an independent predictor of unfavourable. *Aims:* To investigate the frequency, the types of mutations, the molecular abnormalities, the correlation with known molecular alterations (FLT3, NPM) and the prognostic role TP53 mutations in adult AML pts. *Patients and Methods:* 236 adult AML pts were examined for TP53 mutations using several methods, including Sanger sequencing, NGS (20/236) and HiSeq 2000. 42 samples were genotyped with SNP arrays (Affymetrix). *Results:* Mutations of TP53 were detected in 33 cases. 75.8% of all mutated pts had CK (25/33) by contrast the frequency of mutations was lower in "no CK-AML" pts (24.2%). 38 TP53 point mutations (classified by the IARC database as deleterious) and 4 TP53 deletions were found. WES analysis done 31 TP53 wild-type (wt) and 6 TP53 mutated pts revealed no genes exclusively mutated in the 6 TP53 mutated pts and that NRAS/KRAS mutations are mutually exclusive with TP53 mutations. Preliminarily, 6 TP53 mutated and 36 TP53 wt pts were also compared for Copy Number Alterations. Of relevance, gains located on chr 8 were statistically associated with TP53 mutations (p=0.001), as well as losses on chr 5q14.3. 174 and 117 pts were analysed for concomitant presence of TP53 mutations and FLT3/NPM1 disruption/mutation revealing a significant relation between pts with FLT3-ITD or NPM1 and TP53 wt (p=0.015 and 0.009). Of note, alterations of TP53 were significantly associated with poor outcome in terms of both overall survival (Figure 1A; P<0.0001) and disease free-survival and that TP53 compare to FLT3 mutations have worse impact on prognosis (Figure 1B; P<0.0001). TP53 mutations influence survival more than CK/TP53 wt (P<0.0001). *Conclusions:* Our data demonstrated that TP53 mutations occur in 14% of AML with a higher frequency in the subgroup of CK-AML (P<0.0001) and are mutually exclusive with FLT3 and/or NPM1; they predicted to be deleterious and significantly correlated with worse prognosis and may confer resistance to conventional and innovative therapy. For these reasons, TP53 mutation screening should be recommended at least in CK-AML pts. Supported by: ELN, AIL, AIRC, Progetto Reg-Univ 2010-12 (L. Bolondi), FP7 NGS-PTL project.

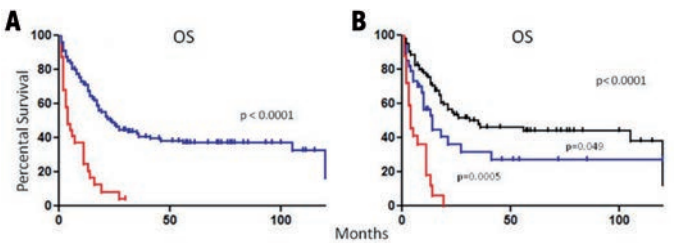

Figure 1. Overall Survival in AML adult patients considering *TP53* mutational status alone (A) and in combination with *FLT3* mutations (B); 120 month follow-up.

C008

NPM1-MUTATED-SPECIFIC T-CELL RESPONSES OCCURRING IN PERIPHERAL BLOOD AND BONE MARROW OF PATIENTS WITH INTENSIVELY TREATED NPM1-MUTATED ACUTE MYELOID LEUKEMIA

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Peptides derived from NPM1-mutated (NPM1mut) protein, a leukemia-specific antigen, may elicit *in vitro* specific immune responses by CD8+ and CD4+ T-cells, obtained from PB of either healthy subjects or patients with NPMc+ AML, leading to antigen-specific lysis of blasts, as documented by cytotoxicity assays, and potentially contributing to favorable clinical outcome (Greiner *et al.*, Blood 2012). We performed an immunological study to investigate the occurrence of NPM1mut-specific T-cells, at different timepoints, in 46 BM and 26 PB samples obtained from 17 adults (median age 58 years, range 21-67) with intensively treated NPMc+ AML, using, as antigenic stimulation, a mixture of short/long peptides (9-18mers), derived from the complete spanning of C-terminal of NPM1mut protein. Eight and 4 patients received either autoHSCT or alloHSCT, respectively. At a median follow-up of 26 months (range 5-84), 12 patients are alive. ELISPOT assay documented NPM1mut-specific T-cells producing IFNg in 45/46 BM samples (median 170 SFC/10^6 cells, range 8-860) and 22/26 PB samples (median 157 SFC/10^6 cells, range 22-736), while NPM1mut-specific T-cells producing IL17 were observed in 30/37 informative BM samples (median 29 SFC/10^6 cells, range 6-210) and in 14/20 PB informative samples (median 14 SFC/10^6 cells, range 6-84). Of interest, high amounts of IFNg-producing specific immune response were early observed in the 7 BM samples collected after induction chemotherapy, whereas IL17-secreting specific T-cells occurred in only 3 of these 7 samples. MRD analysis for NPM1mut by RQ-PCR has been performed on 49 samples obtained from 11 patients. In 20 samples collected from patients in long-term morphologic CR, MRD was undetectable. Interestingly, high frequencies of either IFNg- or IL17-producing NPM1mut-specific T-cells were persistently found in either BM or PB samples collected from 7 patients more than 12 months after AML diagnosis. Moreover, cytokine production and memory T-cell profiles, analyzed by flow cytometry, showed expansion of NPM1mut-specific cytotoxic BM T-cells producing IFNg and TNFa, mainly Effector Memory T-cells, both CD8+ and CD4+. We observed for the first time the spontaneous development of NPM1mut-specific T-cell immunity, regardless of MHC-restriction, in the BM of patients with NPMc+ AML, which may contribute in maintaining long-lasting remission. Further studies are warranted to define a potential role of immunotherapeutic approaches in NPMc+ AML.

C009

LOW-DOSE LENALIDOMIDE AND CYTARABINE COMBO PRODUCES AN HIGH COMPLETE REMISSION RATE THAT CAN BE PREDICTED BY GENETIC PROFILING IN AML PATIENTS AGED ≥70 YEARS: FINAL RESULTS OF A PHASE II STUDY

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We designed a phase II study to assess the efficacy of low-dose lenalidomide (10 mg/day orally, days 1-21) and cytarabine (20mg twice day sc, days 1-15) combo in patients with AML aged ≥70 years. The primary outcome was complete remission rate (CR+CRi) according to the SWOG criteria. Fixing P0 as 17% and P1 as 30% (α=0.05, 1-β=0.90), according to the MiniMax design, the sample size was estimated to be 66 patients. Sixty-six patients (median age: 76 years, median WBC at diagnosis: 3.9x10 9/l) were enrolled. 36/66 patients had an intermediate, 25/66 an unfavorable karyotype and 5/66 were not evaluable. Twenty-eight patients had a *de novo*, whereas 38 patients had a secondary AML. Therapy was repeated every 6 weeks, up to 6 cycles. To identify possible biomarkers associated to sensitivity/resistance, global gene and miRNA

expression profiling (Affymetrix Transciptome Array 2.0) was performed on purified AML cells obtained from 26 patients. The cumulative induction-period mortality was 13%. According to intent-to-treat, CR rate was 36.3% (24/66 patients), PR rate was 3% (2/66) with an overall response rate (ORR) of 39.3% (26/66). Five patients died in CR/PR, whereas 6/26 responding patients are still in CR after a median follow-up 358,5 days (range: 89-1787). Responding patients had a longer median overall survival than non-responders (375 *vs* 70.5 days, P <0.0001). Cytogenetic risk and BM blats at diagnosis were not predictive of CR. Conversely, by studying the miRNA and gene expression profile in 26 patients, we identify a molecular signature, including 306 genes and 3 miRNA associated with the clinical response (CR *vs* no CR). Noteworthy, the involved genes belonged to relevant functional categories. Based on the expression of such genes/miRNA, treatment response could be predicted with high accuracy (88.5%, 23/26 samples correctly classified), with 87% sensitivity, 91% specificity, and 93% positive predictive value for responders. Finally, we developed an algorithm to predict response based on the expression of 16 genes only, that correctly identified 14/14 CR patients. In conclusion, the low-dose lenalidomide and cytarabine combo met the primary endpoint of study. Moreover, the achievement of CR could be efficiently predicted by the gene expression pattern. The study was registered at EMA with the EUDRACT no 2008-006790-33. *Acknowledgements:* Celgene is acknowledged for providing Lenalidomide. *The study was supported in part by AIL Pesaro Onlus*.

C010

PML/RAR KINETICS AND IMPACT OF FLT3-ITD MUTATIONS IN NEWLY DIAGNOSED LOW-INTERMEDIATE RISK ACUTE PROMYELOCYTIC LEUKEMIA TREATED WITH ATRA AND ATO OR ATRA AND CHEMOTHERAPY

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The results of the APL0406 Italian-German randomized trial recently showed the arsenic trioxide (ATO) and All-trans retinoic acid (ATRA) combination is superior to the standard ATRA plus chemotherapy (CHT) in newly diagnosed low-intermediate risk acute promyelocytic leukemia (APL). We analysed by RQ-PCR the kinetics of PML-RAR transcripts and assessed the prognostic impact of FLT3-ITD mutations in patients enrolled by the Gruppo Italiano Malattie EMatologiche dell'Adulto (GIMEMA) in the same trial. All molecular studies were centralized and carried out at a single reference laboratory. By analysing 100 patients for PML/RAR kinetics (51 and 49 in the ATRA-ATO and ATRA-CHT arms respectively), a higher rate of PML-RAR RQ-PCR positivity (78.4% *vs* 59%=p 0.05) and a slower post-induction PML-RAR clearance were detected in the ATRA-ATO, compared to the ATRA-CHT group. No correlations were found in either arms between the log-reduction of PML-RAR transcripts after induction and probability of relapse. By contrast, a significantly greater log-reduction of PML/RAR transcripts was detected in the ATRA-ATO compared to the ATRA-CHT cohort after the 3rd consolidation course (5.8 logs *vs* 5.0 logs; p=0.05). FLT3-ITD mutations had no significant impact on EFS and CIR in either arms; however, a trend for inferior event-free survival (EFS) and higher cumulative incidence of relapse (CIR) was observed in the FLT3-ITD-positive group receiving ATRA-CHT. Overall, our results show at the molecular level a greater anti-leukemic efficacy of ATRA-ATO compared to ATRA-CHT in patients with low-intermiated risk APL and confirm the lack of prognostic significance of post-induction RQ-PCR evaluation in both treatment contexts. Finally, our data indicate that ATRA-ATO treatment is equally effective in these patients regardless of FLT3 mutational status.

C011

TOSEDOSTAT PLUS LOW DOSE CYTARABINE COMBO INDUCES A HIGH RATE OF RESPONSES THAT CAN BE PREDICTED BY GENETIC PROFILING IN AML: FINAL RESULTS OF A PHASE II MULTICENTER STUDY

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We designed a phase II study to evaluate the efficacy of Tosedostat (120 mg/day orally, days 1- 240) and low-dose cytarabine (20mg twice day sc, days 1-10) combo in patients with AML aged ≥60 years. The primary outcome was complete remission rate (CR+CRi) according to the SWOG criteria. Fixing P0 as 10% and P1 as 25% (α=0.05, 1-β=0.90), according to the Fleming's method, the sample size was estimated to be 33 patients. Thirty-three patients (median age: 75 years, median WBC at diagnosis: 3.05x10 9/l) were enrolled. 17/33 patients had an intermediate, 13/33 an unfavorable karyotype and 3/33 were not evaluable. Sixteen patients had a *de novo*, whereas 17 had a secondary AML. Therapy was repeated every 4 weeks, up to 8 cycles. To identify possible biomarkers associated to sensitivity/resistance, global gene expression profiling (GEP, Affymetrix Transciptome Array 2.0) was performed on purified AML cells obtained from 29 patients. Induction-period mortality was 12%. According to intent-to-treat, CR rate was 45.4% (15/33 patients). Interestingly, 3/33 additional patients obtained a PR, translating in an impressive overall response rate of 54.4%. In addition, 4/33 patients remained in stable disease for a median time of 9 months (range: 4-14). Seven patients did not respond at all and died with progressive disease after having received a median of 2 cycles of cytarabine and 45 days of tosedostat. The median time for the achievement of best response was 74 days (range 22-145). Responding patients (CR+PR) had a longer median overall survival than non-responders (P=0.018). Ten out of 18 (55%) responding patients are still in remission (8 CR, 2 PR) after a median follow-up of 319 days (range: 47-548); 2 patients are still alive with stable disease. Twenty-one patients died so far. As far as GEP analysis was concerned, we studied 29 cases and identified a molecular signature associated with the clinical response (CR *vs* no CR). Based on that, an algorithm to predict the clinical response was developed and validated. The tosedostat and low-dose cytarabine combo met the primary endpoint of study (CR rate: 45.4% *versus* 25% expected). Moreover, the achievement of CR could be efficiently predicted by the gene expression pattern. Further, potential biomarkers were identified by GEP. The study was registered at EMA with the EUDRACT n.2012-000334-19. *Acknowledgements:* Cell Therapeutics for providing Tosedostat; AIL Pesaro Onlus for supporting the study.

C012

ACTIVATION OF P2X7 RECEPTOR BY ATP AS A NOVEL TARGET FOR THE DEVELOPMENT OF NOVEL THERAPEUTIC STRATEGIES IN ACUTE MYELOID LEUKEMIA

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ATP is the key energy currency as well as a ubiquitous extracellular messenger. Depending on its dose and the purinergic P2 receptor (P2R) subtype engaged, ATP can trigger many different cell responses, including proliferation and cell death. Among the receptor engaged by ATP, P2X7 is the most consistently expressed by tumor cells and its overexpression is related to tumor growth and progression. Interestingly, high ATP level have been reported to exhibit direct cytotoxicity on many tumor cell type. Recently, we showed that ATP is a potent stimulator of normal stem cell compartment while it has an inhibitory effect on acute myeloid leukemia (AML) cells. Since it is a potent stimulator of HSCs while it has an inhibitory effect on leukemic cells, ATP could be a good candidate for developing innovative therapy with low toxicity on normal stem cell compartment, essential for a complete hematologic recovery after therapy. Our hypothesis is that ATP dosage may be particularly useful for the treatment of P2X7 positive leukemias, *i.e.* AML. In this study, AML samples (n=20) were collected from bone marrow and peripheral blood of patient at diagnosis before treatment (percentage of circulating blasts >90%) and normal HSC were isolated from leukapheresis products of 5 healthy donors receiving GM-CSF. Our data demonstrate that AML cells express high level of P2X7 and that its activation with high dose of ATP induces cell death of blast cells while is not effective on normal CD34+ viability. Interestingly, we demonstrated that P2X7 is also expressed by leukemic stem cells and ATP treatment causes apoptosis through caspase cascade in each subset analysed: CD34-CD38-, CD34+CD38-, CD34+CD38+ and CD34-CD38+. Of note, a direct cytotoxicity on HSC subpopulations was not observed. The efficacy of ATP treatment is also confirmed by *in vivo* experiment. In particular, ATP i.p. administration significantly reduces the growth of leukemia cells in immunodeficient mice transplanted with human AML cells. Novel therapeutic approaches that aim to reduce toxicity and to improve the efficacy of treatment are expected to greatly improve long-term outcomes in AML patients. Overall, our results may provide the biological rationale to use P2X7 as a target for novel anti-leukemia therapeutical approaches.

Infections

C013

FINAL RESULTS OF THE PROSPECTIVE STUDY ON THE ROLE OF BED-SIDE ULTRASOUND IN NEUTROPOENIC ENTEROCOLITIS: 76 NEC OUT OF 1680 NEUTROPENIC EPISODES. EARLY ULTRASUOND REDUCE MORTALITY AND CHANGE DIAGNOSTIC CRITERIA WITH A NEW DISCRIMINANT STATISTICAL MODEL

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Neutropenic enterocolitis (NEC) is a life threatening complication of patients (pts) treated with chemotherapy (CHT) with mortality rate up to 50%. It is characterized by abdominal pain (AP), fever (F) and diarrhoea (D). Ultrasound (US) was used to evaluate bowel-wall thickening (BWT), and >4 mm is considered diagnostic of NEC. Early diagnosis is crucial to start conservative medical management (CMM) which appears the optimal strategy for most cases. Objective: evaluate prospectively if Bed-side-US(BUS) can detect early signs of NEC and guide a prompt treatment (CMM or surgical) in order to reduce mortality. *Methods:* in the last 7 years all pts admitted in Our Hematology/BMT Unit at University of Pisa (Italy), undergoing chemotherapy (CHT), autologous or allogeneic transplant (AutoTx, AlloTx) were enrolled. Abdominal US was performed, baseline before treatment, and as only one symptom (or a combination) appeared within 12h from onset: F and/or D and/or AP in neutropenic pts. *Results:* out of 1680 neutropenic pts 76 episodes were identified (4.7%). Disease diagnosis were HD (N=10), ALL (N=8), AML(N=21),MM (N=9) and NHL (N=28). Treatment received was intensive CHT (N=35), AutoTx (N=37) and AlloTx (N=4). At time of diagnosis (Dx) symptoms were: F+AP+D 48%, F+D 4%, F+AP 1%, AP+D 34%,D 3%,AP 9%. At Dx, F was absent in 46% of pts. As control group (CG) we considered pts with CHT related mucositis and pts restaged with US during neutropenia in absence of symptoms. A total of N=509 pts were randomly chosen and none of them had BWT. Overall 11 pts died (14.5%). In pts treated with CMM (92%) mortality was 11.5%. Six pts underwent surgery and 50% are alive. The likelihood of NEC Dx in a discriminant St model (Bayes theoreme) for pts with BWT and AP=98.8%, AP+D=99.9%, AP+D+F=100%, AP+F=99.9%, D+F=5%. In *Conclusions:* BUS allowed to detect early signs of NEC and to start prompt treatment which was CMM in 92% with a 88.5% survival rate. With BUS pts do not live the isolation room. Early diagnosis and intervention allowed to reduce mortality. Images of BUS and CT were superimposable. Fever is not a condition sine qua non for NEC diagnosis. A prompt BUS in neutropenic patients as just one symptom presents allows to make early diagnosis of NEC, guide prompt treatment (conservative or surgical) reducing mortality.

C014

INVASIVE FUNGAL INFECTIONS IN LYMPHOPROLIFERATIVE DISORDERS: A MONOCENTRIC RETROSPECTIVE EXPERIENCE OF "NON HIGH RISK" HEMATOLOGIC MALIGNANCIES

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Invasive fungal infections (IFIs) are serious, life-threatening complications of hematological malignancies, in particular for patients (pts) affected by acute myeloid leukemia (AML) and after HSCT. While data on pts with AML have been updated during the past few years, little information is available on IFIs in lymphoproliferative malignancies. The aim of this study was to examine our clinical experience with IFIs in "lower risk" hematologic malignancies. A retrospective analysis was performed on 1050 adult pts affected from lymphoproliferative disorders

(CLL–307 pts, HL–190 pts, NHL, aggressive–350 pts, NHL, indolent–100 pts, MM–103 pts) diagnosed and treated in our hospital from 1995 to 2013. We included only probable or proven IFI. Data were collected from routine clinical practice in order to provide information regarding fungal infections and in particular epidemiology, diagnosis, treatment and outcome. Underlying hematological disease characteristics were also considered, with particular attention to the onset, status of the disease and multiple lines of chemotherapy or HSCT and the drugs administered. We reviewed the records of 1050 patients with lymphoproliferative disorders; we recorded 226 contaminations (identified as crop positivity, or a GM detection) in 183 patients; we observed a major concentration of positivity in MM and aggressive lymphoma patients (26% in MM and 24% in aggressive lymphomas). After this preliminary screening we identified 40 patients (3.8%) with probable and 3 patients (0.2%) with proven IFI. In our entire population (1050 patients), the incidence of probable/ proven IFI was 4% (molds 1.7%, yeasts 2%, mixed infections 0.3%) (Table I). The most suitable population for IFI were MM patients (incidence of IFI 13%), followed from NHL aggressive (4%) and HL (4%). According to our experience indolent NHL and CLL patients were less exposed to IFI (3% and 2% respectively). Recent studies identify chronic lymphoproliferative disease as an "emergent" high risk population for IFI. Our data are in line to the recent Italian study published (Nosari et al), who identified a 3% of incidence of IFI among the same category of patients. Despite the lower frequency of neutropenia and the different standard approach treatment to AML, new treatment strategies could enhance the risk of mycoses and require continuous revision of the specific risk factors for fungal infections also among patients with lymphoproliferative disorders.

Table 1. Incidence of fungal infections in different lymphoproliferative disorders.

Underlying disease	Population	IFI incidence (%)	Molds (%)	Yeasts (%)	Mixed etiology (%)
CLL	307	5 (2%)	0%	2%	0%
HD	190	8 (4%)	2%	2%	0%
NHL aggressive	350	14 (4%)	2%	2%	0%
NHL indolent	100	3 (3%)	1%	2%	0%
MM	103	13 (13%)	6%	5%	2%
Total	1050	4%	1,7%	2%	0.3%

C015

PREDICTORS OF MORTALITY IN 100 PATIENTS WITH NEUTROPENIC ENTEROCOLITIS AFTER CYTOTOXIC AGENTS FOR ACUTE MYELOID LEUKEMIA

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Background: Neutropenic enterocolitis (NEC) is a life-threatening complication in patients with acute myeloid leukemia (AML) treated with intensive chemotherapy for obtaining hematological remission. Antimicrobial management of this intestinal infection is a major challenge for clinicians. *Aims:* We analyzed the predictors of mortality for NEC occurring in AML patients undergone to intensive chemotherapy, in order to evaluate the optimal management and the risk factors for NEC. *Methods:* In this mono-centric retrospective cohort study, during the period 2002-2012, we examined 100 patients with NEC which was preceded by cytotoxic agent administration for AML containing different dosages of cytarabine (CY): 30 patients received standard doses of CY (100 mg/m^2 daily), 19 intermediate CY doses (1000 mg/m^2 daily) and 51 high CY doses (6000 mg/m^2 daily). The outcome measured was death within 30 days of NEC onset. Survivor and nonsurvivor subgroups were compared. Empirical treatment regimen was defined as the initial antibiotic therapy of neutropenic fever while post-US treatment regimen was the antibiotics used at the time of ultrasonographic (US) diagnosis of NEC. *Results:* The overall 30-day mortality rate was 23%. A significantly increased mortality rate was observed among patients who have received cytotoxic agent schedule based on high-dose CY (p<0.001) and among patients undergone to tigecycline-sparing empirical antimicrobial regimens (27% *vs* 9% in those who received this drug; P=0.019) and post-US antimicrobial regimens including non more of 2 antibiotics (P=0.02). Whereas, post-US combination treatment with tigecycline, daptomycin and meropenem was predictive of a favorable outcome (P=0.036). In logistic regression analysis, 30-day mortality was independently associated with high-dose CY-containing induction chemotherapy (OR: 0.089; 95% CI: 0.024-0.329; P<0.001). The post-US treatment regimen of NEC with tigecycline, daptomycin and meropenem was associated with lower mortality (OR: 5.096; 95% CI: 1.516-17.131; P=0.008). *Conclusions:* NEC following chemotherapy containing increased dosage of cytarabine is associated with high mortality. To improve 30-day survival, combined treatment with 3 antibiotics with specific activity against complicated intra-abdominal infections, especially those also including tigecycline, may be particularly effective.

C016

INVASIVE PULMONARY ASPERGILLOSIS IN ALLOGENEIC BONE MARROW RECIPIENTS WITH - GLOBIN GENE DIRORDERS: INCIDENCE AND OUTCOME

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Background: Invasive Pulmonary Aspergillosis (IPA) is a devastating opportunistic infection and remains a significant cause of morbidity and mortality in allogeneic Haematopoietic Stem Cell Transplantation (HSCT) recipients. IPA has been well characterized in adults and in the setting of oncological transplant. No data are available regarding IPA in patients with - globin gene disorders undergoing bone marrow transplant (BMT). OBJECTIVE: To evaluate the incidence and the outcome of IPA among BMT recipients with -Thalassaemia Major or Sicke cell Anaemia (SCA). *Methods:* We evaluated the occurrence, the clinical setting and the clinical outcome of IPA in pediatric patients affected by Thalassaemia major or SCA transplanted at our institution. *Results:* A total of 276 consecutive patients (median age 9,3, range 11,7-28 years) with - globin gene disorders who underwent BMT (198 HLA-identical, related donor; 52 haplotype-identical donor, 22 HLA-mismatch, related donor and 4 matched, unrelated donor) were studied. Overall, the incidence of proven or probable IPA was 2.1% (6 out of 276 cases). The median time to onset IPA infection after transplantation was 68 days (range, 13-183 days). In particular, 3 cases (50%) were diagnosed after post-BMT day 60 and 3 (50%) were diagnosed during the post-BMT neutropenic period before engraftment. Graft-*versus*-host-disease (GVHD) was present in 5 (83.3%) of 6 patients with IPA, compared with 70 (25.9%) of 270 patients without fungal infection (P=0.01). Among 6 cases with IPA an alternative donor (matched unrelated and haplotype-identical) was used in 3 patients (50.0%) compared with 53 cases (19.6%) of 270 recipients without IPA (P=0.06). The infection remained confined in the lunge in 5 (83.3%) of 6 IPA cases, in 2 cases surgical intervention was adopted in addition to the adequate systemic anti-fungal medical therapy; only in 1 case the infection was multifocal with CSN involvement. The overall mortality rate for IPA was 0.74% (2 of 276 patients) whereas the IPA attributable mortality rate observed in our population was 33.3% (2 of 6 cases). *Conclusions:* Our data show that in a population affected by - globin gene disorders who undergoing allogeneic BMT, the IPA rarely develop (2,1%) and the IPA attributable mortality rate (33.3%) is markedly lower then the one observed in the setting of haematological malignancies. In our cohort, a significant risk factors for IPA was GVHD.

C017

RISK FACTORS AND OUTCOME OF INFECTIONS BY MULTIDRUG RESISTANT GRAM NEGATIVE BACTERIA IN PATIENTS UNDERGOING HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Infection and colonization by gram negative (GN) bacteria showing acquired non-susceptibility to at least one agent in three or more antimicrobial categories represents a challenging problem in recipients of hematopoietic stem cell transplantation (HSCT) for the management of post-transplant complications and also for the eligibility to transplant in colonized patients. Between January 2013 and December 2014 we prospectively evaluated risk factors and outcome of infections by multidrug resistant (MDR) GN bacteria in 199 consecutive patients receiving auto- (99) or allo-SCT (100). Throat, nasal and rectal swabs and urine culture were collected at admission in all patients. Strict isolation measures were adopted and targeted antibiotic therapy was promptly instituted in case of fever in colonized patients. Cumulative incidence of MDR infections was 3% after auto-SCT and 16% after allo-SCT, with a median time between transplant and MDR infections of 8 days (1-41). Overall, 21 patients developed 27 infective episodes, involving blood (41%), urogenital tract (29%), lung (15%) and bowel (15%). The pathogens were: Pseudomonas Aeruginosa (70%), carbapenem resistant Klebsiella Pneumoniae (26%), E. Coli (4%). In univariate analysis development of MDR infections was significantly associated with acute leukemia (p<0.001), allo-SCT (p=0.001), reduced-intensity conditioning (p=0.04), delayed platelets recovery (p=0.04), history of infection or colonization by MDRGN bacteria before transplant (p<0.001), positive swabs at transplant (p=0.04) and concomitant probable/proven invasive mycoses (p=0.01). The development of MDR infections influenced the outcome after transplant only in the allo-SCT setting. In fact, patients who developed MDR infections after allo-SCT had a significantly higher NRM and a significantly lower OS in comparison with unaffected patients (100 days-NRM=49% *vs* 18%, p=0.01; 1 year-OS=39% *vs* 71%, p=0.009) (Figure 1 and Figure 2). In multivariate analysis independent predictors for poor OS were: refractory disease at transplant (p=0.023), severe acute GVHD (p=0.009) and development of MDR infections after transplant (p=0.025). We conclude that despite surveillance swabs, strict isolation and adequate first-line treatment of MDR GN carriers, MDR infections had a significant and independent negative impact on outcome of allo-SCT recipients. A careful evaluation of the risk-benefit ratio for performing SCT is needed in colonized patients.

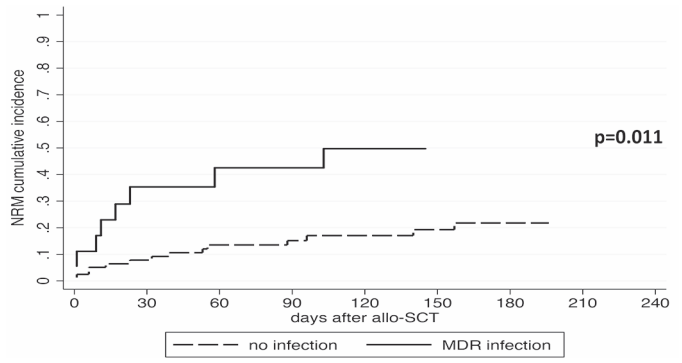

Figure 1. NRM according to MDR infection.

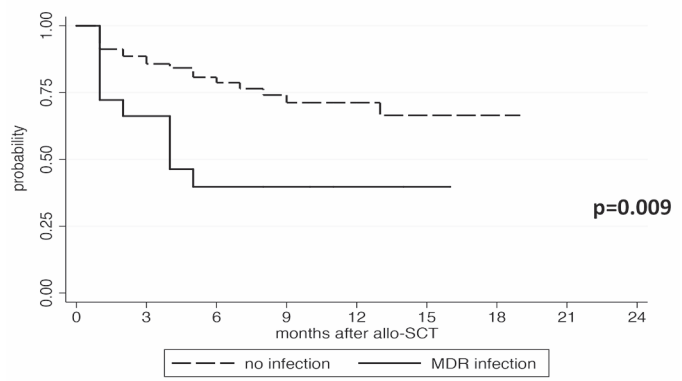

Figure 2. OS according to MDR infections.

C018

PROSPECTIVE MULTICENTER STUDY ON AZOLE RESISTANCE IN ASPERGILLUS ISOLATES FROM SURVEILLANCE CULTURES IN HAEMATOLOGICAL AND HEMATOPOIETIC STEM CELL TRANSPLANTATED PATIENTS (ARTE STUDY)

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Invasive aspergillosis (IA) is a severe complication in patients with haematological malignancy and in those undergoing hematopoietic stem cell transplantation (HSCT). Azole derivatives are the antifungals most used both in prophylaxis and in empirical and targeted therapy. The diagnosis of IA relies mainly on indirect methods (galactomannan). The difficulty in obtaining biological samples from deep tissues in critically ill patients prevents the isolation of the fungus. The colonization of the upper airways represents the first step in the development of pulmonary infection. Surveillance cultures (nasal and oropharynx) are carried out in several centers of hematology and transplantation. The emergence of multi-azole-resistance has been recognized in Europe and also in Italy with resistant Aspergillus fumigatus isolates from both patients on antifungal treatment and naive patients and from the environment. It was shown that patients with IA caused by a multiazole-resistant strain have a mortality rate of 88% compared to a rate of 30-50% for patients infected by a sensitive strain. The dominant mechanism of resistance involves point mutations of CYP51A, gene that codifies for the target enzyme of antifungal azoles. A prospective multicenter study (ARTE) was set up: 1) to centralize Aspergillus isolates from surveillance swabs or bronchial secretions of patients with haematological malignancies or submitted to HSCT; 2) to analyze the pattern of susceptibility of isolates to antifungal azoles; 3) to investigate, in the presence of resistance, the molecular mechanism; 4) to correlate the resistance to demographic and behavior variables and previous antifungal treatments. Twenty-five hematology centers agreed to the ARTE project and up to now 8 centers collected 50 isolates from 41 patients (21-85 year old; 57% living in rural area or exposed to other risk factors). The species more frequently isolated were: A. fumigatus (23), A. flavus (8), A. niger (7), A. terreus (4). All isolates were susceptible to the tested azoles except one A. niger resistant to itraconazole. The continuation of the study will add data useful for a best management of IA in this patient setting.

Thalassemias and Hemoglobinopathies

C019

PULMONARY DYSFUNCTION IN THALASSEMIA MAJOR AND CORRELATIONS WITH BODY IRON STORES

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Background: Although abnormalities in pulmonary function in thalassemia major (TM) patients have been described since 1980, the exact pathogenetic mechanism for the development has not been elucidated. Furthermore, literature data are, to some extent, contradictory regarding the type of lung dysfunction: even if the majority describes a restrictive pattern, some have found a predominant obstructive disease. These discrepancies could be attributed to the heterogeneity of the studies as well as to the multifactorial nature of the pathogenesis. *Aims:* The aim of this study was to evaluate the prevalence and pattern of pulmonary dysfunction in adult TM patients and to investigate possible correlations with iron parameters (serum ferritin and heart and liver T2* values). *Methods:* We retrospectively analyzed 73 TM patients followed at our Rare Disease Center at Policlinico Hospital in Milan, who performed pulmonary function test (PFT) between January 2012 and December 2014. All patients underwent body plethismograph and almost all of them (63 patients) carbon monoxide diffusion (DLCO, single breath method). We also performed complete blood tests and T2* MRI to assess myocardial and liver iron load. *Results:* Overall 73 TM patients (24 males, 49 females) underwent PFT. Mean age was 37±7 years. Restrictive lung disease was present in 26 (35.6%) patients associated with obstructive lung disease in 2 of them. Serum ferritin levels were higher in patients with restrictive pulmonary pattern compared to patients with normal pulmonary function (1526 ng/ml *vs* 975.17 ng/ml, p <0.05). Restrictive lung disease did not correlate with cardiac or liver iron overload. No significant differences were observed in PFT considering age. Twenty-five (25/63, 39.7%) patients had decreased DLCO after correction for lung volume and hemoglobin. No significant correlation was observed between DLCO and ferritin or MRI liver or cardiac T2*values. *Conclusions:* In our data restrictive pattern was predominant in TM patients; we observed a correlation with serum ferritin levels suggesting that iron, particularly its chronic effect, could play a role in the pathogenesis of pulmonary disease in thalassemia. However, as for literature, we could not find a correlation between restrictive pulmonary pattern and heart or liver iron overload. It is possible that differences in iron kinetics and local acting factors as well as the chelation history may underlie these results.

C020

CHRONIC ADMINISTRATION OF HYDROXYUREA AND OUTCOMES IN PATIENTS WITH SICKLE CELL DISEASE AT A SINGLE REFERRAL INSTITUTION

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Introduction: Since HU has been approved for patients with SCD, it is not clear what proportion is taking a therapeutic dose (≥15mg/kg/day) or whether those who were treated with subtherapeutic doses (<15mg/kg/day) benefit from HU. We conducted this analysis to answer these important questions in a single center in Palermo. *Methods:* Patients were enrolled at the Haematology Department of Ospedale V. Cervello between January 2000 and April 2014. Laboratory parameters and frequency of vaso-occlusive crisis (VOC) and acute chest syndrome (ACS) were recorded. Blood counts, fetal hemoglobin (HbF), and changes in SCD complications were compared between the first and last visits and across 3 groups: patients who never took HU were combined with those who suspended HU (no HU group, n=50, 36%); HU <15mg/kg (n=30, 21%); or HU ≥15mg/kg (n=60, 43%). *Results:* There were a total of 140 patients: 25 HbSS, 54 HbS 0thal, and 61 HbS +thal. Median follow-up was 6.6 years. The median age was 35 years (range 0.4-61 years). 28% of patients never took HU, and 8% suspended HU treatment during the follow-up. Among patients taking <15mg/kg HU at first visit, about half stayed in the same dose range (<15mg/Kg/day) and half increased to the ≥15mg/kg dose range. Among patient taking ≥15 mg/kg, 17% decreased to <15mg/Kg/day due to cytopenia; 83% stayed on the ≥15 mg/kg. White blood cell (WBC) counts were lower in both HU groups, but comparing first and last visits, the change in WBC within each group was insignificant (P all >0.05, Table 1). Similarly, the change in total hemoglobin levels within each group was also insignificant (P all >0.05). HbF decreased in the no HU group. Both HU treatment groups had modest increases in HbF (P=0.004, 0.001). With respect to SCD complications, the no HU group had less severe disease at the first visit, with lower percent of subjects with and fewer episodes of VOC and ACS (Table 2). While there was an increase in both VOC and ACS with time, this increase was not statistically significant. Both HU treatment groups had a significant reduction in both complications (p<0.0001 in both), and the magnitude of reduction was similar. *Conclusions:* About one third of patients with SCD never took or discontinued HU. While these patients may have less severe disease initially, their rates of complications increased during follow-up. Among those taking HU, dose adjustment was common. HU increased HbF and is associated with reducing VOC and ACS.

Table 1. Hematologic parameters based on HU status.

	First Visit			Last Visit		
	NO HU	HU <15mg/kg	HU >15mg/kg	NO HU	HU <15mg/kg	HU >15mg/kg
WBC (k/uL)	11.2	8.74*	10.9	10.7	7.8*	8.3*
Hgb (g/dL)	10.0	10.2	10.0	10.2	9.7	9.9
HbF (%)	11.9**	9.4*	10.7*	7.7	11.7*	12.8*

*P<0.05 compared to no HU group
**includes 4 subjects with hereditary persistent of HbF and 2 children aged 3 months.

Table 2. SCD complications based on HU status.

	VOC				ACS			
	% of subjects		Crisis per patient per year		% of subjects		Mean episodes per patient	
	F V	L V	F V	L V	F V	L V	F V	L V
No HU	60	70	2	2.5	22	28	0.2	0.28
HU<15mg/kg	90	56.6	4.3*	1.2*	50	20	0.7*	0.23
HU>15mg/kg	96.6	60	4.1*	1.1*	51	25	1.1*	0.32

FV, first visit; LV, last visit
* P<0.05 compared to no HU group

C021

STABLE AND FULL PRODUCTION OF FETAL HAEMOGLOBIN AFTER ALLOGENEIC BONE MARROW TRANSPLANT IN PATIENTS WITH THALASSAEMIA MAJOR: CLINICAL REMISSION WITHOUT TRANSFUSION SUPPORT

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Introduction: High fetal haemoglobin (HbF) levels ameliorate morbidity and mortality in Sickle Cell Anaemia (SCA) and β-thalassaemia. The variability of HbF levels is genetically controlled by multiple genes and recent studies provide new insight into the molecular mechanisms in order to induce the HbF production in adult haemopoietic cells as a promising therapeutic approach to ameliorate the severity high levels of HbF ameliorate the severity of the β-disorders. A strong support to such novel approaches comes from recent clinical observations carried

out by our Group. *Methods:* Out of 276 consecutive patients with β-haemoglobin disorders undergoing allogeneic Bone Marrow Transplant (BMT) at our institution, we observed 3 BMT recipients who developed the reactivation of HbF synthesis after BMT failure and autologous reconstitution. *Results:* Three patients with β°-thalassaemia major underwent BMT and rejected at +40, +90 and +18 days after transplant respectively. The autologous recovery was documented (0% residual donor cells) in all cases. Transfusion therapy was required to support anaemia until +118, +162 and +178 days after transplant respectively. Afterwards the Hb levels were steadily over 10.2 g/dl (range 10.2-11.8 gr/dL) without the use of transfusion support and the Hb electrophoresis revealed HbF 99.8% in all 3 cases. At +93, +82 and +17 months respectively of ongoing follow-up after graft failure, all 3 patients maintain the sustained and full (99.8%) production of HbF and are transfusion-free. The genetic analysis documented that all patients were carrier of the non-deletion form of hereditary persistence of HbF. The 3 thalassaemic patients exhibit the homozygosity for the –158 (C->T) point mutation in the G promoter sequence. *Conclusions:* Our study showed that the reactivation of HbF synthesis can occur in the adult age and the high levels of HbF provide a therapeutic benefit to the β-disorders. It is likely that the favorable genetic background in these patients concurred the full HbF production.

C022

SUCCESSFUL TREATMENT OF "HARD-TO-CHELATE" β-THALASSAEMIA MAJOR PATIENTS WITH DAILY ALTERNATING DEFERASIROX AND DEFERIPRONE

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Chelation therapy for treating iron overload has changed the prognosis for patients with β-thalassemia major. Three chelating agents are currently available for the treatment of iron overload in patients with β-thalassaemia major: deferoxamine (DFO), deferiprone (DFP), and deferasirox (DFX). Here we report the safe and effective treatment of iron overload with an alternating regimen of deferiprone and deferasirox, this regimen may represent a viable treatment option for patients who are unable to tolerate daily monotherapy. Here we present long-term experience with five such "hard-to-chelate" patients that present a practical challenge to the treating physician. Causes of not eligibilty to mono- and or dual DFO-DFP therapy were: systemic skin reactions (pz 1-2-4-5), significant proteinuria (pz 1-3), arthralgia (pz 1-5), neutropenia (pz 2). *Patients:* Mean age was 33.4 +/-7.7 years, 4 female and 1 male. Mean follow-up of the alternating daily therapy was 54 months +/- 22.2 months. All patients were splenectomized. Liver and cardiac Iron Overload were monitored by MRI-T2star. At the start of the therapy cardiac iron-overload was present in three of five patients, grade severe (T2star<10 msec) in one patient, liver iron-overload was present in two patients. *Results:* The alternating regimen has been found effective in removing iron from the heart and the liver as well as preventing (Figure 1). The patient has not experienced any adverse events related to therapy.

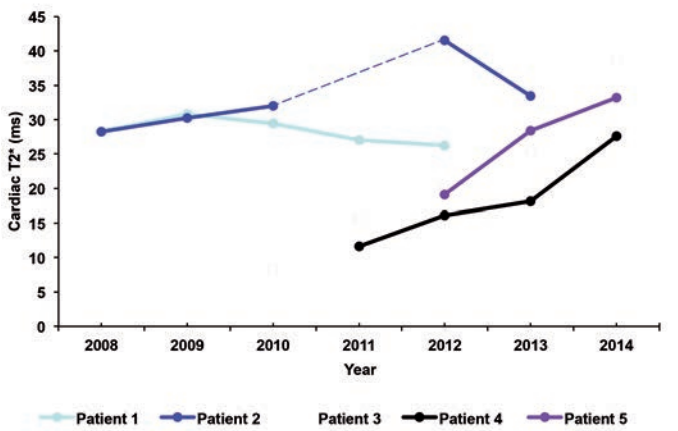

Figure 1.

Conclusions: In this case series, an alternating regimen of deferiprone and deferasirox has shown tolerability and proven effective in reducing iron overload or maintaining safe iron levels in the heart, liver, and even pancreas, in five patients who were unable to tolerate monotherapy and thus continued to accumulate iron up to high risk levels. Larger studies are warranted to investigate this regimen as a potential treatment option for a broader spectrum of -thalassaemia major patients.

C023

POTENTIAL ROLE OF HEMATOPOIETIC PERIPHERAL CIRCULATING BLOOD STEM CELLS AS AN INDEPENDENT MARKER OF GOOD CLINICAL OUTCOME IN PATIENTS WITH β-THALASSEMIA

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Aims: Aim of the current study was to prospectively evaluate the potential role of peripheral circulating CD34+ stem cells as new independent markers of appropriate hemopoietic balance in patients with thalassemia major and intermedia. *Materials and Methods:* Peripheral circulating CD34+ stem cells, CF-GEMM, CFU-GM and BFU-GM were assayed with monoclonal antibodies for CD34 and clonogenic tests, according to standard procedures and ISHAGE method (BD stem cell enumeration kit, Becton Dickinson; H4434, Stem Cell Technology). Peripheral blood samples from patients with thalassemia major (TM) and intermedia (TI) were taken, results were compared with healthy controls. Demographic and clinical data were recorded from each enrolled subject. *Results:* Overall, 56 patients with thalassemia major (median age: 35 years, range: 13-52 years) and 13 with thalassemia intermedia (median age:44 years, range: 27-67) were evaluated. Annual red blood cells transfusion requirements ranged from 10 to 65 in all the patients except three with thalassemia intermedia, that were transfusion independent. One patient with TM did not accept transfusion for religious reasons. A statistically significant increase in peripheral circulating stem cells was observed in all the patients, in comparison with healthy controls. CD34+cells were 6.9±4.5/mmc in patients with TM (p=0.014) and 11.8±14.8/mmc (p=0.051) in patients with TI. Furthermore, only in patients with TI, an increase in CFU-GEMM (3.0±4.8 *vs* 0.75±2.05, p=0.0001) was observed. BFU-E and CFU-GM levels did not show any significant difference. Patients not treated with transfusions showed the mean highest levels of stem cells (CD34: 32.5±14.8/mmc, BFU-E: 41.3±22.8, CFU-GM: 19.6±5.6, CFU-GEMM 9.0±6.1). At multivariate analysis, peripheral circulating CD34+ stem cells did not correlate with age, sex, number of red blood cells units transfused, hemoglobin levels, history of splenectomy and hipotyroidism. *Conclusions:* Circulating peripheral CD34+stem cells are increased in patients with the highest level hemopoietic stress (TI, transfusion independent), thus their determination could represent a useful independent marker of clinical response to transfusion in patients with thalassemia.

C024

MYELOID DYSFUNCTION IN SICKLE CELL DISEASE

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Background: In sickle cell disease (SCD), profound anaemia and severe hemosiderosis cause functional and physiological abnormalities in various organ systems, including immune system. Infectious complications are common, constitute the second most common cause of mortality and a main cause of morbidity. During the haemolytic crisis, large amount of arginase (s-Arg-1) are released, potentially acting as immunosuppressor molecule. Despite its clinical impact, only a few is known about myeloid dysregulation in SCD. *Aims:* Detecting immunological impairment at the steady state evaluating myeloid and lymphoid cells in peripheral blood of SCD patients. *Materials and Methods:* Between May and June 2014,peripheral blood obtained from 30 consecutive SCD patients at the steady state plus 30 healthy subjects was studied for evaluation of myeloid subpopulations and lymphoid paresis. Myeloiddys function was evaluated as percentage and absolute count of circulating myeloid suppressor cells (MDSC) in peripheral blood assessed by flow cytometry as follows: im-MDSC (CD34+/CD11b+/CD13+/CD14-/HLA-DR-/CD45+), neutrophilic-like N-MDSC (CD11b+/CD13+/CD15+/CD14-/HLA-DR-/Lin-) and monocytic-likemo-MDSC (CD14+/HLA-DRlow/-), phagocytic activityof granulocytes using a commercially available kit (Phagotest R), amount of Arg-1 expressed in mature granulocytes by RT-PCR and circulating s-Arg-1 using a commercially available ELISA kit (Biovendor). *Results:* The capability of phagocytosis of granulocytes wassignificantly reduced compared to healthy subjects (p=0.001). G-MDSC subset was not increased, while mo-MDSC subpopulation was increased in SCD (p=0.001) but not in thalasso-SCD. s-ARG-1 was increased in both SCD and thalasso-SCD (respectively 203±3 ng/mL and 248±6ng/mL, p=0.003) and positively correlated with the amount of HbS (r=0.7, p=0.002). Arg-1 expression in granulocytes was increased (20 times higher than healthy controls, p=0.002) *Conclusions:* SCD and thalasso-SCD caucasian patients exhibiti mmunosuppressive myeloid markers including reduced phagocytosis, increased amount of mo-MDSC, Arg-1 expression in granulocytes and circulating s-Arg-1. Further analysis are ongoing to detect if the same myeloid impairment occurs during vase occlusive crisis and in a different genetic background, like in Afro-Americans.

Non-Hodgkin Lymphoma 1

C025

RITUXIMAB, LENALIDOMIDE AND BENDAMUSTINE AS SECOND LINE THERAPY IN PATIENTS WITH MANTLE CELL LYMPHOMA: A PHASE II STUDY OF THE FONDAZIONE ITALIANA LINFOMI

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Background: Lenalidomide (L), rituximab (R) and bendamustine (B) showed significant activity and good safety profile in patients with relapsed or refractory (R/R) MCL. We performed a prospective, multicenter, phase II single arm study, to evaluate in this setting the combination of R, L and B (R2B regimen) 2L therapy. *Methods:* During the induction phase (cycle 1 to 4, every 28 days), patients received R 375 mg/m^2 on day 1, L 10 mg/day on days 1 to 14, B 70 mg/m^2 on days 2 and 3. Patients on CR or partial response (PR) continued to the consolidation phase, which consisted of treatment with R 375 mg/m^2 on day 1 and L 15 mg/day on days 1 to 21 for two cycles every 28 days. Patients in CR and PR at the end of the consolidation phase continued to the maintenance phase with L 15 mg/day on days 1 to 21 every 28 days for additional 18 cycles. The primary objective was CRR. MRD was analyzed by IGH or Bcl-1 based nested (n) and quantitative (q) PCR in peripheral blood (PB) and bone marrow (BM) after the induction and consolidation phase and during L maintenance. Cereblon expression was evaluated by immunohistochemistry on the baseline biopsies and the results were correlated with the outcome (Figure 1).

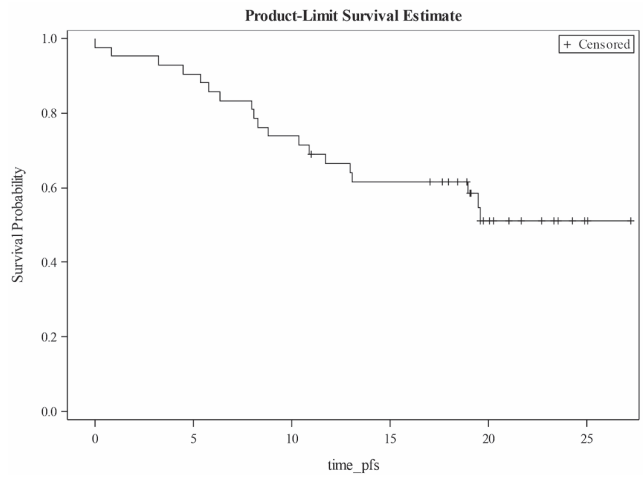

Figure 1. Progression free survival.

Results: Forty-two patients, median age 70 years, were enrolled. ORR and CRR after the induction plus consolidation phases were 79% (33/42) and 55% (23/42), respectively. MRD by n-PCR on PB and BM were 62%

(18/29) and 52% (15/29) after induction and 67% (18/27) and 43% (12/28) after consolidation. After a median follow-up of 20 months the 12 and 24-months PFS and OS were 66% and 51%, 83% and 66%, respectively. At present, no clinical or laboratory parameters, Cereblon expression included, related with the response rate or PFS; at a preliminary analysis there is a trend for better PFS in patients achieving MRD negativity. Grade 3-4 neutropenia and thrombocytopenia were documented during the induction and consolidation phases in 69% and 14% of patients, respectively; 65% of patients experienced grade 3-4 neutropenia also during the maintenance. Non-hematologic toxicity was low. Three patients developed grade 3 (1) and 4 (2) infectious complications during the induction and maintenance phases, respectively. *Conclusions:* The results from this study indicate that R2B therapy has a high therapeutic activity in R/R MCL even in terms of molecular response and is feasible also in elderly pre-treated patients. The final results will be updated in July 2015.

C026

THE IELSG-32 TRIAL: RANDOMIZED PHASE II TRIAL ON PRIMARY CHEMOTHERAPY WITH HIGH-DOSE METHOTREXATE AND HIGH-DOSE CYTARABINE WITH OR WITHOUT THIOTEPA, AND WITH OR WITHOUT RITUXIMAB, FOLLOWED BY BRAIN IRRADIATION *VS* HIGH-DOSE CHEMOTHERAPY SUPPORTED BY AUTOLOGOUS STEM CELLS TRANSPLANTATION FOR IMMUNOCOMPETENT PATIENTS WITH NEWLY DIAGNOSED PRIMARY CNS LYMPHOMA

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Introduction: IELSG #32 is an international randomized phase II trial addressing the tolerability and efficacy of adding rituximab (R)±thiotepa (TT) to methotrexate (MTX)-cytarabine (ARAC) combination, followed by a 2nd randomization comparing consolidation with WBRT or HDC/ASCT in pts with PCNSL (NCT01011920). Herein, we report results of the first randomization. *Methods:* HIV-neg pts 18-70 yo and ECOG PS ≤3 (PS ≤2 if age 66-70) with new histology-proven PCNSL and measurable disease were randomly assigned to receive 4 courses of MTX 3.5 g/m^2 d1+ARAC 2 g/m^2 x2/d d2-3 (arm A); or MTX-ARAC+R 375 mg/m^2 d-5 & 0 (arm B); or MTX-ARAC-R+TT 30 mg/m^2 d4 (arm C). ASC were collected after the 2nd course. Response was assessed after 2nd & 4th courses; pts with responsive disease were further randomized between WBRT and BCNU-TT conditioned/ASCT. Histology and neuroimaging were centrally reviewed. Primary endpoints were CRR (1st random) and 2-year FFS (2nd random). Sample size was estimated on the basis of 2nd random: with P0 65% and P1 85% (one-sided test; 5%; 95%), 52 patients/arm required. *Results:* 227 pts (median age 58 ys; 18-70) were enrolled in 52 centers of 5 countries; 8 pts were excluded due to misdiagnosis, systemic disease or concomitant cancer. No differences in clinical presentation among 3 arms (A 75; B 69; C 75) were observed (Table 1). 733/876 (84%) planned courses were delivered. G4 hematological toxicity was more common in arm C, but infective complications were similar in the 3 arms. G4 non-hematological toxicities were rare. Chemotherapy was interrupted due to toxicity in 21 (9%) pts; 13 (6%) pts died of toxicity. ASC were successfully collected in 152/161 (94%) pts. Arm C was significantly more active, with a CRR of 49% and an ORR of 87%; 118 pts (A 35; B 35; C 48) were referred to 2nd random. At a median follow-up of 20 months (7-60), 111 pts remain failure-free (A 25; B 37; C 49), with 2-yr FFS of 34±6%, 52±6% and 64±6% (p=0.0006), respectively. 124 pts are alive (A 31; B 41; C 52), with 2-yr OS of 40±6%, 58±6% and 66±6% (p=0.01), respectively. *Conclusions:* The addition of TT and R to MTX-ARAC (MATRIX regimen) is associated with significantly improved response, FFS and OS rates in PCNSL pts. With the exception of greater hematological toxicity, MATRIX was not associated with higher rates of severe complications, allowed preservation of antimetabolites dose intensity, and permitted high rates of successful ASC collection.

Table 1.

	A (n= 75)	B (n= 69)	C (n= 75)	p
Median age (range)	58 (18-70)	57 (24-70)	57 (29-70)	NS
Male	46 (61%)	44 (64%)	46 (61%)	NS
ECOG PS >1	27 (36%)	23 (33%)	24 (32%)	NS
Low IELSG risk	14 (19%)	12 (17%)	13 (17%)	NS
Intermediate IELSG risk	47 (63%)	44 (64%)	47 (63%)	NS
High IELSG risk	14 (19%)	13 (19%)	15 (20%)	NS
Intraocular disease	5 (7%)	1 (1%)	1 (1%)	NS
Meningeal involvement	2 (3%)	2 (3%)	3 (4%)	NS
Feasibility and Toxicity				
Actually delivered courses*	223 (74%)	236 (86%)	274 (91%)	
Relative dose intensity MTX	92%	84%	85%	NS
Relative dose intensity ARAC	87%	81%	80%	NS
Relative dose intensity R	-	82%	83%	NS
Relative dose intensity TT	-	-	76%	-
G4 neutropenia*	99 (44%)	119 (50%)	153 (56%)	0.01
G4 thrombocytopenia*	116 (52%)	140 (59%)	200 (73%)	0.0001
G4 anemia*	9 (4%)	6 (3%)	14 (5%)	NS
G≥3 febrile neutrop./infections*	43 (19%)	31 (13%)	45 (16%)	NS
G4 hepatotoxicity*	6 (3%)	3 (1%)	1 (1%)	NS
G4 nephrotoxicity*	0 (0%)	0 (0%)	1 (1%)	NS
Toxic deaths§	7 (9%)	3 (4%)	3 (4%)	NS
Autologous stem cell collection	48/51 (94%)	44/46 (96%)	60/64 (94%)	NS
Median of collected stem cells (x 10^6 CD34+ cells/kg bw)	12.3	15	8.2	NS
Activity and efficacy				
Complete remission rate (95%CI)	23% (14-31)	31% (21-42)	49% (38-60)	A vs. B: 0.29 A vs. C: 0.0007 B vs. C: 0.02
Overall response rate (95%CI)	53% (42-64)	74% (64-84)	87% (80-94)	A vs. B: 0.01 A vs. C: 0.00001 B vs. C: 0.05
2-year Failure-Free Survival 5-year Failure-Free Survival	34 ± 6% 14 ±10%	52 ± 6% 43 ± 8%	64 ± 6% 54 ±11%	A vs. B: 0.01 A vs. C: 0.0001 B vs. C: 0.17
2-year Overall Survival 5-year Overall Survival	40 ± 6% 27 ± 7%	58 ± 6% 50 ± 7%	66 ± 6% 66 ± 6%	A vs. B: 0.05 A vs. C: 0.003 B vs. C: 0.30

C027

IDELALISIB EFFICACY AND SAFETY IN FOLLICULAR LYMPHOMA PATIENTS FROM A PHASE 2 STUDY

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Background: There is an unmet need for new treatment options in fol-

licular lymphoma (FL), particularly for heavily pretreated, high-risk patients refractory to anti-CD20 and chemotherapy. Idelalisib, a PI3K inhibitor, showed antitumor activity and acceptable tolerability as monotherapy in a pivotal phase 2, open-label study in indolent non-Hodgkin lymphoma (iNHL) refractory to rituximab (R) and an alkylating agent (NCT01282424). This post hoc analysis evaluated efficacy and safety in the FL patient subset. *Methods:* Double refractory patients with histologically confirmed iNHL received oral idelalisib 150 mg BID until disease progression (PD) or unacceptable tolerability; patients with FL (grade 1, 2, or 3a; n=72) were included in this analysis. Responses were evaluated by an independent review committee using standardized criteria. The primary endpoint was the overall response rate (ORR). *Results:* At study entry, patients' median age was 62 y, 54% had a high-risk FLIPI score, 22% had bulky disease, and 17% had FL grade 3a. Median (range) number of prior treatments was 4 (2–12); 86% were refractory to their last therapy (32/50 to bendamustine). At data cutoff, median (range) treatment duration was 6.5 (0.6–31.0) mo, with 65 (90%) patients off treatment (PD, 38; adverse events [AEs], 15; investigator decision, 7; death, 5). Lymph node size decreased during treatment by ≥50% SPD in 57%. The ORR (95% CI) was 56% (43–67; P<0.001), including 10 complete responses (CR) and 30 partial responses. Kaplan-Meier (KM)-estimated median (range) time to response was 2.6 (1.6–11.0) mo, median response duration was 11 mo (27 mo in patients with CR), and progression-free survival was 11 mo, substantially longer *vs* the last regimen. Median overall survival (OS) was not reached; KM-estimated OS at 1, 1.5, and 2 y was 87%, 74%, and 68%. The most common AEs (any/grade ≥3,%) were diarrhea (51/14), cough (32/0), pyrexia (29/4), fatigue (28/0), and nausea (28/3). Rates of grade ≥3 transaminase elevation, pneumonitis, neutropenia, anemia, and thrombocytopenia were 14%, 4%, 22%, 3%, and 6%. *Conclusions:* Idelalisib demonstrated rapid, durable responses and acceptable safety in highly refractory, relapsed FL patients with limited treatment options.

C028

THE CODING GENOME OF NODAL MARGINAL ZONE LYMPHOMA REVEALS RECURRENT MOLECULAR ALTERATIONS OF PTPRD AND OTHER JAK/STAT SIGNALING GENES

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Nodal marginal zone lymphoma (NMZL) is one of the few B-cell tumors still remaining orphan of cancer gene lesions. Here we aim at disclosing the pathways that are molecularly deregulated in this lymphoma. The study was based on 35 NMZL with a diagnosis confirmed by pathological revision and lack of clinico-radiological evidence of extranodal or splenic disease. WES (HiSeq 2500, Illumina) and high density SNP array (Cytoscan HD, Affymetrix) of tumor/normal DNA pairs from 18 discovery NMZL, identified 557 non-synonymous somatic mutations affecting 504 genes and 51 copy number abnormalities (CNA). To further characterize mutation recurrence, the 504 discovered genes were investigated in an independent validation panel of 17 NMZL by targeted sequencing of tumor/normal DNA pairs (MiSeq, Illumina). The 17 validation NMZL were also assessed for CNA by high density SNP arrays. By compiling the results of WES and high resolution SNP array, 39 genes were recurrently affected in >3/35 (9%) NMZL by mutations or focal CNA. Among these, MLL2 (34%), PTPRD (20%) and NOTCH2 (20%) were the most frequently mutated genes. Overall, recurrently mutated genes pointed to the molecular deregulation of specific programs in NMZL, including epigenetic modifiers (71% of NMZL), NF-kB signaling (54% of NMZL), cell cycle (43% of NMZL), NOTCH signaling and TLR signaling (40% and 17% NMZL, respectively) (Figure 1A). JAK/STAT signaling was targeted by mutually exclusive lesions in 43% of NMZL, and the protein tyrosine phosphatase receptor δ (PTPRD) tumor suppressor was the most frequently affected gene of this system in 20% of NMZL (Figure 1B-E). PTPRD inhibits JAK/STAT signaling through the dephosphorylation of active pSTAT3. PTPRD lesions in NMZL were represented by somatic mutations that truncated or modified the tyrosine phosphatase domain, as well as deletions of the entire gene locus, including focal and biallelic losses (Figure 1B-C). Interrogation of institutional and public genomic datasets revealed that PTPRD mutations are specific for NMZL, being rare or absent in other mature B-cell tumors, including splenic marginal zone lymphoma (Figure 1D). In conclusion, a number of actionable cellular programs are molecularly deregulated in NMZL. PTPRD lesions are among the most recurrent alterations in NMZL and appear to be specific for this lymphoma type across mature B-cell tumors.

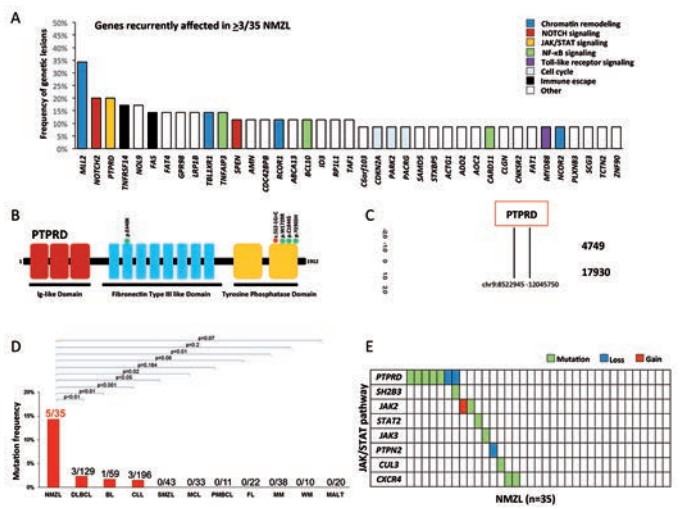

Figure 1.

C029

INTERIM RESULTS OF A PHASE II STUDY OF FONDAZIONE ITALIANA LINFOMI ON GEMCITABINE PLUS ROMIDEPSIN IN RELAPSED/REFRACTORY PERIPHERAL T CELL LYMPHOMA PATIENTS

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Introduction: Relapsed and primary refractory peripheral T-cell lymphomas (PTCL) show a dismal outcome, with 5-years overall survival of 30%. There is no standard salvage chemotherapy for these patients. Gemcitabine has demonstrated to be an effective monotherapy, yielding 60-70% overall response rates in patients with advanced heavily pretreated disease. Romidepsin, a recently Food and Drug Administration-approved histone deacetylase inhibitor, has demonstrated an overall response rate (ORR) of 30% and a complete response (CR) rate of 16%.

A multicentric prospective phase II trial is ongoing to investigate the role of the combination of gemcitabine plus romidepsin (GEMRO regimen) in relapsed or refractory PTCL, looking for a potential synergistic effect of the two drugs. *Methods:* GEMRO regimen contemplates an induction with romidepsin 12 mg/m^2 intravenously (i.v.) on days 1,8,15, and gemcitabine, 800 mg/m^2 i.v. on day 1 and 15, for 6 cycles, every 28 days. After the induction phase, patients in at least a partial remission (PR) proceed onto romidepsin maintenance at the dose of 14 mg/m^2 i.v. until disease progression. The primary endpoint is to evaluate the efficacy, as assessed by CR rate; safety assessment was regarded as a secondary objective. EudraCT number 2012-001404-38. *Results:* Twenty patient were included. At present time, 4 (20%) patients are still on treatment in induction phase and 16 (80%) are evaluable for response and toxicity. The median age of patients was 55 (range, 24-77) years. According to histology, 10 patients had PTCL not otherwise specified, 9 angioimmunoblastic T cell lymphoma, 1 anaplastic large cell lymphoma kinase negative. The median number of prior therapies was 2 (range, 1-4); 7/20 (35%) patients had failed a prior stem cell transplantat. Nineteen out of 20 patients presented with advanced stage. Among the 16 evaluable patients, the ORR was 31% including 2 CRs and 3 PRs. One of the 2 CR patients discontinued the treatment after 4 cycles due to cardiac toxicity, however maintaining a continuous CR with a follow-up of 2 years. Grade >3 adverse events were represented by trombocytopenia (45%), neutropenia (26%), and anemia (9%). *Conclusions:* To date, GEMRO combination regimen shows efficacy data similar to single agent romidepsin as salvage therapy for refractory or relapsed PTCL. More mature data and an adequate follow-up will be required to better understand the role of this combination regimen.

C030

SAFETY AND EFFICACY OF A BRIEF RITUXIMAB, BENDAMUSTINE AND MITOXANTRONE REGIMEN FOLLOWED BY RITUXIMAB CONSOLIDATION IN ELDERLY PATIENTS WITH UNTREATED ADVANCED STAGE FOLLICULAR LYMPHOMA: FINAL RESULTS OF A PROSPECTIVE PHASE II STUDY BY FONDAZIONE ITALIANA LINFOMI

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On the behalf of Fondazione Italiana Linfomi (F.I.L)

Introduction: based on favorable safety and efficacy profile of Bendamustine, we adapted our previous FIL brief chemoimmunotherapy scheme (Vitolo U, JCO 2013) for the treatment of elderly FL patients (pts) by substituting this agent to Fludarabine and Dexametasone, also extending the upper limit of age to 80 yrs. *Patients and Methods:* as already reported (Boccomini C, Haematologica 2013: abs), 76 pts (age 65-80) were enrolled. Inclusion criteria were: stage II-IV disease requiring treatment; "FIT" pts according to geriatric assessment. Treatment plan was: 4 monthly courses of R-BM (375 mg/sqm Rituximab day 1, 90 mg/sqm Bendamustine days 1-2, 8 mg/sqm Mitoxantrone day 1) followed by 4 weekly Rituximab consolidation. Minimal Residual Disease (MRD) analysis by PCR for BCL2 was performed on bone marrow (BM) at diagnosis and during follow-up. *Results:* Median age was 71 (range 65-79); seventy (92%) pts completed the treatment and overall response was 94%: 78% complete remission, 16% partial remission and 6% stable or progressive disease (PD).Overall the regimen was well-tolerated; the most frequent grade 3-4 toxicity was neutropenia, reported in 18% of the cycles, but few cases of infections occurred (68% of pts received G-CSF). At a median follow-up of 44 months, 3-yrs OS was 90.2% and 3-yrs PFS was 67.1%. No significant difference was recorded in 3-yrs PFS according to FLIPI: 60% (95% CI, 40.5% to 75%) for low/intermediate risk pts *vs* 71.7% (95% CI, 56.4% to 82.5%) for high risk pts (p=0.158). Nine deaths were recorded: 3 PD, 4 pneumonia, 1 occult and 1 metastatic pancreatic carcinoma. A molecular marker was found in 53% of pts (40/75): 37 showed the breakpoint in MBR and 3 in mcr regions. No difference in PFS was detected in pts with available marker *vs* pts who had not. The R-BM regimen was highly active in eradicating MRD, with molecular remission (MR) rates >90% up to 12 months after the completion of consolidation, both with nested and RQ-PCR. Moreover, the BM median molecular tumor burden dropped down from 1.91 E-02 at baseline to 0 after R-BM and subsequent time points. Finally, most pts, 21/25 (84%), in MR at the end of treatment were also in clinical CR (Figure 1). *Conclusions:* R-BM is an effective and safe treatment strategy in elderly FL pts inducing high CR and MR rates with a prolonged PFS. This study indicates that Bendamustine can be safely associated to other cytotoxic agents, such as Mitoxantrone, in the context of a brief chemoimmunotherapy schedule.

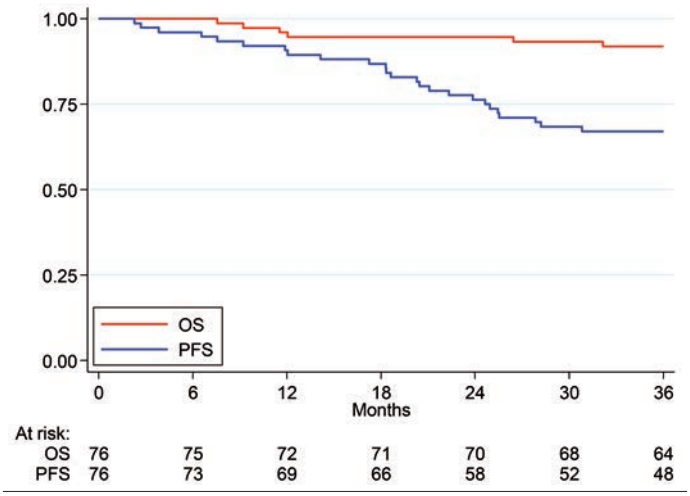

Figure 1.

Acute Leukemia 2

C031

RAS/RTK PATHWAY MUTATIONS IN B-LINEAGE ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS WITHOUT KNOWN FUSION TRANSCRIPTS: PROGNOSTIC ROLE AND POTENTIAL THERAPEUTIC TARGET

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B-lineage acute lymphoblastic leukemia without known fusion transcripts (B-NEG ALL) frequently carries RAS/RTK pathway mutations, being detected in 27% of cases. The most common mutations are those targeting FLT3 – detected in 12.3% of adult cases, 9.8% of adolescents/young adults (AYA) and only 2% of children – and KRAS/NRAS that collectively accounted for 30% of pediatric cases and only 14.7% of AYA and 14% of adults (ABSSUB-4428 EHA 2014). To investigate the prognostic and therapeutic role of RAS/RTK pathway mutations, we 1) evaluated their impact on the overall and disease-free survival (OS, DFS) of 150 B-NEG ALL patients, and 2) assessed the sensitivity of B-NEG ALL primary cells to gene or pathway specific inhibitors. Survival analyses showed that AYA (N=55) and adult (N=46) B-NEG ALL patients harboring RAS/RTK gain-of-function mutations displayed a shorter OS at 4 years when compared to WT cases (18% *vs* 49.5%, 95% CI 5.4-59.3 and 38.1-64.4, p=0.074) and also a significantly shorter DFS (p=0.020), being 20% at 4 years (95% CI 5.8-69.1) compared to 49% (95% CI 35.8-67) for WT cases. On the contrary, in the pediatric cohort (N=49) the survival of RAS/RTK mutated cases resembled that observed in WT cases. To explore the therapeutic impact of these findings, primary B-NEG ALL cells carrying mutations of FLT3, KRAS, NRAS were exposed to tyrosine kinase (dasatinib, ponatinib), FLT3 (quizartinib, crenolanib) and PI3K/mTOR/MEK (rapamycin, BEZ235, selumetinib) inhibitors and the proliferative/apoptotic rates were evaluated by MTT, 3H-thymidine and Annexin V assays. These experiments showed that quizartinib and crenolanib (0.1 microM) reduced significantly the proliferation rate of ALL primary cells from 3 patients carrying FLT3 mutations. Indeed, after 72 hours, quizartinib reduced the percentage of proliferating cells to 31.4±7.9% and crenolanib to 44.1±23.1%; remarkably, also the 3rd generation pan-inhibitor ponatinib was highly active. Also, primary cells from 4 samples harboring NRAS or KRAS mutations were sensitive to PI3K/mTOR/MEK inhibitors (0.1 microM): BEZ235 reduced the percentage of proliferating cells to 21.3±9%, rapamycin to 37.8±21.1%; and selumetinib to 62.4±16.9%. Taken together, our data indicate that RAS/RTK signaling mutations - the most frequent genetic alterations in B-NEG ALL - identify a group of AYA and adult patients with an inferior outcome, whose management might be impacted by the addition of genetics-driven targeted therapies.

C032

PROGNOSTIC IMPACT OF JAK/STAT, RAS/AKT AND NOTCH1/FBXW7 MUTATIONS IN T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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Relapsed or refractory T-cell acute lymphoblastic leukemia (T-ALL) patients have a poor outcome. The identification of oncogenic lesions should lead to a better prognostic classification and to the design of tailored therapeutic strategies. We performed Sanger sequencing of JAK3, JAK1, IL7R and STAT5B (for the JAK/STAT pathway), N/K-RAS, PTEN and FLT3 (for RAS/AKT) and NOTCH1/FBXW7 hotspot exons to assess their prognostic value. The study was performed on 49 T-ALL cases (median age 37 years, range 16-59) enrolled in the GIMEMA LAL 2000 and LAL 0904 protocols for which genomic material was available. Refractory and responsive cases were included. Overall survival (OS) and disease-free survival (DFS) were estimated using the Kaplan-Meier method. The MTT assay was performed to assess the sensitivity of primary cells to selected JAK (ruxolitinib, tofacitinib) and RAS/AKT inhibitors (rapamycin, BEZ235). JAK/STAT and RAS/AKT alterations were identified in 8 (16%) and 10 (20%) cases, respectively, and occurred mainly in relapsed or refractory patients (6/8 and 7/10 cases, respectively). Twenty-eight cases (57%) harbored NOTCH1/FBXW7 mutations, 16 without additional alterations, while 12 with a concomitant JAK/STAT (n=8) or N/K-RAS (n=4) alteration. A significantly shorter OS and DFS were observed in cases carrying JAK/STAT mutations compared to patients without alterations in the pathway (p=0.004 and p=0.002, respectively). Similarly, a significantly shorter OS and a worse DFS were observed in RAS/AKT positive cases compared to negative patients (p=0.0032 and p=0.0001, respectively). Conversely, OS and DFS were significantly better in cases with NOTCH1/FBXW7 mutations alone compared to wild-type patients or to cases with concomitant JAK/STAT or RAS/AKT mutations (p=0.0325 and p=0.0015, respectively). Primary cells from patients with JAK1 or PTEN alteration revealed a selective sensitivity to Ruxolitinib or RAS/AKT inhibitors, respectively. In contrast, cells carrying concomitant JAK1, JAK3 and STAT5B alteration were poorly sensitive both to Ruxolitinib and Tofacitinib. This study documents the negative prognostic impact of JAK/STAT and RAS/AKT mutations in T-ALL, at least when standard chemotherapy regimens are used, and highlights that the favorable impact of NOTCH1/FBXW7 is overcome by the presence of concomitant JAK/STAT or RAS/AKT mutations. Finally, *in vitro* experiments demonstrate that specific inhibitors may be effective, depending on the underlying lesions.

C033

PROSPECTIVE STUDY ON 220 CHEMOTHERAPY-RELATED-NEUTROPENIC EPISODES IN 109 AML PATIENTS. ROLE OF BED-SIDE ULTRASOUND IN NEUTROPENIC ENTEROCOLITIS: EARLY DIAGNOSIS, INCIDENCE AND SURVIVAL WITH DIFFERENT CHEMOTHERAPY REGIMEN

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Neutropenic enterocolitis (NEC) is a life threatening complication of patients (pts) treated with chemotherapy (CHT) with mortality rate up to 50%. It is characterized by abdominal pain (AP), fever (F) and diarrhoea (D). Ultrasound (US) was used to evaluate bowel-wall thickening (BWT), and >4 mm is considered diagnostic of NEC. Early diagnosis is crucial to start conservative medical management (CMM) which appears the optimal strategy for most cases. *Objective:* Evaluate prospectively if BUS can detect early signs of NEC and guide a prompt treatment (CMM or surgical) in order to reduce mortality and to evaluate the impact of different CHT regimens on mucosal damage and NEC occurrence. *Methods:* in the last 7 years all AML pts admitted in Our Hematology Unit wards at University of Pisa (Italy), undergoing chemotherapy (CHT) were enrolled (n=109). Abdominal US was performed, baseline before treatment, and as only one symptom (or a combination) appeared within 12h from onset: F and/or D and/or AP in CHT-related neutropenic pts. *Results:* N=220 chemotherapy-related neutropenic episodes (NE) occurred in 109 pts. N=17 episodes of NEC were diagnosed (7.7%incidence rate). CHT regimens received (total number of cycles/NEC episodes) was: 3+7 (Idarubicin+ARAC) N=62/10, Idarubicin (AML-M3) N=9/1, 3+3+5 (Idarubicin,VP-16,ARAC) N=48/3; 2+5 (Idarubicin+ ARAC) N=24/1; Clofarabine (Clofa) (40mg) N=5/0; Clofa (20mg) 14/0; Clofa+ARA-C (Clofa 20mg) N=18/1; FLANG N=13/1; HD-ARA-C (3gr/mq for 3 consecutive days) N=26/0. Overall 3 pts died out of 17 NEC episodes (mortality rate 17.6%). Statistically (St) CHT regimens mostly associated with NEC were: 3+7 odds ratio (OD) 6.00 (P<0.0001), 3+3+5 OD=1.75 (P<0.0001). There was not a St significant association of the following CHT regimens with NEC occurrence: Clofa (20 or 40mg) N=0 NEC, 2+5

(P=0.125), Idarubicin (AML-M3) (P=0.111), Clofa 20mg+ARAC (P=0.167), HD-ARAC N=0 NEC, FLANG (P=0.143). The association of ARAC to Clofa *vs* Clofa (20 or 40mg) alone was not St significant in NEC occurrence (P=0.9). The likelihood of NEC Dx in a discriminant model (Bayes theoreme) for pts with BWT and AP=100%, AP+D=100%, AP+D+F=100%,AP+F=100%. In *Conclusions:* BUS allowed to detect early signs of NEC and to start prompt treatment with a 76% survival rate. With BUS pts do not live the isolation room. Fever is not a condition sine qua non for NEC diagnosis. Different chemotherapy regimens do have a different impact on mucosal damage.

C034

CLONAL EVOLUTION IN PATIENTS AFFECTED BY THERAPY-RELATED MYELOID NEOPLASM

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Therapy-related myeloid neoplasms (t-MN) include MDS and AML occurring as a late effect of chemo and/or radiotherapy for a primary malignancy or for autoimmune diseases. Cytotoxic therapy may induce chromosomal alterations and genetic mutations in hematopoietic progenitors leading to a high incidence of complex karyotypes and Tp53 mutations. Moreover, Tp53 mutations in the founding clone probably contribute to the frequent cytogenetic abnormalities and poor response to chemotherapy that are typical of t-MN. New sequencing technologies have enabled large screening of somatic mutations in genes candidate for leukemic transformation. Some mutations are localized in specific gene regions and are defined as hot spot mutations, whereas others are spanned in the whole coding region. The aim of our work was to study the role of common somatic mutations during clonal evolution in a cohort of 13 t-MN patients for whom bone marrow (BM) DNA samples collected at the time of primary cancer diagnosis or during follow-up and t-MN onset were available. We studied hot spot mutations in genes belonging to epigenetic regulators (DNMT3A, IDH1 and IDH2), spliceosome enzymes (U2AF1, SF3B1and SRSF2) and SETBP1 using Sanger sequencing for the screening and Pyrosequencing for the quantification of the mutated clone. Spanned mutations in the Tp53 gene were studied by NGS and confirmed by Pyrosequencing. We identified 5 mutations (IDH1 R132H, SRSF2 P95H, SF3B1 K700E, SETBP1 G870R andTp53 Y220C) in 4 patients. One patient with a previous APL was identified as carrier of a Tp53 Y220C mutation, one with AML of a SF3B1 K700E mutation and one with a B-LAL of a SETBP1 G870R mutation. One patient with NHL as primary cancer carried IDH1 and SRSF2 mutated genes. IDH1 R132H mutation was already present in the BM at the time of NHL diagnosis, whereas the SRSF2 P95H mutation was acquired at the time of t-MN diagnosis (Figure 1).

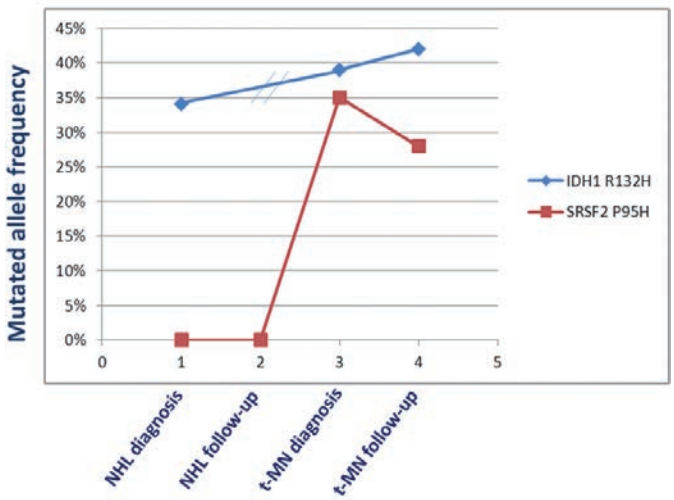

Figure 1.

Pyrosequencing evaluation of identified mutations showed that the frequency of mutated allele was greater than 20% at the time of t-MN diagnosis in three out of four patients, whereas the Tp53 mutation was detected in 6.75% of cells only. In conclusion, most mutations were acquired at the time of t-MN diagnosis, suggesting their pivotal role in the development of t-MN. Since the sensitivity of pyrosequencing is about 5%, we cannot exclude the presence of minor clones onsetting very early after cytotoxic therapy of the primary malignancy and contributing to t-MN development.

C035

ABSOLUTE QUANTIFICATION OF THE PRETREATMENT PML-RARA MOLECULAR TRANSCRIPT DEFINES THE RELAPSE RISK IN ACUTE PROMYELOCYTIC LEUKEMIA

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The persistence of resistant leukemic cells after treatment is responsible for relapse in 10-20% of acute promyelocytic leukemia (APL) patients. Until now, the white blood cells count at diagnosis has been considered the most important prognostic factor in APL, able to better identify those patients at higher risk of relapse. In this study we performed absolute quantification of the PML-RARA transcript by droplet digital polymerase chain reaction (ddPCR) in 76 newly diagnosed APL patients to verify the prognostic impact of the PML-RARA initial molecular burden. ddPCR analysis revealed that the amount of PML-RARA transcript at diagnosis in the group of patients who relapsed was higher than in that with continuous complete remission (CCR) (272 *vs* 89.2 PML-RARA copies/ng, p=0.0004). Moreover, considering the arbitrary cut-off of 124 PML-RARA/ng (the median value of our APL series), a higher proportion of patients who relapsed (85.7%) had >124 PML-RARA/ng compared to the CCR group (39.6%) (odds ratio 0.10; p=0.002). Further parameters (age, sex, WBC count, M3/M3v, bcr transcript type, FLT3 mutation status, CD34 and CD2 expression, relapse risk score) were assessed to verify the presence of different amounts of the fusion gene transcript at diagnosis among these different categories but yielded no statistically significant differences. ROC analysis detected the optimal PML-RARA concentration threshold as 209.6 PML-RARA/ng (AUC, 0.78; p<0.0001) for discriminating between outcomes (CCR *versus* relapse). Among the 67 APL patients who achieved CR after the induction treatment, those with >209.6 PML-RARA/ng had a worse relapse-free survival (RFS) (p=0.0006). At 5-year follow-up, patients with >209.6 PML-RARA/ng had a cumulative incidence of relapse of 50.3% whereas 7.5% of the patients with suffered a relapse (p<0.0001). There was no difference in terms of overall survival (OS) between patients with <209.6 PML-RARA/ng and those with >209.6 PML-RARA/ng. When the group of patients with early death was excluded from the OS analysis, the difference between the two groups was statistically significant (p=0.02). Cox proportional hazards regression model analysis was performed for RFS identifying the amount of PML-RARA before induction treatment as the sole independent prognostic factor for APL relapse (HR 9.26, p=0.0009). We conclude that the amount of PML-RARA before induction treatment represents the sole independent prognostic factor for APL relapse.

C036

TARGET CHECKPOINT KINASE 1 TO IMPROVE THE CYTOTOXICITY OF CONVENTIONAL THERAPIES IN B-/T-ACUTE LYMPHOBLASTIC LEUKEMIA

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The efficacy of the Checkpoint Kinase (Chk)1/Chk2 inhibitors has been assessed for the treatment of different type of cancers but only few studies have been performed on hematological diseases. We evaluated the *in vitro* efficacy of the Chk1 inhibitor, LY2606368, as single agent and in combination with tyrosine kinase inhibitors (imatinib and dasatinib) or with the purine nucleoside antimetabolite clofarabine in B-/T- acute lymphoblastic leukemia (ALL) cell lines and in primary blasts. Human B (BV-173, SUPB-15, NALM-6, NALM-19 and REH) and T (MOLT-4, RPMI-8402 and CEM) ALL cell lines were incubated with increasing concentrations of drug (1-100 nM) for 24 and 48 hours. LY2606368 deeply reduced the cell viability in a dose and time dependent manner in all the cell lines, with the BV-173 (6.33 nM IC50 24hrs) and RPMI-8402 (8.07 nM IC50 24hrs) being the most sensitive while SUP-B15 (61.4 nM IC50 24hrs) and REH (96.7 nM IC50 24hrs) being the less sensitive cell lines. In addiction the sensitivity was not correlated with the different subtypes of ALL or with the mutational status of p53. The cytotoxic activity was confirmed by the significant increment of apoptotic cells (Annexin V/PI), by the increment of H2AX foci and by the activation of different apoptotic markers (Parp-1 and pro-Caspase3). To better understand the relationship between the activation of apoptosis and the effect on cell cycle, different analyses were performed (Propidium Iodide staining). The inhibition of Chk1 deeply changed the cell cycle profile. Indeed in all the cell lines the percentage of cells in S phase and in G2/M phase were reduced by the treatment while the numbers of cells in debris and G1 phase were increased. Furthermore the cytotoxicity of LY2606368 was evaluated in combination with imatinib, dasatinib and clofarabine. For each drug the combination strongly reduced the cell viability when compared to the effect of the single drugs. Moreover, the combination showed an additive efficacy in term of induction of DNA damages as showed by the increase number of H2AX foci and the activation of pChk1 (ser 317). In conclusion, LY2606368 showed a strong cytotoxic activity in B-/T-All cell lines as single agent and in combination with imatinib, dasatinib and clofarabine. In our opinion these data are the basis for a future clinical evaluation of this compound in the treatment of leukemia.Supported by ELN, AIL, AIRC, progetto Regione-Università 2010-12 (L. Bolondi), FP7 NGS-PTL project.

Allogeneic and Autologous Transplantation

C037

LEUKEMIA RELAPSE AFTER ALLOGENENIC HSCT DISPLAYS A DISTINCTIVE IMMUNE-RELATED SIGNATURE, WITH FUNCTIONALLY RELEVANT ALTERATIONS IN HLA CLASS II ANTIGEN PRESENTATION AND T CELL COSTIMULATION

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After allogeneic Hematopoietic Stem Cell Transplantation (allo-HSCT) for Acute Myeloid Leukemia (AML), relapses are frequently observed, and current strategies to prevent or treat them are largely unsatisfactory. According to the "leukemia immunoediting" hypothesis, relapses might be an expression of the selection of leukemic variants that have gained resistance to the immune control mediated by the donor-derived immune system. To verify this hypothesis and provide new biological insights into the mechanisms of post-transplantation relapse, serial AML samples harvested from 9 patients at diagnosis, relapse after chemotherapy and relapse after allo-HSCT were FACS-purified and leukemic blast gene expression profile was analyzed using Illumina microarrays. Most of the transcripts selectively deregulated at relapse after allo-HSCT were involved in immune-related processes, and in particular in T cell costimulation and antigen presentation. No significant enrichment in immune processes was documented at relapse after sole chemotherapy. In a 22-patient validation cohort we confirmed the significant upregulation of the PDL1 coinhibitory ligand on AML blasts harvested at relapse, accompanied by high levels of PD1 on the respective donor-derived T cells. By blocking this inhibitory axis with an anti-PDL1 monoclonal antibody, we could rescue the ability of donor T cells to proliferate in response to patient AML blasts. Moreover, in 4/22 cases of post-transplantation relapse we documented the selective loss of expression of HLA Class II molecules on AML blasts, due to significant downregulation of the CIITA transcription factor. Notably, loss of HLA-II expression occurred more frequently after partially-incompatible allo-HSCT, and abolished T cell-mediated leukemia recognition and killing both *in vitro* and an immunodeficient mouse *in vivo* model. HLA Class II expression and, as a consequence, T cell recognition, could be recovered upon exposure of AML blasts to interferon-. Taken together, our results provide a further proof that the deregulation of immune-related processes, and in particular of the pathways involved in T cell-mediated allorecognition, is a prevalent feature of AML relapses after allo-HSCT. Importantly, the two novel and highly patient-specific immune evasion mechanisms that we identified are actionable, and might thus be rapidly translated into personalized therapeutic approaches.

C038

XRCC1 399GG GENOTYPE PREDICTS SIGNIFICANTLY LONGER OVERALL SURVIVAL AND LONG LASTING CR IN RESISTANT LYMPHOMA TREATED WITH BENDA-BEAM AND ASCT

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We previously demonstrated (Visani *et al.*, Blood 2011, Blood 2014) the efficacy of Bendamustine-BEAM conditioning regimen prior to autologous stem cell transplant (ASCT) in heavily pretreated lymphoma patients (72% CR at 41 months after ASCT; 3 years PFS 75%). Biological markers predicting response to Benda-BEAM would significantly impact the clinical decision making. Cytotoxic activity of Bendamustine is enhanced by inhibition of the base excision repair (BER) DNA-damage response pathway, suggesting that the BER enzymes play a key role in the repair of DNA damage caused by Bendamustine. Accordingly, we evaluated the clinical impact of the BER family genetic variants in 43 patients with resistant/relapsed NHL (n=28) or HD (n=15) treated with Benda-BEAM prior to ASCT. Three SNPs of BER genes [XRCC1 399 (rs25487 G/A, Arg/Gln), XRCC3 241 (rs861539 C/T, Thr/Met), ADPRT T2444C (rs1136410 T/C, Val/Ala)] were analyzed by PCR-HRM assay and restriction enzymes. The association of candidate genotypes with overall survival (OS) was investigated by long-rank test and Cox regression model. First, we found and association between ADPRT 2444 CC or CT genotype and chemoresistant disease, whereas ADPRT TT genotype was associated with chemosensitive disease. Hence, XRCC1 399 GG and XRCC3 241 TT genotype were associated with significantly longer OS in univariate analysis (p=0.035, p=.0.025). Multivariate analysis confirmed the prognostic role of XRCC1 GG in predicting OS (p=0.005). Interestingly, all patients carrying XRCC1 399 GG genotype reached CR, independently from disease type (HD and NHL), suggesting a possible positive predictive role for the XRCC1 399 GG genotype in influencing both CR and OS. On the other hand, CR rate for patients bearing the XRCC1 399 AA or GA genotype were 75% and 84%, respectively. Interestingly, the disease status at transplant was a strong predictor of both PFS and OS in the clinical trial. In conclusion, XRCC1 399GG genotype seems to be predictive for longer OS in lymphoma patients treated with Benda-BEAM and ASCT. Moreover, the presence of XRCC1 399 GG genotypes was associated with the achievement of CR in 100% of patients carrying this genotype. XRCC1 399 genotype could, thus, represent a biomarker in lymphoma patients treated with Benda-BEAM and ASCT. *Acknowledgements: supported in part by AIL Pesaro Onlus*.

C039

EVALUATION OF NILOTINIB SAFETY IN PATIENTS WITH STEROID-REFRACTORY CHRONIC GRAFT-*VERSUS*-HOST DISEASE: A PHASE I-II GITMO STUDY

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Background: Chronic Graft *versus* Host Disease (cGvHD) is still the leading cause of late mortality in transplanted patients. Imatinib has shown activity in steroid-refractory GVHD (SR-cGVHD);however, a higher incidence of adverse events (AE) was reported with daily doses above 200 mg/die. Nilotinib (NIL) has higher affinity than Imatinib to intracellular tyrosine kinases (IC50=20nM for p210BCR-ABL, IC50=71nM for PDGFR) and has a favourable tolerability profile. We conducted a phase I-II study (standard 3+3 design) aimed to define the MTD and the activity of NIL in SR-cGVHD (NCT01810718).A secondary endpoint of the study was pharmacokinetic evaluation of NIL, to be related with efficacy and toxicity measures. *Results:*Twenty-one patients were enrolled (median age 46 years, range 23-66). After dose-escalation up to 600 mg/d, the MTD was not reached. Six patients received NIL at 200mg/d,6 at 300 mg/d,6 at 400/mg/d and 3 at 600 mg/d (300 mg BID). Observed extrahematological AE with a frequency >20% were: asthenia, headache, nausea, pruritus, cramps, constipation; mostly were mild. The most frequent hematological AE was anemia (grade 1-2: 9/21 pts, grade 3: 1/21 pts);other hematological AE were mild and uncommon. Pharmacokinetic evaluation was planned 15 days and 1, 3, 6 months after start of NIL treatment. Samples were drawn in the morning, at least 8h after last NIL administration. Data were available for 17 pts (total of 61 measurements):there were no data for pts receiving NIL 600 mg/d. During the first 6 months of NIL treatment, there were only 4 grade 3 AE and none grade 4 AE. Mean and median plasma concentrations of NIL (C-NIL) were 817(SD±450) and 773 ng/ml in all patients;C-NIL were significantly different by NIL dosing (p=0.016, anova);pairwise correlations revealed a difference between the 300 mg/d and the 200 mg/d group, but not between the 400 mg/d and the 200 mg/d group. All but 3 measurements exceeded the IC50 of PDGFR (36 ng/ml): in these 3 cases poor compliance to NIL was probable. We also checked for associations between lower C-NIL and lack of response (according to simplified NIH criteria) but we did not find significant results;or between higher C-NIL and more frequent or severe AE, but again we did not find significant correlations (Figure 1). *Conclusions:* NIL was well tolerated in this cohort of SR-cGVHD pts, even at higher dosages. The favourable safety profile of NIL makes this drug an attractive option in SR-cGVHD pts unable to tolerate Imatinib.

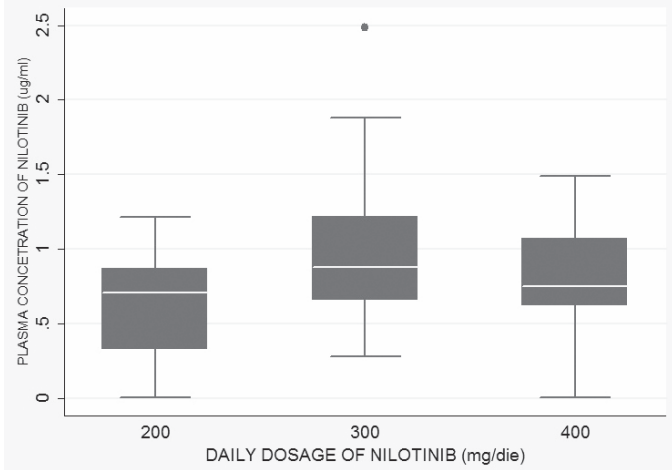

Figure 1.

C040

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

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Allogeneic bone marrow transplantation (BMT) offers the greatest chance of cure for most patients affected by acute myeloid leukemia (AML). Persistence of disease or high levels of pre BMT minimal residual disease (MRD) have been reported to predict relapse risk after BMT. WT1

expression levels and multicolor flow cytometry (MFC) are the most widely used tools to evaluate MRD. We retrospectively analyzed outcome of 257 AML patients with both WT1-based and MFC-based MRD evaluation on bone marrow samples before transplant. Median age at transplant was 44 years. One hundred thirty-three patients were transplanted in first, 54 in second and 72 in third or subsequent complete remission. Induction regimens included fludarabine-containing regimens or standard "3+7" induction. Median follow-up was 48 months (95% CI 37.5 – 50.5 months). Eighty-nine relapses (35%) were reported. Median RFS was 84 months the probability of disease relapse was significantly affected only by disease status (first or subsequent CR) and MRD status before transplantation, measured with any method. Multivariate RFS analysis revealed that the combined MRD evaluation was the only independent predictor of RFS (p <0.001) Specifically, MFC-MRD was the strongest predictor of longer relapse free survival (p <0.001)since only two relapses occurred in the eleven MFC-MRD negative patients. Among MFC-MRD positive patients a further stratification of risk is obtained by the evaluation of WT1 MRD status that was able toidentify patients with significantly worse RFS. (p <0.01). The predictive value of MRD resulted independent from induction schedules, donor type, disease status at BMT and risk group. Similarly, OS analysis disclosed that combined MRD evaluation was a strong predictor of long survival (p <0.001). Multivariate OS analysis showed that BMT year, disease status at BMT and combined MRD evaluation significantly influenced OS duration (p <0.001, <0.002 and <0.003, respectively) Pre transplant MRD evaluation by WT1 and MFC on bone marrow samples is a reliable predictor of relapse risk. Patients with both negative pre-BMT MRD markers have a significantly longer DFS, while patients with both positive MRD markers display an higher risk of relapse. Identifying patients who are at higher relapse risk may allow to modulate post BMT follow up, with the aim of detecting disease recurrence earlier and/ or applying pre-emptive therapeutic strategies in order to delay or prevent AML relapse (Figure 1).

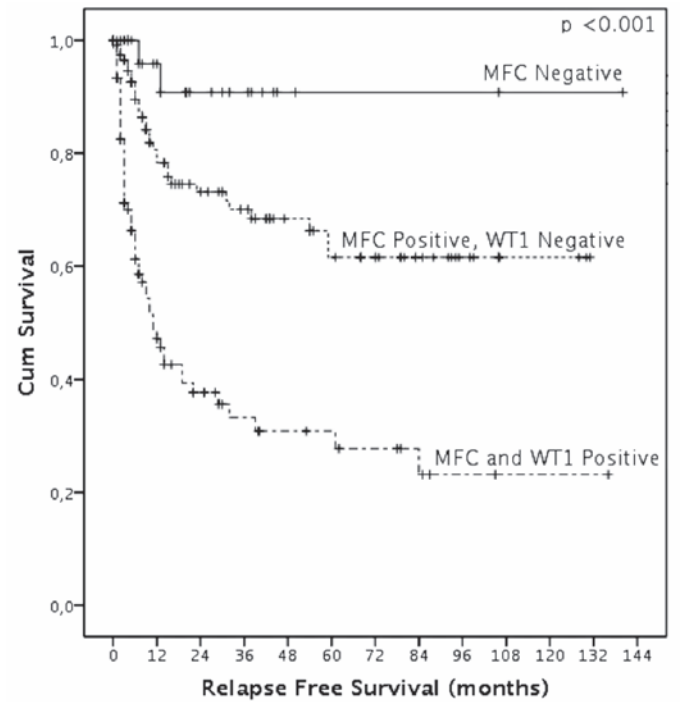

Figure 1. RFS according to risk group.

C041

IMMUNOLOGICAL PROFILE OF PATIENTS AFTER ALEMTUZUMAB-BASED HSCT FOR SEVERE APLASTIC ANEMIA INDICATES POTENTIAL BASIS FOR TOLERANCE WITH MIXED T-CELL CHIMERISM AND EXTREMELY LOW INCIDENCE OF GVHD

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Alemtuzumab-based conditioning regimens for allogeneic HSCT are associated with low rates of GvHD. We investigated clinical outcomes of 37 patients transplanted for acquired idiopathic SAA with Fludarabine, Cyclophosphamide and Alemtuzumab from 2007 to 2014 at King's College Hospital. Median age was 37 (range 15-63), with BM as stem cell source in 7(19%) pts and PBSC in 30(81%). Median cell dose was 6.85(1.9-12.4) CD34+ cells x10^6/Kg. Donor was sibling in 11(29,7%) and unrelated in 26(70,3%) pts, 6 of whom also received 2 Gy TBI for 9/10 mismatch. Primary graft failure occurred in 1 pt (2,5%). Acute GvHD was seen in 6(17%) pts (all grade I-II), and chronic GvHD in 5(14%) pts (all skin, only 1 severe). Factors associated with cGVHD were previous aGVHD (p=0.035) and CD3+ chimerism at day+100 ≥90%(p=0.006). Median follow-up was 30 months (4.3-86.8), with 94% overall survival and 90% event-free survival. Majority of patients were persistently T-cell mixed chimeric 1 year after HSCT, with median CD3+ chimerism level of 70,5%. To investigate the basis for favourable outcomes, lymphocyte composition was compared to 11 healthy age-matched individuals. Lymphopenia persisted to day 360 (p<0,005) and beyond. Proportion of T cells was significantly low (2.6% of lymphocytes at day 30, rising to 41% by day 360, (p=0,018). Although deficiency was greatest for CD4+ T cells, subset composition was normal by one year and naïve CD4+ T cells expressed CD31 indicative of renewed thymopoiesis and implementation of central tolerance in pts aged <50 years. CD8+ T-cell recovery was more rapid, but composition was abnormal due to high effector (CD45RA-/+ CD27- CD62L-) cell numbers. In patients with sustained mixed T cell chimerism, these CD8+ effector T cells were predominantly recipient-derived and associated with CMV/EBV infection suggesting virus-driven expansion. Normal frequencies of regulatory T cells (CD25^high, CD27+, FoxP3+; normal range 4,2%-7,4%) in CD4+ T-cell population compared to 5,3% in SAA pts, were present together with robust recovery of B cells containing an increased percentage of IL-10 producing immunosuppressive cells (day 90, p<0,01). Low proportion of T cells with establishment of naïve T-cell central tolerance in younger pts, persistent mixed chimerism due to effector CD8+ T cells, and presence of regulatory T and B cells subsets, all contribute to creating a tolerant post-HSCT environment, with low rates of GvHD and excellent outcome in SAA pts.

C042

THE ROME TRANSPLANT NETWORK MODEL COMPARED TO THE ITALIAN BONE MARROW DONOR REGISTRY FOR ALTERNATIVE DONOR SEARCH AND SELECTION PROCESS FOR ADULT PATIENTS WITH HEMATOLOGICAL MALIGNANCY

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Background: Patients with high risk hematological malignancy eligible to an allogeneic stem cell transplant (ASCT) should undergo quickly the transplant procedure. From 2011, 3834 adult patients underwent an unrelated donor search at the IBMDR. According to the IBMDR standards, the identification rate for a 7/8 - 8/8 HLA matched Volunteer Unrelated Donor (VUD) within 3 months is 60%, which increases to 71%, when out of the 3 month limit Cord Blood Unit (CBU) 4/6 HLA matched is considered. However, according to the general policy of Transplant Centers, these rates decrease to 43%. These results correspond to an overall unrelated transplant efficiency of 44% regarless of the identification time. Study design: From April 2006, the RTN has adopted a unique policy for the alternative donor search with a hierarchical selection (1st VUD, 2nd CB and 3rd Haplo). Using a low resolution HLA A,B,DRB1 typing, a preliminary query is performed in all cases with the assignment of a good or poor result if more or less than 10 matched donors were identified in BMDW. Selection criteria for VUD consist of at least 8/10 HLA matching, while a single CBU was selected based on cell dose and

HLA matching. All patients received the same conditioning regimen. *Aims:* To compare the RTN transplant efficiency resulted from an established policy of donor selection and a hierarchical algorithm including the haploidentical transplant as third option with data provided by the IBMDR. *Results:* Our data referred to all 417 eligible adult patients show that the RTN policy has led to a timely identification of an alternative donor in 78% of cases with 61% of patients definitively transplanted in comparison to 71% and 44% of unrelated donor (VUD+CB) selection and transplant, respectively reported by IBMDR (p=0.008; p<0.0001). Furthermore, between 2011 and 2014, 117 out of 149 patients (79%) met criteria of a good preliminary query and 50% of them identified a 8/8 matched VUD at high resolution level. A poor preliminary query corresponded to an availability of 8/8 matched VUD for only 12.5% of patients. *Conclusions:* Despite of the more restrictive RTN selection criteria respect of IBMDR, the overall alternative donor identification and transplant efficiency in adequate timing result significantly higher when haploidentical donor is considered. Moreover, the preliminary query represents a useful tool to address the donor choice since the start of the search process at the best.

Hemostasis and Thrombosis

C043

GOOD EFFICACY AND SAFETY OF DESMOPRESSIN USED TO MANAGE BLEEDS, DELIVERIES AND SURGERIES IN VON WILLEBRAND DISEASE: RESULTS OF THE INTERNATIONAL PROSPECTIVE STUDY IN A COHORT OF 225 PATIENTS

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Background: Despite the fact that desmopressin (DDAVP) is considered the treatment of choice in most patients with von Willebrand disease (VWD), there are still open questions about the efficacy and safety of DDAVP in repeated bleeding episodes and during surgery. Indeed most hematologists prefer to use VWF concentrates during recurrent bleeds, deliveries and surgery also in VWD responsive to DDAVP to avoid the risk of tachyphylaxis and possible side effects. *Materials and Methods:* This is an investigator-driven prospective study on the Desmopressin efficacy and safety of patients with von Willebrand disease (ProDesWil) previously assessed for biological response to DDAVP during a challenge test. To evaluate efficacy and safety of DDAVP used to manage bleeds, deliveries, oral and minor/major surgeries in VWD, 268 patients were included in this 24-month prospective study. Inclusion criteria: VWD without age restriction, with bleeding score >3.5, VWF:RCo levels <46 U/dL, and at least another affected member in the family. *Results:* 225/268 (85%) patients met inclusion criteria as VWD1(n=198), VWD2A(n=15), VWD2M(n=12). DDAVP biological response was complete, partial and absent in 89%, 10% and 1% VWD. Fourteen patients with C1130F and R1205H mutations showed accelerated clearance (VWD1C). During the 24-month follow-up 84/225 (37.3%) received DDAVP for bleeds (n=102), deliveries (n=13), oral surgeries (n=27), all other minor/major surgeries (n=46). Total DDAVP injections were 652 with median,range/episode during bleeds (2,1-12), deliveries (3,1-3), dental extractions (1,1-10), surgeries (3.6,1-16). Efficacy was excellent/good in bleeds (92.1%), deliveries (84.6%), oral (100%) and other surgeries (90.6%) being poor in 6 cases with VWD2A(n=4) during 3 GI bleeds or 1 abdominal surgery and VWD1(n=2) during 1 delivery and 1 surgery. Side effects (16 cases) were mainly minor with water retention reported in 2 cases (1 delivery, 1 surgery). *Conclusions:* DDAVP is very effective to manage or prevent bleeding in most patients with VWD proven to be drug-responsive. Since major side effects are relative rare after repeated injections, the use of DDAVP should be always recommended also during delivery and major surgery in VWD

C044

NEW DATA ON THE SAFETY AND EFFICACY OF RECOMBINANT FXIII IN PATIENTS WITH CONGENITAL FXIII A- SUBUNIT DEFICIENCY

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Introduction: Recombinant FXIII represents a new treatment opportunity for patients with congenital FXIII A-subunit deficiency. Monthly prophylaxis with 35 IU/kg rFXIII was shown to effectively control bleeding with ABR of 0.138 bleeds/patient/year and an excellent safety profile. PK analysis revealed first-order elimination of rFXIII with a geometric mean half-life of 13.6 days. All patients had a mean FXIII trough activity level of >0.1 IU/mL. The PK profile was independent of age, supporting that monthly dosing with a fixed 35 IU/Kg rFXIII regimen for all patients with FXIII A-subunit deficiency was adequate for prophylaxis. *Methods:* The mentorTM2 trial is an ongoing safety extension trial to the pivotal mentorTM1 trial (Figure 1). All patients were dosed with 35 IU/kg rFXIII every 4th week. *Results:* Baseline patient demographics are presented in the Table

1. Of 60 patients enrolled,34 come from the mentorTM1 trial,and 26 new patients were enrolled.In mentorTM 2,60 patients had 2,157 exposures (monthly dosing), corresponding to a total of 168 patient years. The ABR was 0.042 bleeds/patient/year overall, 0.012 bleeds/patient/year for spontaneous bleeds, and 0.030 bleeds/patient/year for traumatic bleeds.In 6 patients experienced 7 bleeds requiring FXIII treatment (5 trauma-induced and 2 spontaneous) experienced requiring FXIII treatment. No intracranial, internal organ or severe gastrointestinal bleeds occurred during the trial period. Mean FXIII trough levels were greater than 0.10 IU/mL in all patients. No thromboembolic events, fatal adverse events or adverse events leading to withdrawal were reported. No anti-rFXIII antibodies were detected. *Discussion:* Prophylaxis of bleeding of patients with congenital FXIII A-subunit deficiency with rFXIII in this study has demonstrated very effective bleed control, with an excellent safety profile. The ABR in the ongoing mentorTM2 safety extension trial was 0.042, which is lower than the rate of 0.138 seen in the mentorTM1 pivotal study. An ABR 0.042 corresponds to an average patient having 1 bleed approximately every 24 years. One patient who had a traumatic breakthrough bleed was treated with rFXIII with excellent outcome. These efficacy data, combined with comprehensive PK- and safety data, represent the largest data collection in congenital FXIII A-subunit deficiency in the world, and provide extensive evidence for the safety and efficacy of monthly prophylaxis with 35 IU/kg rFXIII.

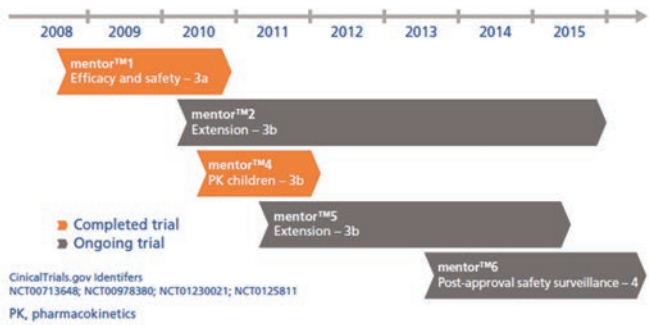

Figure 1. The Novo Nordisk clinical trial program for recombinant Factor 13.

Table 1.

Age, median (range)	26 (7-77)
Age, mean (range)	31 (7-77)
Male sex, n (%)	38 (63)
Race, n (%)	
Black or African American	6 (10)
American Indian or Alaska Native	1 (2)
White	34 (57)
Asian*	9 (15)
Other	6 (10)
Unknown**	4 (7)
Body Mass Index, median (range)	23.7 (12.8-36.9)
Height in cm, median (range)	167.0 (131.0-187.5)
Weight in kg, median (range)	67.5 (22.0-119.4)

* Including 5 Japanese
** French patients are marked as unknown as per the French authorities guideline

C045

THE PREDICTIVE ROLE OF ADAMTS-13 IN PATIENTS WITH IDIOPATHIC THROMBOTIC THROMBOCYTOPENIC PURPURA

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TTP is a rare life-threatening disorder characterized by a severe deficiency of ADAMTS-13 (*i.e.* <5%), a metalloproteinase that cleaves the large multimers of von Willebrand Factor (vWF). Several studies are evaluating the predictive value for disease recurrence of ADAMTS-13 activity and its inhibitors. Aims of the study are to characterize, in patients with TTP diagnosis, the levels of ADAMTS-13 activity and its inhibitors and to establish the prognostic value of these biomarkers for disease recurrences. Blood samples were collected from 54 TTP patients (40F/14M): 48 with idiopathic TTP, 3 with drug-induced TTP and 3 with bone marrow transplantation (BMT)-associated TTP. In a subgroup of 37 TTP patients blood samples were collected at the onset and upon remission. ADAMTS-13 activity, inhibitors, and anti-ADAMTS-13 antibodies were measured in plasma by commercial kit (Technoclone). A complete ADAMTS-13 activity deficiency (*i.e.* <5%) was detected in patients at the onset of idiopathic TTP. In 83% of patients, the deficiency was associated to a significant inhibitory activity against ADAMTS-13 and high anti-ADAMTS-13 antibody titer. At remission, 19 of them showed normal levels (*i.e.* >50%) of ADAMTS-13, while 5 had moderate (*i.e.* 21-50%), 4 severe (*i.e.* 5-20%), and 5 complete (*i.e.* <5%) deficiency of ADAMTS-13 activity. In the majority of cases, the normalization or the partial recovery of ADAMTS-13 activity was associated to the reduction of the antibody levels. Eight of the 9 patients with ADAMTS-13 activity <20% during remission had at least one relapse, as compared to only 8 out of the 16 patients with ADAMTS-13 activity >20% (p=0.004). In 4 TTP patients, we could not detect any inhibitory activity and anti-ADAMTS13 antibodies either in the acute phase, nor upon clinical remission or TTP relapse. DNA sequence analysis demonstrated that these patients were carriers of mutations in ADAMTS13 gene. Patients with BMT-associated TTP showed moderate reduction in plasma ADAMTS-13 activity, without measurable anti-ADAMTS-13 antibodies. Differently, patients with drug-associated TTP, showed severe ADAMTS-13 deficiency in the acute phase with anti-ADAMTS-13 antibody positivity. In conclusion, our data demonstrate that an ADAMTS-13 activity deficiency (*i.e.* <20%) during remission is predictive of recurrences in autoimmune TTP patients. Due to the risk of relapse, TTP patients require follow-up with ADAMTS-13 monitoring and an early management can be predicted.

C046

SHORT AND LONG-TERM OUTCOMES IN HEMOPHILIA PATIENTS WITH INHIBITORS UNDERGOING ORTHOPEDIC PROSTHETIC SURGERY

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Introduction: Major orthopedic surgery is still a difficult challenge in hemophilic patients with high titer of inhibitor. In addition to possible perioperative problems, the long-term outcomes are anecdotal. *Aim of the study:* To evaluate the short and long term outcomes in prosthetic orthopedic procedures done in hemophilic patients with inhibitor from 1998 to 2014. *Results:* Fifteen knee replacements have been performed in 11 patients affected by hemophilia A with inhibitors. At the time of surgery 10 out of 11 patients had an inhibitor titer >5UB. A preoperative pharmacokinetic with rFVIIa was made in 8 out of 11 patients, with the following mean values of clearance rate: 35 ml/h/kg and half-life: 2.85 hours. The average age was 46 years (range 29-55). All patients were treated with 2-3 doses of rFVIIa (90-120 g/kg) every two hours during surgery up to suturing of the wound, followed by continuous infusion of rFVIIa at dose of 30-50 g/kg/h on days 1-3 and 15 g/kg/h on days 4-14. Plasma levels of FVII:C were maintained >15U/mL. All but one patients were transfused with 2 units of red blood cells (RBC), according to standard procedure. Two peri-operative complications occurred: 1 pulmonary embolism and 1 major bleeding at the operated site, poorly responsive to rFVIIa and aPCC, treated with 6 units of RBC. Only one two patients underwent to prosthesis revision for infection, while another due to rupture of the prosthesis metal cup. After surgery all patients showed a significant improvement in pain and a favorable

functional recovery. After a median follow-up of 6 years (1-17 years), a good quality of life as to pain and function of the replaced knee were recorded. *Conclusions:* The orthopedic prosthetic surgery in hemophilic patients with inhibitors can be done, with a good outcome. The continuous infusion of rFVIIa provides stable haemostatic coverage, saving on the rFVIIa total dose compared to the bolus standard dose. However, costs remain high and the possible risks of thromboembolic complications should not be overlooked

C047

THE INFLUENCE OF JAK2 V617F, CALRETICULIN AND MPL MUTATION ON PLATELET ADHESION UNDER FLOW CONDITION IN ESSENTIAL THROMBOCYTHEMIA AND POLYCYTHEMIA VERA

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Background: Three somatic mutations are associated with ET and PV, two disorders characterized by a high incidence of both arterial and venous thrombosis, and microcirculatory disturbances. So far, no studies have been performed to evaluate the influence of these mutations on platelet (PLT) adhesion. *Aims:* To evaluate the role of JAK2 V617F, Calreticulin (CalR) and MPL mutations on PLT adhesion in ET and PV patients. Further, the influence of hematological parameters, hydroxyurea (HU) therapy, and circulating von Willebrand Factor (vWF) was also considered. *Patients and Methods:* Forty-four ET, 26 PV and 25 healthy controls were analyzed. Venous blood was perfused over collagen for 4' at 1,000/second shear rate in a parallel flow chamber and PLT adhesion evaluated under an EVOS fluorescence microscope. After perfusion, PLT were stained with annexin V to evaluate procoagulant phospholipid (*i.e.* phosphatidylserine, PS) exposure. *Results:* PLT adhesion was significantly ($p<0.05$) greater in ET (44.2±13.6%) and PV patients (44.6±13.7%) compared to controls (37.1±8.5%). According to the mutational status, in ET patients PLT adhesion was significantly increased in JAK2 V617F positive patients compared to either triple negative (n=6), or CalR- (n=15) or MPL- (n=3) mutated patients. The highest PLT adhesion was observed in >50% JAK2 V617F allele carriers, while the lowest in MPL mutation carriers. In PV patients, >50% JAK2 V617F allele carriers (n=18) presented a higher PLT adhesion than <50% ones (n=7). Differently, PS expression on PLTs was significantly reduced in ET and PV patients compared to controls. Futhermore, PLT adhesion was significantly related to PLT count ($p<0.001$), but not to leukocyte count or vWF levels. Regarding therapy, HU-treated patients showed significantly ($p<0.05$) lower PLT adhesion (48.5±11.3% *vs* 41.6±14.3%) and higher PS levels (8.5±3.9 *vs* 11.5±6.8%) as compared to non-HU treated patients. *Conclusions:* ET and PV platelets show a greater thrombus formation capacity *in vitro* in dynamic flow conditions. This capacity is significantly influenced by JAK2 V617F mutation burden and HU therapy. On the basis of these results, a prospective study of the PLT thrombus formation potential in a dynamic model is worth to evaluate the predictive value of this parameter on the thrombotic risk of ET and PV patients.

C048

INTERACTIONS BETWEEN ANALGESIC OR ANTINFLAMMATORY DRUGS AND WARFARIN IN PATIENTS RECEIVING LONG-TERM ORAL ANTICOAGULATION: RESULTS FROM THE "FARMAMICO" STUDY

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Treatment with warfarin requires careful consideration of multifold food and drug interactions. In the frame of a multicenter Italian prospective observational study of pharmacovigilance named FARMAMICO, all interactions of oral anticoagulant therapy are recorded. The possible interaction between warfarin and analgesics or anti-inflammatory drugs (NSAID) has been increasingly documented. We aimed to evaluate the effect of analgesics or NSAID on INR in stable patients treated with warfarin in two of the recruitment centers. After adding a new medication, we defined as case an INR variation ≥1 above or below the target range and as a control an INR variation <1. Patients filled self-reporting questionnaires on concomitant drugs. 2370 patients (1415 cases and 955 controls) from centers of Bergamo and Brescia were enrolled. To investigate the association between analgesics (*i.e.* paracetamol) or NSAID (*i.e.* ibuprofen) and the INR variation or adverse event, the Relative Risk (RR) with 95% confidence intervals (95% CI) was estimated, first crudely and then adjusted for age and sex. 9.2% (130/1415) of patients on concomitant therapy with analgesics or NSAID showed ≥1 INR variations. Among them 70.7% had an INR above the therapeutic range, with a 15.4% of them with INR >6. A 5.4% of total patients had instead an INR reduction. Analysis according to the drug classes showed that 56.3% of patients taking paracetamol had a ≥1 INR variations compared to 20.2% of patients taking ibuprofen. These latter had a 5 –fold lower risk of INR variation compared to patients taking paracetamol [RR: 0.2 (95%CI: 0.11-0.36)]. This result is statistically significant and persists after adjustment for age and sex. Only one patient, during paracetamol, reported an adverse event (minor bleeding). Our analysis shows that the use of paracetamol, considered the first choice analgesic for anticoagulated patients, increased the anticoagulant effect of warfarin in stable patients, while the anti-inflammatory drug ibuprofen was safer in these subjects. We therefore recommend close INR monitoring (*i.e.*, every 3–5 days) in patients taking paracetamol during warfarin therapy, especially in those with INR values close to the upper target range, as dosage adjustment may be required.

Chronic Lymphocytic Leukemia and Lymphoproliferative Disorders

C049

SMALL SUBCLONES HARBORING NOTCH1, SF3B1 OR BIRC3 MUTATIONS ARE CLINICALLY IRRELEVANT IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Ultra-deep next generation sequencing (NGS) allows sensitive detection of mutations and estimation of their clonal abundance in tumor cell populations. TP53 mutations identified by ultra-deep NGS significantly impact on survival of chronic lymphocytic leukemia (CLL) patients, independent of their representation in the tumor clone and even if restricted to a small fraction of leukemic cells. Here we aim at assessing the frequency, prognostic impact and evolution during disease course of NOTCH1, SF3B1 and BIRC3 mutations identified by ultra-deep NGS in newly diagnosed CLL. The study was based on a consecutive series of 304 newly diagnosed and previously untreated CLL. NOTCH1, SF3B1 and BIRC3 mutation hotspots were screened on peripheral blood samples by amplicon-based deep-NGS (454 Life Sciences) (average depth: 2437). A bioinformatic algorithm was applied to call non-silent variants out of background NGS noise. Variant calling by the algorithm was validated by Sanger sequencing or, if the variant was below the sensitivity threshold of Sanger sequencing, by both duplicate deep NGS and allele specific PCR (AS-PCR). Variant allele frequency (VAF) was corrected for tumor representation. Ultra-deep NGS identified 46 NOTCH1 mutations (median VAF 24%; range: 1.4-64%) in 14% (43/304) CLL, 43 SF3B1 mutations (median VAF 16%; range: 0.5-48%) in 11% (35/304) CLL and 37 BIRC3 mutations (median VAF 5.6%; range: 0.2-47%) in 8% (26/304) CLL. Out of the variants identified by ultra-deep NGS, a significant fraction of NOTCH1, SF3B1 and BIRC3 mutations (n=69; median VAF:2.95%; range: 0.2-17%) were missed by Sanger sequencing because their VAF was below the sensitivity threshold of the assay (~10%). The overall survival of cases harboring solely small subclonal mutations of NOTCH1, SF3B1 and BIRC3 not detectable by Sanger sequencing was similar to that of wild type cases and longer than that of cases with clonal lesions. Consistently, application of outcome-driven statistical approaches identified 25% (NOTCH1), 35% (SF3B1) and 1% (BIRC3) as the best VAF cut-off values for outcome prediction, thus suggesting that small subclones harboring NOTCH1, SF3B1 or BIRC3 mutations are clinically irrelevant. In conclusion, mutations of NOTCH1 and SF3B1 require a substantial clonal representation to be harmful (Figure 1).

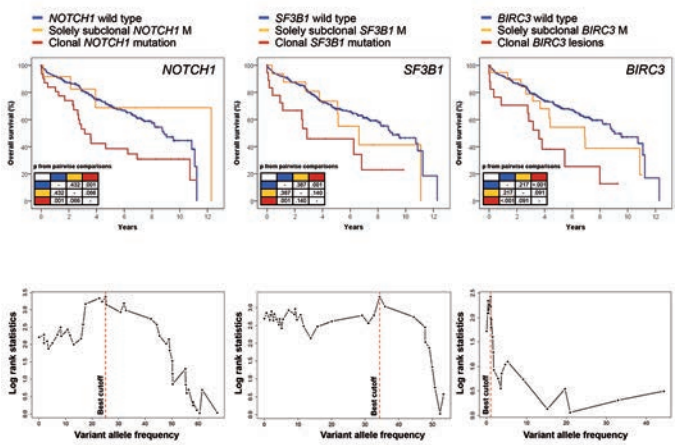

Figure 1.

C050

FRONT-LINE FLUDARABINE, CYCLOPHOSPHAMIDE, OFATUMUMAB CHEMOIMMUNOTHERAPY IN YOUNG (≤65 YRS) AND FIT PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA. PRELIMINARY RESULTS OF THE PHASE 2, MULTICENTER GIMEMA STUDY LLC0911

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The fludarabine, cyclophosphamide, rituximab (FC-R) regimen is associated with a high complete response (CR) rate and is considered the optimal front-line treatment for fit patients with chronic lymphocytic leukemia (CLL). FC combined with ofatumumab (O; FC-O) has also been associated with a high CR rate. The aim of this study was to evaluate whether an increased dose of ofatumumab combined with FC could improve the CR rate in young (≤65 yrs) and fit patients with CLL. Fifty-three fit patients with CLL from 15 Italian institutions were enrolled in this study and treated with the FC-O2 regimen including the FC schedule (F 25 mg/sqm i.v. d1–3, C 250 mg/sqm i.v. d1–3) combined with 13 doses of O (300 mg i.v. d14; 1000 mg d21 at the first cycle; 1000 mg d1 and d15 at cycles 2-6 and d28 at cycle 6). As infection prophylaxis, patients received bactrim and peg-filgrastim as primary prophylaxis of granulocytopenia. CLL diagnosis, treatment requirement and response were assessed according to the 2008 iwCLL guidelines. Minimal residual disease (MRD) was evaluated by flow-cytometry in the peripheral blood (PB) and bone marrow (BM), and also by PCR in negative cases. CT scan evaluation was included in the response assessment. The median age of patients was 58 years (range: 36.2-65.8), Binet stages B and C were recorded in 87% of cases, B-symptoms in 18%, increased B2M values in 73% and bulky nodes (≥5 cm) in 12%. An IGVH unmutated status was recorded in 60% of cases, deletion 13q in 37%, no aberrations in 32%, deletion 11q in 16%, trisomy 12 in 11%, deletion 17p in 2% and TP53 mutations in 9%. At present, in the 25 patients evaluable for response the overall response rate is 92%, with a CR rate of 64%. Among the 16 CR patients the rate of cases with no cytometric MRD in the PB and BM were 94% and 81% respectively. A grade 3-4 granulocytopenia was observed in 13 patients (26%), a severe infection in 7 (7.5%) and 6 (11%) experienced a severe infusion-related reaction during ofatumumab administration leading to treatment discontinuation in 1. After a median follow-up of 6 months (range, 1-14), one patient showed disease progression, while no deaths have been recorded. Taken together, the first analysis of this ongoing study suggests that the combination of FC with an increased dose of ofatumumab shows acceptable toxicity and is associated with a high MRD-negative CR rate in young and fit patients with previously untreated CLL.

C051

OUTCOMES OF ANTICOAGULANT OR ANTIPLATELET USE IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA OR INDOLENT NON-HODGKIN'S LYMPHOMA IN IDELALISIB TRIALS

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Background: IDELA, a selective oral PI3K inhibitor, is approved for use in relapsed CLL (in combination with rituximab [R]) and iNHL (as monotherapy). Both diseases occur mainly in the elderly, who have comorbidities that increase thrombotic risk. This post hoc analysis characterized the use and outcomes of AC/AP therapy, which was allowed in IDELA registrational clinical trials. *Methods:* In the phase 3 Study 312-116 (NCT01539512), frail pts with relapsed CLL (including those with any degree of thrombocytopenia) were randomized to receive a combination of continuous IDELA 150 mg BID or placebo (PBO) with 8 R doses. In the phase 2 Study 101-09 (NCT01282424), pts with refractory iNHL received IDELA 150 mg BID until disease progression or unacceptable toxicity. Grade 1, 2, and ≥3 bleeding events were analyzed using MedDRA preferred terms and CTCAE. *Results:* The 2 trials included 343 pts. In the CLL study, 18 pts (16%) on IDELA+R and 31 (29%) on PBO+R had grade ≥3 thrombocytopenia at baseline. Concomitant AC/AP use was frequent (45% in each study); the most common were aspirin, enoxaparin, and warfarin. AC/AP use was more frequent in pts treated with IDELA+R *vs* PBO+R. The incidence of bleeding events was similar with IDELA, IDELA+R, and PBO+R. Grade ≥3 bleeding events occurred in 1 IDELA+R, 1 PBO+R, and 3 IDELA pts. *Conclusions:* AC/AP use involved 45% of the IDELA registrational trial population. Overall, rates of bleeding events were moderate and similar with IDELA+R *vs* IDELA+PBO; grade ≥3 events were uncommon. There was no specific trend with regard to AC/AP and bleeding events in the 2 arms of the CLL study.

Table 1.

n (%)	CLL		iNHL
	IDELA + R n=110	PBO + R n=108	IDELA Monotherapy n=125
Pts receiving AC/AP	60 (55)	38 (35)	56 (45)
Aspirin	42 (38)	21 (19)	30 (24)
Enoxaparin	11 (10)	6 (6)	19 (15)
Warfarin	8 (7)	9 (8)	11 (9)
Pts with ≥1 bleeding event (any grade)			
Overall	15 (14)	20 (19)	17 (14)
Grade 1/2	14 (13)	19 (18)	14 (11)
Pts on AC/AP	n=60	n=38	n=56
Event at any time	10 (17)	6 (16)	14 (25)
Event on AC/AP	7 (12)	5 (13)	8 (14)
AC at any time	9	4	13
AP at any time	5	3	6
Patients not on AC/AP	n=50	n=70	n=69
Event at any time	5 (10)	14 (20)	3 (4)

C052

CD4+T-LGLL AND CD8+ T-LGLL: TWO DISTINCT DISEASES WITH DIFFERENT BIOLOGICAL AND CLINICAL FEATURES

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T-Large Granular Lymphocyte Leukemia (T-LGLL) is a chronic and heterogeneous lymphoproliferative disorder characterized by clonal expansion of CD3+ Large Granular Lymphocytes (LGLs). T-LGLs derive from CD4-/CD8+ T-Lymphocyte although rare forms of CD4+ LGLL are described and characterized by CD4+/CD8^{-}/dim expression. Most patients present an indolent course but can become symptomatic due to the development of cytopenia, in particular neutropenia. Aim of this study is the characterization of different clinical and biological subtypes of T-LGLL through evaluation of biological features, namely NK receptors and Vβ expression, coupled to clinical features. A cohort of 101 patients with T-LGLL was analysed by flow cytometry using antibody for CD3, CD4, CD8, Vβ, KIR, and NKG2 receptors and was studied for the presence of neutropenia (ANC<1,500/mm^3), severe neutropenia (ANC<500/mm^3), association with autoimmune disorders or secondary neoplasia and treatment requirement. By FACS analysis, two distinct subgroups of patients could be defined: 68 were CD8+ T-LGLL, while remnant 33 were CD4+ T-LGLL. KIR and NKG2 receptor expression was significantly higher in CD8+ LGLL than CD4+ LGLL (KIR: 32% *vs* 12%, p<0.05; NKG2: 43% *vs* 15%, p<0.01). Vβ13.1 and Vβ5.1 were the principal Vβ expressed in CD4+ patients (24% and 15% respectively) while CD8+ patients did not present a skewed Vβ expression pattern. Autoimmune disorders were significantly associated to CD8+ subgroup (32% *vs* 9%, p<0.05) while secondary neoplasia were more common in CD4+ subgroup (36% *vs* 18%, p<0.05). Neutropenia and severe neutropenia were significantly more frequent in CD8+ T-LGLL subtype (neutropenia: 57% *vs* 3%, p<0.01; severe neutropenia: 25% *vs* 0%); interestingly, female patients had significantly higher recurrence of neutropenia than males (51% *vs* 27%, p<0.05). Finally, only 13 out of 101 patients (13%) required treatment during the natural course of the disease and all these patients were CD8+ T-LGLL while no one of CD4+ T-LGLL patients was treated over a median follow up of 9 years. In conclusion, in our cohort of patients, two distinct biological and clinical subgroups of T-LGLL were clearly identified; in particular, CD8+ T-LGLL patients were characterized by high KIR and NKG2 expression, association with autoimmune diseases, presence of neutropenia and treatment requirement, while CD4+ T-LGLL presented a more indolent course of disease.

C053

CHROMOSOME ABERRATIONS DETECTED BY CONVENTIONAL KARYOTYPING USING NOVEL MITOGENS IN CHRONIC LYMPHOCYTIC LEUKEMIA: CLINICAL AND BIOLOGIC CORRELATIONS

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In vitro stimulation with CpG-oligonucleotide DSP30 plus IL2 (DSP30/IL2) improves the proliferation of CLL cells, yielding assessable metaphases in most patients. By this method, karyotypic aberrations (KA) are detected in 80% of CLL with some patients showing KA in regions not covered by the classical 4-probe FISH panel. We designed this study to analyze the prognostic impact of KA as detected by stimulation with DSP30/IL2 in newly diagnosed CLL that had received FISH and genetic characterization. The clinical and prognostic significance of KA was first evaluated in a retrospective series of patients (learning cohort, LC) diagnosed between 1998 and 2006 and then validated prospectively in a series of cases (validation cohort, VC) diagnosed and analyzed for karyotype with DSP30/IL2 stimulation between 2007 and 2014. Each patient was categorized into a FISH risk group according to the following classification: favorable (isolated 13q14 deletion or absence of FISH aberrations), unfavorable (deletions of 11q22 or of 17p13), intermediate (trisomy 12). Each patient was also categorized into a cytogenetic risk group according to the following classification: favorable (isolated 13q14 deletion or normal karyotype), unfavorable (deletions of 11q22 or 17p13, or complex karyotype, ie, at least 3 chromosome aberrations), interme-

diate (all other KA). In the VC, we also evaluated IGHV, TP53, NOTCH1 and SF3B1. KA undetected by FISH were found in 34.5% and 35.3% of the cases in the LC and VC, respectively. In addition to FISH, KA allowed to reclassify 22.8% and 25.6% of cases in the LC and VC, respectively, into a higher cytogenetic risk group. By multivariate analysis, both in the LC and VC, KA other than isolated del13q correlated with a shorter time to first treatment (TFT; p<0.001 and 0.003, respectively), while a complex karyotype predicted a worse overall survival (OS, p=0.015 and 0.010, respectively). In the VC a shorter TFT was also predicted by stage (p<0.001), IGHV (p=0.05) and del(17p)/TP53 mutations (p=0.033) while stage (p=0.023) and del(17p)/TP53 mutations (p=0.024) independently predicted a shorter OS. FISH results did not independently affect TFT and OS, in both the LC and VC, as was the case for NOTCH1 and SF3B1 mutations in the VC. This study suggests that in CLL, conventional karyotyping with DSP30/IL2 stimulation is more effective than FISH for the detection of KA allowing for a more precise refinement of the prognostic risk.

C054

mRNA EXPRESSION AND *IN VITRO* MODULATION OF PROGRAMMED DEATH-1 RECEPTOR AND ITS LIGANDS PDL1 AND PDL2 PROTEINS IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: PD1 and its ligands PDL1 and PDL2 form a prominent immune checkpoint ligand/receptor axis in maintaining an immunosuppressive tumor microenvironment. We report: PD1, PDL1, and PDL2 mRNA expression in early-stage CLL (clinicaltrials.gov:NCT00917549); baseline protein expression and effects of exposure to autologous activated T-cells (AAT); effects of ibrutinib (IBR), a covalent inhibitor of BTK, on this ligand/receptor axis in AAT co-culture conditions. *Methods:* GEP analysis was performed on highly purified B-cells (N=211). PD1, PDL1 and PDL2 expression (N=8) was evaluated in B and T cells by flow cytometry (FACSCantoII, BD Biosciences). AAT cells prepared using Dynabeads Human T-Activator CD3/CD28/IL2 for T Cell Expansion and Activation (Life Technologies), were identified by visual inspection of cluster formation (CF). B-CLL/AAT co-cultures were exposed to IBR (1/10µm) for 24/48h (1) prior to (pCF) or (2) after (aCF) AAT formation. *Results:* GEP analysis showed 1) higher PD1, PDL1, and PDL2 expression in CLL B-cells *vs* normal B-lymphocytes subsets; 2) correlation between PD1 expression and unmutated IGVH (p<.005) and high risk cytogenetics [del(17p) and del(11q)]; 3) significantly shorter TTFT for cases having PDL2 above median value. ATT co-cultured with CLL B-cells showed 1) upregulation in expression of PD1 and its ligands, particularly PDL1 *vs* baseline (%PD1 19.5 *vs* 33.5, p=.012;%PDL1 0.55% *vs* 17.9, p=.012;%PDL2 9.9 *vs* 25.48, p=.035); 2) a similar up-regulation in CD3+ cells of PD1 and PDL1 (%PD1 28.7 *vs* 72.4, p=.012;%PDL1 1.9 *vs* 67.2, p=.012) also in ATT with no change for PDL2 (14.1 *vs* 28.4, p=NS). The above experiments with/without IBR treatment in culture pCF with 1,10µM IBR and for 24h, 48h showed 1) dose/time-dependent IBR inhibition of CF; 2) a marked decrease in PD1 and PDL1, already after 24h and significantly after 48h of culture in both B and T-cells; 3) inhibition of PDL2 protein expression in B-cells after 48h of IBR, phenomenon already visible at 24h with 1nM IBR, while a modest change was observed for T-cells; 4) similar changes observed in PD1 proteins in T-cell subsets CD8+, CD4+. Experiments aCF indicated no relevant changes in PD1, PDL1 and PDL2 expression in either B or T-cells. *Conclusions:* Microenvironment-derived signals regulate expression of PD1 and its ligands PDL1/PDL2 in neoplastic B/T-cells in CLL. IBR inhibits AAT-activation *in vitro* and PD1/PDL1/PDL2 protein expression suggesting a rationale for target therapy.

Myeloma and Monoclonal Gammopathies 1

C055

MYELOMA CELLS FROM THE BONE MARROW TO THE SKIN: HOW?

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Multiple myeloma (MM) is a plasma cell (PC) malignancy characterized by the tight dependence on the bone marrow (BM) microenvironment due to the high expression of cell adhesion molecules including CD44, CD54, CD56, CD49d and chemokines receptors such as CXCR4. Secondary extramedullary involvement without a leukemic phase is a rare complication of MM that may occur at the end stage of the disease. Among the extramedullary localizations, the skin is one of the possible sites due to the physiological homing of PCs. However the mechanisms of extramedullary spread are not well understood. In this study, we analyzed the gene expression profiles (GEPs) by GeneChip U133 Plus 2.0, Affymetrix, and the immunophenotypic and immunohistochemistry (IHC) profiles of MM cells across the course of the disease in a patient with Bortezomib (BOR) refractory MM who developed a cutaneous localization after 16 months from the diagnosis. IHC profiles were also analyzed to confirm the results in 4 more MM patients with secondary skin involvement. By GEP analysis, performed on diagnosis and BM relapse samples, 742 genes were differentially expressed. A more stringent analysis identified 19 down-regulated and 42 up-regulated genes. Data were confirmed by Real Time PCR on selected genes mainly involved in PC homing. At the BM relapse we found that MM cells highly expressed CCR10, a receptor for CCL27 (Cutaneous T-cell-attracting chemokine) and CXCR4 and were negative for CD56. A significant down-regulation of ICAM1 and ALCAM was observed together with the up-regulation of MAGE family genes, DKK1 and SEMA3A in the BM relapse sample compared to diagnosis one. At the development of the cutaneous involvement, 4 months after BM relapse, the immunophenotype of MM cells showed the lack of CXCR4 expression and the presence of CD56. On the other hand the IHC profile of skin MM cells showed the expression of both CD56 and CD54. Consistently the loss of CXCR4 expression was recently associated to BOR resistance and extramedullary disease in a mouse model of MM. In conclusion, the skin homing of MM cells could be related to the high expression of CCR10 and the loss of CXCR4 expression across the progression of the disease. Moreover the down-regulation of CD54 and the lack of expression of CD56 by MM cells in the BM relapse together with the re-acquisition of both antigens during the cutaneous relapse may be the mechanism that drives the escape from BM of PCs and their localization into the skin.

C056

A PHASE 3, RANDOMIZED CLINICAL STUDY OF AUTOLOGOUS TRANSPLANTATION VS CYCLOPHOSPHAMIDE-LENALIDOMIDE-DEXAMETHASONE AT DIAGNOSIS

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High-dose chemotherapy followed by autologous stem cell transplantation (ASCT) is the standard approach for patients ≤65 years of age with multiple myeloma (MM). However, novel agents have challenged the role of ASCT. In this randomized, phase 3 trial, we compared ASCT with cyclophosphamide-lenalidomide-dexamethasone (CRD) in with newly diagnosed MM patients ≤65 years. The primary endpoint was progression-free survival (PFS), the secondary endpoints were safety and overall survival (OS). All patients received induction therapy with four 28-day cycles of lenalidomide-dexamethasone (lenalidomide 25 mg day 1–21 and low-dose dexamethasone 40 mg day 1, 8, 15, 22) followed by stem cell mobilization. Subsequently, patients were randomized to consolidation with 2 cycles of melphalan 200 mg/m^2 with stem-cell support (MEL200-ASCT) or six 28-day cycles of CRD (cyclophosphamide 300 mg/m^2 day 1, 8, 15; dexamethasone 40 mg days 1, 8, 15, 22; and lenalidomide 25 mg days 1–21). A total of 389 patients were enrolled. Patient characteristics were well balanced between the two arms. After a median follow-up of 4 years, the median PFS was 42 months with MEL200-ASCT *vs* 28 months with CRD (HR 0.67, P=0.014). The 4-year OS was 87% with MEL200-ASCT *vs* 71% with CRD (HR 0.51, P=0.028). MEL200-ASCT showed to be superior to CRD in terms of PFS and OS in most of the analysed subgroups. MEL200-ASCT induced a higher rate of grade 3-4 hematologic (84% *vs* 26%, P<0.001) and non-hematologic (39% *vs* 22%, P=0.008) adverse events (AEs) compared with CRD. Infections (19% *vs* 6%, P=0.004) and gastrointestinal AEs (20% *vs* 5%, P<0.001) were the major non-hematologic AEs. Although MEL200-ASCT increased the rate of grade 3-4 AEs, serious hematologic (0% *vs* 2%, P=0.49) and non-hematologic AEs (7% *vs* 10%, P=0.393) were similar between the two groups. No toxic deaths occurred with MEL200-ASCT; 1 patient died of septic shock in the CRD group. second primary malignancies (SPM) occurred in 4 patients who went off protocol before consolidation (1 gastrointestinal cancer, 1 renal cancer, 1 breast cancer, 1 squamous cell carcinoma), 8 patients in the MEL200-ASCT group (6 squamous cell carcinomas, 1 prostate cancer, 1 melanoma), and 5 patients in the CRD group (1 squamous cell carcinoma, 1 gastrointestinal cancer, 1 renal cancer, 1 breast cancer, and 1 glioblastoma). In conclusion, MEL200-ASCT significantly prolonged PFS and OS compared with CRD, without increasing serious AEs, toxic deaths, and SPMs.

C057

SUPERIOR EFFICACY OF VTD OVER VCD BEFORE AND AFTER AUTOLOGOUS STEM-CELL TRANSPLANTATION IN NEWLY DIAGNOSED MULTIPLE MYELOMA

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Bortezomib-thalidomide-dexamethasone (VTD) has been approved by EMA as induction therapy for autotransplantation (ASCT)-eligible patients (pts) with newly diagnosed multiple myeloma (MM). Bortezomib-cyclophosphamide-dexamethasone (VCD) is an alternative to VTD, but efficacy results of this regimen have not been backed by phase III studies and no prospective comparison of VTD *vs* VCD has been performed so far. We analyzed the outcomes of 117 pts randomized to the VTD arm of the GIMEMA-MMY-3006 study with those of an equal number of pair mates [matching criteria: age (±2 years), ISS stage (1 *vs* 2 *vs* 3), presence of t(4;14) and/or del(17p) positivity] who were treated with VCD and ASCT as part of the EMN02 trial. Induction treatment consisted of three 21-day cycles of VTD and VCD (bortezomib and dexamethasone doses as in VTD, cyclophosphamide 500 mg/m^2 on d 1 and 8). In comparison with VCD, VTD yielded a higher rate of ≥VGPR (IMWG criteria) before (38% *vs* 60%, p=0.001) and after ASCT (63% *vs* 78%, p=0.014), including a higher CR rate (5% *vs* 19%, p=0.001, and 14% *vs* 39%, p<0.001, respectively). The superior efficacy of VTD over VCD was retained before ASCT (CR: 25% *vs* 5%, p=0.002; ≥VGPR: 67% *vs* 38%, p=0.001) and after ASCT (CR: 43% *vs* 11%, p<0.001; ≥VGPR: 85% *vs* 64%, p=0.007) in pts with ISS stage 2-3. Pts with t(4;14) and/or del(17p) treated with VTD had a significantly higher probability to achieve ≥VGPR than the same subgroup receiving VCD (86% *vs* 43%, p=0.004). Any grade 3-4 hematological toxicity was higher with VCD *vs* VTD (12% *vs* 5%). Grade ≥2 peripheral neuropathy (PN) (NCI CTCAE, version 3.0) was not significantly higher for pts on VTD compared to those on VCD (12% *vs* 9%, p=0.19), but the difference between the two groups was statistically significant when grade 3-4 PN was considered (2% *vs* 7%, p=0.004). No difference in the median number of CD34+ cells/kg infused to support Mel-200 was observed between pts receiving VTD or VCD (4 x10^6 *vs* 4.5 x10^6/kg). In conclusion, VTD induction therapy was associated with significantly higher pre-ASCT and post-ASCT CR and ≥VGPR rates compared to VCD, a gain retained across high-risk subgroups of pts. These data confirm that VTD is one of the most active induction regimens currently in use in preparation for subsequent ASCT. Possible benefits of VTD *vs* VCD in terms of longer post-ASCT PFS will be reported at the meeting.

C058

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

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The probability of progression of SMM into symptomatic disease (MM) is highly variable and therefore the identification of sub-groups of patients (pts) at different risk is a relevant end point. The presence of osteolytic lesions identified by FDG-PET/CT has been recently incorporated in the updated criteria for the diagnosis of MM. However, no data are available on the impact of PET/CT FLs in the absence of underlying osteolyses in SMM on time to progression (TTP) into MM. To address this issue, we prospectively studied a cohort of 120 pts with SMM with PET/CT at presentation of the disease. Pts with osteolyses underlying FLs were excluded from the study, as they were considered as having MM. Laboratory follow-up took place every 3-4 months. Skeletal progression was defined by the appearance of one or more sites of osteolytic bone destruction, pathological fractures and/or soft masses at PET/CT or MRI. The start of systemic therapy was defined as the date of event for the analysis of TTP. PET/CT was pos in 19/120 (16%) of the pts; 8 pts had 1 FLs, 3 pts 2 FLs, 6 pts more than 3 FLs and 2 pts a diffuse bone marrow involvement. Median SUV max value was 4.7. Ten per cent, 11% and 13% of the pts were having more than 60% BMPC, FLC ratio ≥100 and more than 1 FL at MRI, respectively. With a median follow-up of 2.2 years, 38% of the pts progressed to MM, in a median time of 4 years, including 21% with skeleton involvement, with/without the appearance of other CRAB symptoms, and 17% with exclusive serological progression. The RR of progression of the pts with a pos PET/CT was 3.00 (95% CI 1.58 - 5.69, P=0.001). Moreover, the RR of skeletal progression was 4.44 (95% CI 1.97 – 10.02, P <0.001); median TTP PET/CT pos *vs* neg pts: 2.2 *vs* 7 years (Figure 1). The probability of progression within 2 and 3 years for pts with pos PET/CT was 58% and 66%, respectively, *vs* 33% and 42% for neg pts. Results did not significantly differ after pts with early MM according to the updated criteria were excluded from the analysis. A multinomial logistic regression analysis showed that PET positivity was significantly related to progression with skeletal involvement while BMPC>60% to the exclusive sero-

logical one. In conclusion, PET/CT could become a new risk factor to define high risk SMM. Further studies are warranted to find and optimal cut off of FLs and/or SUVmax to capture the higher risk of progression at 2 years and to merge with other prognostic factors.

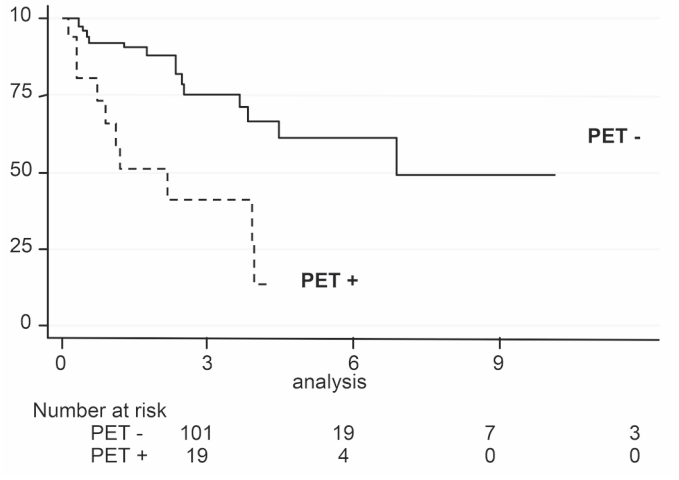

Figure 1. Kaplan-Meier survival estimates.

C059

EFFICACY OF BIOSIMILAR G-CSF *VERSUS* ORIGINATOR G-CSF IN PERIPHERAL BLOOD STEM CELL MOBILIZATION IN *de novo* MULTIPLE MYELOMA PATIENTS

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Filgrastim and lenograstim are the standard granulocyte colony-stimulating factor (G-CSF) agents for peripheral blood stem cell mobilization (PBSC) in patients who undergo autologous stem cell transplantation. To assess whether biosimilars are effective, we conducted a single-center prospective study that included 40 consecutive *de novo* multiple myeloma (MM) patients who received cyclophosphamide 4 g/m^2/day plus biosimilar filgrastim G-CSF to mobilize PBSC. These patients were compared with a group of 37 patients matched for age, diagnosis, previous chemotherapy, and mobilization who had been treated with originator G-CSF. The mean number of CD34+ cells/ L in the peripheral blood was 199.6±207.4 in the biosimilar and 192.8±154.7 in the originator group (P=0.87). The median number of CD34+ cell/kg recipient collected was 11.5±5.8 and 12.3±5.3 in the biosimilar and originator group, respectively (P=0.51). The mobilization failure rate was 2.5% and 2.7% in the biosimilar filgrastim and originator filgrastim cohorts (P=n.s.), respectively. Twenty-nine patients in the biosimilar and 28 patients in the originator group underwent autologous transplantation. There were no statistically significant differences between the biosimilar and originator G-CSF cohort in terms of hematopoietic recovery parameters and transplant-related toxicities. In conclusion, the efficacy of biosimilar G-CSF seems equivalent to the reference G-CSF.

C060

SALVAGE AUTOLOGOUS STEM CELL TRANSPLANTATION FOR RELAPSED MULTIPLE MYELOMA

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There is not a standard of care for patients (pts) with multiple myeloma (MM) relapsed after autologous stem cell transplantation (ASCT). We retrospectively analyzed 66 MM pts who relapsed after upfront single or double ASCT and received a salvage ASCT (sASCT) at four italian centres. Median age at sASCT was 60 years. Median progression free survival (PFS1) from upfront ASCT to relapse was 44 months (mos). 72.7% of pts received sASCT at first disease progression. Reinduction regimens were bortezomib (bort)-based (84%) and IMiDs-based (16%). ORR was higher and median time to response was shorter with bort-based in comparison to IMiDs-based regimens (88% *vs* 60%, p=0.049; 2.2 *vs* 4.2 mos, p=0.01, respectively). 64% of pts already had harvested stem cells for sASCT while 24 pts needed a further peripheral blood stem cells (PBSC) mobilization. High-dose melphalan was the standard regimen before sASCT and was used at 200 mg/sm in 64% of pts. Responses to sASCT included: CR 43.9%, VGPR 33.3%, ≥PR 93.9%. 29% of pts improved from <CR before sASCT to CR after sASCT (p=0.0001). With a median follow up of 2 years after sASCT, 39 pts (59%) experienced progression. Median PFS from sASCT (PFS2) was 17.3 mos. PFS2 was significantly shorter in pts with PFS1 ≤24 mos (9.6 *vs* 18.3 mos, p=0.003), in pts who did not receive sASCT at first disease progression (9.7 *vs* 18.3 mos, p=0.03), in pts with extramedullary disease (EMD) (9.9 *vs* 18.3 mos, p=0.008) and in pts who received reinduction therapy with an IMiDs-based regimen (9.9 *vs* 18 mos, p=0.01). In multivariate analysis PFS1 ≤24 mos (HR 0.21, CI 0.08-0.56) and the presence of EMD (HR 6.6, CI 1.8-24.2) were associated with a shorter PFS2. 23 pts (35%) died after sASCT, mainly due to disease progression (74%). TRM at day +100 was 3%. Median OS from ASCT (OS1) and sASCT (OS2) was 166 and 43 mos, respectively. OS2 was significantly shorter in pts with PFS1 ≤24 mos (14.2 *vs* 58.3 mos, p=0.003), in pts who did not receive sASCT at first disease progression (13.7 *vs* 58.3 mos, p=0.008), in pts with EMD (13.9 *vs* 58.3 mos, p=0.03) and in pts who failed CR after sASCT (30 mos *vs* not reached, p=0.006). In multivariate analysis PFS1 ≤24 mos (HR 0.26, CI 0.09-0.80) and <CR after sASCT (HR 0.27, CI 0.09-0.80) were associated with a shorter OS2. Novel agent-based sASCT is safe and effective for relapsed MM. Patients in first relapse, with PFS1 >24 mos and achieving CR benefit the most from sASCT (Figure 1).

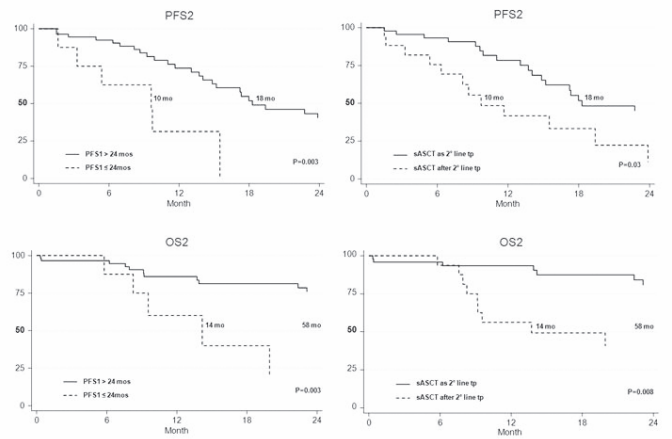

Figure 1.

Cytogenetics and Molecular Genetics. Laboratory Diagnostics

C061

DROPLET DIGITAL PCR FOR DNMT3A AND IDH1/2 MUTATIONS TO IMPROVE EARLY DETECTION OF ACUTE MYELOID LEUKEMIA RELAPSE AFTER ALLOGENEIC HSCT

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Background: Leukemia relapse after allogeneic Hematopoietic Stem Cell Transplantation (allo-HSCT) is a major unsolved issue, and efforts are aimed to anticipate relapse detection to the Minimal Residual Disease (MRD) stage. Mutations in DNMT3A and IDH1/2 occur early during the step-wise process of Acute Myeloid Leukemia (AML) tumorigenesis, possibly representing valid markers for MRD tracking. *Materials and Methods:* By conventional Sanger sequencing, we screened for the mutations of interest (DNMT3A R882H, DNMT3A R882C, IDH1 R132C, IDH1 R132H, IDH2 R140Q and IDH2 R172K) 89 AML samples harvested at diagnosis from patients who underwent myeloablative allo-HSCT. 140 bone marrow samples collected longitudinally over time after transplant from the 26 patients who carried at least one of the mutations were analyzed by ultra-sensitive droplet digital PCR (ddPCR) assays, using the Bio-Rad QX100 system. ddPCR results were compared to those obtained testing the same samples by quantitative PCR (qPCR) assessment of the WT1 gene transcript and of hematopoietic chimerism. *Results:* All the samples which resulted positive by conventional sequencing were confirmed by ddPCR, and in 23/25 (92%) the population carrying the mutant allele, quantified by ddPCR, consistently exceeded the morphological count of leukemic blasts, suggesting the presence of the mutation also in apparently normal hematopoietic cells. All the samples tested at post-transplantation relapse resulted positive for the mutations present at diagnosis, except for one case, originally carrying both DNMT3A and IDH2 mutations and typing negative for the latter at relapse. When post-transplantation remission samples were tested, 71/77 (98%) of those harvested from 8 patients who remained long-term leukemia-free (median follow-up 23 months) resulted negative for the mutations of interest. 8/9 patients who subsequently relapsed presented at least one sample harvested during apparent disease remission which resulted positive by ddPCR, anticipating relapse of at least one month. Of notice, only 5 of those 9 patients displayed WT1 transcript overexpression before relapse and only 2 patients displayed host chimerism above the 1% threshold. *Conclusions:* Based on our data, ddPCR for DNMT3A and IDH1/2 mutations appears extremely promising, displaying very high specificity and sensitivity in relapse prediction, and comparing favorably with currently in use qPCR-based post-transplantation monitoring techniques.

C062

COMBINED INTERPHASE FISH RELIABLY DETECTS NEW DRIVING AND DRUGGABLE REARRANGEMENTS IN THE DIAGNOSTIC WORK-UP OF T-ALL

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The advent of whole genome technologies has extended the identification of rearranged and/or aberrantly expressed genes. The major challenge today is to translate these new biological insights into clinical practice to improve diagnostic work-up and patient management. We developed a flexible CI-FISH assay which can be adapted to investigate new genes/loci as soon as they appear to be implicated in the pathogenesis and/or progression of T-ALL (La Starza R, Leuk Res 2013). CI-FISH with 26 probes (NCBI and UCSC databases) validated 6 new chromosome rearrangements detected by RNAseq (HiSeq2000, Illumina) in adult patients enrolled into the LAL 2000 and 0904 GIMEMA protocols (Table 1), and allowed a deeper insight into the underlying molecular mechanisms. New rearrangements of known oncogenes reclassified 3 T-ALL into the HOXA category. A new HOXA-RP11-455F11 involved the HOXA cluster with a non-TCR partner at 1q32. Interestingly, the 1q32 region holds actively transcribed sequences which are also involved in a non-TCR MYC translocation (La Starza R, Blood 2014). The rare NUP98-PSIP1 fusion occurred as isolated molecular event, confirming previous observations in NUP98-positive leukemias. So far, NUP98-PSIP1 has been associated only with AML, MDS, and blastic phase CML. A new DDX3X-MLLT10 pointed to MLLT10 as another promiscuous gene. Interestingly, MLLT10 fusions are characterized by a distinct gene expression profile within the HOXA category (Brandimarte L, Blood 2013). Two new fusions were identified in 2 TAL/LMO-positive cases. In one case, the SSBP2-FER, underlying a cryptic inversion/deletion at 5q14.1-q21.3, joined the FER tyrosine-kinase domain to the SSBP2 dimerization domain. SSBP2-FER transformed Ba/F3 cells to IL-3–independent growth and induced autophosphorylation of the downstream STAT proteins (Atak KZ, PLOS-Genet 2013). In the other case, a cryptic TRAC-SOX8 rearrangement occurred at chromosome 16p13 and caused SOX8 overexpression. Finally, a complex intrachromosomal 19p13 rearrangement produced an out-of-frame MAST3-C19orf10 fusion, probably causing PTEN inactivation, and amplification and overexpression of NOTCH3/JAK3 in a case of NOTCH1-positive T-ALL also bearing 6q15-q16, 9p21 and 17q11.2 deletion. CI-FISH is a powerful tool to validate RNAseq results and it represents a suitable test for diagnostic screening. A precise genetic diagnosis is mandatory to properly stratify patients and identify targetable leukemogenic pathways.

Table 1.

RNASeq cod	S/A	Immunophenotype	Karyotype	NOTCH1/FBXW7	New rearrangements	Genetic group	Follow-up (months)
R27	F/16	early	46,XX	mut	*MIR181A1HG-HOXA11*	*HOXA*	relapse,+63 died
R5	M/20	not available	not available	wt	*NUP98-PSIP1*	*HOXA*	CR, +56 alive
R10	M/26	cortical	46,XY,t(X;10)(p11;p12)	mut	*DDX3X-MLLT10*	*HOXA*	CR,+80 alive
R4	F/24	not available	not available	wt	*SSBP2-FER*	*TAL/LMO*	relapse,+30 died
R24	M/22	cortical	not available	wt	*TRAC-SOX8*	*TAL/LMO*	relapse,+13 died
R15	M/39	early	47-48,XY,+8,-9,der(11)(9;11)(q13;p15),-19,-19,-22,+4mar	mut	*MAST3-C19orf10* *NOTCH3/JAK3 amplification*	Unclassified	refractory,+12 died

C063

NOVEL CALR SOMATIC MUTATIONS IN ESSENTIAL THROMBOCYTEMIA

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Essential Thrombocythemia (ET), Primary Myelofibrosis (PMF), and Polycythemia Vera (PV) are Philadelphia-negative (Ph-neg) classical myeloproliferative neoplasms (MPN). In 2005, diagnosis and treatment of Ph-neg MPNs were revolutionised by the discovery of the Janus Kinase 2 (JAK2) V617F mutation. Approximately 50-60% of ET patients harbored the JAK2V617F mutation while Thrombopoietin receptor (MPL) mutation was detected in 3-5% of cases. Recently, mutations in the Calreticulin (CALR) gene, encoding a multi-functional Ca2+ binding protein chaperone, were discovered in MPN patients. ET was characterised by thrombocytosis and absence of a relevant bone marrow fibrosis. In ET patients CALR mutations were associated with male gender, younger age, lower hemoglobin level and leukocyte count, higher platelet count and a reduced risk of thrombotic events. We retrospectively analysed JAK2, CALR and MPL mutations in 212 ET patients at the Section of Pathology of AO Città della Salute e della Scienza of Turin. The aim of this study was to provide additional details on the molecular characterisation of ET. JAK2, CALR and MPL mutations were detected in 51.4%, 28.3% and 3.8% of ET patients respectively. Accord-

ing to previous studies, CALR mutational frequency was 63% within both JAK2V617F and MPL W515L/K wild-type cases. The sequence characterisation of the 60 CALR mutated samples, showed a prevalence of type 1 (63.3%), followed by type 2 (20%), type 3 (5%) and type 14 (1.7%). The remaining 6 mutated cases (10%) consisted of 6 novel CALR mutations not yet described in COSMIC catalogue at March 2015. Despite the evidence that the resultant C-terminal is the same for all variants, different CALR mutations seem to correlate with clinical characteristics of the patients. As expected, in our series the male gender was associated with type 1 and younger age with type 2 variants. Furthermore, platelet count was significantly higher in type 2 *vs* type 1 mutated patients. Moreover, the new mutations found seem to be associated with a different phenotype (female gender but platelet count similar to type 1) even if the number of infrequent variants is too low to reach statistical significance. A detailed assignment of CALR genotype must be included for an accurate ET diagnosis; it can correlate with some clinical features and may have implications for patients risk stratification and management.

C064

EVALUATION OF THE DIASORIN Q-LAMP TECHNOLOGY FOR THE MOLECULAR DIAGNOSIS OF THE PHILADELPHIA POSITIVE LEUKEMIAS: AN ITALIAN MULTICENTER STUDY

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Introduction: molecular detection of BCR-ABL mRNA is routinely performed to confirm Chronic Myeloid Leukemia and for risk stratification of B-precursor Acute Lymphoblastic Leukemia. Conventional RT-PCR is the method of choice for this purpose. In this study we have evaluated an alternative technique, the Q-LAMP Iam BCR-ABL (DiaSorin), for differential detection of BCR-ABL p190 and p210 fusions. *Methods:* the Q-LAMP Iam BCR-ABL is a non-PCR, isothermal method for simultaneous retro-transcription and amplification of the common isoforms of BCR-ABL. In case of BCR-ABL negative the assay detects the housekeeping GUS mRNA, acting as internal control. The reaction is incubated aat constant temperature for 60 minutes onto the Liaison Iam (DiaSorin), a small size instrument that monitors the amplifications in real time and automatically analyzes final results. Overall 165 archived RNA samples (111 BCR-ABL positive, 54 negative; 500 ng per reaction) have been tested and the results compared with the RT-PCR ones. 11 samples showed a chemical contamination (A260/A230 ratio between 0,24 and 1). In addition, 8 rare isoform samples (e19a2 n=3; e6a2 n=3; e8(-44nt)/+(intr abl1 30nt)/a2 n=1; p210 with a deletion in the fusion region) have been tested. *Results:* The Q-LAMP and RT-PCR data resulted 100% concordant. The amplification of p210 and p190 positive samples was visible in real-time in 22,7 and 18,3 minutes as average. The amplification of the samples chemically contaminated correctly occurred in 20,8 min as average. The rare isoforms e19a2 and e6a2 have been amplified, as well as the p210 positive sample with a deletion in the fusion region, not detectable by quantitative PCR. The rare isoform e8(-44nt)/+(intr abl1 30nt)/a2, detected by nested RT-PCR, did not produce amplification in Q-LAMP. The 54 negative samples have been validated by amplification of GUS mRNA. The assay correctly discriminated the p210 and p190 isoforms, but did not differentiate between b2a2 and b3a2. *Conclusions:* The evaluation study showed that the Q-LAMP Iam BCR-ABL in combination with the Liaison Iam instrument produced concordant results with conventional RT-PCR on archived 165 RNA samples. The strengths identified are the speed, the robustness and the safety of the one-step, close tube format, which allow, united with the ready-to-use lyophilized CE marked reagents, adoption even in not highly specialized laboratories for molecular diagnosis of Philadelphia Positive Leukemias at the onset.

C065

IDENTIFICATION OF A NEW PDGFRB FUSION PARTNER RESPONSIVE TO IMATINIB IN A MYELOID NEOPLASM WITH EOSINOPHILIA

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Fusion genes derived from the platelet derived growth factor receptor β (PDGFRB) or α (PDGFRA) play an important role in the pathogenesis of eosinophilia-associated myeloproliferative neoplasms. Despite more than 20 PDGFRB fusion partners have been reported and the tyrosine kinase inhibitor imatinib mesylate induced rapid and complete hematological remission in the majority of cases. In this study, we report an uncommon case of a myeloproliferative disease with a new PDGFRB rearrangement. A 40 year-old man was admitted with fever and acute renal failure. He complained for itching. Laboratory exams showed leukocytosis with neutrophilia and hypereosinophilia (white blood cell count 16x 10^9/l, 75% neutrophils, 13% eosinophils), mild anemia and normal platelet count. Abdomen ultrasound showed splenomegaly. Bone marrow biopsy showed normal cellularity (60%), with granulocytic hyperplasia, and increased eosinophils and megakaryocytes. Cytogenetic analysis showed a normal (46,XY) male karyotype. Fluorescent *In Situ* Hybridization (FISH) with LSI PDGFRB Dual Color, Break Apart Rearrangement Probe (Abbott) revealed a partial deletion of PDGFRB gene (chr 5q32) in 50-60% of cells. Cosmid 4-1 and 9-4 (Baxter E.J. *et al.*, BJH, 2003, 120, 251-256) confirmed the deletion of the 5' of the gene. Single Nucleotide Polymorphism array (SNPa) with Cytoscan HD platform (Affymetrix Santa Clara, CA) revealed a 662,9 kb CN=1 deletion from region 5q32(149539271bp) to region 5q33.1(150202192bp). Further refinement with FISH probes RP11-915J10(150210459-150364168), RP11-632E9(150444566-150611756), and fosmid G248P85622E10 (150434061-150478062) narrowed 5q deletion between the 3' of PDGFRB and the 5' of TNIP1 gene. The patient in peritoneal dialysis, was treated with imatinib at a daily dose of 100 mg orally. At 6 months since the start of imatinib, the patient had no itching and his peripheral blood counts showed normal leukocytes and disappearance of eosinophilia. The abdomen ultrasound revealed normal spleen size. Bone marrow biopsy showed disappearance of granulocytic hyperplasia and hypereosinophilia. Interphase FISH documented only 3% of cells with the deletion at 5q. In conclusion we identified a new fusion partner of PDGFRB as a driver of successful therapy with tyrosine kinase inhibitor.

C066

A NEW FAMILIAL TINF2 MUTATION IN A TELOMERE SYNDROME WITH APLASTIC ANEMIA

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Background: Since telomere status influences senescence programs, stem cell pool exhaustion and carcinogenesis, it plays a prominent role in disease etiology. Defects in genes that are responsible for telomere length homeostasis, such as TINF2 mutations, underlie telomere syndromes, a spectrum of genetic disorders including diskeratosis congenita, aplastic anemia (AA), idiopathic pulmonary fibrosis, liver cirrhosis (LC); myelodysplastic syndromes and acute myeloid leukemia (Armanios M. *et al.* 2012). *Material and Methods:* A 69-year-old man was referred because of anemia and pancytopenia. Bone marrow aspirate indicated AA with normal karyotype. A multisystem disorder, *i.e.* LC, psoriasis, nail

dystrophy, severe osteoporosis, mild chronic kidney failure and hypertension, suggested a telomere syndrome. Peripheral blood and/or hair bulbs DNA, in the proband and family members was screened for telomerase enzyme components TERT and TERC as well as TINF2 using a PCR based DHPLC assay (Wave MD system; Transgenomic Inc. Omaha, NE). Sequencing of abnormal chromatographs was performed by Sanger method (ABI 3500 Genetic Analyzer, Applied Biosystems). Telomere length analysis by Q-FISH is in progress. *Discussion:* Mutational analysis showed the proband's peripheral blood and hair bulb cells bore a TINF2 exon 2 mutation, c.254 A>G p.I85A, which was also found in his two brothers but not in peripheral blood from 200 healthy donors. Consequently, this new familial TINF2 mutation, which is reported here for the first time, was detected in a patient affected by a telomere syndrome with AA, in one brother who had suffered an acute myocardial infarction, and in a second brother with transient thrombocytopenia and cardiomyopathy. Unlike other TINF2 mutations which affect exon 6 encoding for the C-terminal TERF1 binding domain (Sasa GS. *et al.* 2012) this new mutation is located at exon 2, encoding for the N-terminal domain. This is involved in shelterin-shelterin interaction as it binds TPP1 and TERF2, both of which are members of the shelterin protein complex (the other members being TERF1, POT1, RAP1). TINF2 acts as a bridge to connect between them other shelterins. These, regulates telomerase enzyme activity which, with its reverse transcriptase (TERT) and RNA template (TERC), controls telomere length. Interestingly, sequence alignments (ClustalW 2.1) revealed TINF2 exon2 is well-conserved in vertebrates (NCBI) suggesting its crucial biological role.

Quality of Life, Pain Therapy and Support Therapy

C067

SECOND CANCERS AND MAJOR INFECTIONS OCCUR IN TWO DIFFERENT SUBSETS OF PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Most important and common complications of chronic lymphocytic leukemia (CLL) are major infections (MI, defined as those events requiring inpatient management and/or intravenous antibiotic treatment) and second cancers (SC). *Aims:* The aim of this study was to describe the risk of SC as compared to MI in patients with CLL. *Methods:* We retrospectively analyzed clinical and biological data of 747 patients. FISH analysis (n=492), CD38 expression (n=612), ZAP70 (n=513), IGHV (n=480), TP53, NOTCH1, BIRC3, SF3B1 (199 tests) mutational status were evaluated at diagnosis or before starting treatment. We used Mann-Whiney, Fisher exact or Chi-square, Log-rank test, Kaplan-Meier method and Cox model when appropriated. Time to SC/MI (TTSC or TTMI) were calculated from the date of initial presentation to the SC/MI (event) or last known follow-up (censored). Combined antibody deficiency (CAD) means low levels of IgG and IgA or IgM. Not reached=nr. *Results:* 133 patients (18%) experienced 157 SC (64% after CLL diagnosis) and 86 (11%) experienced 121 MI events. By Kaplan-Meir analysis we observed that the risk of MI (20yy TTMI 44%, 30yy 79%) progressively increase through years while the risk of SC seemed to reach a plateau (20yy TTSC 37%, 30yy 44%, Figure 1). We also observed that SC and MI occurred in a mutually exclusively manner ($p<0.001$), with only 3.6% of the cohort had both complications (Figure 2). Clinical and biological markers associated with a higher risk of SC and MI were Rai III-IV ($p=0.020$ and $p<0.001$), Binet C ($p=0.041$ and $p<0.001$), 17p deletion ($p=0.002$ and $p<0.001$), need for treatment ($p=0.0247$ and $p<0.001$). No differences were found for TP53, NOTCH1, BIRC3, SF3B1 gene mutations. The only different biological marker between patients with SC and MI was TP53 abnormalities, being more common in MI subset ($p=0.002$). Pretreated patients and subjects with CAD had a shorter TTSC and TTMI with respect to patients who did not need a specific CLL therapy (17yy *vs* nr [$p<0.029$] and 20yy *vs* nr [$p<0.001$]) or those without CAD (16 years *vs* nr [$p<0.002$] and 20yy *vs* nr [$p<0.001$]), respectively. These data were confirmed in multivariate analysis. We also showed that patients who experienced MI event had a shorter OS than those with SC or all others ($p=0.013$). *Discussion:* We provide evidence of the existence of two clinical subsets of CLL, one unbalance towards the development of SC the other towards MI, which can only be in part explained by know biological markers.

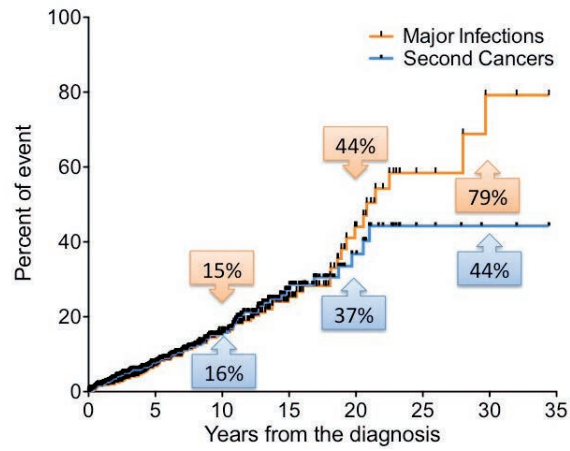

Figure 1.

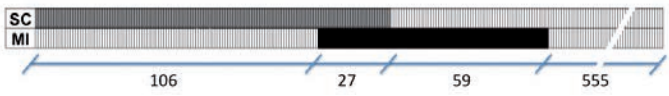

Figure 2.

C068

QUALITY OF LIFE AT DIAGNOSIS IN ELDERLY PATIENTS WITH ACUTE MYELOID LEUKEMIA CONSIDERED FIT FOR INDUCTION OF REMISSION CHEMOTHERAPY

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AO Bianchi-Melacrino-Morelli Reggio Calabria; AOU di Udine Udine; Ospedale Civile Spirito Santo Pescara; Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico, Milano; AO San Giovanni Moscati Avellino; Centro di Riferimento Oncologico di Basilicata, Rionero in Vulture, PZ; IRCCS Casa Sollievo della Sofferenza San Giovanni Rotondo, FG; Ospedale Umberto I, Ancona; Ospedale Ferrarotto Catania; Ospedale Sant'Eugenio, Roma, Italy

Aims: In elderly patients with acute myeloid leukemia (AML), complete remission (CR) rate following intensive chemotherapy is approximately 45% with a shorter duration of remission and high treatment-related mortality (30-50%). Median survival is about 12 months. Intensive chemotherapy is indicated in a small proportion of "fit" elderly patients. In a phase III, prospective, randomized, open-label, multicenter trial designed to assess the efficacy of post-remission treatment with 5-Aza *versus* best supportive care (BSC) in patients >60 years of age with AML in CR after conventional induction "3+7" and consolidation chemotherapy, QoL was assessed in patients at diagnosis and in the 2 years post-remission. We present interim results of QoL assessment at diagnosis.

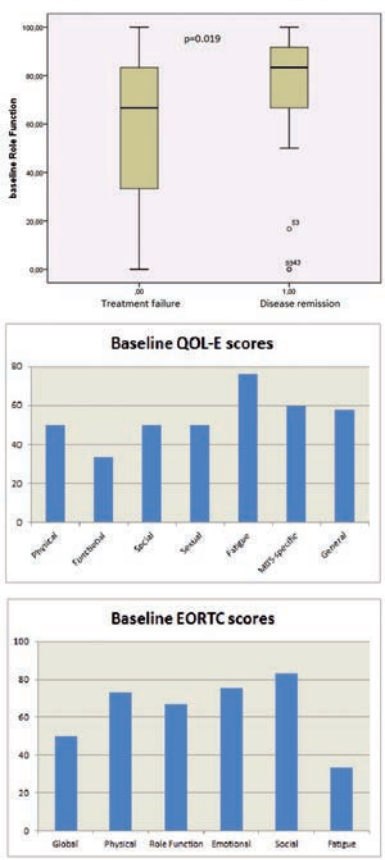

Figure 1. EORTC QLQ-C30 Role function scores at diagnosis in elderly AML patients failing chemotherapy induction treatment *versus* those obtaining complete remission.

Methods: Patients with newly diagnosed AML with >30% bone marrow blasts, "*de novo*" or evolving from myelodysplastic syndrome without contraindications for intensive chemotherapy and with an ECOG performance status <3 are included. Induction chemotherapy consists of two courses of "3+7": daunorubicin 40 mg/m^2 daily days 1-3 and cytarabine 100 mg/m^2 daily continuous IV infusion days 1-7. Patients in CR receive consolidation (cytarabine 800 mg/m^2 3 hour infusion bid days 1-3) and are randomized 1:1 to receive BSC or 5-Aza maintenance therapy until AML recurrence for 4 years and six months. QoL assessment was performed at baseline and during the follow-up period using the EORTC QLQ-C30 and the QOL-E v.3 questionaires. Higher scores reflect better quality of life, except for symptom scores of the EORTC QLQ-C30. *Results:* Ninety-eight patients (male/female 49/49) of median age 70 (IQR 65-74) years have been enrolled. At diagnosis median hemoglobin was 9.1 (8.4-9.9) g/L, leukocytes 7.9 (2.3-29.7)/μL, platelets 54 (29-85) Gi/L and bone marrow blasts 70 (50-86)%. Seventy-five patients had "*de novo*" AML and 23 had comorbidities. Thirty-eight patients obtained a CR following chemotherapy. Median QOL-E scores were poor (<60) in all dimensions, except for fatigue (76, IQR 52-86) and did not distinguish prognosis. Median EORTC QLQ-C30 scores were good in all domains except for global health status (50, IQR 33-67). Role Function was better in patients obtaining CR (Figure 1). *Conclusions:* Elderly patients with AML at diagnosis identified as fit for chemotherapy generally do not present fatigue. Global health status is poor and perception of role function may be associated with response to induction chemotherapy.

C069

QUALITY OF LIFE AND LATE EFFECTS IN 44 LONG-SURVIVING NON M3 ACUTE MYELOID LEUKEMIA PATIENTS

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Acute Myeloid Leukemia (AML) outcome had improved in last decades, conversely late effects and quality of life studies are still lacking. We have administered two questionnaires, EORTC QLQ-C30 and FACT-AN, in 44 cured AML patients (31 <60 years and 13 >60 years) treated at our department between 1997 and 2010 (7 Allogeneic, 16 Autologous Transplant, 21 chemotherapy alone). We stratifyed QoL scores by age at diagnosis, performance status (PS), Sorror Index, kind of leukemia treatment, comorbidity at diagnosis. Multivariate analysis showed that older patients had worse EORTC QLQ-C30 physical and emotional scale scores and higher values of pain symptoms in comparison to younger counterpart, with RR of 20.1 (p=0.001), 22.7 (p <0.04) and 18.4 (p=0.03) respectively. Elderly patients also had lower Total Outcome Index and FACT-An subscale scores (RR: 11.9, p=0.02; and 8.77, p=0.04 respectively). Sorror index >2 was related to lower EORTC QLQ-C30 social scale and dyspnea scores (RR: 32.5; p=0.001 and 21.7; p=0.001 respectively) and FACT-An functional well being values (RR=3.9; p=0.001). Late effects, occurring 3 months after the end of treatment, evaluated in 44 patients, included 12 cases of grade II-IV cardiotoxicity (3 arythmia, 9 cardiomyopathy) with 89% incidence in patients with cardiac comorbidities at diagnosis, 0%, 20% and 55.5% in patients receiving respectively Daunorubicin, Idarubicin and at least two different anthracyclines. Sorror Index>2 was the only factor significantly predicting cardiotoxicity at the multivariate analysis with a RR of 82.7 (p=0.001). We also observed 15 cases of methabolic toxicities (2 hyperglycemia, 12 iron overload and 1 electrolytic alteration), 6 case of gastrointestinal toxicity (gastric ulcer, 2 irritable bowel, 1 parodonthopathy, 1 severe and persistent mucositis, 1 esophagitis). 3 patients (2 males and 1 female) had been fertile; all female patients developped menopause after Transplant. 3 patients had secondary neoplasia consisting of Multiple Myeloma, breast cancer and axillary sarcoma. Our study underlines the importance of Sorror Index at diagnosis in defining patients eligibility to cardio-prophylactic therapy. We also suggest the use of psycho-somatic strategies, also based on physical activities or autogenic training, in long term AML survivors. The analysis of larger series of cured AML patients are strongly needed in order to define guidelines for reducing long term treatment AML toxicity.

C070

DISABILITY AND SURVIVAL OF HOME CARE ONCO-HEMATOLOGICAL PATIENTS

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Introduction: Reduction of capacity to attend to basic activities of daily living (ADL) is a frequent complaint in home care (HC) managed patients (pts) affected by hematological malignancies. Disability may arise both as an effect of comorbidities, hematological disease or its complication, and, lastly, treatment toxicity. Disability itself is a risk factor for complications (thrombosis, accidental falls, pressures sores), so that it could be expected to affect overall survival (OS). Data about this issue are preliminary, suggesting a correlation between disability and OS in HC pts. *Aim:* To confirm and clarify the presence of an effect of disability on OS in home care pts affected by hematological malignancies. *Methods:* At the moment of pts admission in our home care service for hematological pts, disability was assessed with Katz Index, as a part of data registration at pt entry. Pts with diagnosis other than hematological malignancies were excluded from analysis. OS from HC admission was calculated with Kaplan-Meier survival curves analysis; effect on OS of seven variables (age, gender, diagnosis, chemotherapy lines before HC admission, comorbidities, Karnofsky performance status (KPS) and disability at HC admission) was evaluated. *Results:* From September 2011 and February 2015, 132 pts were included to analysis. Anagraphic, disease, comorbidities, KPS and disability data are reported in Table 1 with statistical analysis result. Among the 7 examined variables, AML diagnosis, age<80, treatment lines >0, poor PS (KPS<50) and disability (KI<3) showed statistically significant negative effect on OS. *Conclusions:* OS is strongly influenced by disability and, although trajectory prognostication requires to consider several variables, disability is one of the simplest tool to stratify pts according to survival expectation. Finally, interventions aimed to reduce or prevent disability could have effect on survival, although only clinical trials could address this issue.

Table 1. Patients data and statistical analysis.

					Kaplan-Meier survival analysis		
	n	Median	Min	Max	Category	Median OS	p
Gender							
f	66				f	134	NS
m	66				m	127	
Age		80	21	100			
<80	66				<80	61	**0,01**
≥80	66				≥80	197	
Diagnosis							
AML	42				AML (42)	52	**0,01**
MDS	40				MDS (40)	325	
Lym/CLL	19				Other (50)	117	
MPN	15						
MM	13						
ALL	3						
Treatment lines		1	0	6			
0	53				0	221	**0,004**
1	41				>0	70	
2	16						
3	11						
>3	11						
Comorbidities							
No	61				No	67	NS
Yes	69				Yes	145	
Unk	2						
Disability							
Very severe (KI=0)	12						
Severe (KI=1)	14						
Int 2 (KI=2)	21						
Int 1 (KI=3)	10				Int 2 to very severe (KI=0-2)	67	**0,04**
Mild (KI=4)	12						
Very mild (KI=5)	15				None to Int 1 (KI=3-6)	185	
None (KI=6)	47						
Unk	1						
Perform. status		50	20	90			
<50	51				<50	72	**0,04**
≥50	78				≥50	169	
Unk	3						

AML: acute myeloid leukemia; MDS: myelodysplastic syndrome; Lym/CLL: lymphoma / chronic lymphocytic leukemia; MPN: myeloproliferative neoplasm; MM: Multiple Myeloma; ALL: acute lymphoblastic leukemia; Unk: unknown; Int: intermediate.

C071

THE USE OF INFUSION VASCULAR DEVICES MAY BE IMPROVED, IN HEMATOLOGY, THROUGH THE USE OF A DEDICATED DECISION ALGORITHM

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Adequate venous access is a necessary requirement in chemotherapy (CT) administration. The organizational planning of the placement of such devices may be difficult to manage with delays potentially causing a subsequent delay in CT. The RNAO guidelines (2008) for the "Assessment and Device Selection for Vascular Access", provide an algorithm for patients (pts) requiring a Central Venous Access Device (CVAD), based on clinical variables. This algorithm is not specifically for haematology pts. Based on this work, we adapted the algorithm using clinical variables related to CT for use in our haematology pts (high osmolarity, low or high pH, risk of intimal injury and duration of chemotherapy). The decision algorithm was tested in 126 consecutive pts who accessed our day hospital between 01/01 and 31/08/2014. During CT, a port-a-cath (PAC) was placed in 14 pts (11%) and a Peripherally Inserted Central Catheter (PICC) in 7 pts (5.5%). Analyzing the clinical variables related to CT (cited above), 47 pts (36%) received therapy at risk for high osmolarity, 61 pts (47%) at risk for high or low pH and 78 (60%) at risk for intimal damage. Of the 126 pts starting CT without a CVAD, 73 received CT that would have required a CVAD for presence of at least one of the following risk factors - high osmolarity, low or high pH, or risk of intimal injury. Amongst these 73 pts, only 18 (24%) had CVAD's inserted (13 PAC, 5 PICC); of the remaining 55 pts (76%) without CVAD's, CT was delivered via peripheral venous access devices (PVAD) or by use of a temporary CVAD. Of the 53 pts that were not receiving CT with high osmolarity, low or high pH, or potential risk of intimal injury, assessment of risk should also be based on the duration of CT. In this group, 11 received subcutaneous CT, and of the remaining 42 pts who received intravenous CT, 27 received treatment lasting less than 4 weeks, and as such, via through PVAD or midline. Fifteen pts received treatment lasting >4 weeks, therefore requiring a CVAD, which had not been positioned. Out of a total of 126 pts, only 18 (14%) had a CVAD placed, however, if the proposed algorithm was used in the same population, the number of CVAD insertions would have risen to 88 (70%). The use of this decision algorithm would allow early identification of pts at risk of damage from CT, allowing a programmed insertion of the most appropriate device avoiding, where possible, urgent CVC placements, reducing risks, costs and delays in CT (Figure 1).

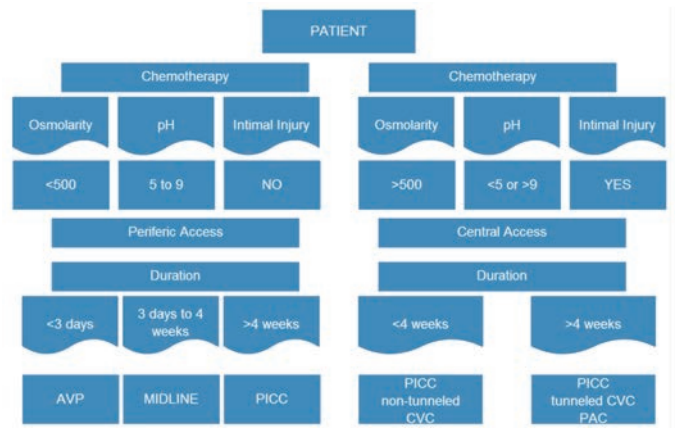

Figure 1.

C072

EFFICACY AND SAFETY OF BIOSIMILAR EPOETIN A IN PATIENTS WITH CHRONIC LYMPHOID NEOPLASMS AND CHEMOTHERAPY-INDUCED ANEMIA: AN OBSERVATIONAL, RETROSPECTIVE, MONOCENTRIC ANALYSIS

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Epoetin (EPO) biosimilars are an alternative to originator erythropoietic agents in the treatment of chemotherapy (CHT)-induced anemia, as they share equivalent mechanism of action, efficacy and safety. Clinical studies specifically evaluating their role in patients with lymphoproliferative disorders undergoing CHT are lacking. This analysis evaluated the response to EPO α biosimilar after a 4 (and 8)-weeks treatment period and the rate of CHT cycles delays/interruptions due to anemia in 65 consecutive adult patients affected by non-Hodgkin's lymphoma and chronic lymphoproliferative syndromes, all receiving CHT (first-line induction in 50 cases, second-line/salvage in 15 cases), and presenting with CHT-induced anemia. Median age was 69 (range 21-90) years, with 43% of the patients older than 70. Response to EPO was defined as an increase in hemoglobin (Hb) after 4 (Hb4) and 8 weeks (Hb8) of treatment of at least +1 g/dL, or as the achievement of Hb>11 g/dL with transfusion independence. Stability was defined as Hb between -1 and +1 g/dL. A Hb<-1 g/dL or an acquired transfusion dependence indicated lack of response. Treatment with EPO was started at first occurrence of Hb<10 g/dL during CHT. Treatment duration was established by treating physician and it was stopped when Hb was at least 11 g/dL, when the CHT program had reached completion and if a patient became transfusion-dependent, as per institutional guidelines. Mean Hb at EPO treatment start was 9.3±0.5 g/dL. After 4 weeks, the reached Hb was 10.7±1.4 g/dL for patients on first-line CHT, and 11.4±1.6 g/dL for those on a second/later line, with mean Hb4 of 1.5±1.4 g/dL and 2.1±1.5 g/dL for each group of patients. Twenty-three patients had their Hb level measured after 8 weeks, with an Hb of 10.6±1.5 g/dL and 9.7±1.3 g/dL for each treatment subgroup, and Hb8 of 1.3±1.4 g/dL and 0.5±1.2 g/dL, respectively. Overall, 46 patients responded to the treatment, yielding to 70.7% hematological improvement. Seventeen patients (26.2%) showed a stable Hb level throughout treatment course, and 2 (3.1%) did not respond. CHT cycles were delayed in 11 cases (16.9%) because of anemia; interruptions occurred in 7 cases (10.8%). No adverse events were documented. The treatment of CHT-induced anemia with biosimilar EPO α in patient with chronic lymphoproliferative disorders is correlated with a high rate of responses and allows a safe completion of the planned CHT programme in most of the cases (Figure 1).

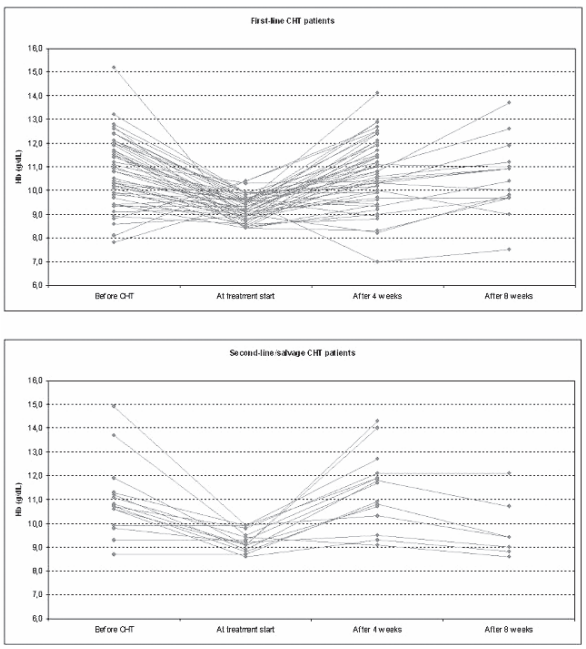

Figure 1.

Non-Hodgkin Lymphoma 2

C073

DIFFUSE LARGE B-CELL LYMPHOMA GENOTYPING ON THE LIQUID BIOPSY

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Diffuse large B-cell lymphoma (DLBCL) harbor mutations of potential clinical relevance in the era of targeted treatments. Cell-free DNA (cfDNA) is shed into the blood by tumor cells undergoing apoptosis and can be used as source of tumor DNA for the identification of cancer-gene somatic mutations. Accessing the blood has clear sampling advantage in the monitoring of mutations in real time. Also, cfDNA is representative of the entire tumor heterogeneity, thus allowing to identify mutations from tumor cells residing in non-biopsied sites. Here we aimed at assessing the accuracy of DLBCL genotyping on cfDNA. The study included 15 consecutive DLBCL provided with paired tumor DNA from the diagnostic tissue biopsy, cfDNA from plasma, and normal germline DNA. Ultra-deep next generation sequencing (NGS) (coverage >2000x in >80% of the target) of the coding exons from 59 genes that are recurrently mutated in B-cell tumors was performed on MiSeq (Illumina) using a SeqCap library preparation strategy (NimbleGen). VarScan2 was used to call non-synonymous somatic mutations and a stringent bioinformatic pipeline was applied to filter out sequencing errors. Genotyping of the DLBCL tissue biopsies disclosed somatic mutations of heterogeneous clonal abundance (median 32% of the alleles, range 0.9%-87%) in known DLBCL-associated genes, including TP53 (46% of cases), MLL2, TBL1XR1 (26% each), EZH2, CREBBP, BCL2 (20% each), STAT6, B2M and TNFAIP3 (13% each). Genotyping of the paired plasma cfDNA correctly identified almost all (90%) the mutations that were clonally represented in >15% of the alleles of the tissue biopsy, and nearly half (43%) of subclonal variants occurring in <15% of the alleles. Consistent with the notion that the amount of tumor-specific cfDNA is low in plasma, DLBCL mutations generally occurred in a small fraction of the cfDNA sequencing reads (median 5%, range 0.2-54%) and their detection needed highly sensitive ultra-deep NGS strategies. Plasma cfDNA genotyping also disclosed a number of additional somatic mutations (~1 per case, range 0-6) that were not detectable in the tissue biopsy. Our results indicate that cfDNA: i) can be used in DLBCL to detect clonally represented somatic mutations, thus validating the concept of "liquid biopsy" in this lymphoma; and ii) allow the identification of DLBCL driver mutations that are otherwise absent in the diagnostic tissue biopsy and conceivably restricted to clones that are hidden in non accessible tumor sites (Figure 1).

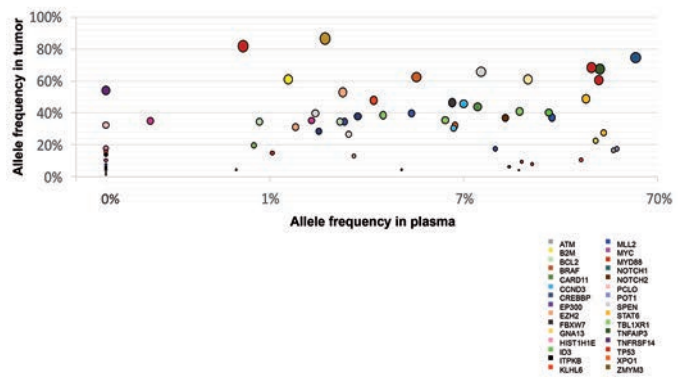

Figure 1.

C074

IDELALISIB MONOTHERAPY RESULTS IN DURABLE RESPONSES IN PATIENTS WITH RELAPSED OR REFRACTORY WALDENSTRÖM MACROGLOBULINEMIA

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Background: Idelalisib (Zydelig®), a selective oral inhibitor of PI3K, demonstrated considerable anti-tumor activity in patients with relapsed/refractory indolent non-Hodgkin lymphomas (iNHL) in phase 1 (Flinn, 2014), and double refractory iNHL in phase 2 trials (Gopal, 2014). The purpose of this analysis was to specifically evaluate safety and efficacy outcomes in the subset of subjects with Waldenström macroglobulinemia (WM). *Methods:* Eligible WM patients (pts) included those with relapsed/refractory disease (p1), or those with disease refractory to both rituximab and an alkylating agent (p2). Idelalisib dosages ranged from 150 mg QD, and 50 mg-200 mg BID (p1), and 150 mg PO BID (p2) and were administered continuously until disease progression. WM response was assessed by IgM levels and CT-imaging (Owen, 2013). *Results:* Enrolled pts, phase 1 (N=9), and phase 2 (N=10), had a median age of 63 and 60 years [range 42-83] and 78% and 80% were male, respectively. Patients had received a median of 4 prior regimens in both groups. Overall response rate (ORR) was 5/9 (56%), and 8/10 (80%). Table 1. Minor responses were noted over a median of 3 months, and most responses continued to deepen over 6 months or longer. Median DOR was 32.8 months (p1), and not yet reached (p2). 67% have continued response at 2 years (p2). Median PFS is 33.3 months, and 22.1 months, respectively. Interestingly, >3 g/dL improvements in hemoglobin were noted in 5/9 and 7/10 subjects respectively over 3-6 month timeframe. Grade ≥3 adverse events included increased ALT/AST 5/9, and 1/10, and diarrhea/colitis 1/9, and 3/10. One G3 ALT elevation and 1 G3 diarrhea resulted in study discontinuation. *Conclusions:* These combined data suggest single agent idelalisib monotherapy is active in WM. Durable responses were seen in the majority of subjects. Marked improvements in hemoglobin level are also associated with response. The safety profile was acceptable and manageable, with no apparent disease specific safety signals. Phase 3 clinical trials of Idelalisib with combination therapy are in progress for iNHL subjects, including WM subjects.

Table 1.

	02 (n=9)	09 (n=10)
ORR, n (%)95% CI	5 (56%) [21-86]	8 (80%) [44-98]
Complete Response	0	0
Partial Response	1 (11%)	7 (70%)
Minor Response	4 (44%)	1 (10%)
Stable Disease	2 (22%)	1 (10%)
Progressive Disease	1 (11%)	1 (10%)
Not Evaluable	1 (11%)	0

C075

THE PROGNOSTIC ROLE OF CELL OF ORIGIN PROFILE AND OVEREXPRESSION OF MYC ASSESSED BY IMMUNOHISTOCHEMISTRY IN YOUNG HIGH-RISK PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA: RESULTS OF FIRST LINE RANDOMIZED BIO-DLCL04 TRIAL OF FONDAZIONE ITALIANA LINFOMI

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[1]On the behalf of Fondazione Italiana Linfomi, Hematology 2, Città della Salute e della Scienza Hospital and University, Torino; [2]Hematopathology Section, Department of Hematology and Oncology L. and A. Seragnoli, S. Orsola-Malpighi Hospital, University of Bologna, Bologna; [3]Unit of Cancer Epidemiology, University and CPO Piemonte, Torino, Italy

The prognostic role of COO assessed by IHC is controversial in Rituximab era. FIL conducted a phase III randomized trial aimed at investigating the benefit of intensification with high dose therapy+autotransplant compared to R-dose-dense therapy as first line in young DLBCL at poor risk (aa-IPI 2-3). (Vitolo, ASH 2012). The aim of BIO-DLCL04 was to correlate the biological markers with PFS. From 2005 to 2010, 412 untreated DLBCL at aa-IPI 2-3 were enrolled. Central histology revision was mandatory and 13 patients were excluded due to different histologies. Biological markers were analyzed on DLBCL NAS; COO analysis was performed by IHC and cases were classified in germinal center (GC) and nonGC according to Hans' algorithm; COO assessed by Nanostring is ongoing; BCL2, BCL6 and MYC anomalies were tested by IHC and by FISH (final analysis ongoing). Cases were deemed positive if at least 30% of lymphoma cells were stained with each antibody (with the exception of at least 40% for MYC). At the time of this analysis, 223 DLBCL NAS were analyzed: 131 non-GC and 92 GC; BCL2, BCL6 and MYC anomalies were tested in 196, 74 and 107 cases respectively. Clinical characteristics for nonGC *vs* GC were: median age 51 years for both, male 49% *vs* 45%, aa-IPI 3 15% *vs* 25%, bone marrow involvement (BM) 16% *vs* 24%. R-HDC was performed in 45% of nonGC patients and in 49% of GC. Complete response was recorded in 105 (80%) nonGC patients and in 62 (67%) GC. At a median follow-up of 49 months, the 3-year PFS for nonGC *vs* GC was 75% (95% CI: 67-82) *vs* 57% (95% CI: 46-67) with crude hazard ratio, HR 0.55 (0.35-0.87), p.01 and adjusted (for age, gender, aa-IPI, BM) aHR 0.56 (0.35-0.88), p.013. No significant differences by treatment were reported. Overexpression of MYC by IHC had a relevant prognostic impact, with aHR 1.84 (0.99-3.44), p.054. By IHC, 3-years PFS for double negative *vs* single BCL2 or MYC overexpression *vs* double positive, was 85% *vs* 68% *vs* 51% respectively, with an aHR for double expressers compared to double negative of 3.91 (1.13-13.53), p.031. Figure 1. The final analysis by FISH is ongoing. In conclusion, with the limit of the analysis performed by IHC based on Hans' algorithm, BIO-DLCL04 showed an unexpected better outcome for non-GC compared to GC, irrespective of treatment arm. The ongoing analysis conducted by Nanostring will be more informative. The overexpression of MYC, single or associate to BCL2 overexpression, was confirmed as relevant prognostic role.

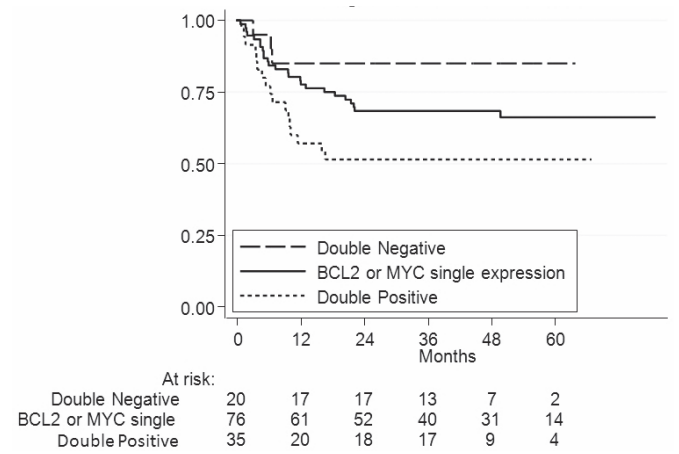

Figure 1. Progression-free survival.

C076

PHASE II TRIAL OF SHORT COURSE FLUDARABINE, MITOXANTRONE AND RITUXIMAB FOLLOWED BY 90Y-IBRITUMOMAB TIUXETAN IN UNTREATED FOLLICULAR LYMPHOMA: SEVEN YEARS FOLLOW-UP

B. Casadei,[1] F. Quirini,[1] C. Pellegrini,[1] A. Pulsoni,[2] G. Annechini,[2] A. De Renzo,[3] V. Stefoni,[1] E. Derenzini,[1] A. Broccoli,[1] L. Gandolfi,[1] L. Tonialini,[1] L. Argnani,[1] M. Cavo,[1] P.L. Zinzani[1]

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Introduction: As follicular lymphoma (FL) is characterized by an indolent clinical course, affected patients generally experience frequent disease relapse with shorter duration of remission at every disease

recurrence. The addition of consolidative radioimmunotherapy (RIT) to standard chemoimmunotherapy approaches has led to enhanced results in terms of response and progression free survival (PFS) rates. The application of short-course inductions has helped in reducing chemo-related toxic effects. Benefits deriving from combined strategies regimens require however a confirmation in the long term. *Methods:* From December 2006 to November 2008, 55 untreated patients with intermediate/high risk follicular non-Hodgkin's Lymphoma (NHL) were enrolled in a non-randomized multicenter phase II trial of four induction cycles of fludarabine, mitoxantrone and rituximab (FMR) and a subsequent consolidating single administration of 90Y-ibritumomab tiuxetan, given 8-14 weeks later (Zinzani *et al.* Annals of Oncology, 2011). Patients' median age was 56 (range, 26-84) years; 19 of 55 (34.5%) patients had an high risk (>=3) FLIPI score. All patients obtaining at least a partial response (PR) have been followed through a whole body computed tomography (CT) scan every 6 months for the first 2 years and every year thereafter. *Results:* The complete response (CR) rate after the entire treatment regimen was 89.0% (49/55 patients). With a median follow-up of 7 years, 30/49 (61.2%) patients are still in continuous CR, whereas 21/55 (38.1%) experienced a disease progression (PD). The PFS at 6.7 years was 50.1% with a disease free survival (DFS) at 6.4 years of 68%. The overall survival (OS) at 7.8 years was 72.7%. Death occurred in 8 (14.5%) patients; among them, 4 died of a secondary acute myeloid leukemia, developed after 3 (in 3 cases) and 4 years since enrollment. *Conclusions:* Short-course FMR with 90Y-ibritumomab tiuxetan RIT consolidation confirms its efficacy in patients with intermediate/high-risk follicular lymphoma also in the long-term, as demonstrated by favourable 7-years DFS and PFS rates. These results are comparable with those obtained with 6 FMR cycles in larger patients series.

C077

HIGH DOSES OF ANTIMETABOLITES FOLLOWED BY HIGH-DOSE SEQUENTIAL CHEMOIMMUNOTHERAPY AND AUTOLOGOUS STEM CELL TRANSPLANT IN PATIENTS WITH SYSTEMIC B-CELL LYMPHOMA AND SECONDARY CENTRAL NERVOUS SYSTEM INVOLVEMENT: FINAL RESULTS OF A MULTICENTER PHASE II TRIAL

A.J.M. Ferreri, M. Foppoli, G. Citterio, M.G. Cabras, C. Patti, M. Mian, R. Zambello, C. Tarella, M. Di Nicola, A.M. D'Arco, G. Doa, M. Bruno-Ventre, M. Sassone, L. Scarfò, A. Fanni, A. Mulè, T. Calimeri F. Ciceri, F. Caligaris-Cappio

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Introduction: Treatment of secondary central nervous system (CNS) dissemination in patients with aggressive lymphomas remains an important, unmet clinical need. Herein, we report the final results of a multicenter phase II trial addressing a new treatment for secondary CNS lymphoma (SCNSL) based on encouraging experiences with high doses of antimetabolites in primary CNS lymphoma (Ferreri *et al.* Lancet 2009), and with high-dose sequential chemoimmunotherapy (R-HDS) in relapsed aggressive lymphoma (Tarella *et al.* JCO 2008) (clinicaltrials.gov NCT00801216). *Methods:* HIV-negative patients with aggressive B-cell lymphoma and secondary CNS involvement at diagnosis or relapse, age 18-70 years, and ECOG performance status ≤3 were enrolled, and treated with high doses of methotrexate and cytarabine, followed by R-HDS (cyclophosphamide, cytarabine, etoposide) supported by autologous stem cell transplantation (ASCT). Treatment included 8 doses of rituximab and 4 of intrathecal liposomal cytarabine. The primary endpoint was 2-year even-free survival (EFS); the planned accrual was 38 patients. *Results:* 38 pts were registered (age 32-70 ys, median 59; M/F ratio 1.5): 33 had DLBCL, 3 MCLb, 3 FL. CNS disease was detected at diagnosis in 16 pts (all with extra-CNS disease) and at relapse in 23 (8 with extra-CNS disease). Thirty-four pts completed the induction phase; 73 (93%) of 78 planned courses were actually delivered; Toxicity was usually haematological and manageable, with grade-4 febrile neutropenia in 3% of delivered courses, and grade-4 non-hematological toxicity in 2%. Drugs dose reduction was indicated in 3 pts. Four patients died because of toxicity. ASCs were successfully collected in 24/27 (89%) patients (median 10 x 10^6/kg); 20 patients underwent ASCT. Response at the end of the whole program was complete in 24 patients (CRR=63%, 95%CI=48-78%). At a median follow-up of 48 months, 17 patients remain relapse-free, with a 2-year EFS of 50±8%; 16 patients are alive, with a 5-year OS of 41±8% for the whole series and 68±11% for transplanted patients. Extra-CNS and/or meningeal disease did not affect outcome. No pt was irradiated to the brain to achieve lymphoma remission. *Conclusions:* This radiotherapy-free combination of high doses of antimetabolites, R-HDS and ASCT is feasible and effective in pts ≤70 ys with SCNSL. Toxicity is usually haematological and manageable. Survival benefit is attainable also in pts with meningeal and/or concomitant extra-CNS disease.

C078

PHARMACOKINETIC DATA, CLINICAL CHARACTERISTICS AND OUTCOME IN FOLLICULAR LYMPHOMA PATIENTS IN MAINTENANCE WITH RITUXIMAB: AN ANALYSIS OF THE FONDAZIONE ITALIANA LINFOMI

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Introduction: Rituximab (R)-chemotherapy induction followed by R maintenance is the standard of care for patients (pts) with follicular lymphoma (FL). Several studies proposed a presumptive 'active' R level of 25 µg/mL. However, scanty data are available with regard to the R pharmacokinetic (PK) during maintenance and its possible relationship with pts' characteristics and outcome. *Methods:* Pts with grade 1, 2 or 3a FL in maintenance therapy with i.v. R (375 mg/m^2) administered every 2 (pts in front line) or 3 months (after salvage therapy) were investigated. R plasma trough concentrations (Ctr) and the area under the curve (AUC) were determined using a sensitive validated ELISA assay. PK data were correlated with the pts' clinical characteristics and outcome. *Results:* From March 2013 to March 2014, 101 pts have been recruited: median age was 60 years (IQR 30-84) and 50 (48.5%) were males. Forty-four/101 (43%) pts were overweight (BMI: 25 to <30) and 10.8% were obese (BMI: >30). At study entry, 92 pts were in first complete response (CR), 19/92 in second CR and 9 in partial response (PR), of whom 2 after salvage therapy. In 85/101 (group 1) and in 16/101 (group 2) pts, R was administered every 2 or 3 months, respectively. At a median follow-up of 23 months from the start of R maintenance, 91 patients were in CR, 3 in PR and 7 relapsed. The median Ctr level was 32 µg/ml (0.0 – 200) in all pts, 33 µg/ml (0-188) in pts treated every 2 months and 22.8 µg/ml (0-200) in pts treated every 3 months, respectively (p 0.7). A higher median R Ctr level (36 µg/ml *vs* 22 µg/ml, p 0.04) and a higher AUC (61.23 *vs* 41.9, p 0.011) were found in females. No correlation between Ctr, AUC and BMI were documented, but a trend towards higher levels were found in pts with a BMI <30 (Ctr=40.38 µg/ml *vs* 31.96 µg/ml, p 0.23; AUC=54.19 *vs* 42.55, p 0.13). At this preliminary analysis, no relationship between the quality of response (CR *vs* PR), outcome (response *vs* relapse), the Ctr levels and AUC was evident; however in a subanalysis of group 1, more pts with lower Ctr relapsed during R maintenance (38.8 µg/ml *vs* 33.9 µg/ml, p 0.01) and, notably, 4/6 who relapsed had Ctr levels under 25 µg/ml. *Conclusions:* Our study confirms the favorable R PK profile in females and in the 2 months administration scheme. Probably, because of the relatively short follow-up and low number of events during maintenance, no relationship between PK data and outcome has so far emerged and a longer observation time is required.

Acute Leukemia 3

C079

WHOLE EXOME SEQUENCING IDENTIFIES MUTATIONS OF ALDH2, IMPDH2, RETSAT, HSPG2, CHPF AND OTHER METABOLIC GENES AS A NOVEL FUNCTIONAL CATEGORY OF GENOMIC ALTERATIONS IN ACUTE MYELOID LEUKEMIA

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Next Generation Sequencing identified 9 functional categories of mutations in acute myeloid leukemia (AML). However, multiple genetic hits participate to AML pathogenesis and metabolic dysregulations, as IDH1/2 mutations, play oncogenic functions. The study aims to define novel functional categories of AML mutations affecting relevant and druggable biological processes. We performed whole exome sequencing (WES) of 37 AML cases (HiSeq2000, Illumina). Variants where called with MuTect or GATK for single nucleotide variant (SNV) and indels detection, respectively. We analyzed 59 bone marrow samples (AML, controls) by gene expression profile (HTA2.0, Affymetrix). We detected an average of 26 somatic variants per patient by WES. We identified 8 novel functional categories of mutations including transcription, translation, protein degradation, cytoskeleton and metabolism. Since metabolic pathways are promising therapeutic targets, we focused on them. We identified 82 variants targeting 70 metabolic genes, with 78% of patients having at least one mutation in a metabolic gene and 35 variants rated as damaging by CONDEL. The most represented pathways were amino acids, lipids, CoA and nucleotides metabolism, transport and bioenergetics. IMPDH2, a mediator of MYC-induced proliferation involved in nucleotide interconversion, was mutated and overexpressed in AML, suggesting a potential oncogenic function. ALDH2, a regulator of hematopoietic stem cell functions associated with metabolic remodeling was mutated and downregulated in AML. Seven genes were mutated in 5-8% of samples: RETSAT, HSPG2, CHPF, ABCA2, ND1, APOBR, NAAA. RETSAT, HSPG2, CHPF mutations were predicted as drivers by DOTS-Finder. Bioenergetic pathways were affected by mutations in glycolysis and gluconeogenesis (GPI, ITPA), oxidative phosphorylation (ND1, ND4, ND5, CYTB), pentose phosphate pathway (H6PD, PGLS). Patients carrying mutations in the bioenergetic pathway showed a strong trend towards reduced overall survival. Metabolism is the most represented class of mutated genes in our AML cohort after signaling, suggesting a novel functional category. Our data indicate that genetic determinants participate to leukemia metabolic plasticity and oncogenic mutations of metabolic enzymes may drive leukemogenesis, impact on survival and become novel targets for personalized therapies. *Acknowledgements:* ELN, AIL, AIRC, progetto Regione-Università 2010-12 (Bolondi), FP7 NGS-PTL project GS & AP: equal contribution.

C080

NEW DELETION OF JAK2 DETECTED BY SNP ARRAY: A ROLE IN OVERALL SURVIVAL IN ACUTE MYELOID LEUKEMIA PATIENTS

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Introduction: In Acute Myeloid Leukemia (AML) there is a strong need to develop new diagnostic and therapeutic options. Single nucleotide polymorphism (SNP) microarray can detect cytogenetic lesions which may plays a role in oncogenesis. *Aims:* Our objective is to evaluate the prognostic impact of these genetic alterations on clinical outcome. *Materials and Methods:* We analyzed 272 AML patients (pts): 229 performed by SNP Array 6.0 (Affymetrix), 6 by CytoScan HD Array (Affymetrix) and 37 performed by Cytoscan HD Array and also by Next Generation Sequencing (NGS)-WES HiSeq 2000 (Illumina). SNP Array data were analyzed by Nexus Copy Number™ v7.5 (BioDiscovery). *Results:* The median age of our cohort of pts was of 52 years and they had a median age at diagnosis of 51,3 years with a female/male ratio of 51% and 49%. Copy Number Alterations (CNAs) were detected in all patients affecting all the chromosomes, in particular our cohort of pts present a percentage of CNA, divided as follow: 42% of UPD (Uniparental Disomy), 19% of Copy Number (CN) gain, 39% of CN loss. By SNP array analysis we found that several genes were preferentially deleted, for example ADAM3A (62,2% pts), TP53 (15% pts), HRAS (8,6% pts), ETV6 (7,8% pts) and JAK2 (7% pts), while genes preferentially involved in amplifications are: FLT3 (42,5% pts), KIT (36,5% pts), KRAS (34,2% pts) and SIRPB1 (32,5% pts). We focused on a particular heterozygosis loss of JAK2, detected in 8% of pts, which goes from 5080280 bp to 5098786 bp, involving the second tyrosine-kinase domain (part of exon 17 and exons 18, 19, 20, 21 and 22). We showed that the group of patients which present this deletion had an overall survival rate better than the group with an amplification of the gene (p-value <0,01). None CN loss in this region of JAK2 had been described in hematopoietic tissue before. Moreover, we studied the deletome profile of our cohort of pts (Figure 1) in order to correlate the minimal common deletion region with the mutational data obtained by NGS-WES. *Conclusions:* By SNP arrays we have identified CNAs involving important cancer genes in AML and we showed that a new deletion in JAK2 may play a role in the overall survival. Future prospectives will be to confirm and correlate other cancer genes aberrations and mutations with the prognosis of AML, in order to identify new biomarkers relevant for the disease. *Acknowledgement:* ELN, AIL, AIRC, PRIN, progetto Regione-Università 2010-12 (L. Bolondi), FP7 NGS-PTL project.

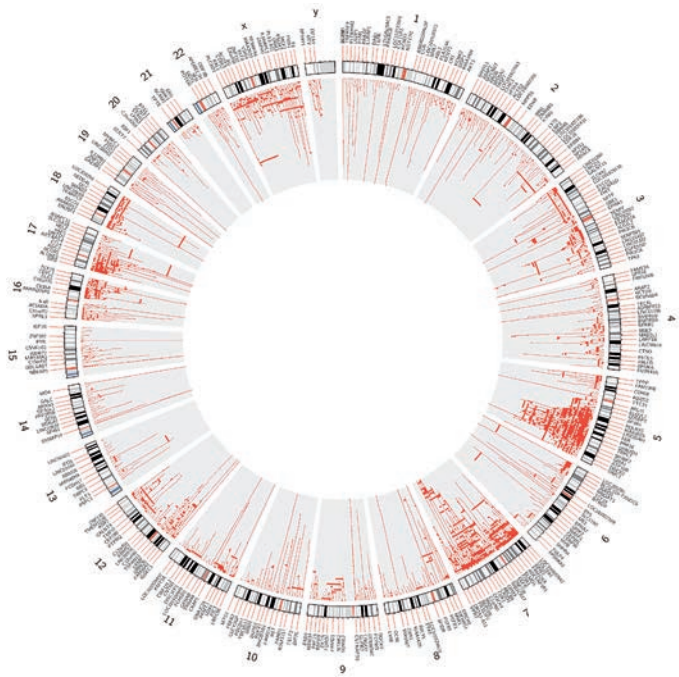

Figure 1.

C081

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

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Background: Most prognostic factors in acute myeloid leukemia (AML) are surrogate for disease chemosensitivity; among them, genetics is the most relevant and is the framework of European Leukemia Net (ELN) stratification. At any rate, response to induction remains a powerful prognostic parameter, expressing actual chemosensitivity. In responsive pts, minimal residual disease (MRD) is an accurate tool to refine prognosis. We have previously demonstrated that an early assessment of the speed of peripheral blast clearance (PBC) by flow cytometry (FC) during induction strictly correlates with outcome. Aim. To correlate PBC after the first 3 days of induction with MRD after induction. *Methods:* Pts had untreated non-M3 AML according to WHO and were treated according to standard induction therapy. At diagnosis, bone marrow (BM) aberrant leukemia-associated immuno-phenotypes (LAIP) were identified by FC. We quantified LAIP+ cells by single platform before treatment (day 1) and following 3 days of therapy (day 4). PBC of each pt was expressed as the ratio between absolute LAIP cell count on days 1 and 4 converted to a log scale. By ROC curves, 1.5 log was the most accurate cut-off separating pts with high and low PBC, respectively (PBC-hi, PBC-lo). In pts achieving CR after a cycle, we searched for LAIP+ cells in BM expressing MRD as percentage on overall BM cells; pts were MRD+ when LAIP+ cells were detected as at least 0.01% of total BM cells. *Results:* Between 2007-2013, 104 pts achieving CR had available LAIP and were studied both by PBC and MRD. As per PBC, 34 (32.7%) pts were PBC-lo; 70 (67.3%) were PBC-hi. PBC status significantly correlated with ELN stratification (p=0.0007). Regarding MRD, 50 (48.1%) pts were MRD+ and 54 (51.9%) MRD-. MRD status correlated with ELN categories as well (p=0.0067). Both PBC and MRD influenced DFS and OS. Most important, PBC predicted MRD status; specifically, 45/70 (64.3%) PBC-hi and 9/34 (26.4%) PBC-lo pts were MRD-negative, respectively (Fisher's test p=0.0004). The combination of both parameters separated 2 categories with favorable (PBC-hi/MRD-) and very poor (PBC-lo/MRD+) outcome (Figure 1). Discordant cases had intermediate prognosis. *Conclusions:* PBC is very early and powerful outcome predictor in AML, providing a real time quantification of chemosensitivity in the first days of therapy. PBC correlates with MRD status after induction and the combination of both parameters identifies pts with strikingly different outcome.

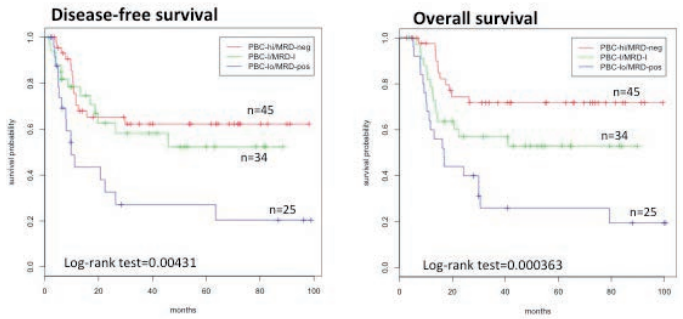

Figure 1.

C082

INHIBITION OF NOTCH SIGNALLING OVERCOMES BONE MARROW STROMAL-MEDIATED ANTI-APOPTOTIC AND CHEMORESISTANCE EFFECTS IN AML

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Both preclinical and clinical investigations suggest that Notch signaling is critical for the development of many cancers and for their response to chemotherapy. We previously showed that Notch inhibition may abrogate stromal-induced chemoresistance in lymphoid neoplasms. However, the role of Notch in acute myeloid leukemia (AML) and its contribution to the crosstalk between blast cells and bone marrow stromal cells remain controversial. AML blast cells were obtained from bone marrow samples (n=28) and peripheral blood (n=16) of AML patients. BM-MSCs were expanded from bone marrow of 12 healthy donors (BM-MSCs) and of 12 AML patients (BM-MSCs*). *In vitro* and in silico analysis showed that AML cells expressed Notch receptors and ligands, regardless the FAB classification or molecular pattern. Notably, 50% of AML samples showed basal Notch signalling activation as demonstrated by the detection, at mRNA and protein level, of HES-1. The activation of Notch signalling by using recombinant Jagged-1 or Jagged-2 did not lead to any change either in AML cell survival or in cell proliferation. On the contrary, the pan-blockade of Notch signalling, both at the membrane level by GSIs or the combination of Notch blocking antibodies, and at the transcriptional level using SAHM1, abolished AML cell proliferation in culture. Notably, BM-MSCs* showed higher level of Notch1 and Jagged1 as compared to BM-MSCs. Interestingly, we observed that BM-MSCs*, but not BM-MSCs, stimulated AML proliferation and the pan-Notch inhibition was sufficient to revert this phenomenon. In addition BM-MSCs* were more efficient in rescuing AML cells from apoptosis induced by chemotherapeutic agents. Pan-Notch signalling blockage by either GSI-XII or combination of Notch receptor-blocking antibodies in presence of chemotherapeutic agents significant lowered the supportive role of BM-MSCs towards AML blasts; conversely SAHM1 has a slight effect. The specific blockade of Notch-1, -2, -3 or Jagged-1, -2 reduced partially the chemoresistance, while blockade of Notch-4 or DLL-3 rescued totally the chemosensitivity of primary AML cells in co-culture with BM-MSCs. These results suggest that the inhibition of Notch signalling may represent a potential therapeutic strategy to improve AML treatment by overcoming bone marrow stromal-mediated anti-apoptotic and chemoresistance effects.

C083

MAPPING WHOLE-EXOME SEQUENCING DATA INTO KEGG PATHWAYS GIVES INSIGHTS FOR RISK STRATIFICATION OF ACUTE MYELOID LEUKEMIA PATIENTS

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Acute Myeloid Leukemia (AML) is a heterogeneous disease and a complex network of events contribute to the pathogenesis. How genomic alterations cooperate to induce AML, the pathways affected by the mutated genes and their prognostic value are still unknown. Aim of the study is to better stratify AML patients, identify novel molecular biomarkers by mapping genomic alterations in biological pathways and correlate them with clinical outcome. We sequenced the exome of 33 AML cases at diagnosis (Illumina Hiseq2000). Mutations were called with GATK and MuTect (validation rate=89%). For each patient, we mapped the mutated genes into KEGG pathways and we performed unsupervised clustering analysis of the pathways. We identified 4 groups of patients characterized by significant differences in the number of mutated pathways (p=0.032), with an average of 74, 58, 28 and 9 per patient, respectively. PI3K-Akt signalling was the top mutated pathway (23/33 patients). Notably, 100% of patients in group 1 and 2 were mutated in that pathway, while 62% and 20% were mutated in group 3 and 4, respectively. Moreover, KRAS was mutated in all group 1 patients and was defined a driver gene by DOTS-finder and MutSig. Six/8 patients of group 2 had TP53 mutations. The top mutated pathways in group 3 were the

PI3K and Metabolic pathways, (75% patients); whereas the latter was mutated in 50% of group 4 patients. Klapan-Meier survival curves showed a significant correlation of the 4 groups with clinical outcome (p=0.047). Group 2 had an extremely unfavourable prognosis, as expected from TP53 mutated cases. By mapping WES data into KEGG pathways, we distinguished 4 groups of patients. Two were characterized by distinct molecular biomarkers able to stratify patients with different prognosis. KRAS was identified as driver gene by two tools, underling its relevance in leukemogenesis. Our analysis defined a potential association between two TP53-WT and the TP53-mutated cases, in terms of KEGG pathway clusterization, which could be defined only by omics analysis. Our results confirm the different prognostic risk of AML patients with KRAS or TP53 mutations and highlight the relevance of WES data interpretation according to mutated pathways for patient stratification. *Acknowledgements:* ELN, AIL, AIRC, progetto Regione-Università 2010-12 (L. Bolondi), FP7 NGS-PTL project. AP&GS equal contribution.

C084

THE DOUBLE FACE OF NPM1. FOCUSING ON GENE DELETION AND COMPLEX KARYOTYPES

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We previously reported that NPM1/5q35.1 monoallelic loss is a very rare event in MDS/AML with isolated del(5q) whereas present in ~45% of cases with complex del(5q) (La Starza R. *et al.* 2010). The association of NPM1 deletion/haploinsufficiency with gross chromosomal changes suggested NPM1 gene plays a major role in the origin of genomic instability observed in t-MDS/AML with complex del(5q) (Pedersen-Bjergaard J. *et al.* 2006). Furthermore, in defining del(5q) boundaries, we showed NPM1 loss significantly associated with large proximal deletions in complex del(5q) (Nofrini V. *et al.* 2010). Here we report on the results of a prospective study on 86 MDS/AML with isolated (17 cases) and complex (69 cases) del(5q) enrolled from March 2011 to December 2014 in the Laboratory of Cytogenetics of the Hematology at Perugia University. In addition we describe an *in vitro* model of haploinsufficiency obtained by NPM1 gene silencing in a human cell line. FISH probes RP11-117L6/5q35.1 and RP11-80K5/5q14.1 investigated respectively del(5q) telomeric and centromeric boundaries. The MRC5 human fibroblast cell line was silenced with lentiviral particles (Santa Cruz). Silencing was confirmed by RT-qPCR. MRC-5 and siNPM1-MRC5 cell lines were treated with camptothecin (CPT), ultraviolet light (UV) and ionizing radiation (IR). Chromosomal damage was evaluated by testing for chromosomal aberrations (CA) and sister chromatid exchanges (SCE). Primary DNA damage and repair kinetics were investigated by comet assay. NPM1/5q35.1 deletion was found in 5.9% (1/17 cases) of isolated del(5q) and in 40.6% (28/69 cases) of complex del(5q)/-5 MDS/AML. RP11-80K5 was retained in all isolated del(5q) cases and in 23/69 (33.3%) patients with complex del(5q). Combining analysis of centromeric and telomeric breakpoints, 7/41 (17%) NPM1+/+ cases bore the RP11-80K5 deletion, while 16/28 (57.1%) NPM1+/- cases lost RP11-80K5 (Fisher exact test, p=0.0007). siNPM1-MRC5 showed a significant increase in chromosomal damage compared with MRC5, regardless of the chemical or physical agent. Greater sensitivity did not correlate with cell cycle delay or cell death. The comet assay did not show differences in primary DNA damage induction or in repair kinetics. Altogether, these results provide strong evidence that NPM1 deletion is associated with complex karyoptypes and large genomic losses at chromosome 5q in MDS/AML. Moreover NPM1 haploinsufficiency underlies increased sensitivity to mutagenic insults.

Myeloproliferative Diseases

C085

TREATMENT OF ESSENTIAL THROMBOCYTHAEMIA IN EUROPE: AN OBSERVATIONAL STUDY OF 3649 HIGH-RISK PATIENTS IN EXELS

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Introduction: The Evaluation of Xagrid Efficacy and Long-term Safety (EXELS) study (NCT00567502) is the largest prospective observational cohort of high-risk patients (pts) with essential thrombocythaemia (ET) reported to date. The primary objective was safety and pregnancy outcomes of anagrelide (ANA) compared with other cytoreductive therapies (CRT). Secondary objectives included efficacy, measured by the incidence of thrombohaemorrhagic events and platelet reduction. *Patients and Methods:* High-risk pts (≥1 of age >60 yrs, previous thrombotic event, platelet count >1000 x 109/L) with ET were enrolled across 13 countries in Europe between 2005 and 2009 and followed every 6 months for 5 years. Event rates are presented as number of pts per 100 pt years exposure and by treatment at time of event. *Results:* 3649 pts were categorised according to treatment at registration as follows: ANA (n=804), ANA+other CRT (n=141), other CRT (n=2666) and no CRT (n=38). Over 80% of pts received either hydroxycarbamide (HC) or ANA, and 69.8% of pts received anti-aggregatory therapy. At registration, median age was lower in the ANA (55.5 yrs, range 18 89) and ANA+other CRT (59.0 yrs, range 22–88) groups *vs* the other CRT group (70.0 yrs, range 17 95). The arterial thrombotic rate was similar in ANA (1.63) and other CRT (1.62) groups, whereas the venous thrombotic rate was lower in the ANA group *vs* the other CRT group (0.35 *vs* 0.57). In contrast, the major haemorrhagic event rate was higher in the ANA *vs* other CRT group, especially in patients treated with anti-aggregatory therapy (1.24 *vs* 0.41, respectively). 105 pts transformed to myelofibrosis (MF) and 62 to acute leukaemia (AL). In pts who had only ever received either ANA or HC, rate of transformation to MF was higher in the ANA *vs* HC group (0.78 *vs* 0.17) whereas transformation to AL was higher in the HC *vs* ANA group (0.22 *vs* 0). The rate of non-haematological malignancies was higher in the other CRT group compared with the ANA group (1.35 *vs* 0.49). No unexpected side effects were noted. *Discussion:* Pts who received ANA were younger than those who received other CRT. The rates of thrombohaemorrhagic events were generally low, with differences in rates of venous thrombotic and haemorrhagic events between the ANA and other CRT group. Transformation to MF was more frequent in the ANA group while transformation to AL was more common with other CRTs.

C086

A MOLECULAR INTERNATIONAL PROGNOSTIC SCORING SYSTEM FOR PRIMARY MYELOFIBROSIS: AN AGIMM PROJECT

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Background: PMF is associated with a median survival of 5–7yrs ranging from more than 15yrs to less than 2yrs. The IPSS, DIPSS or DIPSS-plus are commonly used to predict survival. However, more recently the mutational status and the number of driver (JAK2/CALR/MPL) or other prognostically-relevant mutated genes (ASXL1, SRSF2, EZH2, IDH1/2) provide IPSS/DIPSS-plus independent prognostic information. The aim of this study was to develop a new prognostic score by including clinical and mutation-relevant prognostic information. *Methods:* The study included 588 PMF from 6 Italian institutions. Previously published methods were used to sequence JAK2, MPL, CALR, EZH2, ASXL1, IDH1/2 and SRSF2. Clinical and molecular data were recorded at diagnosis or within 1 yrs. The prognostic model was developed through a stepwise selection process, based on a z-test of the regression coefficients, and its relative quality was measured by Akaike information criterion (AIC) and c-statistics (AUC) methods. *Results:* During the follow-up (median 3.7yrs), 206 (35.0%) pts died and 67 (11.4%) progressed to leukemia. Mutational frequencies were 63% for JAK2, 20% CALR (73% type1 and 21% type2), 6% MPL, 7% EZH2, 22% ASXL1, 2.5% IDH1/2 and 9% SRSF2; 7% of pts presented more than 2 HMR mutated genes. Univariate analysis identified these risk factors for inferior survival: age>65yrs, constitutional symptoms, Hb <100g/L, leukocytes >20x109/L, platelets <150x109/L, circulating blasts ≥1%, splenomegaly >10cm, grade 3 fibrosis, triple-negativity for JAK2, MPL and CALR (TN), JAK2 or MPL mutation, CALR type2 and mutations in ASXL1, SRSF2, EZH2 or IDH1/2, number of mutated genes>2 (mutation>2). A HR-weighted adverse point was assigned to variables remaining significant in multivariable analysis: age>65yrs, TN and CALR type2: 2 points; mutation>2 1.5 points; leukocytes>20x109/L, Hb<100g/L, platelets<150x109/L, JAK2 or MPL mutation and grade 3 fibrosis: 1 point; constitutional symptoms (0.5 point). Accordingly, four risk groups were delineated (Figure 1a): low (score 0-1); Int-1 (score 1.5-3); Int-2 (score 3.5-5); and high (score >5). AIC and AUC indicated that MIPSS performed better than IPSS in predicting survival. Leukemia-free survival was also predicted by MIPSS categories (p<0.0001, Figure 1b). *Conclusions:* The MIPSS retained continuity with the IPSS and was shown to possess an improved prognostic ability power for survival and leukemic evolution in PMF patients at diagnosis compared with the IPSS.

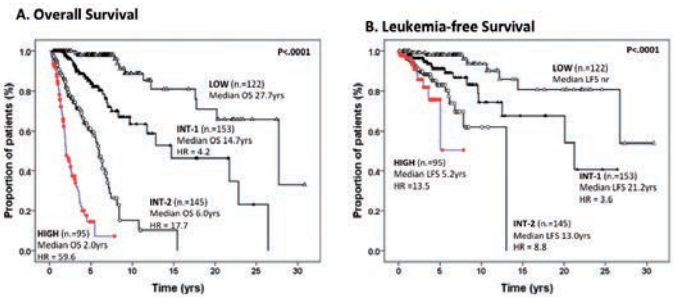

Figure 1.

C087

A PHASE 2 STUDY OF RUXOLITINIB IN PATIENTS WITH SPLANCHNIC VEIN THROMBOSIS ASSOCIATED WITH MYELOPROLIFERATIVE NEOPLASM-FINAL RESULTS

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Philadelphia-negative Myeloproliferative Neoplasms (MPN), including Polycythemia Vera (PV), Essential Thrombocythemia (ET) and Myelofibrosis, both Primary (PMF) and secondary to PV or ET (PPV-MF and PET-MF), are frequently underlying cause of splanchnic vein thrombosis (SVT). Ruxolitinib reduces spleen volume (SV) and improves symptoms in patients (pts) with MF, PV and ET. This is an investigator-initiated phase 2 study of ruxolitinib in pts with splenomegaly and SVT associated with MPN. The drug was provided free of charge by Novartis. 21/21 patients completed 24 weeks of treatment; diagnosis of MPN were PMF 8 (38.1%), PV 5 (23.8%), ET 4 (19.1%), PPV-MF 3 (14.3%), PET-MF 1 (4.8%). 19 pts had spleno-porto-mesenteric thrombosis and 3 Budd Chiari Syndrome; one pt had both sites involved. Initial dose of Ruxolitinib was 10 mg BID for PV, 25 mg BID for ET, 15 mg BID for MF pts if baseline (bl) platelet count (PLT) 100 to 200x10^9/L and 20 mg BID if bl PLT >200x10^9/L. Median values at enrolment: hemoglobin 12.9 gr/dL (9.4-16.7), PLT 212 x10^9/L(100-389), white blood cell count 7.3 x10^9/L(1.8-16.4). Primary objective was the proportion of pts achieving reduction in spleen size: 67% of pts obtained a ≥50% spleen length reduction by palpation; 29% achieved a SV reduction ≥35% by imaging, comparable to previous studies in MF/PV pts without SVT. Secondary objectives included evaluation of splanchnic circulation: no changes in venous thrombosis status nor in resistive or pulsatility index of splanchnic artery were noted; cardiac output decreased mainly due to reduction of heart rate; spleen stiffness was evaluable in 4/21 pts and these pts obtained a mean reduction of 17%; oesophageal varices remained stable; symptoms related to MPN, evaluated by MPN-SAF, reduced from a median total symptom score of 65 at bl to 42 at w24. Safety assessment: no serious adverse events (AE) occurred; hematologic toxicities included thrombocytopenia (6 events, only 1 G3), anemia (5 events, only1 G3) and neutropenia (3 events, only 1 G3), that led to temporary withdrawal or dose reduction in 7 pts, each. Seven infectious AE occurred, all G1-2. Exploratory objectives: JAK2V617F allelic burden did not show significant changes at w24; serum VEGF at w4 and the absolute number of peripheral endothelial progenitor cells decreased in pts with PMF at w24 *vs* bl. *Conclusions:* Ruxolitinib was safe in pts with MPN associated to SVT and effective in reducing spleen size.

C088

IMPACT OF DRIVER AND HIGH MOLECULAR RISK MUTATIONS ON CLINICAL PHENOTYPE AND PROGNOSIS IN PATIENTS WITH POST-POLYCYTHEMIA AND POST-ESSENTIAL THROMBOCYTHEMIA MYELOFIBROSIS: AN AGIMM STUDY

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Recently mutations in JAK2,CALR and MPL (defined"drivers") defined different outcomes in PMF. CALR+ PMF pts had better overall survival (OS) compared with JAK2+,MPL+ and triple-negative (TN) pts, while TN PMF had the worst prognosis. Mutation(s) in at least one of ASXL1, EZH2, IDH1/2, SRSF2 (high molecular risk (HMR)) in PMF is associated with negative outcome. Whether the same applies to secondary myelofibrosis (sMF) is still unknown. We aimed at evaluating the correlations of driver and HMR mutational genes status with hematologic characteristics and clinical presentation, and the role for outcome prediction, in sMF. sMF were diagnosed by IWG-MRT criteria. Previously published methods were used to screen mutations in JAK2, MPL, CALR, EZH2, ASXL1, IDH1/2 and SRSF2. The prognostic value of the molecular variables with regard to OS was estimated by the Kaplan-Meier method and Cox regression. 333 sMF pts from 4 Italian centres was collected: 182 PPV-MF and 151 PET-MF (45.3%). PPV-MF cohort: Median follow up was 3.6y (0.6-26.8y) and median time from PV to PPV 10.2y (1.1-30.7y). Death occurred in 55 pts (30.2%), 13 pts (7.1%) developed leukemia. Median OS from PPV diagnosis was 8y (5.6-10.4y). Mutational status is reported in Table1. PET-MF cohort: Median follow up was 3.4y (0.7-18.8y), median time from ET to PET 11.5y (0.9-30.6y). Death occurred in 40 pts (26.5%), 15 pts(9.9%) developed leukemia. Median OS from PET diagnosis was 14.5y (7.7-24.3y). Mutational status is reported in Table1. Median CALR allele burden in PET was 56% with no significant differences in the CALR mutation subtypes (57.5% in type1, 47.5% in type2 and 45.0% in others). There was a reduced rate of death in type1CALR+(15.6%) compared with type2CALR+(25%), JAK2+(31%), MPL+(20%) and TN(67%) pts (P=0.041), although Kaplan Meier estimates did not reach a statistically significance. Finally, there were less AML transformation in CALR+ pts(1.9%) compared with JAK2+(13.9%), MPL+(7.1%) and TN(22.2%) (P=0.04). HMR status was more frequent in PET (35.3%) compared with PPV (25%) pts (P=0.046). Analysis of the impact of driver mutations and HMR status on clinical and hematological features did not show any significance in all comparison. Univariate analysis for OS did not confirmed significant correlations considering the separate cohort or the entire population. In conclusion, driver mutational status defines a milder disease in PET, while HMR does not impact on prognosis of sMF pts unlike in PMF.

Table 1.

		PET-MF N=151	PPV-MF N=182
JAK2V617F,%		49	100
JAK2V617F allele burden, median % (range)		52 (5-100)	79 (23-100)
CALR n (%)	Type1*	32 (60.4)	-
	Type2 *	12 (22.6)	-
	atypical	9 (17)	-
CALR allele burden, median %,(range)		56 (20-100)	-
MPLW515, n (%)		15 (10.6)	-
TRIPLE NEGATIVE, n (%)		9 (6.3)	-
ASXL1**, n (%)		31 (26.6)	24 (16.6)
EZH2**, n (%)		9 (7.6)	6 (4.1)
IDH**, n (%)		2 (1.7)	8 (5.5)
SRSF2**, n (%)		4 (3.4)	2 (1.3)
HMR (at least one mutation)**, n (%)		42(35.3)	36 (24.8)

*type1=del52,type2=ins5; **on available samples: PET N=119, PPV N=145;

C089

CLONAL EVOLUTION ANALYSIS IDENTIFIES ETNK1 AS AN EARLY EVENT AND SETBP1 AS A LATE EVENT IN ATYPICAL CHRONIC MYELOID LEUKEMIA

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Atypical Chronic Myeloid Leukemia (aCML) is a clonal disease belonging to the myeloproliferative/myelodysplastic disorders. In previous studies our group identified recurrent, somatic mutations in SETBP1 and ETNK1 genes in aCML. Other recurrently mutated genes such as ASXL1, EZH2, CBL, TET2, NRAS and U2AF1 were already reported in previous studies. The presence of a large set of variants in aCML suggests that this clonal disorder is heterogeneous. The hierarchical reconstruction of the different mutations can have important biological, prognostic and therapeutic repercussions; therefore we started a project focused on the identification of the aCML clonal evolution steps through the analysis of individual leukemic clones in samples whose mutational status was previously analyzed by matched whole-exome sequencing (WES). One of our patients, CMLPh-019, was characterized by the presence of a complex mutational status, with somatic mutations occurring in SETBP1, ETNK1, ASXL1 and CBL genes. Clonal analysis of this sample was very informative, allowing the reconstruction of a large part of his evolution. In particular, in 44/60 (73.3%) clones we detected the presence of all the 4 variants; in 15/60 (25%) mutations on ETNK1, ASXL1 and CBL and wild-type (WT) SETBP1. Of these clones, 33% carried heterozygous and 67% homozygous CBL mutations. In one clone (1.7%) we detected heterozygous ETNK1 and homozygous CBL variants and WT sequences for ASXL1 and SETBP1. Taken globally, these data indicate that ETKN1 and CBL mutations represent early events, ASXL1 mutations intermediate ones and SETBP1 a late event in the history of this aCML patient (Figure 1a). Moreover, the identification of homozygous CBL mutations in all the main clonal phases suggests that the evolution from hetero to homozygosity occurred independently in at least 3 different clones and therefore that a significant positive selective pressure is associated with this event. Allelic imbalance analysis of CMLPh-019 exome using CEQer revealed that the mechanism leading to CBL homozygosity is represented by a somatic uniparental disomy event occurring in the telomeric region of the long arm of chromosome 11 from CEP164 locus to the end of the chromosome (Figure 1b). In summary our study, albeit preliminary, suggests that ETNK1 and CBL variants are early and SETBP1 a late event in aCML.

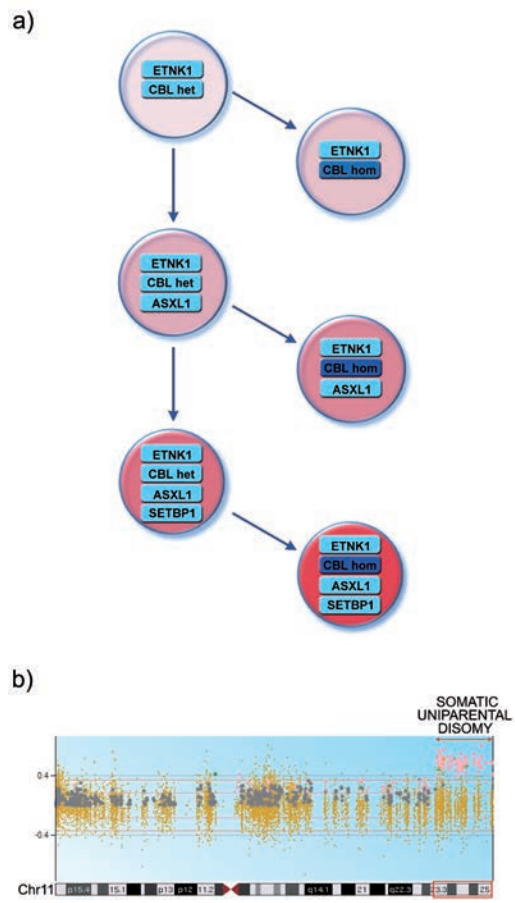

Figure 1.

C090

CLINICAL SIGNIFICANCE OF LOW JAK2V617F ALLELE BURDEN: A MONOCENTRIC STUDY

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Thanks to increased sensitivity of quantitative PCR (qPCR) techniques, the detection of low allele burden (AB) of the JAK2V617F mutation is becoming rather frequent in laboratory practice. However, the interpretation of these results is challenging, since JAK2V617F mutation has been detected also in healthy blood donors at low level. To evaluate the significance of JAK2V617F low AB in clinical practice, we reviewed the results of the JAK2V617F tests performed at the Institute of Hematology "L. e A. Seràgnoli", Bologna. Between January 2013 and January 2015, 1087 JAK2V617F analysis were performed. DNA was obtained from granulocytes and analyzed with ipsogen JAK2 MutaQuant Kit. All samples with low allele burden (AB) were tested in duplicate. In selected cases, JAK2 exon12, CALR and MPL mutations were also tested. JAK2 exon12 was assessed by DHPLC, followed by Sanger sequencing; CALR analysis was performed by NGS; MPL mutations were investigated by ipsogen MPLW515K/L MutaScreen and by Sanger sequencing (for MPLS505N). Forty-six (4.2%) patients had a low JAK2V617F allele burden (0.1-3%). Clinical suspicions that motivated the molecular evaluation were: essential thrombocytemia/thrombocytosis (23); erythrocytosis/polycythemia vera (14); myelodisplasia (7); atypical thrombosis (1); myelofibrosis (1). The clinical suspicion of ET was investigated by CALR and MPL molecular evaluation and, in selected cases, by bone marrow biopsy. Overall, 15 out of 23 (65.2%) patients with thrombocytosis were diagnosed with WHO-defined ET; in one case, a Type1 CALR mutation was detected (42% AB). In the other 8 patients, thrombocytosis was reactive to an inflammatory or neoplastic disease (Table 1).

Table 1. Main characteristics of 46 patients with low JAK2V617F allele burden.

Patient n.	Initial clinical suspicion	%JAK2 V617F (AB)	*CALR* mutation	*MPL* mutation	*JAK2* exon 12	Bone marrow histology	Final clinical diagnosis
1	Erythrocytosis	0.39	WT	WT	WT	n.a.	BPCO
2	Erythrocytosis	0.16	WT	WT	WT	n.a.	BPCO
3	Erythrocytosis	0.12	WT	WT	WT	n.a.	BPCO
4	Erythrocytosis	0.74	WT	WT	WT	n.a.	BPCO
5	Erythrocytosis	0.59	WT	WT	WT	n.a.	BPCO
6	Erythrocytosis	0.12	WT	WT	WT	n.a.	PV
7	Erythrocytosis	0.49	WT	WT	WT	n.a.	PV
8	Erythrocytosis	1.84	WT	WT	WT	n.a.	PV
9	Erythrocytosis	2.23	WT	WT	WT	n.a.	PV
10	Erythrocytosis	0.40	WT	WT	WT	n.a.	PV
11	Erythrocytosis	0.72	WT	WT	WT	n.a.	PV
12	Erythrocytosis	0.15	WT	WT	WT	n.a.	PV
13	Erythrocytosis	0.50	WT	WT	WT	n.a.	PV
14	Erythrocytosis	0.16	WT	WT	WT	n.a.	PV
15	Thrombocytosis	0.64	WT	WT	n.a.	n.a.	ET
16	Thrombocytosis	3.04	WT	WT	n.a.	ET	ET
17	Thrombocytosis	2.98	WT	WT	n.a.	ET	ET
18	Thrombocytosis	2.37	WT	WT	n.a.	ET	ET
19	Thrombocytosis	2.00	WT	WT	n.a.	ET	ET
20	Thrombocytosis	1.56	WT	WT	n.a.	n.a.	ET
21	Thrombocytosis	1.46	WT	WT	n.a.	n.a.	ET
22	Thrombocytosis	1.05	WT	WT	n.a.	n.a.	ET
23	Thrombocytosis	0.79	WT	WT	n.a.	ET	ET
24	Thrombocytosis	0.56	Type1 (42%)	WT	n.a.	n.a.	ET
25	Thrombocytosis	0.36	WT	WT	n.a.	ET	ET
26	Thrombocytosis	0.15	WT	WT	n.a.	n.a.	ET
27	Thrombocytosis	0.43	WT	WT	n.a.	PMF-0	ET
28	Thrombocytosis	2.36	WT	WT	n.a.	PMF-0	ET
29	Thrombocytosis	2.38	WT	WT	n.a.	n.a.	ET
30	Thrombocytosis	0.34	WT	WT	n.a.	n.a.	Arthritis
31	Thrombocytosis	0.32	WT	WT	n.a.	n.a.	Arthritis
32	Thrombocytosis	0.86	WT	WT	n.a.	n.a.	Reactive thrombocytosis in prostate cancer
33	Thrombocytosis	0.16	WT	WT	n.a.	n.a.	Reactive thrombocytosis in prostate cancer
34	Thrombocytosis	0.61	WT	WT	n.a.	NORMAL	Reactive thrombocytosis in prostate cancer
35	Thrombocytosis	0.12	WT	WT	n.a.	NORMAL	Reactive thrombocytosis in erysipelas
36	Thrombocytosis	0.59	WT	WT	n.a.	n.a.	Arthritis
37	Thrombocytosis	0.41	WT	WT	n.a.	n.a.	Probable reactive thrombocytosis
38	MDS	0.14	WT	WT	n.a.	MDS	MDS
39	MDS	0.12	WT	WT	n.a.	MDS	MDS
40	MDS	2.79	WT	WT	n.a.	MDS	MDS
41	MDS	0.55	WT	WT	n.a.	MDS	MDS
42	MDS	0.46	WT	WT	n.a.	MDS	MDS
43	MDS	0.63	WT	WT	n.a.	MDS	MDS
44	MDS	0.34	WT	WT	n.a.	MDS	MDS
45	MF	2.29	WT	WT	n.a.	MDS	PMF
46	Atipycal thrombosis	1.30	WT	WT	n.a.	MPN NOS	MPN NOS

All 14 patients with erythrocytosis were unmutated for exon12. Nine (64,3%) patients had a final diagnosis of PV according to WHO criteria. In the remaining 5 cases, a chronic pulmonary obstructive disease was diagnosed. In patients with a suspected MDS/MF, diagnosis was confirmed, while the pt with atypical (portal) thrombosis was diagnosed with a MPN NOS. In 13 out of 1087 (1.2%) tested patients, JAK2V617F low AB did not result in a hematological diagnosis. Notably, all subjects with reactive thrombocytosis/erythrocytosis carried AB <1%. The integration of clinical and histological data is therefore particularly advisable in this setting. *Supported by:* ELN, AIL, AIRC, progetto Regione-Università 2010-12 (L. Bolondi), FP7 NGS-PTL project.

Anemias and Red Cells Disorders

C091

CLINICAL, HAEMATOLOGICAL AND MOLECULAR CHARACTERIZATION OF 10 PATIENTS AFFECTED BY GLUCOSE PHOSPHATE ISOMERASE DEFICIENCY

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Glucose-6-phosphate isomerase (GPI) deficiency (OMIM 172400), is the second most common erythro-enzymopathy of anaerobic glycolysis, after pyruvate kinase. The disease, transmitted as an autosomal recessive trait, is chacterised by chronic nonspherocytic hemolytic anemia of variable severity; in rare cases mental retardation or neuromuscular symptoms have also been reported. So far about 60 cases of GPI deficiency have been described; 35 different mutations, mostly missense, have been identified in the GPI gene located on chromosome 19q13.1. In this study we characterized at clinical, haematological and molecular level 10 GPI deficient patients (6 males, 4 females) from 9 families; eight patients were of Italian origin and 2 were Turkish. Median age at admission was 7 yrs (range 1-51) and onset of anemia was at birth or in the early infancy; neonatal jaundice was observed in 4 patients, 3 of whom required exchange transfusion. Hb levels ranged from 2.7g /dL during haemolytic crises to 11,7 g/dL. All patients needed blood transfusion in childhood: 3 regularly (every 4-8 weeks), the remaining occasionally during infection-associated haemolytic crises. Splenectomy, performed in 5 patients before the diagnosis of GPI deficiency, did not lead to recovery from anaemia, only resulting in a slightly increase of Hb levels (0.5-1 g/dL). Transfusion requirement was greatly reduced with ageing and also after splenectomy. Two patients underwent chelation therapy for iron overload. None showed neurological signs. Genetic analysis confirmed the wide molecular heterogeneity of GPI deficiency: 11 different mutations were found, 4 of them never described before (c.311 G>A, c.307C>G, c.269T>C, c.1066G>A); all the new mutations affect highly conserved residues, were not detected in Ensembl and 1000 genomes database, and were predicted to have pathogenic effects by Polyphen, SIFT analysis. In particular, mutation Asp356Asn is part of active site and falls in a region involved in the sugar isomerase domain and dimer-dimer interface, further suggesting its drastic effect. In one patient we were unable to find the second mutation; however, the loss of heterozygosity at the cDNA level suggested the presence of a null mutation of the paternal GPI allele (Table 1).

Table 1. Main clinical, haematological and molecular data of the 10 GPI deficient patients.

Pt	Hb g/dL	Retics 10^9/L	Tx	SF ng/ml	Splenect. (age)	Hb g/dL	Retics 10^9/L	Mutations	Effect
	pre splenectomy					post splenectomy			
1	6,1*-10,2	231	Occas.	n.a.	no	-	-	C 145G>C/c.921C>A	Gly49Arg /Phe307Leu
2	6,2*-11,6	166	Occas.	n.a.	no	-	-	**c.311 G>A/ c.584C>T**	**p.Arg104Gln/ p.Thr195Ile**
3	n.a.	n.a.	Occas.	2356	yes (9)	11.5	445	**c.307C>G / c.307C>G**	**p.Leu103Val/ p.Leu103Val**
4	8.4-10	113	Occas.	488	yes (7)	9.4-10.5	364	c.301G>A/ c.1009G>A	p.Val101Met/ p.Ala337Thr
5	10	n.a.	Occas.	202	no	-	-	c.1009G>A/ c.1009G>A	p.Ala337Thr/ p.Ala337Thr
6	n.a.	n.a.	Occas.	353	yes (17)	10,5	170	c.584C>T/ c.584C>T	p.Thr195Ile/ p.Thr195Ile
7	11,7	347	Occas.	210	no	-	-	?/ c.1415G>A	LOH/ p.Arg472His
8	8.5	410	regular	n.a.	no	-	-	**c.269T>C/ c.1066G>A**	**p.Ile90Thr/ p.Asp356Asn**
9	5,4*-8,9	210	regular	1123	yes (6)	9,4	1420	c.1040G>A/ c.1040G>A	p.Arg347His/ p.Arg347His
10	2,7*-8,4	660	regular	185	yes (3)	9,2	1740	c.1040G>A/ c.1040G>A	p.Arg347His/ p.Arg347His

Tx: transfusions; SF: Serum Ferritin, n.a.: not available; *= during hemolytic crises
new mutations are reported in bold.

C092

BIOSIMILAR EPOETIN A IN THE MANAGEMENT OF CHEMOTHERAPY-INDUCED ANAEMIA: RESULTS FROM ANEMONE OBSERVATIONAL STUDY

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Background: A large proportion of patients with solid tumours or non-myeloid haematopoietic tumours develop symptomatic anaemia, with relevant impact on quality of life (QoL).The efficacy of erythropoiesis-stimulating agents (ESA) in improving QoL and reducing blood transfusions has been widely demonstrated. Several biosimilar epoetins have been approved by the European Medicines Agency. Biosimilar is a regulatory term used to describe medicines with similar properties to that of an approved biological medicine for which the patent has expired. Binocrit® is a biosimilar epoetin α approved by EMA in 2007 for several indications, including the treatment of chemotherapy-induced anaemia (CIA). The aim of this retrospective study was to verify, in the Italian clinical practice, the trend of haemoglobin (Hb) levels in anaemic cancer patients for whom physicians deemed to use Binocrit®. *Patients and Methods:* The ANEMONE study was a national, longitudinal, retrospective, multicentre observational study. Patients had to be 18 years or older, presenting solid tumour or non-Hodgkin's lymphoma, Hodgkin's disease or multiple myeloma, receiving chemotherapy,treated with Binocrit® to manage CIA. The primary outcomes were the proportion of patients with an increased level of haemoglobin ≥1 g/dL during the first 4 weeks and with an increased level of Hb ≥2 g/dL during the first 12 weeks. *Results:* 245 patients were enrolled and 215 patients were evaluable for statistical analysis. In the first 4 weeks, 49.3% of patients showed increased Hb=1 g/dL: 45.5% in patients with solid tumours, 52.1% in patients with haematologic malignancies. In the first 12 weeks, 51.6% of patients showed increased Hb=2 g/dL (48.4% solid tumours, 54.2% haematologic malignancies) (Figure 1). Treatment with Binocrit® was well tolerated and no unexpected adverse drug reactions were reported. Interestingly, iron supplementation was adopted less than expected according actual guideline indications. *Conclusions:* Despite the fact that biosimilar epoetins are widely used at European level, few publications currently have reported data about their efficacy and safety in real world clinical practice. This multicentric study represent, therefore, an important step to create and increase awareness of biosimilar epoetin clinical data. These results confirm efficacy and safety on biosimilar epoetin α (Binocrit®) for the treatment of CIA in routine practice in patients with solid tumors, lymphoma and myeloma.

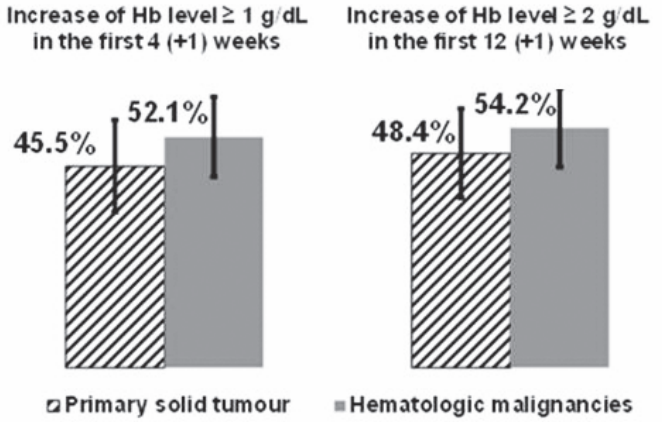

Figure 1. Increase of HB level during the observation period.

C093

THREE CASES OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA WITH SPONTANEOUS CLINICAL REMISSION

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PNH is a rare disorder characterized by haemolytic anaemia, marrow failure and thrombosis due to a somatic mutation which cause GPI–anchored proteins deficiency. We describe 3 PNH patients (pts) who had spontaneous clinical remission. PNH was diagnosed before 1998 by Ham and Sucrose Haemolysis test and thereafter by flow cytometric (FC). Pt 1 was referred in 1970 (22 yrs) with Hb 6.8 g/dL and reticulo-

cytes 24%. Her transfusion requirement was about 2 RBC units/month and several episodes of severe haemolysis occurred until 1975. In 1977 Hb normalised. In 1988 Ham and Sucrose haemolysis test were negative. In 1999 FC showed 0.48% and 0.2% CD55 and CD59 deficient erythrocytes, respectively; molecular analysis showed PNH clone bearing c.341Gdel mutation. Blood counts and haemolytic markers were normal. Pt 2 was diagnosed in 1971 (28 yrs). She presented with Hb 6 g/dl and transfusion requirement of 2 RBC units/month until 1980. From 1981 to 1988 the transfusion requirement increased to 2 RBC units every 20 days. Since 1990 Hb remained stable (9-10 g/dL) and she was no longer transfused. Since 1996 Hb increased to 12-13 g/dL and haemolytic markers normalized. In 2001 the Ham and Sucrose haemolysis tests became negative and FC showed 3.4% and 1.2% CD55 and CD59 deficient erythrocytes, respectively. Molecular analysis showed PNH clone c.1306Tdel mutation. Hb level and haemolytic indices were normal at the last follow up in 2013. Pt 3 (Figure 1)was diagnosed in 2003 (67 yrs). Blood values were: Hb 8.4 g/dL, retyculocytes 65300/mmc, and 8.4% and 6.8% CD55 and CD59 deficient granulocytes; molecular analysis showed a PNH clone c.553T>C mutation. During the first year he was transfused (1 RBC unit every 2 months) to maintain Hb>10g/dL for concomitant ischemic heart disease. Subsequently (2012) the PNH clone increased (23% erythrocytes, 11% granulocytes, and 63% monocytes by FLAER), intravascular haemolysis worsened and transfusion requirement augmented (2/3 RBC units/month). In the following years blood analysis and PNH clone were unchanged and the pt repeatedly refused eculizumab. From January 2014 Hb normalized, intravascular hemolysis decreased, and transfusions were no more required. In July 2014 GPI-negative cells were reduced in erythrocytes (11.2%) and unchanged in granulocytes and monocytes. Spontaneous clinical remission in PNH may occur underlining the bizarre clinical course of this rare disease and the need for carefully monitoring of GPI-negative cells.

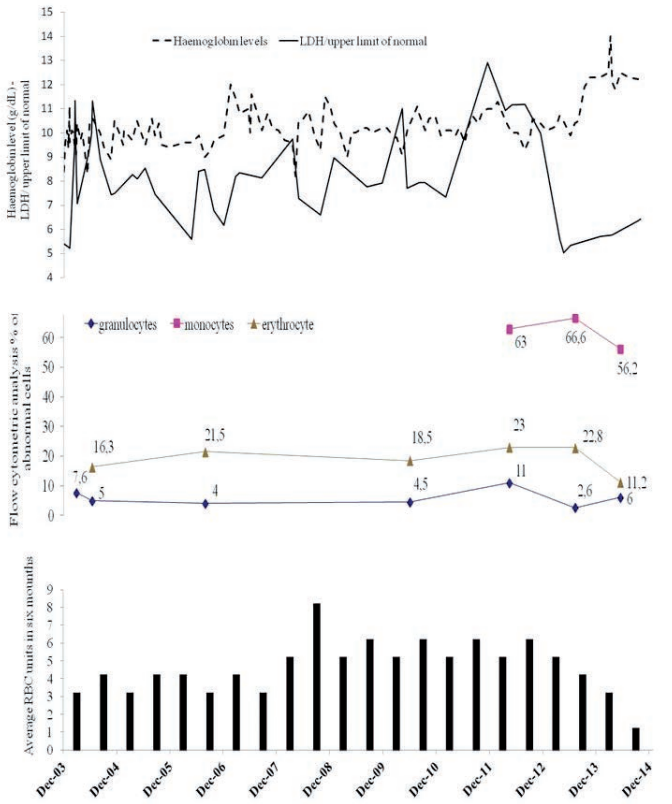

Figure 1.

C094

LASER ASSISTED OPTICAL ROTATIONAL CELL ANALYZER: A USEFUL TOOL IN THE DIAGNOSIS OF HEREDITARY HAEMOLYTIC ANAEMIAS

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Haemolytic anaemias are a heterogeneous group of hereditary disorders caused by defects of the red blood cell (RBC) membrane and of enzymes resulting in reduced erythrocyte lifespan. The more common membrane protein defects are hereditary spherocytosis (HS) and elliptocytosis (HE), followed by hereditary stomatocytosis (HSt), due to alteration of membrane permeability. Among enzymopathies the most frequent is piruvate kinase deficiency (PK) followed by glucose-6-phosphate isomerase (GPI). We evaluated the diagnostic power of laser-assisted optical rotational cell analyser (LoRRca MaxSis, Mechatronics Instruments, NL), able to measure RBC deformability in osmotic gradient conditions (Osmoscan). We analysed 73 cases with RBC membrane disorders (61 HS, 5 HE, 7 Dehydrated HSt) and 22 enzymopathies (18 PK, 4 GPI). A reference curve interval was obtained from 75 healthy controls. The evaluated parameters were: Omin (coinciding with the 50% haemolysis in osmotic fragility assays), EImax (maximal deformability), and Ohyper (representing cellular hydration status). All the 61 HS, regardless the biochemical membrane defect, showed typical altered ektacytometry curves, with a decreased EImax and a shift to the right of the Omin (Figure 1a), the 5 HE cases displayed the classical trapezoidal feature and a decreased EImax (Figure 1b), and the 7 DHSt were characterized by the typical left-shift of the curve (Figure 1c). All GPI cases showed altered enlarged Osmoscan curves associated with significant increased Ohyper (Figure 1d). Not-splenectomised PK deficiency cases (n=13) displayed normal Osmoscan (Figure 1e), whereas splenectomised patients gave Osmoscan curves in a defined atypical area (Figure 1f). On the whole our results demonstrated that all the Osmoscan curves were diagnostic in HS, HE and DHSt. In fact, the parameters Omin and EImax were more significantly altered in HS and HE patients, and the left-shift of all the parameters was typical for DHSt. Interestingly, Ohyper was firstly described increased in GPI deficiency, offering a new diagnostic tool for this rare enzyme defect. The interpretation of Osmoscan requires caution in splenectomised cases as splenectomy may interfere with the RBC deformability. In conclusion, the Osmoscan analysis performed by LoRRca Maxsis represents a useful and feasible first step screening test in the diagnosis of RBC membrane disorders and other rare haemolytic anaemias.

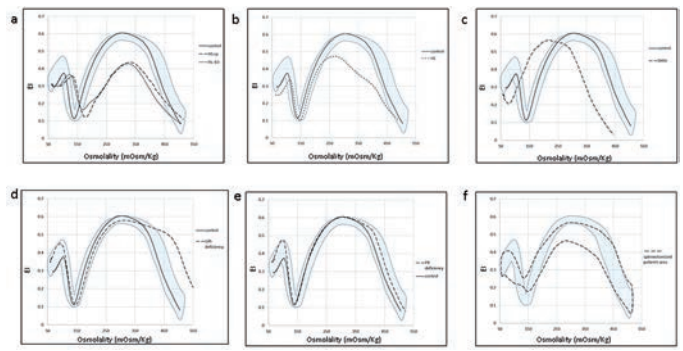

Figure 1.

C095

ORAL HIGH DOSE LIPOSOMIAL IRON THERAPY IS SAFE, FAST, WELL TOLERATED AND COST-EFFECTIVE AS INTRAVENOUS IRON IN PATIENTS WITH SIDEROPENIC ANEMIA INTOLERANT/REFRACTORY TO IRON SULPHATE. MULTICENTRIC RANDOMIZED STUDY

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Background: In iron deficiency anemia support with intravenous iron allows a faster anaemia correction and a faster ferritin increase than iron sulfate. Frequently iron sulfate and intravenous iron generate adverse events as hypotension, urticarioyd reactions, shock, epygastralgia, con-

stipation or diarrhea. High doses of oral iron frequently are poorly tolerated because of adverse events. *Aims:* Aim of this study is to verify if high doses of oral liposomial iron are safe, cost-effective and well tolerated as standard doses of intravenous ferrigluconate in patients with iron deficiency anemia intolerant/refractory to iron sulphate. *Patients and Methods:* We considered two group of patients(RANDOMIZED 1:1) with iron deficiency anemia without other relevant comorbidities. In group A M/F was 2/3, 7 patients had haemorragic gastritis, 3 hemorragic enteric bleeding angiodysplasia, 10 hypermenorrhaea, median level of hemoglobin (Hb) was 8 g/dl(R 7-10), median ferritin level was 10 ng/ml (R 3-20), with a normal level of CRP or ESR, and received liposomial iron 30 mg 4 tablet/day. In group B M/F was 1/3, 9 patients had haemorragic gastritis, 1 hemorragic enteric bleeding angiodysplasia, 10 hypermenorrhaea, median level of Hb was 8.5 g/dl(R 8-9.5), median ferritin level was 8 ng/ml (R 2-18), with a normal level of CRP or ESR, and received iv sodium ferrigluconate 62.5 mg iv in NS 100 ml in 3 h/day. The median treatment costs in each group were calculated considering the monthly global treatment cost for each patients in the treatment period. This provided an estimate of the costs, independent of the precise cost of the drug, but tied to the final outcome (efficacy) of the therapeutic strategy used during the observation period. *Results:* In group A,1g Hb increase was observed after a median of 8 days(R 7-12), a target Hb level of 12 g/dl was achieved in a median time of 4 weeks(R 2-4) with a median cost of € 110/months (R 92-162), 6 (30%) patients showed adverse events(3 epigastralgia, 3 diarrhoea). In group B,1 g Hb increase was observed after a median of 7 days(R 6-10), a target Hb level of12 g/dl was achieved in a median time of 3.5 weeks(R 1.5-4)with a median cost of €326/months(R 250-360), 4(20%) patients showed adverse events(2 hypotension, 2urticaria and headhace). *Conclusions:* Oral high dose liposomial iron support is safe, fast, well tolerated and cost-effective as intravenous iron in sideropenic anemia. This study needs confirmation on a larger cohort of patients.

C096

FERRIC CARBOXYMALTOSE ALLOWS TO OBTAIN TRANSFUSION INDEPENDENCE IN PATIENTS WITH MODERATE IRON DEFICIENCY ANEMIA SECONDARY TO CHRONIC BLOOD LOSS

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Introduction: Chronic blood loss (CBL) is often cause of iron deficiency anemia (IDA) in the elderly population. Such patients are often refractory or intolerant to oral iron therapy, forcing the intravenous administration. The older formulations (ferric gluconate or sucrose) require multiple infusions, which may be impossible to be used for multiple reasons in the elderly population, forcing therefore to proceed incorrectly with blood transfusion. The ferric carboxymaltose (FCM) is a new generation iron formulation that allows to administrate once a time a high dose of iron. The aim of this study was to evaluate the effectiveness of FCM in patients with IDA secondary to CBL in transfusion support. *Methods:* We retrospectively evaluated 30 patients with moderate IDA (hemoglobin - Hb - <10 g/dl and ferritin <12 ng/ml or transferrin saturation - TSAT - <16%) refractory or intolerant to oral iron therapy that necessitated transfusion support in the previous 12 months. They were treated with FCM (0.5-2 g in 1-4 sessions). The primary end point was to evaluate the reduction of transfusion requirements (units of packed red blood cells - RBC - transfused) after FCM treatment. *Results:* The mean age of the cohort was 76 (range 40-97) years, with a M/F ratio 15/15. The causes of CBL were gynecological in 3 and gastrointestinal in the remaining 27 patients. In the previous 12 months a mean of 8 RBC had been transfused, with an average interval between transfusions of 81 days. At the treatment (T0) the mean (±standard deviation) value of Hb was 8.4 (±0.9) g/dl, the TSAT 5.9% (±3.5%) and ferritin 17 (±16) ng/ml. The median FCM dose was 1 gr. During the treatment we observed only one mild adverse event (fever). At 6 weeks from T0 the mean Hb was 10.6 (±2.1) g/dL, with an average increase of 2.2 g/dl. With a median follow-up of 196 days from T0 transfusion requirements was 2 RBC, statistically significant lower than before T0 (p <0.001, Figure 1). Overall 8 patients necessitated again transfusion support after T0. After a median time of 80 days 11 patients needed retreatment with FCM for recurrence of IDA: 5 of them obtained again a response. The percentage of transfusion independent patients at median follow up was equal to 81%. *Conclusions:* In patients with moderate IDA secondary to the CBL FCM significantly reduces the need of transfusions and achieves transfusion independence in a high percentage of cases. If the IDA would reappear the retreatment is equally effective.

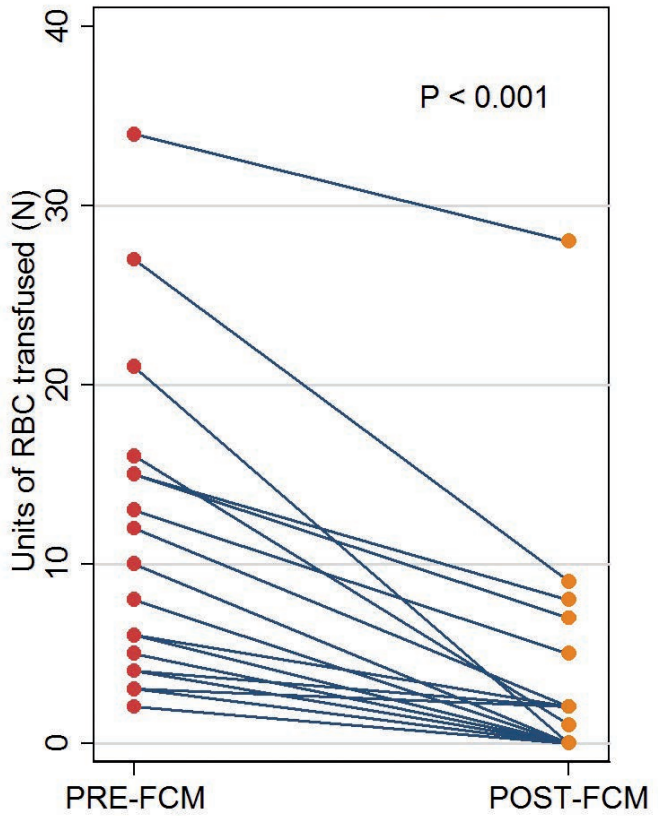

Figure 1.

Myeloma and Monoclonal Gammopathies 2

C097

UP-REGULATION OF PROK-2/BV8 IN GRANULOCYTES IS PRESENT BOTH IN MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE AND MULTIPLE MYELOMA

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Introduction: Our previous work showed that in Multiple Myeloma (MM) the immune function is impaired, including immunosuppressive properties of granulocytes due to their increased amount of arginase-1 and reduced phagocytic activity (Parrinello, manuscript in preparation). It is currently unknown if granulocyte dysfunction occurs in progression from MGUS to MM. Aim Providing a gene expression profile of mature granulocytes isolated from peripheral blood at the steady-statein MGUS and MM. *Methods:* Using oligonucleotide microarrays we first evaluated the gene expression profile of granulocytes at the steady state in 10 MM, 8 MGUS and 8 healthy subjects matched for sex and age. Then, we validated the first up-regulated gene PROK-2, obtained from preliminary findings in granulocytes from peripheral blood in 85 consecutive newly diagnosed MGUS (N=45), MM (N=40) and 15 healthy subjects, in RT-PCR (validation set). *Results:* We found 708 genes differentially expressed (467 up- and 241 down regulated) in MGUS *versus* healthy granulocytes at the steady state. The set of annotated, differentially expressed genes could befunctionally organized by "gene ontology" (http://www.geneontology.org/) into the following major categories: i) receptors and signal transduction (including up-regulation of CD14, Toll-like receptor 5 (TLR-5), IL-7 Receptor (CD127), IL-11 receptor, TGF-β receptor 2, hematopoietic cells kinase (HCK), IFNAR1); ii) negative regulation of adaptive immune response (including up regulation of CD127, STAT6, IFNAR1, OSCAR, PROK-2/BV8 and down regulation of p50, p65,NFKBIA, IL8, ELK-1, HIF-1 α, CEBP-β, CEBP-zeta). In MM samples we confirmed a statistically significant up-regulation of PROK-2/BV8 (a key molecule of VEGF-independent angiogenesis), CD14 (mediator hypersensitive innate immune response to lipopolysaccharide) and HCK (the hematopoietic cell kinase, involved in neutrophil migration and degranulation). In the validation set, PROK-2/BV8 expression was ten times higher in MGUS than healthy subjects (p=.02) and up to hundred times higher in MM (p=.001). In MM patients, increased levels of PROK-2/BV8 were positively associated with advanced bone disease and unfavourable cytogenetics. *Conclusions:* Granulocytic impairment is present in MGUS and worsened in MM patients due to increased expression of genes that negatively regulate adaptive immune response. PROK-2/BV8 is a key molecule involved in the granulocyte dysfunction and could be involved in the progression from MGUS to MM.

C098

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

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

Table 1.

CNA	locus	gene (Kb)	positive	full gene	partial	LOH	response prediction
P53 CN loss	17p13.1	25	15/170 (8.8%)	13/170	2/170	4/170	no
Rb1 CN loss	13q14.2	232	85/170 (50%)	74/170	11/17'	-	no
CS1B CN gain	1q32.1	6	52/170 (30.5%)	52/170	-	12/170	no
DM4 CN gain	1q32	144	57/170 (33.5%)	55/170	2/170	-	no
KN2C CN loss	1p32.3	7.7	20/170 (11.7%)	19/170	1/170	29/170	no
4F1 CN loss	1p33	675	24/170 (14.1%)	16/170	8/170	30/170	no
M46C CN loss	1p12	29	37/170 (21.7%)	37/170	-	5/170	no
NOX CN loss	16q23	1400	35/170 (20.6%)	26/170	9/170	12/170	no
CND1 CNA	11q13	17	79/170 (46.5%)	-	-	1/170	no
CND3 CNA	6p21	148	27/170 (15.9%)	-	-	3/170	no
MAFB CNA	20q11.2	4.4	23/170 (13.5%)	-	-	6/170	no
no CNAs	-	-	13/170 (7.6%)	-	-	+	no
AP1 CN gain	11q22.1	160	71/170 (42%)	63/170	8/170	-	yes
CN loss	14q13.1	-	23/170 (13.5%)	-	-	3/170	yes

C099

REVISED INTERNATIONAL STAGING SYSTEM: A NEW PROGNOSTIC STRATIFICATION MODEL FOR MULTIPLE MYELOMA PATIENTS

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Multiple myeloma (MM) is a heterogeneous disease, with survival duration ranging from a few months to more than 10 years. A simple and reliable tool is needed to stratify MM patients. We combined the International Staging System (ISS) with chromosomal abnormalities (CA) detected by interphase Fluorescence *In Situ* Hybridization (iFISH) after CD138 plasma cells purification and serum lactate dehydrogenase (LDH) to evaluate their prognostic value in newly diagnosed MM (NDMM). Clinical and laboratory data from 4445 NDMM patients enrolled in eleven international, multicentre, clinical trials were pooled together. The K-adaptive partitioning algorithm was used to define the most appropriate subgroups with homogeneous survival. Median age was 62 years and 65% of patients were ≤65 years. In the overall population of 4445 patients, 60% received autologous stem cell transplanta-

tion (ASCT), 44% proteasome inhibitors (PIs) and 66% immunomodulatory drugs (IMIDs); only 5% of patients did not receive any novel agent upfront. ISS, CA, and LDH data were simultaneously available in 3060/4445 patients. Patient characteristics and treatments were similar when compared to the overall population and a final analysis was performed in this cohort. We defined three groups: revised ISS (R-ISS) I (N=871), including ISS I (serum 2-microglobulin level <3.5mg/L and serum albumin level ≥3.5g/dL), no high-risk CA [del(17p) and/or t(4;14) and/or t(14;16)] and normal LDH level (below the upper limit of normal range); R-ISS III (N=295), including ISS III (serum 2-microglobulin level >5.5mg/L) and high-risk CA or high LDH level; R-ISS II (N=1894), including all the other possible combinations. After a median follow-up of 46 months, the 5-year OS was 82% in the R-ISS I, 62% in the R-ISS II, and 40% in the R-ISS III groups; the 5-year PFS was 55%, 36% and 24%, respectively. Multivariate analyses confirmed R-ISS as a strong prognostic factor for both OS (R-ISS stage III *vs* I hazard ratio: 9.64, p<0.001) and PFS (R-ISS stage III *vs* I hazard ratio: 3.37, p<0.001). In subgroup analyses, R-ISS maintained its prognostic role in patients younger and older than 65 years of age as well as in patients -who did and those who did not receive ASCT, PIs, and IMIDS. In conclusion, the R-ISS staging system is a new risk stratification algorithm which includes simple, reliable and widely used prognostic markers, and it allows the identification of three different MM entities with clearly different outcome.

C100

DIFFERENT EXPRESSION AND METHYLATION PROFILES IN PRIMARY AND SECONDARY PLASMA CELL LEUKEMIA BY HIGH-RESOLUTION MICROARRAY ANALYSES

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Plasma cell leukemia (PCL) is a very aggressive and rare hematologic malignancy that can be distinguished into primary (pPCL), originating *de novo*, or secondary (sPCL) malignancy, arising as a leukemic transformation of multiple myeloma (MM). Global gene (GEP), miRNA expression and methylation profiles were generated in pPCL and sPCL patients, in order to elucidate the molecular features of these two clinical forms. GEP of highly purified plasma cells (PCs) from 24 pPCL, enrolled in an Italian GIMEMA multicenter clinical trial, and 12 sPCL patients were generated on Affymetrix Gene ST 1.0 array. A subset of them were analyzed on miRNA 3.0 (19 pPCLs-11 sPCLs) and Illumina Infinium Methylation 450K BeadChip (9 pPCLs - 4 sPCLs) arrays, respectively. These studies included also MM, human myeloma cell lines (HMCLs) and bone marrow normal samples in larger datasets. Expression analyses were carried out by dChip and SAM software, methylation analyses by means of Minfi Bioconductor package and functional annotation studies by DAVID 6.7 and miRBase tools. Main genomic aberrations were investigated by FISH analysis. Hierarchical clustering of the most variable genes on PCL cases distinguished sPCLs from pPCLs. Considering the entire dataset, almost all sPCLs were grouped with HMCLs, whereas most of pPCLs were in a separate cluster together with all MAF-translocated and the majority of t(4;14) MMs. Supervised analysis revealed 173 genes specifically upregulated in sPCL *vs* pPCL cases. These transcripts were mostly involved in mitotic cell cycle, such as chromosome segregation and regulatory checkpoints, or in DNA recombination, replication and packaging, showing the strongest upregulation in HMCLs, when evaluated in the entire PC dataset. Nineteen miRNAs resulted differentially expressed in sPCL *vs* pPCL cases: several members of miR-106a-363 and miR-17-92 clusters, known to promote proliferation, angiogenesis and cell survival, were up-regulated, whereas miR-152 and miR-29b, with a known pathogenetic role in MM, resulted down-regulated. Furthermore, a differential methylation pattern (48 differential methylated positions) was evidenced in PCL comparison, involving immune system and embryonal development genes. The finding of specific gene, miRNA expression and methylation profiles in PCL may be useful to understand the molecular alterations discriminating the two forms of PC dyscrasias, thus providing a contribution to the identification of novel therapeutic targets.

C101

EARLY DEATH IN ELDERLY PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA TREATED WITH NEW DRUGS: A POOLED ANALYSIS OF TWO RANDOMIZED PHASE 3 TRIALS

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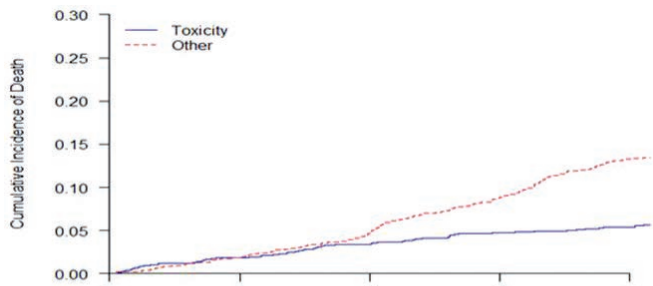
Early mortality in elderly patients with multiple myeloma (MM) is commonly due to combined effects of active disease and co-morbid factors. Although novel agents have considerably improved MM outcome, toxicities should be carefully considered. In this study, we analyzed early deaths not related to disease progression during treatments with lenalidomide or bortezomib. We used individual patient data from two large randomized studies. A total of 1173 newly diagnosed MM patients ineligible for transplantation were analyzed: 511 were enrolled in the Gruppo Italiano Malattie EMatologiche dell'Adulto (GIMEMA) MM-03-05 study and received bortezomib-containing regimens, 662 were enrolled in the European Myeloma Network (EMN) EMN01 study and received lenalidomide-containing regimens. A total of 1146 patients could be evaluated for this analysis. Within 24 months of start of therapy, 207 patients (18%) died for any causes, 61 (5%) died due to toxicities. One percent (12 patients) of toxic deaths occurred within 60 days, with a linear increase over time of 1% every 6 months (Figure 1).



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

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

The rate of toxic deaths was similar between patients receiving bortezomib *vs* lenalidomide (6% *vs* 5%, p=0.32), and it was significantly higher in patients ≥80 years (11/107 [10%], p=0.005). Causes of deaths were cardiac complications (18 pts [29%]), infections (17 pts [18%]) and vascular complications (9 pts [15%]), with no difference between the 2 different treatment regimes. In a multivariate analysis, age (HR 1.09 per 1 year increase, p=0.002) and ISS score (HR 3.81, p=0.01 ISS 2 *vs* ISS 1;

HR 5.69, p=0.002 ISS 3 *vs* ISS 1) increased the risk of death, poor performance status did not (HR 1.25, p=0.59). Greater tumor burden and activity (ISS) increased the risk of toxic deaths as these deaths occurred before the maximal benefit of therapy could be obtained: 92% of patients who died from toxicity within 2 months of start of therapy had achieved a suboptimal response (8 not available, 3 SD, 1 PR, ORR 8%). Novel, more effective and rapid therapies have reduced the risk of toxic mortality. Yet, one-third of early deaths occurred primarily due to cumulative specific drug-related toxicities. Improved supportive therapy, prevention and prompt management of complications are crucial to reduce the risk of toxic deaths. The 2-fold higher risk of toxic deaths in octogenarians suggests the need for a careful assessment of frail patients who may benefit from a gentle or palliative approach.

C102

DIFFERENTIAL MIRNA EXPRESSION PROFILES IN DIFFERENT FORMS OF PLASMA CELL DYSCRASIAS

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Multiple myeloma (MM) is a plasma cell (PC) malignancy characterized by a marked genetic heterogeneity, which may progress to leukemic transformation in secondary plasma cell leukemia (sPCL), whereas primary PCL (pPCL) presents *de novo* in the leukemic phase. In order to investigate the involvement of miRNAs in MM onset and progression to PCL condition, global miRNA expression profiles were generated on samples of different PC dyscrasias and healthy donors. The entire dataset comprising 4 healthy (N) donors, 97 MM at onset, 11 sPCL and 19 pPCL cases at diagnosis, enrolled in an italian GIMEMA multicenter clinical trial, was profiled on the Affymetrix GeneChip miRNA 3.0 array. Expression analyses were carried out by dChip and SAM softwares and functional annotation studies by miRBase and TAM tools. Hierarchical clustering on most variable miRNAs (281/1733) in the entire dataset grouped almost all PCL together with MM cases, carrying t(4;14) or MAF-translocations. The remaining MM cases were clustered according to TC classification (IgH translocations/cyclin D expression), whereas N samples were in a distinct sub-cluster. In order to find miRNAs discriminating disease from healthy condition, each PC dyscrasia group was compared to N controls, thus globally evidencing 67 modulated miRNAs. Embryonic stem cell regulation, inflammation and immune response, cell cycle and apoptosis resulted the most enriched functions. To identify specific miRNA expression patterns in PC dyscrasias, a three-class comparison was performed evidencing 107 differentially expressed miRNAs. In particular, several members of hsa-miR-17 and hsa-miR-106a clusters, known to have an oncogenic role and implicated in immune system function, together with members of hsa-miR-506 cluster showed the highest upregulation in sPCL cases, whereas miR-29a and miR29b clusters, implicated in hormones regulation and apoptosis functions, were specifically downregulated in this group. In pPCL cases, miR-221 cluster members were at the lowest and onco-miRNAs, as miR-21 and miR-155, at the highest expression level, respectively. Furthermore, miR-143 cluster and miR-199 family members, associated to cell differentiation and inflammation, were downregulated in both PCL dyscrasias *vs* MM. Overall, our study better define the miRNA expression profiles that distinguish PC dyscrasias from healthy condition and involved in MM disease progression, thus contributing to the identification of novel therapeutic targets.

Myelodysplastic Syndromes

C103

LOW RPS14 EXPRESSION IS FREQUENTLY FOUND IN NON 5Q- MYELODYSPLASTIC SYNDROMES

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Background and Aims: The RPS14 gene, located on chromosome 5 and involved in the ribosomal protein synthesis, has been reported as a causal factor in the 5q- syndrome, where its up-regulation during treatment with lenalidomide has been associated with best responses. RPS14 expression in non-5q-MDS was reported in 53%-71% of cases. Interestingly, in low and intermediate-1 IPSS subgroups, patients with lower RPS14 expression had longer OS.Thus, the aim of this study was to assess the RPS14 expression in a larger series of non-5q- MDS patients. *Patients and Methods:* A total of 112 patients, 45% females and 55% males, with a median age of 71-year (range 19-89), were enrolled in 5 different Italian institutions from March 2010 to October 2014. Nine patients were affected by CMMoL; the prognosis of the remaining 103 cases was determined according to the IPSS in low (36%), intermediate-1 (31%), intermediate-2 (21%), and high risk (12%). About 40% of cases were affected by RCMD, 24% by RAEB-2, and 13% by RA. Twelve bone marrow samples from healthy donors have been used as normal references. *Results:* In healthy donors, the mean RPS14 expression was 0.94±0.26; in the whole MDS series, it was 0.57±0.42. In comparison with healthy controls, the 52% of MDS cases showed lower RPS14 expression levels: 79% in the RA, 56% in the RAEB1, 44% in the RAEB-2, and 41% in the RCMD subgroups. When patients were stratified according to the IPSS, in the half of the low, intermediate-1 and intermediate-2 cases RPS14 was under-expressed, opposite to one third of the high risk and 13% of the CMMoL patients only. No relationships with age or sex were observed. To evaluate if the haploinsufficiency would be responsible of the low expression of RPS14 gene, we performed the copy number assay on 32 MDS, 15 healthy donors, and 3 patients with 5q- syndrome: in 91% of cases the copy number assay excluded the haploinsufficiency. *Conclusions:* Our study showed in a large series of patients that a lower RPS14 expression interests the half of the non-5q- cases, especially those affected by RA and at low and intermediate IPSS risk. Other authors previously reported that low expression of RPS14 was not due to promoter hypermethylation. Here we demonstrated that also the haploinsufficience is not the cause of the RPS14 low expression. Moreover, our findings suggest a possible role for lenalidomide in non-5q- MDS, especially in low risk patients.

C104

ASSOCIATION OF AZACITIDINE AND LENALIDOMIDE (COMBINATION *VS* SEQUENTIAL TREATMENT) FOR HIGH-RISK MYELODYSPLASTIC SYNDROMES (IPSS RISK: HIGH OR INT-2): A PHASE II CLINICAL AND BIOLOGICAL STUDY

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Introduction: Recently, several studies have evaluated the efficacy and safety of combining, in high-risk Myelodysplastic Syndromes (MDS) patients (pts), azacitidine (AZA) with Lenalidomide (LEN), either administered concurrently (Sekeres, 2010; 2012), or sequentially (Platzbecker, 2013). The aim of this study was to evaluate the efficacy and safety of the combination *vs* the sequential use of AZA and LEN in high-risk MDS pts (IPSS score risk: High or INT-2). Primary endpoint: Overall Response Rate (ORR), defined as the Rate of Complete Remission (CR), Partial Remission (PR), Marrow Complete Remission (mCR), and Hematological Improvement (HI), following the International Working Group (IWG) criteria (Cheson, 2006). *Methods:* A randomized, phase II, multicenter, open label study, including pts with MDS with International Prognostic Scoring System (IPSS) risk High or Intermediate-2, without previous treatment with AZA or LEN. ARM 1 (combined treatment): AZA: 75 mg/m^2/day (days 1-5) I.C.+LEN: 10 mg/day (days 1-21), orally, every 4 weeks. ARM 2 (sequential treatment): AZA: 75 mg/m^2/day (days 1-5) I.C.+LEN: 10 mg/day (days 6-21), orally, every 4 weeks. A sample size of 44 pts was planned. *Results:* From March 2013, 44 pts (27 males), with a median age of 72 (48-83 yrs) were enrolled, from 13 hematologic italian Centers. At baseline, IPSS risk was: Intermediate-2: 32 pts; High: 9 pts; not determined (N.D.) (because of lack of cytogenetic data): 3 pts. (all with RAEB-2). 21 pts were randomly assigned to ARM 1, and 23 pts to ARM 2. At the time of this analysis 33/44 pts (75%) are evaluable for response (≥6 cycles of treatment). 25/33 pts (ORR: 75.7%) showed a favourable response: 8 CR, 1 PR, 2 mCR, 9 HI, 5 HI+mCR. Responder pts were: 13/17 (ORR: 76.5%) in ARM 1 (3 CR; 1 PR; 1 mCR; 5 HI, 3 HI-mCR), and 12/16 (ORR: 75%) in ARM 2 (5 CR; 1 mCR; 4 HI, 2 HI+mCR), respectively. A grade >2 non hematologic toxicity was observed in 23/44 (52.3%) pts. 25/44 pts (56.8%) had a dose reduction of LEN because of hematologic or non-hematologic toxicity. 18 pts died; 8 pts showed progression to AML. At a molecular level, a significant increase of phosphoinositide-specific phospholipase C (PI-PLC) β1 and/or PI-PLCγ1 expression, and an increase of β-globin expression was associated with a favourable clinical response. *Conclusions:* Our results seem to confirm the feasibility of the association of AZA and LEN in high-risk MDS pts.

C105

TREATMENT WITH AZACITIDINE REVERSES MESENCHYMAL STEM CELLS DERIVED FROM HIGH RISK MYELODYSPLASTIC PATIENTS TO A NORMAL PHENOTYPE

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Introduction: Mesenchymal stem cells (MSCs) isolated from bone marrow of patients affected by myelodysplastic syndromes (MDS) play a critical role in myelodysplastic microenvironment. *Methods:* We studied bone marrow (BM)-derived MSC isolated from 30 high risk IPSS MDS patients at diagnosis and after azacitidine (AZA) treatment (MSC-MDS-AZA) comparing them with 10 old healthy donors. We analyzed structural properties and proliferative potential of MSC-MDS in addition to the global DNA methylation and molecular pathways expression changes involved into MDS pathogenesis. *Results:* Our previous data showed that the MDS microenvironment is characterized by a significant loss of MSC proliferation with morphological changes and impaired hematopoietic support *in vitro*. The reduced proliferation potential may be at least partially associated with a negative regulation of the cell cycle and an up-regulation of CDKN2B. Among patients studied after AZA treatment, 47% achieved complete hematological and karyotypic remission (CR) and interestingly, only MSC of these patients changed their phenotype and molecular properties and reverted their features to healthy MSC. Other samples maintained the altered morphology and impaired cell cycle regulation such as MSC at the diagnosis. CDKN2B was up-regulated in the MSC-MDS at diagnosis 11.5 times respect to MSC-MDS-CR ($p<0.05$). It is still unclear whether methylation reversal involves not only hematopoietic cells but also cells of bone marrow microenvironment. MSC-MDS at diagnosis showed aberrant DNA hypermethylation status compared with controls that significantly decreased after AZA treatment similarly to old donors, regardless of hematological response. These data suggested that AZA treatment involved methylation reversal of MSCs despite their low proliferation rate. Then, we studied signaling pathway that could be involved in the epigenetic alterations demonstrated after AZA treatment. Molecular data showed an increased level of sFRP1, FRZB, WIF1 and DKK1 in MSC-MDS-CR respect to MSC-MDS at diagnosis, suggesting that the functional expression of Wnt inhibitors could be contributing to normalize Wnt pathway in MDS. *Conclusions:* MSC-MDS at diagnosis was structurally, functionally and epigenetically altered while MSC-MDS-CR after AZA treatment revert to a normal phenotype. Deregulated Wnt signaling pathway in MSC-MDS may have a role in the abnormal functioning BM niche that fails to support normal hematopoiesis.

C106

SIRPB1: BIOMARKER OF RESPONSE TO 5-AZACITIDINE TREATMENT IN MDS AND AML PATIENTS

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Introduction: MDS and AML are a group of diseases of the elderly that initiates in a hematopoietic stem cell and are characterized by clonal hematopoiesis and uncertain prognosis, mostly due to cytogenetic background. In both diseases, 5-Azacitidine (5-Aza) has been successful, inducing prolonged survival and delayed AML evolution. *Aims:* To identify the genes mostly predictive of treatment response we use SNP arrays. *Materials and Methods:* SNPs array was performed by CytoScan HD Array (Affymetrix, Inc) The results obtained were analyzed by Chromosome Analysis Suite v1.2 (Affymetrix Inc.) and Nexus Copy Number™ v7.5 (BioDiscovery). *Results:* We treated 246 pts: 214 AML and 32 MDS with a median age of 59 and 70 years, respectively. Forty-five pts (32 MDS/13 AML) were treated with 5-Aza (75 mg/sqm/daily), while 201 AML were treated with conventional chemotherapy. SNP arrays was done in 22/45 (49%), 13 pts were defined "insensitive/resistant". Macroscopic CNAs affecting a complete chromosome or its arms were detected in 5 of 22 pts (23%), while classical cytogenetic was able to detect only two cases of trisomy 8 (9%) suggesting superiority of SNPs array for CNAs identifications. Chromosomic aberrations are more statistically frequent on patients "insensitive" *versus* patiens "sensitive" (64% *vs* 35%) ($p\leq0.01$). Moreover we found that, from the median of chromosomic alterations lenghts (in kbp), the group of "insensitive" MDS/AML patients to 5-Aza therapy present more losses than "sensitive" ones. By Nexus Copy Number software we identify 137 genes highly differentially gain, loss or LOH between "insensitive" *versus* "sensitive" to 5-Aza ($p \leq 0.05$). Among these genes we focused on SIRPB1 (cytoband 20p13, 56Kbps) since it was loss on 14/22 (64%) "insensitive" pts (p=0,023) and gain on 7/22 (32%) "sensitive" ones (p=0,0324), respectively. SIRPB1 common deletion region length is 27 kbps and the common amplified region length is 30 kbps. By NGS-WES we analyzed 35/214 (16%) AML samples at diagnosis. We found mutations in SF3B1, NPM1, CBL, RUNX1, BCOR, KIT, GATA2, IDH2, KDM6A, KIAA1324L, PRIM2, RRN3, APOBR and again in SIRPB1 an heterozygous missense variant (rs45545343; p. H/D). *Conclusions:* We conclude that SIRPB1 is a promising marker of response in patients with myelodysplastic syndromes and acute myeloid leukemia treated with 5-Azaciditine. Acknowledgement: Celgene, ELN, AIL, AIRC, progetto Regione-Università 2010-12 (L. Bolondi), FP7 NGS-PTL project.

C107

COMPREHENSIVE ANALYSIS OF RECURRENT GENE MUTATIONS IN CHRONIC MYELOMONOCYTIC LEUKEMIA: ASSOCIATION WITH DISEASE CHARACTERISTICS AND PROGNOSTIC IMPACT

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Chronic myelomonocytic leukemia (CMML) represent the most frequent among the rare myelodysplastic/myeloproliferative neoplasms. At diagnosis, pts may present a myelodysplastic (MD-CMML) or a myeloproliferative phenotype (MP-CMML) depending on the blood leukocyte count (<*vs.*≥13x10e9/L, respectively). The clinical course is very heterogeneous, eventually characterized by disease progression in a significant proportion of patients. To discriminate between molecular lesions involved in disease onset and progression, we performed a comprehensive analysis of recurrent gene mutations in primary cell samples collected at different time points in 42 patients (22 with MD-CMML and 20 with MP-CMML). DNA was extracted from different cells subtypes sorted by FACS. The following regions were analyzed by deep and/or direct sequencing: the whole coding sequence of TET2, N-and K-RAS exons 1-2, CBL exons 8-9, SRSF2 hot spot P95 and ASXL1 exon 12. Allele-specific PCR for the JAK2 V617F was also performed. At least one mutation was found in 33/42 (79%) patients: TET2 in 68%, SRSF2 and ASXL1 in 26%, other proliferation-associated genes (N-and K-RAS, CBL and the JAK2V617F allele) in 33%. Analysis of sequential samples showed that TET2, SRSF2, ASXL1 and CBL lesions were present at the time of diagnosis and retained during disease course in all cases. Conversely, RAS and JAK2 mutations were acquired during CMML course in 21% of cases. While ASXL1 and proliferation genes mutations associated with shorter survival (OS), a significantly longer OS was observed in patients with 2 *vs* those with 0 or 1 TET2 mutations. TET2 mutations were more common in MD-CMML, while ASXL1, SRSF2 and proliferation genes mutations were more common in MP-CMML pts. Of note, a high proportion of MD-CMML patients carrying these alterations at diagnosis progressed to MP-CMML and/or AML later on. Overall in line with previous reports, our results support the observation that TET2 mutations correlate with a better outcome. We provide evidence for the first time of the positive role of double TET2 mutants *vs* single or wt TET2, which would significantly impact CMML management with regard to disease monitoring and risk stratification assessment.

C108

MOLECULAR ALTERATIONS ASSOCIATED WITH PROGRESSION OF MYELODYSPLASTIC SYNDROMES TO MYELODYSPLASTIC SYNDROMES/MYELOPROLIFERATIVE NEOPLASMS

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Myelodysplastic syndromes (MDS) are heterogeneous disorders characterized by ineffective hematopoiesis and risk of progression to acute myeloid leukemia. However, MDS sometimes develop during their clinical history features of myeloproliferative neoplasms (MPN). The knowledge of the mechanisms at the basis of proliferative-non leukemic progression could better define the prognosis of this group of MDS patients and support identification of new therapeutic strategies. The molecular bases of this progression have not been investigated so far. We postulated that specific acquired somatic mutations could be associated with the transformation from MDS to MDS/MPN. Six cytopenic MDS patients that progress to MDS/MPN were included in the study. The mutational profile was characterized using a TruSeq Custom Amplicon panel (Illumina) targeting the 57 recurrently mutated genes in myeloid malignancies. Around 250 ng of genomic DNA extracted from mononuclear cells obtained at diagnosis and at progression diagnosis were used to prepare sequencing libraries following the Illumina standard protocol. Samples were run on an Illumina MiSeq and variants were annotated by ANNOVAR. Detected variants were distilled on the basis of their exonic function, allele frequency, the presence in variants databases, and several prediction scores. Five of the six patients (pts) presented at least two mutations (median number per patient: 3, range 0-4). The most frequently mutated genes were TET2 and ASXL1 (mutated in 4 pts each of them). Mutations of DNMT3A, SETBP1, CUX1, U2AF1, EZH2, and FLT3 were present in just one patient. A total of 16 and 18 mutations across the 57 genes were identified in the pre- and post-progression samples, respectively. Therefore, two pts presented new mutations in the post-progression sample: one patient showed a new mutation in the TP53 gene and the other patient in the DNMT3A gene (Table 1). This is the first study to investigate the mutation status of a group of cytopenic MDS patients showing a progression to MDS/MPN by the study of serial samples using a NGS myeloid gene panel. This study informs the new mutations can appear during disease progression to MDS/MPN but it seems that these genetic events are not closely related conditions for progression as in 4 of the 6 progression samples new mutation were not found.

Table 1.

Patients	MDS	MDS/MPN
1	**ASXL1**_Q592X,**TET2**_Q913X	**ASXL1**_Q592X,**TET2**_Q913X
2	**SETBP1**_I871T,**DNMT3A**_G607A **ASXL1**_L762X, **CUX**_R725C	**SETBP1**_I871T,**DNMT3A**_G607A **ASXL1**_L762X, **CUX**_R725C **TP53**_D49N
3	**TET2**_E1026fs, **EZH2**_V626fs **ASXL1**_G642fs, **RUNX1**_P350fs,	**TET2**_E1026fs, **EZH2**_V626fs **ASXL1**_G642fs, **RUNX1**_P350fs, **DNMT3A**_R693C
4	**ASXL1**_R693X, **U2AF1**_Q157P **TET2**_S1050L	**ASXL1**_R693X, **U2AF1**_Q157P
5	No mutations	No mutations
6	**TET2**_R1452X, **FLT3**_D835Y **EZH2**_E210X	**TET2**_R1452X, **FLT3**_D835Y **EZH2**_E210X

Chronic Myeloid Leukemia

C109

DEEP MOLECULAR RESPONSE TO NILOTINIB AS FIRST-LINE TREATMENT OF BCR-ABL+ CHRONIC MYELOID LEUKEMIA

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Background: The treatment-free remission (TFR) is an emerging treatment goal in CML and a sustained deep molecular response (DMR, MR4 or better) is a pre-requisite to achieve TFR. The 5-year update from the ENESTnd trial showed a superiority of NIL over IM in terms of achievement of DMR, but differences concerning the stability of DMR have not been reported yet. Independent studies are important to confirm and to extend the results of company-sponsored trials. *Aims:* To assess the efficacy of NIL as first-line treatment in terms of achievement of DMR and stability of DMR. *Methods:* Phase 3b study conducted by the GIMEMA CML WP (NCT01535391). Primary endpoint: MR4 at 24 months. Key secondary endpoints: kinetics of molecular response and stability of DMR. Starting NIL dose: 300 mg BID (dose escalation to 400 mg BID for ELN 2009 suboptimal response or failure). Molecular response assessed in GIMEMA standardized molecular laboratories (Labnet network): MR4 and MR4.5, according to EUTOS 2015 definitions; sustained MR4 or MR4.5, MR4 or MR4.5 for at least 1 year with at least 3 evaluable analysis. All the analysis were performed according to the ITT principle. *Results:* 130 CML patients in early chronic phase have been enrolled: median age, 50 (18-85) years; high risk patients, 22% (Sokal), 6% (Euro) and 8% (EUTOS); CCA in Ph+ cells at baseline, 5%; median follow-up, 29 (24-37) months. At the last contact the patients still on treatment with NIL were 100/130, 77%, while 30/130 patients, 23%, permanently interrupted the study drug: 3% progression, 5% failure or suboptimal response, 8% adverse events, 1% TFR, 5% other reasons. At 3 months, 80% of patients had BCR-ABL transcript levels <10%; at 6 months, 78% of patients had BCR-ABL transcript levels <1%. The major molecular response rates at 12 and 24 months were 57% and 65%, respectively. The rates of MR4 at 6, 12, 18 and 24 months were 12%, 28, 31% and 46%, respectively. Seventy-six patients (58%) achieved a MR4 at least once; the patients with a sustained MR4 were 39/76 (51%, or 30% of the total). The rates of MR4.5 at 6, 12, 18 and 24 months were 2%, 7%, 11% and 17%, respectively. Eleven patients (8%) achieved a sustained MR4.5. All the patients are still alive. *Conclusions:* After 2 to 3 years of treatment, 30% and 8% of all the enrolled patients were in stable MR 4.0 and in stable MR4.5, respectively. It is likely that a longer treatment time is required to bring more patients to treatment-free remission.

C110

MESENCHYMAL STEM CELLS FROM CML PATIENTS REGULATE GRANULOCYTE-LIKE MYELOID DERIVED SUPPRESSOR CELLS ACTIVATION

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The complex interplay between cancer cells and immune system allows neoplastic cells to evade immune surveillance and expand. Recently, our and other groups have been demonstrated that a subpopulation of myeloid cells, defined as "myeloid-derived suppressor cells" (MDSCs), plays an important role of immune escape in chronic myeloid leukemia (CML) patients inducing T cell tolerance. Mesenchymal stem cells (MSCs) are a heterogeneous population of stromal adult stem cells with immunomodulatory properties that contribute to form a cancer stem niche where tumor cells are protected and sustained in their growth. The aim of this study was to evaluate the influence of MSCs on expansion and activation of MDSCs in CML patients. In a first instance, using real time PCR, we evaluated the expression of immune modulatory factors (arginase 1, NOS2, COX2, TNF, TGF, IL6, IL10, IL1) by CML MSCs (n=10) compared with healthy donors (HD) ones (n=6). CML MSCs showed higher COX2, TGF and IL6 expression ($p<0.05$). Subsequently, we investigated the capacity of MSCs from CML patients and HD to generate MDSCs. Human peripheral blood mononucleated cells (PBMCs) isolated from healthy volunteer donors were cultured alone and with CML or HD MSCs (1:100 ratio). After one week, PBMCs were collected and MDSCs were then isolated using anti-CD66b magnetic microbeads. The phenotype of MDSCs (identified as CD11b+CD33+CD14-HLADR- cells) was confirmed by cytofluorimetric analysis. The immunosuppressive capacity of the generated MDSCs was analyzed. Myeloid cells were co-cultured with autologous CFSE-labeled T cells stimulated by phytohaemagglutinin (PHA). Only MDSCs generated by co-culture with CML MSCs were suppressive, decreasing T cell proliferation of 31±12% ($p<0.01$) while myeloid cells generated by co-culture with HD MSCs and control (isolated from PBMCs cultured in medium alone) did not show any suppressive effect on T cell proliferation. Analyzing the expression of immune modulatory factors by MSCs after 48 h of co-culture with PBMCs, we observed higher expression of IL10 and TGF ($p<0.05$) and IL6 ($p<0.01$) by CML MSCs than HD ones. These data suggest that multiple mechanisms are involved in MDSCs induction by CML MSCs. In conclusion, we demonstrate that MSCs from CML patients are able to generate and activate MDSCs and might favor cancer immune evasion in CML patients.

C111

FOXM1 POST-TRANSLATIONAL MODIFICATIONS ASSOCIATED WITH THE BCR-ABL1 FUSION GENE OF CHRONIC MYELOID LEUKEMIA

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Signals promoting proliferation and survival of BCR-ABL1+ leukemic stem cells (LSC) are subject to intense investigation. Our work was focused on FoxM1, a member of the Forkhead transcription factor family and a central component of β-catenin activation. β-catenin is a key signal for BCR-ABL1+ LSC self-renewal and persistence to tyrosine kinase (TK) inhibitors. As following its nuclear import, it binds the T-cell factor/lymphoid enhancer factor 1 (TCF/LEF1) to form a transcriptionally active complex which triggers critical genes for leukemic hematopoiesis proliferative advantage. FoxM1 activation in chronic myeloid leukemia (CML) may be contingent upon multiple BCR-ABL1 TK-associated events, involving Polo-like kinase 1 (Plk1), p53 and Cdh1. It might be therefore regarded as a putative target for the selective eradication of BCR-ABL1+ LSC. The investigation was carried out in cell lines (K562, K562 IM resistant) and bone marrow mononuclear cells (MNCs) from

CML patients. Plk1 inhibitor BI-2767 was used to define the signal transducing pathway leading to FoxM1 activation in BCR-ABL1+ cells. PCR amplification was performed to assess the expression of FoxM1 and Cyclin D1, a β-catenin downstream gene critical for BCR-ABL1+ cell proliferation. Western blot and immunoprecipitation analyses were performed to study FoxM1, β-catenin, Cyclin D1 and Plk1 protein levels and phosphorylation in sub-cellular compartments. Our results suggest that: 1.FoxM1 activation associated with BCR-ABL1 TK proceeds from post-transcriptional events encompassing the protein phosphorylation at serine-threonine residues and driving Plk1 activating phosphorylation; 2.phosphorylation is a key event for FoxM1 interaction with β-catenin and Plk1; 3.Plk1 inhibition results in a significant reduction of FoxM1 transcript, suggesting a feedback mechanism in the regulation of FoxM1 expression in BCR-ABL1+ cell context; 4.FoxM1 is overexpressed in MNCs from bone marrow samples of CML patients at diagnosis compared to healthy donors. Such increment disappeared in patients who reached major molecular response to TK inhibitors. 5.FoxM1 overexpression is more evident in CD34+ compartment of CML patients than in MNCs fraction. In conclusion BCR-ABL1 induces FoxM1 post-translational modifications possibly involved in the β-catenin nuclear import and transcriptional activation. Accordingly, FoxM1 may be considered as a promising drug target useful to eradicate LSC compartment in CML.

C112

CARDIOVASCULAR EVENTS IN CHRONIC MYELOID LEUKEMIA PATIENTS TREATED WITH NILOTINIB FIRST-LINE: AN ANALISYS OF THE GIMEMA CML WP

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Nilotinib (NIL) is approved for the first-line treatment of CML based on the results of the ENESTnd study that demonstrated a higher efficacy compared to imatinib (IM). However, there are concerns on the vascular toxicity of NIL, disclosed by an increased rate of cardiovascular adverse events (CVAEs) with respect to imatinib. For this reason, we investigated the CVAEs in 2 studies of the GIMEMA CML WP that included NIL as first-line treatment of CML: the GIMEMA CML 0307 trial (73 pts; NIL 400 mg BID), and the GIMEMA CML 0408 trial (123 pts; 3-months alternating regime of NIL 400 mg BID and IM 400 mg QD). The median age at CML diagnosis of all 196 pts was 55 (18-84) years; 59 (30%) pts were ≥65 years; 52% were males; cardiovascular risk factors (CVRF: hypertension, dyslipidemia, diabetes, BMI ≥30, and prior ischemic disease) were present in 74 (38%) pts (median 1, range 1-4). There were no significant differences between the pts characteristics in the 2 studies. The median f-up was 61 months. Nineteen CVAEs occurred in 17 (8.6%) pts: 7 acute myocardial infarctions (MI); 5 PAODs; 2 carotid stenosis, 2 aortic atherosclerosis, 1 stroke, 1 unstable angina, and 1 stable angina (1 patient had 1 PAOD, 1 stroke, and 1 MI). In pts with CVAEs, the median age at CML diagnosis was 67 (43-84 years), and the median interval from CML diagnosis to CVAE was 38(1-76) months. Out of the 17 pts with CVAEs, 53% were males; 70% were ≥65 years at CML diagnosis; 76% had at least 1 CVRF, and 65% were treated with NIL alone. All pts but one (who died at 90 years for congestive heart failure post MI) are alive. Treatment of CVAEs included coronary angioplasty in 5 pts, lower limb amputation in 2 pts, and peripheral vascular surgery in 2 pts; all other pts received medical treatment; 12/17 pts permanently discontinued NIL. In univariate analysis, the occurrence of CVAEs was associated with age≥65 years (12/59[20%] *vs* 5/137[3.6%]; p=0.0004), the presence of at least one CVRF (13/74[18%] *vs* 4/122[3.3%]; p=0.001), and the monotherapy with NIL (11/73[15%] *vs* 6/123[4.8%]; p=0.018). CVAEs occurred at a significant rate in pts treated with NIL-based regimes, and in particularly in pts ≥65 years and/or with CVRF. Noteworthy, the alternating schedule of NIL and IM resulted in a lower incidence of CVAEs compared to NIL monotherapy. Thus, considering the morbidity associated to CVAEs, the risk/benefit ratio of NIL monotherapy should be carefully evaluated in selected groups of patients.

C113

NEW ALTERNATIVE PROTEIN Bcr/Abl-OOF SHOWS ONCOGENIC ACTIVITY BY ACTIVATION OF RAC AND COOPERATION WITH Bcr/Abl IN CHRONIC MYELOID LEUKEMIA.

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Introduction: In Chronic Myeloid Leukemia, beside the common major BCR/ABL transcripts, we previously reported that the chromosome Philadelphia is associated with alternative BCR/ABL splice variants involving BCR exons 1, 13 or 14 and ABL exon 4 in about 80% of patients. The translations of these transcripts are characterized by the impairment in the reading frame of ABL exon 4 leading to the generation of an early stop codon within ABL exon 5. Therefore Bcr/Abl fusion proteins (Bcr/Abl OOF) are characterized by a correct Bcr portion followed by a sequence of amino acids arising from the out of frame (OOF) reading of the c-Abl exon 4 and 5 gene sequence. The product of this new transcript contains the characteristic Bcr domains but its hallmark is the absence of COOH-terminal Rho GTPase GAP domain. *Aims:* the aim of this work was to determine the role of the Bcr/Abl OOF. We analyzed the effects of the protein in term of cytoskeleton modelling, adhesion and activation of oncogenic pathways. *Results:* By immunofluorescence and western blot assay we observed that the localization of Bcr/Abl-OOF is predominantly cytoplasmatic and that it colocalizes in endosomal vescicles with Bcr protein with a rule on EGFr turnover. Bcr/Abl-OOF confers an altered adhesion if compared to empty pcDNA, with a morphology characterized by activated Rac, which assume a peculiar submembrane localization typical of Rho proteins when present in the activated form. This result suggest that Rac activation could be one of the responsible of Bcr/Abl-OOF cell morphology during cell adhesion on fibronectin. We also analysed a possible involvement of Bcr/Abl-OOF in the most important oncogenic pathway. Apoptosis assay showed that transfected cells with Bcr/Abl-OOF presented lower apoptotic rate than control cells, with 67% of reduction (p=0,01). Proliferation assay shown a 2 fold of induction of proliferation rate (p=0,001) in Bcr/Abl-OOF cells, which is restored after Rac inhibitor treatment. Next we analyzed the level of expression of Bcr/Abl OOF in 16 patients at diagnosis and after three month of therapy with Imatinib, in order to find a correlation with our *in vitro* results. Interesting only the four patients with no significantly reduction of both transcripts had a bad clinical response to drug. These analyses confirme the possibility that Bcr/Abl-OOF cooperate with the oncogenic Bcr/Abl pathway, suggesting a new attractive possibility to treat patients with a suboptimal response.

C114

MOLECULAR EVALUATION OF ZNF224 MRNA EXPRESSION IN CML PATIENTS AS A NOVEL DETERMINANT OF TKI RESPONSIVENESS

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Introduction: The transcription factor Wilms' tumor gene 1, WT1, is implicated both in normal developmental processes and in the generation of a variety of solid tumors and hematological malignancies. WT1 is highly expressed in leukemia cells and its overexpression is associated with a poor response to therapy. Recently the Krüppel-like zinc-finger protein, ZNF224 was identified as a novel WT1-interacting factor involved in WT1 transcriptional regulation. ZNF224 itself could be modulated by cytosine arabinoside (ara-C), a drug widely used in the treatment of myeloid leukemia and that ZNF224 overexpression increases susceptibility to apoptosis of Ph+ K562 cell lines. In our retrospective analysis we evaluated the relative expression of ZNF224 mRNA in 30 adult patients with BCR-ABL–positive chronic phase chronic myeloid leukaemia (CP-CML) as a determinant of imatinib sensitivity. *Methods:* Response to tyrosine kinase inhibitor (TKI) imatinib is assessed with standardized real quantitative polymerase chain reaction and/or cytogenetics at 3, 6, and 12 months. Response to the therapy was classified as optimal, warning, and failure, according to the recent ELN criteria. We compared the ZNF224 expression at diagnosis with molecular response over the first 12 month of imatinib therapy. Sample have been selected, for retrospective analysis, for them interim molecular results a 12 month, showing 15 patients in optimal response (OR), 10 patients in a warning response (WR) and 5 patients in failure response (FR). 5 healthy donors (HDs) were included to the study. All patients signed informed consent in accordance with the Declaration of Helsinki. RT-qPCR results were normalized by the expression of ABL mRNA (Normalized mRNA copy Number: NCN). *Results:* ZNF224 mRNA were significantly up-regulated in PB samples at diagnosis of patients with OR compared to patients with WR/FR, (1.13±0.76 *vs* 0.62±0.25 NCN, respectively; p=0.05). Interesting the ZNF224 mRNA expression in HDs was significantly higher (2.11±0.98 NCN *vs* OR patients, p=0.05 and WR/FR patients; p=0.0005). The treatment for 12 month with imatinib increase the ZNF224 expression in both CML categories (2.91±1.72 NCN in OR and1.77±1.52 NCN in WR/FR; p=0.05). *Conclusions:* We observed that the OR patients express a significantly higher number of copies of the ZNF224 transcript than WR/FR. Furthermore, in both groups of patients at diagnosis, ZNF224 protein levels are lower than those after therapy with TKI at 12 months.

Platelet Disorders

C115

THROMBOPOIETIN RECEPTOR AGONIST SWITCHING IN ADULT IMMUNE THROMBOCYTOPENIA PATIENTS: A RETROSPECTIVE COLLABORATIVE SURVEY FROM 8 ITALIAN CENTERS

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Introduction: The TPO-RAs Romiplostim (Rom) -weekly subcutaneous administration- and Eltrombopag (El) -once daily oral administration- are an effective therapy for ITP patients (pts). However, some pts either are non-responders or lose response - *i.e.* desired platelet (plt) count achieved but not sustained – or experience fluctuations in plt counts. Finally, untoward effects or pt's preference may be an issue considering the different route and timing of administration of both agents. Availability of 2 TPO-RAs, with different site of binding within the TPO receptor, makes it appealing to try switching with the aim of overcoming treatment limitations of either agent. *Patients:* Charts of ITP pts receiving TPO-RAs at 8 Centers were reviewed. *Results:* Between Jan 2009-Feb 2015, 57 of 248 pts on TPO-RA (23%) underwent switch: El Rom 27/57 (47.4%), Rom El 30/57. Median age at 1st TPO-RA administration was 57 yrs (range 16-81). Median lines of previous therapy 3 (range 1-6; splenectomy: 22/57). Table 1 summarizes reasons for TPO-RA switch and outcome.

Table 1.

REASON for SWITCHING	n (%)	NR (%)	R (%)	CR (%)
All	57 (100)	25 (43.8)	10 (17.5)	22 (39)
El->Rom	27 (47.4)	10 (37)	5 (18.5)	12 (44.5)
Rom->El	30 (52.6)	15 (46.6)	5 (16.6)	10 (33.4)
1st TPO-RA failure	28 (49.1)	16 (55.2)	2 (7.1)	10 (34.5)
**El->Rom	16	7	2	7 *
**Rom->El	12	9	0	3
Loss of response	13 (22.8)	5 (38.4)	2 (16.7)	6 (50)
El->Rom	5	2	1	2
Rom->El	8	3	1	4
Plt count fluctuation	5 (8.7)	1 (20)	2 (40)	2 (40)
El->Rom	1	0	0	1
Rom->El	4	1	2	1
Patient's preference	6 (10.5)	2 (33.3)	2 (33.3)	2 (33.3)
El->Rom	0	0	0	0
Rom->El	6	2°	2	2
Adverse event°°	5 (8.7)	1 (20)	2 (40)	2 (40)
El->Rom	5	1	2	2
Rom->El	0	0	0	0

Rom: romiplostim; El: eltrombopag; CR: complete response; R: response; NR: no response (according to Rodeghiero et al,
*1 pt subsequently lost response to R because of development of neutralizing antibodies
** response El --> Rom (56%) vs Rom --> El (25%): Fisher's exact test p= 0.136
° both NR regained a response when switched back to Rom
°° 1 hepatic enzyme increase, 1 CPK increase; 2 skin rash (all resolved upon discontinuation of El); 1 retinal thrombosis.

The majority of pts (41/57, 71.9%) were switched for efficacy issues, *i.e.* failure or response loss: among these 41 pts, 48.8% responded to the 2nd TPO-RA. Of the 16 pts switched for reasons other than efficacy, 12 (75%) maintained a response on the 2nd TPO-RA: 4/5 switched for plt count instability (counts stabilized=2 pts), 4/6 switched for pt's preference, 4/5

switched for side effects. Age at TPO did not influence response (Student's t test p=0.09878). Higher number of previous therapy lines was associated with higher probability of being a non-responder upon switching in both the whole cohort of pts and in the subgroup switching for efficacy reasons (Mann-Whitney test p=0.0347 and p=0.0349 respectively). Either TPO-RA switch sequence was equally effective in yielding response in both the whole cohort and in pts switching for efficacy reasons (Student's t test p=0 0.425 and p=0.354 respectively). *Discussion:* Switching enables approximately 56% of pts (32/57) to achieve, regain or maintain (depending upon reason for switching) response; switching for inefficacy yields lower response rates (48.8%) compared to switching for reasons other than efficacy (75%). Plt counts fluctuation stabilizes in 40% of pts. These results are in line with those reported by Khellaf (Haematologica 2013) and Gonzales-Porras (BJH 2014), confirming that TPO-RA switch can be a safe and reasonable treatment option for ITP pts.

C116

HOW IMMUNE THROMBOCYTOPENIA TREATMENT HAS CHANGED OVER TIME: A MONOCENTRIC STUDY

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In the last years, the therapeutic landscape of immune thrombocytopenia (ITP) is greatly changed, thanks to the introduction into clinical practice of new treatments, particularly rituximab (RTX) and agonists of the thrombopoietin receptor (TPO-RA) eltrombopag and romiplostim. We report a large retrospective study on 557 ITP patients (pts) that were diagnosed between 1980 and 2015 and homogeneously followed at the "Seràgnoli" Institute of Hematology, Bologna, with the aim to critically analyze the changes in therapeutic choices over time and their impact on clinical outcomes. Median follow-up of living pts was 6.9 yrs (range 0.5-30); 66 (11.8%) had died, after a median time from diagnosis of 6.4 yr; causes of death were certainly related to the hematological disease in 2 (3%) cases. Median age at diagnosis was 51 years (range, 1-95); 208 were male (37,3%). 397 (71%) pts required treatment; 219/397 (55%) subsequently underwent a second-line therapy and 96/219 (44%) required also a third-line therapy. In 18 (4.5%) pts, ≥4 therapies were required. Overall, 307 (77%) pts obtained a stable, off-therapy complete (>100x10^9/l) or partial (>30x10^9/l) response. Steroid-dependent responses were achieved in 69 (17%) pts; 8 (2%) pts did not have a response. Pts were stratified according to year of diagnosis: decade 1980-89: 106 (19%) pts; 1990-99, 133 (24%) pts; 2000-09: 223 (40%); 2010-15: 95 (17%) pts. Over the years, the choice of splenectomy as second-line therapy decreased significantly; however, over 20% of pts still underwent splenectomy third-line (Table 1).

Table 1. Treatment strategies over time.

	Years of ITP diagnosis			
	1980-89 n.106	1990-99 n.133	2000-09 n. 223	2010-15 n. 95
Front-line therapy				
Prednisone (PDN)	100%	100%	96%	90%
Dexametazone (DEX)	0	0	4%	10%
Second-line therapy				
PDN/DEX	18%	38%	28%	42%
Azathioprine (AZA)	28%	44%	26%	19%
splenectomy	46%	12%	8,5%	3%
TPO-RA	0	0	3%	3,5%
RTX	0	6%	28,5%	32,5%
others	8%	0	6,%	0
Third-line therapy				
PDN/DEX	10%	42,5%	28,5%	7,5%
AZA	14%	7,5%	6%	15%
splenectomy	62%	23%	31,5%	23%
TPO-RA	0	4%	6%	38,5%
RTX	4,5%	15,5%	17%	16%
others	9,5%	7,5%	11%	0

Contextually, RTX use progressively increased both as second and third-line treatment; also, TPO-RA have become the main third-line therapy. Overall responses were similar over time, with 97.5% *vs* 99% of pts achieving a response in the periods 1980-89 and 2010-15, respectively. However, the proportion of pts with treatment-dependent responses increased (15% *vs* 26%). Sixty-three ≥Grade2 hemorrhages were recorded in 47 (8.5%) pts. Over the 4 decades, the cumulative incidence of hemorrhage was 1.7%pt/yr (1980-89), 0.9% (1990-99), 0.9% (2000-09) and 0 (2010-15) (p<0.001). Infections occurred in 53 pts (9.5%), for a cumulative incidence of 1.16% pt/yr. Over time, we observed a delay of splenectomy and an increased use of RTX and continuative TPO-RA administration as second and third-line therapies. Notably, the incidence of hemorrhages progressively decreased, while infectious risk remained stable over time.

C117

TPO-RECEPTOR AGONISTS AS TREATMENT PREPARING ADULT PATIENTS WITH IMMUNE THROMBOCYTOPENIA TO SPLENECTOMY. RESULTS OF A RETROSPECTIVE, OBSERVATIONAL STUDY (GIMEMA ITP0614)

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Background: In chronic ITP, splenectomy still represents the most active salvage therapeutic strategy in patients failing medical therapy. For a small proportion of patients who are refractory to steroid, immunoglobulin and to other immune suppressive agents and who have very low platelet count, splenectomy may result at higher risk of perioperative complications and, for this reason, in some cases, is contraindicated. The TPO receptor agonists (TPO-RA) Romiplostim (R) and Eltrombopag (E) have shown high therapeutic activity in ITP but data of efficacy and safety regarding their use as bridge to splenectomy are missing. *Methods:* On this grounds we evaluated, in a multicenter retrospective GIMEMA study, adult patients with persistent or chronic active ITP, with indication to splenectomy, who were treated with TPO-RA with the aim to increase platelet count and allow are more safe execution of splenectomy. *Results:* 32 patients, median age 43 years, 31 with primary and 1 with HCV positive ITP, were enrolled. All patients were previously treated and 21 received 3 or more lines of treatment. 72% of patients resulted refractory to the last medical therapy before the use of TPO-RA. The median platelet count before the start of TPO-RA was 16 x 109/L. 23 patients (72%) were treated with R and 9 (28%) with E for a median duration of 86 days. A concomitant anti-ITP medication was added in 17/23 (74%) and 2/9 (22%) patients treated with R and E, respectively. 29 patients (90%) responded to the use of TPO-RA (96% after R, 78% after E), allowing the obtainment of a median platelet count before splenectomy of 137 x 109/L. During the period of treatment only 1 patient developed an AE (grade 2 bleeding). 30 patients performed splenectomy (refusal in 2 responders), laparoscopic in 26 and laparotomic in 4. Response (platelet count >30 x 109/L and doubling of the baseline count) and CR (platelet count >100 x 109/L) after splenectomy was achieved/mantained in 1 (3%) and 23 patients (77%), respectively. Post-splenectomy complications were characterized by 2 grade 3 thrombotic events (platelet count at the time of thrombosis 121 and 167 x 109/L, respectively) and 1 grade 3 infectious event. *Conclusions:* TPO-RA may represent a therapeutic option to improve platelet count and reduce the risk of peri-operative complications in ITP candidates to splenectomy.

C118

RITUXIMAB TREATMENT IN ITP OF ADULTS: LONG-TERM EFFECT AND CLINICAL PREDICTORS OF RESPONSE

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Background: Rituximab (R) is more and more adopted as second or further line of treatment in adult patients with primary Immune Thrombocytopenia (ITP). Unfortunately, up to date, only scanty data are available as far as the long-term effect and safety profile. Furthermore, clear clinical or biological predictors of response are missing. A recent study (Bussel *et al.* Haematologica 2014) indicated younger age, female status and shorter interval from diagnosis to R as important clinical predictor of response. *Methods:* We retrospectively evaluated the outcome of patients with ITP who were treated with R salvage therapy. Inclusion criteria were: diagnosis of primary ITP, age >16 years old, use of standard dosage R (375 mg/m^2 x 4) as second or further line of therapy from September 1999 to September 2014. *Results:* 38 consecutive patients, median age 47 years (range 16-76), 22 female (58%) were included; the median interval from diagnosis to R was 31 months (range 1-156). 17 and 21 patients received R as second or further line of therapy, respectively; splenectomy was previously performed in only 3 patients. The median period of observation was 58 months. Response (platelet count >30 x 109/L and doubling of the baseline count) and complete response (platelet count >100 x 109/L) soon after R treatment were documented in 24 (63%) and 18 (47%) patients, respectively. Women appeared to have significantly higher probability to achieve response (P=0.008) and complete response (P=0.023). A higher tendency to achieve a complete response was also observed among younger patients (P=0.081). The long-term response rate (LTR, *i.e.* the number of patients who achieved and maintained response during the whole period of observation) was 13/38 (34%). The achievement of LTR was strongly associated with the obtainment of complete response after R (P=0.013); a trend was also observed for younger patients (P=0.082) and women (P=0.096). During the period of R administration 2 patients developed serum sickness syndrome; in the subsequent period of observation 2 patients developed neoplasia, 1 hypogammaglobulinemia and 1 HZV reactivation. *Conclusions:* Rituximab salvage therapy allows to achieve LTR and splenectomy sparing effect in nearly 1/3 of patients with primary ITP. These results appear significantly higher in female and younger patients.

C119

EFFICACY OF TPO-MIMETICS IN PATIENTS WITH CHRONIC IMMUNE THROMBOCYTOPENIA

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Immune thrombocytopenic purpura (ITP) is an autoimmune disorder in which antibodies are produced to circulating platelets. The isolation of TPO and better understanding of its role in thrombopoiesis has led to the development of new highly effective tTPO analogs had some successes in treating highly refractory ITP patients but were taken out of development due to TPO-antibody induction. From November 2008 and April 2015 54 patients (29 M; 25 F) were treated with TPO-mimetics: 38 underwent therapy with Romiplostim and 16 to Eltrombopag. Median age was 71 years (range 39-94 years). 8/54 (14%) patients received both of therapies: 5 (4 F; 1 M) switched from Romiplostim to Eltrombopag and 3 (3 M) switched from Eltrombopag to Romiplostim. In the group of patients treated with Romiplostim, 20/38 had already received more than 4 lines of therapy, while 6 were at the 2nd line of therapy, 12 at the 3rd. Only 2/16 patients who received Eltrombopag were at the 2nd line of therapy, and the others were at least at the 3rd line. The median platelet count was 17.000/µl at the start of Romiplostim and 22.000/µl in patients treated with Eltrombopag. With median follow-up of 36 months (4-74), 33 (86%) patients treated with Romiplostim achieved a response (19 complete response, 14 partial response), 4 patients were no responders and 1 was a loss of response. 7 are out of treatment with a stable platelet count. In our study 16 (42%) patients stopped Romiplostim after a median time of 23 months (1-56): 6 for stable response; 2 for adverse events; 2 for loss of response; 2 underwent splenectomy; 4 for no response. The median platelet count at suspension of Romiplostim was 94.000/µl (2.000-739.000). In patients treated with Eltrombopag 13 (81%) achieved a response (9 complete response, 4 partial response), 3 were no responders. 11 (69%) patients stopped Eltrombopag after a median time of 10 months (1-16): 5 for adverse events; 3 for no response; 2 for stable response; 1 for loss of response. The median platelet count at suspension was 74.000/µl (2.000-739.000). Several studies reported Romiplostim and Eltrombopag to be highly effective against chronic ITP, with average immediate responses exceeding 80% in our study. TPO-mimetics have proved efficacy in patient with ITP and their use can be applied in several conditions (bridge to splenectomy; sustained response; switch and discontinuation).

C120

THROMBOPOIETIN RECEPTOR AGONISTS IN PATIENTS AFFECTED BY PRIMARY IMMUNE THROMBOCYTOPENIA: ANALYSIS OF POSSIBLE PREDICTIVE FACTORS FOR SUSTAINED AND DURABLE RESPONSE FROM A RETROSPECTIVE CASE SERIES

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Background: The efficacy of thrombopoietin receptor agonists (TPO-RAs), Romiplostim and Eltrombopag, for the treatment of primary immune thrombocytopenia (pITP) patients (pts) has been already demonstrated. Case reports on sustained responses (SR) off treatment have been reported but, to date, no prognostic factors for response have been identified. *Aims:* To retrospectively identify possible prognostic factors for SR and durable response (DR) in TPO-RAs treated pITP pts. *Patients and Methods:* Data were examined for pts who received a TPO-RA between February 2009 and March 2014. Response (R) was defined as platelet (plt) count ≥30x10^9/L and at least a 2-fold increase of the baseline value and absence of bleedings; complete response (CR), as a plt count ≥100x10^9/L; fluctuating response (FR) as CR or R maintained without a stable dose of TPO-RAs; durable response (DR) as CR or R persisting >4 weeks with a stable dose of TPO-RAs, without other concomitant/rescue therapy; SR, as CR or R, persisting for a period >4 weeks after TPO-RAs discontinuation, without concomitant/rescue therapy. Differences in the distributions of variables between groups of pts were analyzed by the Fisher exact test; analyses were performed using the SPSS software. *Results:* Thirty-nine pITP pts resistant to one or more therapy lines have been treated with TPO-RAs (28 with Romiplostim, 11 with Eltrombopag). Twentynine/39 pts (74%) were responders. Eighteen/29 (62%) reached a CR (11 with Romiplostim, 7 with Eltrombopag) and 11/29 (38%) a R (10 with Romiplostim, 1 with Eltrombopag). A DR was observed in 16/29 pts (55%) (12 with Romiplostim, 4 with Eltrombopag): 13/16 (81%) had obtained a prior CR, while 3/16 (19%) a R. Seven SR (6 with Romiplostim, 1 with Eltrombopag) were reached: 5/7 pts achieved a SR from a prior DR, while the other 2 interrupted the treatment because of a rapid increase of plt count over the safety threshold for drug administration, without therefore achieving a prior DR. The CR was statistically related with the achievement of a subsequent DR: 13/18 (72%) CR pts obtained a DR, while only 3/11(27%) R ones did (p=0.027). Five/16 (31%) DR pts reached a SR, while none of the 11 FR pts had the same outcome. Although there was a trend, this difference was not statistically significant (p=0.06). *Conclusions:* In our TPO-RAs treated pITP population, CR was a positive prognostic factor for the achievement of a DR (p=0.027). Moreover, we observed a trend, not statistically significant, for DR pts to obtain a subsequent SR.

POSTERS

Anemias and Red Cells Disorders. Thalassemias and Hemoglobinopathies

P001

PRIMARY ACQUIRED CHRONIC PURE RED CELL APLASIA REFRACTORY TO STANDARD TREATMENTS: VERY LONG LASTING REMISSION BY RITUXIMAB

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Introduction: Acquired pure red cell aplasia (PRCA) in adults is a chronic illness with very rare spontaneous remissions and heavy transfusion burden. Although it is often associated with lymphoproliferative disorders, PRCA may occurs also in idiopathic form. *Aims:* We report an idiopathic PRCA 63-year-old woman who was unresponsive/intolerant to three lines of immunosuppressive treatment, achieving a long lasting remission by rituximab given as salvage therapy. *Methods:* The patient was diagnosed in June 2003 as having primary PRCA, being all possible secondary causes been excluded by appropriate investigations. She required 2 red blood cells (RBC) transfusions every 2 to 3 weeks and was initially treated with cyclosporine A plus corticosteroids, achieving a significant disease control until the transfusion independence. So that, cyclosporine A was gradually tapered until suspension. However, in February 2007, PRCA relapse was observed so that she was restarted on cyclosporine A, which dosage was tapered according to the patient's response. However, in January 2011, she experienced a progressive chronic renal failure for which cyclosporine A was withdrawn; a full recovery of the renal function and, on the other hand, the reappearance of transfusion requirement, within the typical frame of PRCA, were observed. Therefore, having the patient a transfusion requirement of approximately 4 units of RBC/ month, steroids were re-challenged without any benefits. Again, the patient received azathioprine without any response. On May 2012, presenting the patient a progressively increased of transfusion burden (about 6 RBC units/month), an anti-erythrocyte agglutinating IgG antibody (strong positivity of direct and indirect Coomb's tests) was detected; therefore, the patient's management became a challenging concern due to the lack of compatible RBC units available for transfusions. In view of this life threatening condition, refractory, anti-CD 20 (rituximab), at the dose of 375 mg/m^2/week for a total of 4 cycles was given, being the patient properly informed and having she given her consent. After the second dose of rituximab, she exhibited normal hemoglobin and reticulocyte levels, without any side effects; 3 years after last rituximab course, the patient is well and still in remission. *Conclusions:* At the best of our knowledge, it is the first description of PRCA, as isolated and idiopathic condition, resolved by rituximab.

P002

IMMIGRATION INFLUENCE ON THE INCIDENCE OF HAEMOGLOBINOPATHIES IN PUGLIA

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Introduction: Haemoglobinopathies are the most common single gene disorders in humans. In Puglia, the incidence of heterozygous β thalassemia is around 7%, with minimal fluctuations among the various provinces of Puglia, while the incidence of α thalassemia is around 1.5%. Haemoglobin variants (HbS) have been found in a few families. In the last decade, the social context of Puglia has changed due to migratory flows; immigrants come in from many different areas, but in recent years there has been a rapid increase in immigration from North Africa and South-East Asia. As the number of immigrants continues to increase, disorders of haemoglobin chains will become increasingly prevalent, making a more efficient triage and diagnosis necessary. *Methods:* In the period 2005-2014, 627 patients were examined at our center, coming mainly from Eastern Europe, Africa and Asia. All patients received first level screening tests for haemoglobinopathies and, when necessary, molecular study of the globin genes. *Results:* Our data show a gradual increase in the percentage of foreigners referred to our center (1.3% in 2005 *versus* 5.23% in 2014). The incidence of α and β thalassemia has not varied significantly, but molecular study of the α and β globin genes showed mutations rarely or never encountered in the population of Puglia. A haemoglobin variant was found in 15.6% of immigrants referred to our Center (HbC, HbE, HbD Punjab, HbD Ouled-Rabah, Hb Constant Spring), although HbS accounted for 82.65% of all haemoglobin variants. *Conclusions:* Some haemoglobin variants such as HbS, HbC and HbE, typical of specific geographic areas, have appeared in the Apulian territory as a result of the migratory flows. The growing presence of non endemic mutations of globin genes requires alterations in the laboratory data methodologies and interpretations to foster correct diagnoses, as well as for prevention purposes. We will continue to see an increase in the number of immigrants as the barriers between countries are gradually removed. Thus, although this new phenomenon is not going to be transitory but is not yet an emergency, we need to adjust our medical procedures and way of thinking.

P003

HEMOGLOBINOPATHIES SCREENING IN CORD BLOOD BANKING: THE FLORENCE EXPERIENCE

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Objectives: Both NetCord-FACT standards and the Italian law established that Cord Blood Banks (CBB) must perform the hemoglobinopathies screening prior to listing cord blood units (CBU) and anyway before releasing them. Such a screening should utilize a method capable to identify both homo and heterozygous status of S- and C- hemoglobin (Hb), in addition to quantify hemoglobin F and A, as the latter can suggest a thalassemic condition in the newborns. A large scale assessment of hemoglobin pattern in normal newborns has been poorly reported in the literature. We aimed to define a two-steps approach to be exploited in the CB banking: each CBU suitable for allogenic banking is submitted to first level (High Performance Liquid Chromatography) analysis. The molecular investigation is performed only if indicated by the hemoglobin pattern. *Methods:* Since 2006, 1323 CBUs suitable for allogenic banking at the Florence CBB were tested on residual red blood cells discarded after the CBU volume reduction. The Hb pattern was performed by HPLC technique (Dual kit, Variant II, BioRad, USA). Molecular test was performed when indicated by the chromatogram (n=27). *Results:* A significant increase in the HbA levels between 38° and 39°, 39° and 40°, 40° and 41° gestational weeks was observed (p<0,05). (Figure 1)

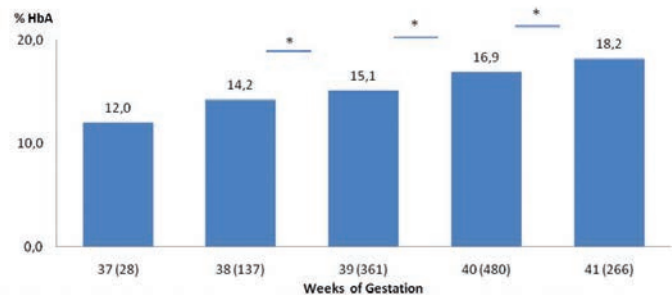

Figure 1. Significant (p <0,05) increase in Hemoglobin A percentage compared to the week of gestation in normal condition.

The analysis of the HbA percentages distribution allowed to define a HbA percentage >12% (97,5th percentile) in lack of abnormal bands as normal condition. Values of HbA including 5-12% and HbA≤5% suggest a condition of either heterozygous or homozygous thalassemia, respectively and have to be investigated with genetic test so as the presence of the Hb variants. Currently the microsatellite analysis is required to exclude a maternal contamination with HbA≥29%. We therefore submitted to molecular investigation 26 cases with a positive Hb pattern

that resulted in 2 HbS carriers, 1 β-thalassemia major (autologous collection), 9 β-thalassemia carriers, 1 Hb G St. Josè carrier, 11 normal profile. Two analysis are currently in progress. In addition, in one case with prenatal diagnosis of β thalassemia carrier we have observed 13% HbA (>cutoff). *Conclusions:* In this retrospective analysis, our HPLC assay resulted as sensitive and reliable in determining the need for molecular investigation. These results may contribute to the standardization of hemoglobinopathies screening in the Cord Blood Banks.

P004

HEMATOLOGICAL RESPONSE INDUCED BY DEFERASIROX IN PATIENT WITH BONE MARROW HYPOPLASIA AND IRON OVERLOAD

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This is a case of an 80 years old man who referred to our department because of normocytic anemia (Hb:7,5 g/dl, McV 83,8 fl); no explanation to this anemia showed in his blood tests; so we performed bone marrow aspiration but it turned out to be hypocellular so it was necessary a bone marrow biopsy conclusive for the diagnosis of Bone Marrow Hypoplasia. Soon after this diagnosis the patient went on α erythropoietin therapy (40.000 IU twice a week) associated to oral prednisone at a daily dose of 25 mg but there was no response and the patient experienced a quite rapid and progressive decline in his white blood cells and platelets count. Consequently it was necessary to provide a frequent RBCs support (four units per month). Six months later the patient showed data consistent with iron overload, in particular serum iron: 227 mcg/dl, serum ferritin: 2117 ng/ml, transferrin saturation 96%. For this reason, according to current guidelines concerning iron transfusional overload and iron chelating therapy, we prescribed Deferasirox treatment at a daily dose of 15 mg/kg. Without modifying or introducing drugs able to stimulate hematopoiesis the patient experienced a progressive and rapid improvement in his blood count that was able to reduce first and then stop RBCs support, to improve iron overload data and in his quality of life. In details after six months of Deferasirox treatment: Hb: 13.1 g/dl, serum ferritin: 884 ng/ml, WBC and Plt in the normal range. The patient stopped deferasirox treatment more than a year ago and has progressively reduced the dose of erythropoietin. No relapse until today. As reported in several studies oxidative stress may play a role in the pathogenesis of some bone marrow failure disorders as aplastic anemia and hypoplastic myelodysplastic syndromes via increased apoptosis of bone marrow precursors mediated by ROS generation, lipid peroxidation and free iron levels. Iron overload, mainly as a consequence of chronic transfusion support, may have a significant role in stimulating oxidative stress. As demonstrated in several *in vitro* models Deferasirox has the ability to reduce oxidative stress in bone marrow precursors and improve or restore normal hematopoiesis. This could be the case of our patient and we are strongly convinced that deferasirox therapy must have a role in the setting of patients who suffer from disorders characterized by hematopoiesis failure and iron overload (Figure 1).

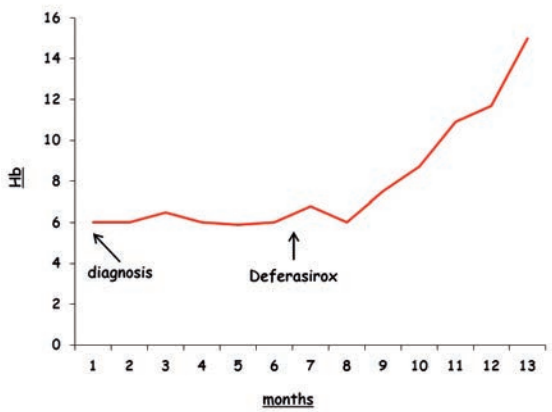

Figure 1. Improvement of Hb level after beginning Deferasirox treatment.

P005

A NOVEL NONSENSE MUTATION [CODON 130 (TAT>TAG)] IN EXON 3 OF THE β-GLOBIN GENE, NOT CAUSING β-THALASSEMIA INTERMEDIA PHENOTYPE

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We report a novel nonsense mutation at codon 130 (c.393 T>G) in exon 3 of the β-globin gene, in the heterozygous state. In the same codon, four different mutations have been previously described: three were Hb variants, and one was a nonsense mutation, in an isoform (TAT>TAA) different respect to the new mutation (TAT>TAG). Some mutations described in the third exon, often, result in high unstable globin chains and are defined as dominantly inherited β-thalassemia. In Hb Filottrano (1), a novel Hb variant we described in 2012, the frameshift mutation at the first nucleotide (-A) at codon 120 gives rise to a β-globin chain elongated to 156 amino acid residues and results extremely unstable. The abnormal chains precipitate in the erythroblasts as inclusion bodies, thus causing inefficient erythropoiesis and, ultimately, resulting in a dominant clinical phenotype. The Italian woman (age 47yrs), carrier of the new mutation at codon 130, showed a clinical picture of β-thalassemia, characterized by a marked anemia (Hb 9.4 g/dL; MCH 19.9 pg; MCV 63 fl) with evident anisocytosis, poikilocytosis and presence of schistocytes in the peripheral blood film. Besides she presented a slight splenomegaly (longitudinal diameter 13.8 cm) but not increased bilirubinemia. The isopropanol stability test resulted negative and the inclusion body in erythrocytes were absent. Her mother (age 74 yrs) appeared as a classical β-thalassemia carrier with erythroblastemia and an increased value of ferritin (447 ng/mL) but with hematological characters less pronounced than those of daughter. Instead the father showed a severe magaloblastic anemia (RBC 2,78 1012/L; MCV 133 fL) and overall he looks very different from his daughter. Therefore, the case described today doesn't seem to result in a phenotype analogous to that in Hb Filottrano and should not be considered part of that group of β-thalassemia intermedia, dominantly inherited. It seems to consist in a β-thalassemia carrier phenotype, with very evident characters, whose clinical evolution, however, would keep under observation. (1) "Hb Filottrano [codon 129 (-A)]: a novel frameshift mutation in exon 3 of the β-globin gene causing dominantly inherited β-thalassemia intermedia." A. Amato *et al.* Hemoglobin, 2012; 36(5):480-4.

P006

ANALYSIS OF ERYTHROCYTE ANTIOXIDANT DEFENSE STATUS IN PATIENTS WITH HEMOGLOBINOPATHIES OR CARRIERS STATUS OR CHRONIC DEGENERATIVE DISEASES. EFFECTS OF *IN VIVO* TREATMENT WITH ANTIOXIDANTS

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Reactive oxygen species (ROS) are a natural products of the normal metabolism of oxygen. Normally cells defend themselves against ROS damage through enzymatic and non-enzymatic mechanisms. Under some conditions, the balance between pro-oxidants and anti-oxidants shifts toward the oxidative stress of the cell. In this situation ROS are generated in excess and oxidizing proteins, lipids and DNA may cause cell death and organ damage. Oxidative stress is believed to aggravate the symptoms of many diseases, including β-thalassemia. The activity of glucose-6-phosphate dehydrogenase (G6PD), gluthatione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD) were assayed to evaluate the status of the antioxidant defense system in the erythrocytes for protection against oxidative stress. Total antioxidant capacity (TAC) of serum was measured to show the antioxidant status of patients. We studied oxidative stress in 2 groups of patients: β-thalassemia group and chronic degenerative diseases group. We evaluated oxidative stress parameters G6PD, GPx, CAT, SOD, TAC in β-thalassemia group (thalassemia intermedia (TI) patients, β-thalassemia trait (T) patients and healty subjects (C)). TI and T patients exhibited an altered antioxidant defense status compared to C. We aimed to assess the effect of a six months treatment with oral antioxidant (tocopherol, ascorbic acid, lipoic acid, ubiquinol, β-carotene, camellia sinensis, vitis vinifera, ginkgo biloba, N-acetylcysteine) on the status of antioxidant defense system in the chronic degenerative diseases group. The data indicated a significantly increment in erythrocyte GPx activity and no patients reported

adverse events. Our data are a starting point for further studies of the state of oxidation in the β-thalassemia group erythrocytes and improve treatments for patients based on antioxidants.

P007

DEFERASIROX INDUCED LIVER DAMAGE IMPROVEMENT IN CHRONICALLY TRANSFUSED MYELODYSPLASTIC SYNDROMES PATIENTS

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Most MDS patients are at diagnosis, or become, transfusion dependent during the course of the disease. Chronic RBCs support is typically accompanied by a progressive iron overload which can be responsible of several organ damage(mainly affecting liver and heart) mediated by increased oxidative stress. Transfusion dependence and iron overload in MDS patients have a negative prognostic significance. Oral iron chelating Deferasirox may significantly improve iron overload and restore organ damage. We have experience of ten low-risk, transfusion dependent (median RBCs support of 2,6 units per month) MDS patients (median age 72,3 years) who showed data consistent with iron overload after having received at least 20 RBCS units. All these patients also presented data of liver damage mainly of AST and ALT serum concentrations above the normal range According to current guidelines concerning iron chelating therapy in chronic transfusion dependent anemias, all these patients went on treatment with oral chelator Deferasirox at doses depending on their monthly transfusional requirements. What we have observed in all the patients is a quite rapid decline of AST and ALT concentration up to normal range levels. What is surprising is that this improvement appeared to be independent by significant modifications of iron overload serum markers, mainly serum ferritin. According to this experience we think that oral chelating therapy must play a significant role in the treatment of chronically transfused anemic patients. The main role of iron chelating therapy in chronic transfusion anemias should be to prevent the iron overload organ damage which could adversely affect the life expectancy of patients (Figure 1).

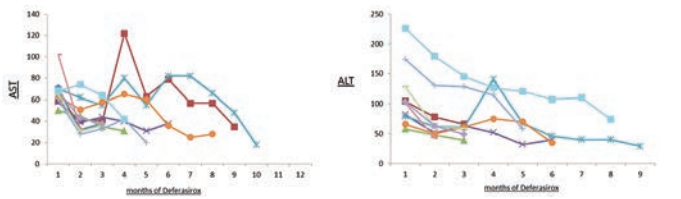

Figure 1.

P008

ECULIZUMAB IN THE TREATMENT OF A LIFE-THREATENING COLD AGGLUTININ DISEASE PATIENT

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Cold agglutinin disease is characterized by antibody-mediated hemagglutination with complement activation and subsequent hemolysis. Although CAD usually has an indolent course with mild extravascular hemolysis, it may be a life-threatening disease in case of massive intravascular hemolysis due to terminal effector complement activation. Dramatic presentations are possible, with very severe anemia, massive hemoglobinuria, fatigue and dyspnoea; response to the treatment exploited in other autoimmune hemolytic anemia is often disappointing. We report the case of a young 34 year-old male patient treated eight months before by ABVD x 6 courses because of a classical Hodgkin's Lymphoma, and admitted on February 2015 with a very severe anemia, jaundice and hemoglobinuria. Blood counts showed: Hb 3,8 g/dL; Reticulocytes 107.000/uL; Platelets 307.000/uL. Unconjugate bilirubin and LDH were 25 mg/dL and 21.000 U/L, respectively. DAT was strongly positive for the presence of complement on red blood cells. High titer - >1:2000 - of cold agglutinin identified as anti-I specificity, was detected. Indeed, a large amount of C3 molecules were detected on RBC by flow cytometry, confirming the pathogenic role of complement activation in the hemolysis. I.v. Methylprednisolone and supportive treatment with packed and washed RBC were ineffective. Patient's condition worsened quickly. After patient's informed consent and local Ethic Committee approval, Eculizumab (Soliris®) – the potent C5 inhibitor approved for the treatment of PNH – was obtained by compassionate off-label use for the treatment of this patient. Before starting therapy, the patient was vaccinated against N Meningiditis. The administration schedule consisted of four 600 mg weekly doses, followed by four 900 mg doses every two weeks. After the first dose hemoglobin was stable on 5 g/dL, LDH fell down to 2000 U/L, and hemoglobinuria progressively reduced. Sustained inhibition of the terminal effector complement led to normalization of Hb and LDH levels at the end of the 5-weks loading phase, with disappearance of hemoglobinuria. Because of the improved clinical conditions and of changes in immunohematological testing (DAT became negative and cold agglutinin titer reduced to <1:16), the use of Rituximab was omitted. Although the off-label use of Eculizumab in this subset of patients has been already reported, we like to underline the effectiveness and good tolerance of this life-saving approach.

P009

ANALYSIS OF RENAL TUBULAR DISFUNCTION IN PATIENTS WITH MAIOR THALASSEMIA TREATED WITH ORAL CHELATION AGENTS

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Introduzione: Con il miglioramento della prognosi e il prolungamento della sopravvivenza nei pazienti affetti da talassemia maior, è stato possibile mettere in luce complicanze multiorgano indotte da tale patologia, in particolare quelle endocrinologiche, cardiache ed epatiche. Le complicanze renali, pur rare, sono effetti avversi della terapia chelante,in particolare del trattamento con deferasirox. *Scopo dello studio:* Dopo l'osservazione di un caso di grave tubulopatia complicata da acidosi metabolica sintomatica, abbiamo deciso di estendere il monitoraggio della funzionalità renale a tutti i pazienti pediatrici affetti da talassemia maior seguiti presso il centro di emato-oncologia pediatrica di Padova, con lo scopo di mettere in luce precocemente le alterazioni renali indotte dalla terapia chelante orale. *Materiali e Metodi:* Il nostro studio comprende 7 pazienti pediatrici (2 maschi, 5 femmine) affetti da β talassemia maior. Tutti sono sottoposti a regime ipertrasfusionale cronico e trattati con terapia chelante orale (deferiprone-DFP, deferasirox-DFX): 2 pazienti trattati con il solo DFP, 4 con DFX, 1 con DFP prima e secondariamente con DFX. DFP è stato utilizzato per un periodo compreso tra 5 e 30 mesi con una mediana di 26. DFX è stato utilizzato per un periodo compreso tra 19 e 44 mesi con una mediana di 28. In tutti i pazienti sono stati monitorati i valori sierici di creatinina,proteinuria, albuminuria e indici urinari di tubulopatia quali l'N-acetilglucosamina (NAG), α1 microglobulina (A1MG) and β2 microglobulina (B2MG). *Risultati:* Proteinuria non nefrosica, microalbuminuria, e incremento del NAG sono stati osservati in tutti i pazienti analizzati, indipendentemente dalla tipologia di chelante utilizzato. Al contrario l'aumento dei valori urinari di A2MG e B1MG si osserva solo nei pazienti trattati con deferasirox. Un solo paziente ha presentato un danno tubulare acuto con importante glicosuria dopo l'inizio della terapia chelante con deferasirox. Non si sono osservate alterazioni della clearance della creatinina. La presenza di danno glomerulare e tubulare evidenziata in tutti i pazienti affetti da talassemia maior dimostra come essi siano riconducibili a processi fisiopatologici comuni quali la microangiopatia indotta dall'emolisi cronica e il sovraccarico marziale. La terapia chelante,in particolare l'utilizzo del deferasirox,parrebbe complicare tale situazione, pur essendo indispensabile per la sopravvivenza dei pazienti con tale patologia.

Respective mean eGFR increases from baseline (BL) were 29.3 and 30.0 mL/min/1.73 m^2 at Wk 26 and 1 yr. 20 of 24 pts (83%) with BL dialysis discontinued by Wk 26 and remained dialysis-free at 1 yr. 4 pts with no BL dialysis began dialysis. 2 pts had meningococcal infections in the first 26 wks–both recovered; 1 continued ECU. The update confirms that ECU continues to inhibit TMA in adult aHUS pts, and maintains or improves upon clinical gains seen at 26 wks.

P013

ADDRESSING PATIENT CONCERNS ABOUT PRODUCT SWITCHING IN HEMOPHILIA A: EVALUATING TUROCTOCOG A DATA FROM THE GUARDIAN™ CLINICAL TRIAL PROGRAM

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Objectives: Patients with haemophilia A are often reluctant to switch their factor VIII(FVIII) product for a variety of reasons. Despite the lack of evidence to support this, the concern that switching may increase the risk of inhibitor development remains.We aimed to address patient concerns and perceptions about product switching using data from clinical trials of turoctocog α (NovoEight(R)), a new B-domain truncated recombinant FVIII(rFVIII). The focus was on the questions: "Will I develop an inhibitor?" "Will I bleed more after switching product?" "If I bleed, can it be treated quickly and effectively?" *Methods:* Safety and efficacy of turoctocog α were studied in phase 3 trials of previously treated patients (PTPs)with severe haemophilia A(n=213)(guardian(TM)1: adults/adolescents 12-65 years; guardian (TM)3: children 0-11 years). All patients switched to turoctocog α prophylaxis (25-50 IU/kg every second day or 25-60 IU/kg three times weekly) from other FVIIIproducts. Breakthrough bleeds were treated on-demand with turoctocog α (aiming for 0.50 IU/mL plasma FVIII activity). Long-term safety and efficacy of turoctocog α are being investigated in patients completing guardian (TM)1 or guardian (TM)3 in the guardian (TM)2 extension trial. *Summary:* No patients receiving turoctocog α developed confirmed inhibitors. In guardian (TM) 1 and guardianTM3, the median annualized bleeding rate (ABR) was 3.7 and 3.0 bleeds/patient/year among adults/adolescents and children,respectively.Three-year interim results from guardian(TM)2 showed that ABR decreased over 6 months and stabilized with continued turoctocog α prophylaxis; overall median ABR across all patients was 1.7 bleeds/patient/year.Furthermore, turoctocog α successfully resolved the majority of bleeding episodes reported by adults/adolescents (84%; 403/477 bleeds) and children (94%; 116/123 bleeds) in guardianTM1 and guardian (TM)3, with most bleeds controlled with 1-2 infusions (89%; 446/499 bleeds [adults/adolescents];95%;120/126 bleeds [children]). *Conclusions:* Patients on turoctocog α prophylaxis did not develop inhibitors after switching from their previous product.Additionally, the ABR was similar to that reported previously for full length rFVIIIs, and was maintained at a low level over time. Breakthrough bleeds were treated effectively with turoctocog α.Turoctocog α was well tolerated and effective with low immunogenicity in PTPs.Overall, the evidence on switching is reassuring for patients.

P014

STABILITY OF TUROCTOCOG A, A NEW RFVIII PRODUCT FROM NOVO NORDISK, WHEN STORED AT HIGH TEMPERATURE AND HUMIDITY

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Introduction and Objectives: Turoctocog α is a new recombinant FVIII product available in six different strengths: 250 IU, 500 IU, 1000 IU, 1500 IU, 2000 IU and 3000 IU. To support worldwide use, stability studies have been performed covering requirements in climate zone I, II, III and IV, including some countries with a very hot and humid climate. To further support the use of turoctocog α in these countries, the effect of extremely hot and humid storage conditions on the quality of the drug product was evaluated. The current approved shelf life temperature for turoctocog α is 5°C, where the drug product may be kept at room temperature (≤30°C) for a certain time period. *Materials and Methods:* Batches of turoctocog α were assessed for long term stability at 5°C±3°C/ambient humidity (AH)/darkness for 18 months followed by storage at 30°C±2°C/75%±5% relative humidity (RH)/darkness for 12 months to support shelf life. Furthermore, the same batches were followed in a stability study at 30°C±2°C/75%±5% RH/darkness for 18 months and in an accelerated stability study at 40°C±2°C/75%±5% RH/darkness for 6 months. Other studies assessed the effect of temperature on the quality (potency) of turoctocog α. Parameters slightly affected by the temperatures were aggregation and oxidation. *Results:* Long term stability results were consistent at high storage temperature and humidity conditions (Table 1). *Conclusions:* In summary, turoctocog α is stable when stored at high temperature and humid conditions.

Table 1.

	Storage conditions, temperature (humidity)			
	5°C/AH 30 months	5°C/AH for 18 months then 30°C /75% RH for 12 months	30°C/75% RH for 18 months	40°C/75% RH for 6 months
Potency % of nominal strength (range)	95–115	93–100	85–93	83–102

Temperature had no effect on drug quality

Strength	Time period/temperature	Result
2000 IU	4 weeks at 50°C	No compromise of quality
2000 IU	12 months at 40°C, transferred to 5°C for 12 months	
250 IU	12 months at 30°C, transferred to 5°C for 9 months	

P015

PERCUTANEOUS TRANSLUMINAL AORTIC VALVE IMPLANTATION FOR SEVERE AORTIC VALVE STENOSIS IN PATIENT WITH HEMOPHILIA A SEVERE, USING FACTOR VIII RECOMBINANT

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Introduction: The life expectancy of haemophilia patients is increasing and now approaches that of the general male population, and they are confronted with age-related co-morbidity, including cardiovascular disease. The management of cardiovascular events in elderly hemophiliacs necessarily differs from that of non-hemophilic patients. Aortic stenosis (SA) is the most common degenerative valve disease in Western countries, and its incidence increases with age and nowadays, the surgical aortic valve replacement is the gold standard. The treatment of valvular disease using transcatether technique, such as TAVI, is today a valid and interesting alternative to conventional cardiac surgery. *Case Report:* An 56-years-old man with a severe hemophilia A with HCV-related chronic liver disease was admitted to hospital due to dyspnea on moderate exercion. A history of arterial hypertension, hyper-thyroidism and diagnosis of severe aortic valve stenosis were present in past anamnesis. Indeed family history of ischemic heart disease was present. Aortic valve replacement was indicated, and because of the contraindication of surgery (high bleeding risk) and his comorbility, the patient was subjected to Transluminal Aortic Valve Implantation (TAVI) that was performed in the cardiac catheterization laboratory, under mild sedation and local anesthesia with fluoroscopic guidance. Overall, the outcome of the procedure was satisfactory. The patient did no experience any adverse events during and after the procedure. During hospitalization, the patient was treated with recombinant FVIII 80 UI/Kg in bolus, before surgery and then at a dose of 40 UI/Kg every 12 hours after surgery. During the procedure has been practiced heparin bolus 5000 UI. The patient has continued home therapy in prophylaxis with recombinant FVIII infusion every 12 hours for 15 days, every 24 hours for others 15 days and for 30 days LMWH 6000 U/I 24h. After six days of hospital stay we discharged the patient in NYHA class I-II on medical therapy without any complications. *Conclusions:* Literature data show that TAVI can effectively treat elderly patients with high surgical risk or inoperable. It showed a reduction in mortality at 1 year of 50% and a reduction in the combined endpoint of mortality and re-hospitalization of 55% in the group that

Hemostasis and Thrombosis

P010

SAFETY AND EFFICACY OF NEW ORAL ANTICOAGULANTS IN A COHORT OF 80 VERY OLD PATIENTS AFFECTED WITH ATRIAL FIBRILLATION: A REAL-LIFE MONOCENTRIC EXPERIENCE

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New oral anticoagulants (NOACs) have been tested in several studies for the treatment and prophylaxis of stroke and thromboembolism (TE) due to non-valvular atrial fibrillation (NVAF). Even if several studies demonstrated that NOACs were not inferior to warfarin in the prevention of ischemic stroke and TE events, very old patients (older than 75 yrs.) are underrepresented in those series (30-40%) and a real-life post-marketing evaluation of the safety profile in this age category is still warranted. The purpose of the study was to evaluate, during the first year of NOACS intake, the efficacy, safety and compliance to therapy in patients aged >75 years affected with NVAF. Due to the renal excretion of NOACs, expecially for the dabigatran, a specific evaluation of renal function was also performed by measuring pre-and post-treatment glomerular filtration rate (GFR).We analyzed 80 patients whose median age was76.6 years (range 75-93), all diagnosed with NVAF. Forty-six (57%) were males and 34 (43%)females. Sixty out of 80 (75%) patients were treated with dabigatran, 8/80 (10%) with rivaroxaban 12/80 (15%) with apixaban, respectively. Treatment was effective and generally well tolerated; overall,adverse event (AE) rate was low (10%) with no grade 3-4 toxicity episodes recorded. Two patients (2.5%)discontinued treatment because of grade 2: gastro-intestinal bleeding (1) and renal failure (1), both the complications promptly resolved after treatment withdrawal. Six patients suffered from dyspepsia which in 5 required a shift to alternative NOACs to resolve. Prolonged exposure to NOACs did not affect renal function and after 1-year of treatment,GFR showed a significant degree of correlation (Spearman's r 0.65, p<0.0001) with the baseline one. This was particularly significant because the large majority of the patients (60%) received dabigatran which has a nearly exclusive renal excretion. These results confirm that treatment with NOACs is safe and effective even in a very old population of patients with a negligible withdrawal rate (<5%) and no significant impact on renal function even for the dabigatran exposed group.

P011

PERIPHERALLY INSERTED CENTRAL CATHETERS RELATED-THROMBOSIS IN ACUTE MYELOID LEUKEMIA: INCIDENCE AND RISK FACTORS

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Background: The use of peripherally inserted central catheters (PICCs) for the delivery of intensive chemotherapy to patients (pts) with acute myeloid leukemia (AML) has become ever more frequent in recent years. Although relatively safe, its use is associated with an increased risk of catheter-related thrombosis (CRT). *Design and Methods:* The aims of our study were: 1) to determine the incidence of CRT in a homogeneous series of AML pts in whom PICCs were inserted, 2) to identify risk factors associated with CRT development. This retrospective analysis included 136 AML pts who underwent placement of PICC from November 2008 till December 2014 because of new diagnosis requiring induction therapy (n 84=62%), delivery of consolidation course (n 27=20%) or salvage for relapse (n 25=18%). PICCs were implanted under ultrasonography guide and age, sex, phase of disease, body mass index (BMI), sepsis, infections at CVC-exit site, white blood cell count (WBCc) and platelets (PLTs) were the risk factors analyzed for a possible causal role in CRT development. Symptomatic CRT were confirmed by ultrasonography. *Results:* We observed 136 consecutive AML pts, 58 females and 78 males, median age 57 years (range 19-82), WBCc 5.1x10<sup>9/L (range 0,380-180x10<sup>9/L). The median duration of PICC indwelling was 92 days (range 2-439). We observed 14/136 (10%) episodes of CRT. Twelve (86%) of 14 episodes of CRT occurred during the induction/salvage phase. Of 136 pts, 97 (71%) experienced an episode of sepsis and 36 (26%) an episode of CVC exit site infection. We did not observe any correlation between CRT and age, sex, BMI, WBCc and PLTs. Instead, the occurrence of CRT was significantly associated with CVC-exit site infection (9/14, p=0.0007) and sepsis (13/14, p=0.05). Although not reaching the statistical significance, the highest frequency of CRT was observed in pts with active disease (during induction/salvage) rather than in CR (during consolidation). In multivariate analysis, only CVC-exit site infections was confirmed to be an independent risk factor for CRT development. In conclusions, CRT was reported in 10% of AML pts in whom a PICC was implanted, being CVC-exit site infection a major determinant for such a complication; presence of active disease may contribute to CRT via coagulation impairment. Further prospective and large studies are warranted to confirm our data.

P012

ECULIZUMAB INHIBITS THROMBOTIC MICORANGIOPATHY AND IMPROVES RENAL FUNCTION IN ADULT ATYPICAL HEMOLYTIC UREMIC SYNDROME PATIENTS: 1-YEAR UPDATE

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In the largest prospective study of adults with atypical hemolytic uremic syndrome (aHUS), eculizumab (ECU) prevented thrombotic microangiopathy (TMA), and improved renal function and hematological parameters by 26 weeks (wks). Here, we report a 1-yr update. Single-arm, Phase 2 trial of ECU in adult aHUS patients (pts); platelets <LLN at screening; prior plasma exchange/infusion and genetic screening were not required. The primary endpoint was complete TMA response at 26 wks. 41 pts treated, 38 (93%) completed 26 wks. At 1 yr, median treatment duration was 11.9 months. At Wk 26, 30 pts (73%) achieved the primary endpoint and at 1 yr, 33 pts (81%) (Table 1).

Table 1.

Baseline Demographics and Disease Characteristics	ITT (N=41)	
Age at first infusion of ECU (years), mean (SD)	40.3 (15.3)	
Female sex, n (%)	28 (68)	
Identified complement abnormalities, n (%)	21 (51)	
Time from aHUS diagnosis until screening (months), median (range)	0.79 (0.03–311.26)	
Newly diagnosed pts, n (%)	30 (73)	
Duration of current clinical manifestation to baseline (months), median (range)	0.52 (0.03–19.15)	
PE/PI during current clinical manifestation of aHUS to baseline, n (%)	35 (85)	
Dialysis at baseline, n (%)	24 (59)	
Prior renal transplant, n (%)	9 (22)	
Platelet count <150x10³/L, n (%)	27 (66)	
LDH >ULN, n (%)	32 (78)	
eGFR ≤60 mL/min/1.73 m², n (%)	41 (100)	
Efficacy Outcomes	**26 weeks**	**1-year update[a]**
Complete TMA response[b], n (%) [95% CI]	30 (73) [57–86]	33 (81) [65–91]
Modified complete TMA response[c], n (%) [95% CI]	23 (56) [40–72]	26 (63) [47–78]
Hematologic normalization[d], n (%) [95% CI]	36 (88) [74–96]	40 (98) [87–100]
Platelet count normalization[e], n (%) [95% CI]	40 (98) [87–100]	41 (100) [91–100]
Platelet count change from baseline (x10⁹/L), mean (SD)	135.1 (113.8) P<0.0001	116.9 (102.1) P<0.0001
eGFR increase from baseline ≥15 mL/min/1.73 m², n (%) [95% CI]	22 (54) [37–69]	25 (61) [45–76]
eGFR increase from baseline (mL/min/1.73 m²), mean (SD)	29.3 (23.6) P<0.0001	30.0 (27.2) P<0.0001
CKD improvement ≥1 stage from baseline, n (%) [95% CI]	26 (63) [47–78]	28 (68) [52–82]

[a]Mean (SD) duration of exposure was 13.2 (7.05) months; median (range) was 11.9 (2.9–28.8) months.
[b]Complete TMA response was defined by normalization of platelet count and LDH, and ≤25% increase in serum creatinine from baseline (renal preservation) on 2 consecutive measurements ≥4 weeks apart.
[c]Defined by normalization of hematological parameters (platelet count and LDH) and ≥25% reduction from baseline in serum creatinine (renal improvement) on 2 consecutive measurements ≥4 weeks apart.
[d]Defined by platelet count and LDH normalization for ≥2 consecutive measurements ≥4 weeks apart.
[e]Defined by platelet count ≥150 x 10⁹/L for ≥2 consecutive measurements ≥4 weeks apart.
CI, confidence interval; CKD, chronic kidney disease; LDH, lactate dehydrogenase; PE/PI, plasma exchange/plasma infusion; SD, standard deviation, ULN, upper limit of normal.

underwent TAVI *vs* "standard therapy" (medical and/or percutaneous valvuloplasty), in patients with haemorragic disorders. The is first case of TAVI to our knowledge in patient with haemophilia.

P016

THROMBIN GENERATION IN MYELOMA AND LYMPHOMA PATIENTS

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Background: Thrombotic complications often occur in patients with lymphoma and myeloma. In literature, evidences concerning with activation of haemostasis in lymphoproliferative diseases are poor. *Aims:* To investigate the haemostasis activation, in patients affected by lymphoma or myeloma, using laboratory tests able to detect an hypercoagulable condition, such as thrombin generation assay, prothrombin fragment (F1+2) and d-dimer. *Patients and Methods:* 21 patients with lymphoma and 17 patients with myeloma were studied and compared to 29 age-matched healthy subjects. Blood withdrawals were always performed before starting chemotherapy. Thrombin generation assay (TGA), F1+2 and D-dimer were evaluated as activation markers of haemostasis. TGA was performed using a fluorometric technique (Technothrombin TGA Kit, Technoclone, Austria) and the TGA RB Low reagent; F1+2 was performed by enzyme immunoassay (Siemens) an D-dimer by immune-turbidimetric assay (Stago). *Results:* About TGA test, patients show a statistically significant reduction of lag time and time to thrombin peak instead maximal concentration of formed thrombin (peak thrombin) was higher in patients than controls. F1+2 plasma levels were statistically higher in patients (301±281 pmol/L) than controls (131±43 pmol/L) (p=0.005). D-dimer was statistically higher in patients (1.6±1.1 ug/ml) than in controls (0.5±0.3ug/ml) (p=0.00005). No direct relationship was observed among respectively TGA, F1+2 and D-dimer plasma levels. Subsequently lymphoma from myeloma patients were splitted: TGA data remained statistically significant only for myeloma patients (p<0.004) while F1+2 and D-dimer plasma levels were statistically significant in myeloma patients (p=0.02; p=0.000001 respectively) and in lymphoma patients (p=0.01; p=0.002 respectively) if compared to control group. *Conclusions:* A hypercoagulability condition was observed in patients with lymphoproliferative diseases before starting chemotherapy; the observed hypercoagulability is more significant in myeloma patients. These data need to be confirmed in a wider group of patients.

P017

EVALUATION OF DIFFERENT INCIDENCE OF TROMBOPHILIC MUTATIONS IN PATIENTS WITH PULMONARY THROMBOEMBOLISM (PTE) AND WITH DEEP VEIN THROMBOSIS (DVT). CONFIRMATION OF THE FACTOR V LEIDEN PARADOX

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Introduction: The importance of thrombophilic mutations in pathogenesis of deep vein thrombosis (DVT) and pulmonary thromboembolism (PTE) is different. In a previous study we found that thrombophilic mutations are more frequent in idiopathic forms of DVT than in secondary forms, while they are distributed equivalently in different forms of PTE. Factor V Leiden (FVL) carries relatively higher risk of DVT, compared to the risk of isolated PTE (FVL paradox), while FVL mutation in PTE secondary to DVT would have an intermediate incidence. *Methods:* We evaluated the incidence of trombophilic mutations (heterozygous and homozygous FVL, prothrombin G20210A and the homozygous C677T in the MTHFR gene) in 89 patients (pts) with DVT and in 49 pts with PTE secondary to DVT. *Results and Discussion:* FVL mutation was present in 23.3% of pts with DVT and in 6.8% of pts with PTE (p:0,01) while FII mutation was present in 13.3% of pts with DVT and in 23.2% of pts with PTE (p: ns). MTHFR homozygous state was present in 26% of pts with DVT and in 25% of pts with PTE. Mechanisms of FVL paradox are not yet well known. Therefore we started a thromboelastogram (TEG) evaluation of the different phases of coagulation in FVL mutation carriers and in normal controls. Especially, analysis of fibrinolysis would be useful to highlight any differences in the subsequent lysis of the thrombus. Pts studied with TEG are currently too few to get results. They will be presented at the conference. Stratifying pts by the presence or not of predisposing factors it was observed, also on a larger series of pts, that presence of at least one of the three mutations is more frequent in idiopathic DVT than in secondary forms (40% *vs* 13.67%) while the same does not happen in PTE (13.95% in idiopathic *vs* 19.56% in secondary forms). In particular FVL mutation is completely absent in idiopathic PTE. Furthermore incidence of concomitant neoplasms was 23% in DVT and only 6.4% in PTE. This result requires confirmation on a larger scale, but it reflects the latest findings of literature. In conclusion, it is confirmed that PTE and DVT have different pathogenesis. FVL mutation and neoplasms incidence are significantly less frequent in PTE, than in DVT.

P018

POTENTIAL NEW MOLECULAR BENEFITS OF CONCOMITANT TREATMENT WITH MESOGLYCAN IN PATIENTS AT HIGH ATHEROTHROMBOTIC RISK

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Purpose: The pathogenetic role of vascular inflammation in the development of atherothrombosis has come to define the key action of endothelial line, particularly the glycocalix of glycosaminoglicans. Mesoglycan consists of natural glycosaminoglicans and it shows laboratory and clinical benefits in central and peripheral artery diseases. The aim of the current study was to compare coagulation, inflammation and endothelial dysfunction plasma markers from patients treated with standard therapy for acute coronary syndrome with those reported also to assume mesoglycan as concomitant medication, in order to evaluate the effects of combined treatments on hemostatic, fibrinolytic, and inflammatory networks. *Methods:* We retrospectively evaluated assays determined in 90 patients with a diagnosis of acute coronary syndromes, comparable for demographic and clinical characteristics, treated with conventional antithrombotic therapy (n=45, group B) and reported to concomitantly take also mesoglycan (n=45, group A) during a 12 months period. Laboratory markers of coagulation activation and inflammation were assayed twice in each patient (in the acute phase, at the beginning of treatment during hospitalization, and at six months follow-up). All the assays were performed in a centralized laboratory; patient demographic data and clinical characteristics were obtained from their medical records. Available records were related to the hospital admission date, primary diagnosis, comorbidities, treatment administered, concomitant treatments, treatment indicated at hospital discharge. *Results:* Laboratory parameters determined in groups A and B showed a statistically significant reduction (p<0.05) of the following coagulation and inflammation activation markers in group A *vs* group B at six months follow-up: fibrinogen (340.5±57.5 *vs* 415±55.5 mg/dL), coagulation factors VIII (145±27.5 *vs* 195±60.5%) and X (155±3 *vs* 5170.5±37.5%), von Willebrand Factor (155±47.3 *vs* 205±55%), activated Factor X (0.45±0.19 *vs* 0.62±0.27 mcg/ml), prothrombin fragment F1+2 (0.90±0.75 *vs* 1.97±1.5 nmol/L), serum adhesion molecules I-CAM (285±60 *vs* 305±62 mcg/mL) and omocysteine (14.3±5.5 *vs* 15.4±3.5 micromol/L). *Conclusions:* The current analysis shows that mesoglycan may improve outcome of patients with a recent history of acute coronary syndromes by reducing coagulation and inflammation activation markers.

P019

CHANGES IN ANNUALIZED BLEEDING RATE OVER TIME AND RELATIONSHIP WITH DOSING OF TUTOCTOCOG A DURING THE GUARDIAN PROGRAM

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Introduction: The guardian (TM) program is a large,multinational trial program designed to support NovoEight (R) registration, a new B-domain-truncated rFVIII. 214patients with severe hemophilia A without inhibitors were enrolled in guardian (TM)1 and guardian (TM)3; upon completion,participants could enter an extension trial (guardian (TM)2) that is ongoing. The trials primary endpoint was incidence of FVIII inhibitors (≥0.6 Bethesda Units) and a key efficacy endpoint was the annualized bleeding rate (ABR). *Methods:*For this post-hoc analysis, PTPs must have participated in guardian (TM)2 and either guardian (TM)1or3, had ≤1 week of surgery treatment in guardian (TM)1/guardian (TM)3, and had ≥3 months of exposure to NovoEight (R) prophylaxis during a selected time period in guardian (TM)2. Starting dose of NovoEight (R) was 20 IU/kg in guardian (TM)1 and was determined by the investigator in guardian (TM)3; doses were adjusted based upon clinical criteria,at the physician discretion. This analysis investigated the relationship between change in ABR from guardian (TM)1/guardian (TM)3 to guardian (TM)2 and average ABR during these periods. Change in ABR *versus* the ratio of mean weekly preventive dose was also investigated. *Results:* 166 patients met inclusion criteria for this analysis(111-guardian (TM)1 and 55-guardian (TM)3). Overall median ABR in guardian (TM)1 was 4.0 bleeds/patient/year (range:0.0–38.4), and in guardian (TM)3 was 3.8 bleeds/patient/year (range:0.0–34.7); overall median ABR during guardian (TM)2 was 1.6 bleeds/patient/year (range: 0.0 18.8). ABR by age is shown in Table1.The majority of patients had a reduction in ABR during the trials (Figure1). The patients with the highest ABR during the initial period were those with the largest reduction in ABR. The majority of patients showed an increase in dose over the analysed period (~20% compared with the initial dose). *Conclusions:* For PTPs on long-term prophylaxis with NovoEight (R), change in ABR over time may be correlated with initial ABR levels. Patients who initiated with high ABR levels tended to have the most pronounced reduction in ABR while, for others, low ABR was maintained.Older patients likewise tended to have a larger reduction in ABR than younger patients. No clear correlation was found between dose and ABR, indicating that dose changes may occur due to considerations other than bleeding frequency. Slight increases in dose over time likely represent individualized dose adjustments by physicians to optimize treatment outcomes.

Table 1. Median annualized bleeding rate and reduction over time.

	guardian™1/ guardian™3	guardian™2	% reduction during extension
Adults	4.2	1.3	69%
Adolescents	4.0	1.5	63%
Children 6–11 years	3.6	2.3	36%
Children ≤5 years	3.8	2.0	47%

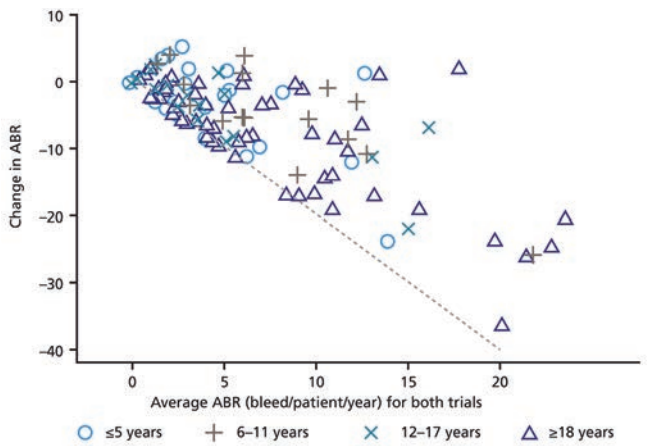

Figure 1. Change in ABR *versus* average ABR for guardian™1/ guardian™3 and guardian™2.

P020

EVALUATION OF SEROLOGICAL AND SYNOVIAL MARKERS, JOINT ULTRASOUND AND CLINICAL SCORES AGAIN SUPPORTS THE HYPOTHESIS THAT HEMOPHILIA B IS LESS SEVERE THAN HEMOPHILIA A

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Background: Historically hemophilia A (HA) and B (HB) have been considered clinically indistinguishable, with musculoskeletal bleeding, particularly joint bleeding, as hallmark of severe hemophilia. Some evidences, however, suggest that patients (pts) with HB may have a less severe bleeding phenotype, with fewer bleeding episodes and better long-term outcomes, compared to severe forms of HA. *Objectives:* To investigate differences in the severity of arthropathy in severe HB and HA. *Patients and Methods:* 35 HB (average age: 34.6 years) and 70 HA (33.5 years) pts with at least one joint bleeding were consecutively enrolled and the following parameters evaluated: joint bleedings (<10, 10-50, >50); regimen of treatment (prophylaxis/on demand); WFH, Pettersson and ultrasound scores; serum soluble RANKL and OPG, expression of RANK, RANKL and OPG in synovial tissue. Statistical analysis was performed by Chi-square test, T-test, Mann-Whitney and Spearman's rank correlation coefficient; a p-value <0.05 was considered statistically significant. *Results:* The percentage of pts with <10 hemarthrosis was significantly higher in HB compared with HA (p<0.0001), while that of pts with either 10-50 or >50 haemarthrosis was significantly greater in HA than in HB (p<0.001 and p=0.03, respectively). HB and HA pts treated with prophylaxis were 37% and 49%, while on-demand 63% and 51%, respectively. Mean WFH clinical score 20.2 *vs* 36.6 (p<0.0001), Pettersson score 5.7 *vs* 6.8 points, and US score 4.3 *vs* 10.9 (p <0.0001) were significantly lower in HB. Serum OPG (p<0.0001) and sRANKL levels (p=0.005 and p=0.006, respectively) were significantly decreased in HA compared to normal controls and HB, who showed similar values. The expression of RANK and RANKL in synovial tissue was similar in HB and HA, while OPG was markedly increased in tissue from HB. *Conclusions:* The reduced number of haemarthrosis at target joint and the lowest number of pts treated with prophylaxis in HB confirm the hypothesis that these patients may have a less severe disease. WFH and US scores suggest show a less severe arthropathy in HB than in HA pts. Reduction of OPG levels could play a pivotal role in arthropathy progression and be used as biomarker of disease severity.

Infections and Quality of Life

P021

EPIDEMIOLOGY AND CLINICAL OUTCOME OF LOWER RESPIRATORY TRACT INFECTIONS BY RESPIRATORY SYNCYTIAL VIRUS OR PARAINFLUENZA VIRUS TYPE 3 IN ADULTS RECEIVING TREATMENT FOR EITHER ACUTE LEUKEMIA OR SEVERE APLASTIC ANEMIA: A RETROSPECTIVE SINGLE CENTER STUDY

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Symptomatic respiratory syncytial virus (RSV) or parainfluenza virus type 3 (PIV3) infections occur in 1-12% and 2-7%, respectively, of adults with hematologic malignancies, mainly during HSCT, with progression to lower respiratory tract infection disease (LRTID) observed in at least one-third of cases and high average mortality (10-30%). We retrospectively analyzed the incidence of lung infiltrates and, subsequently, the frequency of detection of either RSV or PIV3 antigens on bronchoalveolar lavage (BAL) samples in 144 adults (median age 59 years, range 15-80), observed over a period of 5 years with either acute leukemia under intensive chemotherapy (135 patients) or severe aplastic anemia (9 patients) receiving immunosuppression. Lung infections observed during HSCT were not included in this study. Overall, 143 lung infections throughout the 330 administered cycles (namely 316 either induction, or consolidation or salvage chemotherapy regimens+14 immunosuppressive cycles) were observed. Eighty-nine patients experienced at least one pneumonia episode during neutropenia. BAL was performed in 97 cases, yielding positive results for any infectious agent in 64 cases (65.9%), whereas viral agents were documented in 28 cases (28.9%). RSV and PIV3 antigens were found in 5 (5.2%) and 9 (9.3%) BAL samples, respectively, collected from 13 different patients mainly during induction treatment. Among these 14 samples, bacteria and/or fungi were concurrently observed in 7 cases. Overall, LRTID associated with RSV or PIV3 were observed in 5/144 (3.5%) and 8/144 (5.5%) patients, 5/330 (1.5%) and 9/330 (2.7%) treatment cycles, 5/143 (3.5%) and 9/143 (6.3%) pneumonia episodes, respectively. Hypoxemia occurred in 9 cases, but non-invasive ventilation was necessary in only 3 patients, who eventually died, because of concurrent aspergillosis in one case and due to subsequent lung infiltrates, occurred after resolution of the viral infection in the remaining two cases, respectively. Eleven patients received oral ribavirin for a median time of 13 days (range 9-21), until viral infection resolution, without significant toxicities. Overall mortality within 30 days after RSV or PIV3 nosocomially acquired infection was 28.5%, but without direct virus-associated fatalities. In our series of neutropenic subjects with RSV- or PIV3-associated LRTID, a non-severe clinical behavior, with adequate antiviral therapy and in the absence of either co-infections or comorbidities, was observed.

P022

RISK FACTORS FOR SYMPTOMATIC CYTOMEGALOVIRUS REACTIVATION AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION IN MYELOMA AND LYMPHOMA PATIENTS: A SINGLE-INSTITUTION OBSERVATIONAL STUDY

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The huge increase of intensive and pleiotropic immunosuppressive chemo-immunotherapy in patients with lymphoma and the introduction of proteasome inhibitors and/or immunomodulators in the treatment of myeloma has led to an increase of viral infections in these setting of patients, particularly in the Herpesviridae family. However, current data on Cytomegalovirus (CMV) reactivation following autologous hematopoietic stem cell transplantation (ASCT) remain, so far, limited. To address this peculiar aspect, a retrospective observational study on a cohort of 327 adult patients consecutively transplanted for lymphoma (n=126) or myeloma (n=201) was performed in our Institution. Aim of the study was to determine the incidence of and the risk factors for CMV symptomatic infection and/or end-organ disease, defined according to published recommendations. CMV DNA load in the blood has been determined by PCR in the case of a clinical suspicion of reactivation, therefore, no routine monitoring strategy was adopted. Overall, 36 patients (11%) required specific antiviral treatment for a symptomatic reactivation (n=32) or an end-organ disease (n=4). Reactivation rate was of 16% and 8% in lymphoma and myeloma patients, respectively. Transplant-Related Mortality (TRM) was significantly higher in patients who experienced a CMV reactivation (8.3% *vs* 2.4%; P=0.047). In univariate analysis, a pre-transplant HBcIgG seropositivity, a diagnosis of T-cell non-Hodgkin lymphoma and higher median age at transplant were significantly associated with the risk of developing a clinically relevant CMV infection requiring specific antiviral therapy (P<0.001, P=0.042 and P=0.004, respectively). In multivariate analysis, only a pre-transplant HBcIgG seropositivity (OR: 0.112, 95%CI: 0.03-0.501; P=0.023) and a diagnosis of T-cell non-Hodgkin lymphoma (OR: 0.211, 95%CI: 0.09-0.661; P=0.05) proved to be an independent predictor of a post-transplant clinically relevant CMV reactivation. In conclusion, a clinically relevant CMV infection can occur in about 11% of adult patients with lymphoma or myeloma undergoing ASCT. Most of cases of CMV reactivation are easily manageable but it can be a potentially life-threatening complication. From our study, a pre-transplant HBcIgG seropositivity and a diagnosis of T-cell non-Hodgkin lymphoma should be considered as an independent predictor factor of clinically relevant CMV infection after ASCT.

P023

CARBAPENEM-RESISTANT ENTEROBACTERIACEAE INFECTIONS IN HEMATOLOGIC PATIENTS: RESULTS OF A PROSPECTIVE SURVEILLANCE PROGRAM

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Patients with hematologic malignancies are considered at high risk of severe infections sustained by carbapenem-resistant Enterobacteriaceae (CRE), mainly KPC-producing Klebsiella pneumoniae, because of chemotherapy-induced gastrointestinal mucositis, prolonged hospitalization, severe neutropenia and frequent use of broad-spectrum antibacterial agents. Intestinal carriage of CRE may precede infection, and carriers represent an important reservoir for dissemination of CRE in the hospital setting. Prompt identification of carriers is a key step in effective infection control. To evaluate CRE colonizations/infections and clinical outcome in hematologic patients, we conducted a prospective surveillance program from 2013 to 2014. For all patient, rectal swabs were collected using eSwab (Copan, Brescia, Italy) and placed in a transport medium. An aliquot of the transport medium was then plated on chromID CARBA SMART Agar (bioMérieux Marcy L'Etoil, France), and subjected to selective enrichment in broth containing meropenem (final concentration=2µg/mL), followed by plating onto chromID CARBA SMART Agar(bioMérieux). The presence of CRE (KPC-type, VIM-type, OXA-48 and NDM-type) was then confirmed by real-time PCR from suspected colonies grown on selective plates. During the studied period we documented a total of 31 CRE colonizations from rectal swab cultures: 11 in 2013 (11 KPC-type) and 20 in 2014: 16 KPC-type, 3 Citrobacter freundii (KPC-type and VIM-type producer) and 1 E. coli (KPC-type). In all patients with CRE colonization, quinolones prophylaxis was immediately stopped before starting chemotherapy. The incidence rate of CRE sepsis was 23% (7 KPC bacteraemia cases); of those 57% showed evidence of prior colonization. All patients with KPC sepsis were treated with a combination therapy including meropenem (extended infusion 2 g/every 8 h)-tigecycline-amikacin and in some cases also colistin and rifampin. Resistance rate to colistin was 85% and to gentamicin 28%. The mortality rate of KPC sepsis was 57%; these patients, all with severe and prolonged neutropenia, died because of a framework of multiple organ failure, with a median of 4 days from presentation until death. In conclusion CRE and mainly KPC are emerg-

ing life-threatening pathogens in severe neutropenic patients with a high mortality rate. Strict surveillance measures are essential to limit the spread of CRE among neutropenic patients. For this reason active surveillance of intestinal colonization, based on rapid diagnostic tests, is mandatory.

P024

EPIDEMIOLOGY AND OUTCOME OF MULTIDRUG RESISTANT PSEUDOMONAS AERUGINOSA BLOODSTREAM INFECTIONS IN PATIENTS WITH HAEMATOLOGICAL DISEASES

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Background: Multiresistant Gram negative bacteria are an emerging problem in haematological patients (pts). Among them, Multi Drug Resistant (MDR)-Pseudomonas Aeruginosa (PA) is a well-known cause of severe and potentially life-threatening infections, becoming in these last years one of the most worrisome phenomena, due to the scarcity of effective antimicrobial agents against this pathogen. *Methods and Results:* In this study we evaluate the epidemiology and outcome of bloodstream infections (BSI) due to MDR-PA in pts with haematological disease. Data regarding 256 BSI, involving 187 pts with various hematological disorders, were collected at our Division of Hematology and SCT over a 5-year period (Oct 2008-Oct 2013) within an observational prospective surveillance study. All the cases of BSI due to MDR-PA were identified and characterized for antibiotic susceptibility, distribution over time, severity of infection and outcome. We identified 316 bacteria in 256 BSI; PA (MDR and non-MDR) accounted for 36/316 isolates (11%), including 28/36 MDR-PA isolates (9% of the whole isolates and 78% of the PA isolates). Considering the whole MDR isolates (including KPC, E.Coli-ESBL, E. Cloacae-MDR, S. Maltophilia and Enterococci-VRE), MDR-PA isolates were 28/64 (44%). The antibiotic susceptibility of PA changed through the time: resistances (R) to Meropenem, Ceftazidime and Piperacillin-tazobactam increased significantly in the last 12 months of the study (11/2012-10/2013) compared to the previous (10/2008-10/2012) period (Meropenem–R=93% *vs* 48%, Ceftazidime-R=93% *vs* 38% and Pip-Tazo-R=7% *vs* 48%, p value<0,05), while susceptibility to Amikacine remained very good (only 8% of R) in the whole period of observation. Severe sepsis and mortality rates were significantly higher in MDR-PA-BSI compared to BSI due to non MDR Gram-neg bacteria (50% *vs* 12% and 46% *vs* 10% respectively, p<0.0001). The mortality rate for MDR-PA-BSI was higher in pts undergoing SCT compared to other hematologic pts (69% *vs* 17% respectively, P<0,001) *Conclusions:* MDR-PA-BSI are a re-emerging problem in hematological pts in these last years with a dismal outcome particularly in SCT cases (mortality rate of 69%). Taking into account the severity of this infection and the low efficacy of meropenem, ceftazidime and piperacilline-tazobactam, when the MDR-PA-BSI is documented or suspected (in PA colonized cases) a prompt intervention with effective drugs like Amikacine and Colistine is required.

P025

SEIFEM B 2012. A PROSPECTIC, MULTICENTRIC, OBSERVATIONAL STUDY ON FEBRILE EVENTS IN ACUTE LYMPHOBLASTIC LEUKEMIA. FINAL RESULTS:

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Objectives: Our purpose was to update the epidemiology of febrile events (FEs) occurring in acute lymphoblastic leukemia (ALL) patients undergoing different phases of treatment. *Methods:* From April 2012 to December 2013, in 20 Italian haematological centers, all consecutive patients, adult and pediatric, with a diagnosis of ALL were prospectively enlisted. Phase of treatment, chemotherapy, steroids, neutropenia, type and site of infection, treatment for FE and outcome of infection were documented. A statistical analysis was performed to identify risk factors. *Results:* We observed 316 ALL patients. Median age was 44 (range 3-78), M/F 1.2:1. Patients undergoing HSCT were withdrawn from this study. We collected 227 FEs occurring during 498 different phases of treatment in 147 patients (incidence 46%). The main epidemiological data are shown in Table 1.

Table 1. Main epidemiological data.

	FEs	Overall mortality	Attributable mortality
FEs	227(100%)	24/227(10.5%)	18/227 (8%)
Phase of treatment			
Before treatment	10(4%)	1 (4%)	1(5%)
Induction	100 (44%)	5(21%)	5(28%)
Consolidation/Maintenance	68(30%)	2(8%)	2(11%)
Relapse	41(18%)	9 (38%)	7(39%)
Refractory disease *	8(4%)	7(29%)	3(17%)
Treatment			
Aggressive chemotherapy	181(80%)	19 (80%)	14(78%)
Tyrosin-kinase inhibitors	7(3%)	1 (4%)	1(5.5%)
Aggressive chemotherapy+Tyrosin-kinase inhibitors	22(10%)	2 (8%)	2(11%)
Steroids only	1(<1%)	/	/
Other strategies	6 (3%)	1 (4%)	/
No treatment	10 (4%)	1 (4%)	1(5.5%)
Steroids			
Yes	142(63%)	20 (83%)	16(89%)
No	85(37%)	4 (17%)	2(11%)
Neutropenia			
Yes	192 (85%)	24(100%)	18(100%)
No	35(15%)	/	/
Infection			
Bacterial	90(40 %)	10(42%)	9(50%)
Fungal **	20(9%)	3 (13%)	2(11%)
Viral	6(2%)	1 (4%)	1(5.5%)
Mixed	13 (6%)	5 (21%)	3(17%)
Clinically documented infection ***	20 (9%)	2(8%)	1(5.5%)
FUO	78 (34%)	3 (12%)	2(11%)
Site of infection ****			
Blood	77 (52%)	10(48%)	8(50%)
Lungs	39(26%)	6 (28%)	6(38%)
Other	33 (22%)	5 (24%)	2(12%)

*:3rd/4th reinduction, contenitive therapy, salvage therapy.
**: Proven, probable, possible infection according to EORTC criteria.
***: 19 probably bacterial, 1 probably viral.
**** :for non FUO FEs.

Fever of unknown origin (FUO) was observed in 78 cases (34% of FEs, incidence 16%) including also FE arising prior to any treatment. Bacteria caused 109 microbiologically/clinically documented FEs (incidence 22%), fungal infections were 20 (incidence 4%, 14 molds and 6 yeasts: 6 proven, 10 probable, 4 possible), viral infections were 6 (incidence 1.2%). Mixed infections occurred in 13 cases, mostly fungal+bacterial infections (11/13 cases). As regards FEs in the different phases of treatment, 100 FEs (44%) arose when first induction therapy was administered; 68 (30%) during consolidation or maintenance, 41

(18%) during relapse and 8 FEs (4%) throughout therapy for refractory disease. Prolonged neutropenia was present in 192 FEs (85%), in 63% of cases steroid were administered and in 76% of FEs a central venous catheter (CVC) was placed. Overall mortality rate was 10.5% (24/227). 18 deaths occurred with evidence of infection (8%) more specifically, 10 (9.1%) due to bacteria, 2 (10%) to fungi, 1 to viruses (14.2%), 3 (23%) associated with mixed infection, 2 (2.5%) with FUO. Neutropenia, use of steroids and a refractory disease, significantly correlate with infection related mortality (p value 0.043, 0.006, 0.006 respectively). *Conclusions:* ALL patients can frequently develop FEs. Principal pathogens are bacteria but FUO rates remain high. Infectious complications occur frequently, however the mortality rate is lower than observed in other higher risk categories. Most likely the new aggressive therapeutic approaches to ALL could modify the infectious risk in this patients.

P026

OUTPATIENT EXPERIENCE WITH BIOSIMILAR FILGRASTIM IN PATIENTS WITH LYMPHOID NEOPLASMS: LESSONS FROM DAILY CLINICAL PRACTICE

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Filgrastim biosimilars are extensively used in routine clinical practice and they are recommended for the prophylaxis of febrile neutropenia (FN) in cancer patients undergoing chemotherapy. A monocentric observational study was conducted to evaluate the efficacy and safety of biosimilar filgrastim in patients with lymphoid neoplasms treated with different non-myeloablative chemotherapy regimens on an outpatient basis. Data from 141 consecutive patients were obtained from institutional database. Median age was 56 (range 15-86) years. Diffuse large B-cell lymphoma (47 patients), Hodgkin's disease (29) and follicular lymphoma (16) were the prevalent subtypes; among the remaining, there were 12 indolent non-follicular lymphomas, 11 mantle-cell, 7 T-cell and 2 Burkitt's lymphomas; 9 patients had hairy cell leukemia, 7 chronic lymphocytic leukemia, and 1 Langerhans' histiocytosis. A total of 148 chemotherapy lines were administered (7 patients received 2 lines), 123 were delivered first-line. The applied chemotherapies were: CHOP/COMP (38 cases), ABVD (25), fludarabine-containing regimens (22), third-generation CHOP-like regimens (20), cladribine (8) and ifosfamide-containing regimens (14). Other regimens were: bendamustine, ibritumomab, lenalidomide, high-dose cyclophosphamide or cytarabine. Filgrastim biosimilar was applied to prevent or treat FN in accordance with the European Society of Medical Oncology guidelines. Overall, 1,806 vials were used, at an average of 12.9 vials/patient. Biosimilar filgrastim was used for the prophylaxis of FN in 143 cases; in 5 cases (3.4%) it was used for the therapy of FN. All chemotherapy-induced cytopenias were managed on an outpatient basis; hospitalization rate was 5.4%, due to intensive treatment for FN. No treatment was interrupted because of persistent neutropenia or unrecovered myelotoxicity. FN was always transient and easily manageable with biosimilar filgrastim and intravenous antibiotics. No septic shock nor infection-related deaths were documented. Adverse events were infrequent, consisting of bone pain (4%), fever (1.3%) and tachycardia (0.7%). Drug administration was interrupted in 1 case due to an adverse event. Biosimilar filgrastim is easy and safe to use on an outpatient basis, with no clinically relevant adverse events and without significant differences from its originator. It shows efficacy in both prevention and treatment of FN among several chemotherapy regimens (Table 1).

Table 1.

Regimen	Patients treated	Vials used	Vials/patient (range)
CHOP/COMP	38	436	11.5 (3-18)
ABVD	25	442	17.7 (3-40)
Fludarabine-Mitoxantrone	15	184	12.3 (3-18)
VNCOP-B	14	160	11.4 (2-21)
IEV	14	83	5.9 (4-10)
Cladribine	8	150	18.8 (8-42)
Fludarabine-Cyclophosphamide	7	52	7.4 (3-13)
MACOP-B	6	54	9.0 (2-20)

P027

THE PSYCHOLOGICAL DISTRESS IN ONCO-HAEMATOLOGIC INPATIENTS: A STUDY ON ITS SCREENING BY MEANS OF THE DISTRESS THERMOMETER

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Background: The psychological distress screening is a crucial topic in psycho-oncology. The National Comprehensive Cancer Network (NCCN) has published guidelines on the distress management in oncology and has recommended the use of the Distress Thermometer (DT) for the screening. Although many studies concern oncological settings, scarce attention has been paid to psychological distress in onco- haematological patients. The present study involves a sample of onco-haematological adult patients admitted at the Haematological Division at the Cà Foncello Hospital in Treviso (Italy). *Methods:* Participants filled out the Distress Thermometer and the Problem List (usually associated to the DT) during their hospitalization. *Results:* The enrolled sample was composed of 102 consecutive inpatients (60.8% males and 39.2% females), with a median age of 59 years. 31% of the sample had an acute leukaemia, 31% a multiple myeloma, and 31% a lymphoma. The 41.6% of the sample displayed psychological distress: more in detail, the 21.8%, 13.8% and 12.9% of the sample displayed respectively a mild, moderate and severe distress. In the present sample, the distress score was found associated to neither gender nor diagnosis. *Conclusions:* Our data were substantially in line with results provided by Grassi *et al.* (2012) in their large validation study of the DT for our country.

P028

SUCCESSFUL THERAPY OF CHRONIC DISSEMINATED CANDIDIASIS IN HEMATOLOGIC PATIENTS WITH HIGH-DOSE LIPOSOMAL AMPHOTERICIN B: A RETROSPECTIVE STUDY OF SEIFEM-REGISTRY

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Background: Candida spp. is one of the most recurrent cause of invasive fungal infection in hematological patients.CDC is a complication of Candida infection with involvement of liver and spleen and rarely other organs. In this study we reviewed the clinical features of hematologic patients who developed CDC. Particular attention was focused on the identification of the best antifungal therapy for CDC. *Methods:* In this multicentric retrospective study, conducted in 9 Italian hematology units, we examined charts of hematological patients with CDC, according to the recent EORTC criteria, diagnosed from 2003 to 2012.The outcome measured was response to antifungal therapy within 30 days of CDC diagnosis.In addition, we evaluated the possible delay in hematologic disease chemotherapy plan due to the infection. *Results:* During the study period, 20 patients (M/F 11/9; median age 51 years, range 19-78) developed CDC. Patient characteristics are listed in the Table 1. All patients underwent ntensive chemotherapy and 6/20(30%) received cytarabine-based regimens. Before the infection, 14/20(70%) had severe neutropenia for a median time of 14 days (range 0-30). Antifungal prophylaxis was done in 14/20 patients: 65% of them had azoles in oral formulation. All patients were treated by systemic antifungal agents. Liposomal Amphotericin B(L-AmB) was given to 9/20(45%) patients; of them, 3 had standard dose(SD) L-AmB(3 mg/kg/day) and 6 received high dose(HD) L-AmB (5 mg/kg/day). Azoles were used in 6/20(30%) patients. Finally, 5/20(25%) patients were treated with Echinocandins. All patients treated with HD L-AmB (6/6-100%) had complete resolution of CDC infection; only 1 patient(16%) disrupted chemotherapy program for the infection. In the SD L-AmB group, there was 100% treat-

ment failure(1 death; 2 stable responses) and 1/3(33%) patient delayed chemotherapy for the infection. Echinocandins treatment resulted in complete resolution of infection in 2/5(40%) cases, partial response in 2/5 and failure in one case. In this group, 3/5(60%) patients did the planned chemotherapy. Patients who received Azoles had complete resolution of infection in 2/6(33%) cases and treatment failure in 4/6 cases; only3/6(50%) patients performed chemotherapy as planned. *Conclusions:* This study showed that HD L-AmB was the best therapy for CDC in hematologic patients: we observed complete resolution of infection in 100% of patients treated with this schedule and 84% of them continued chemotherapy program.

Table 1. Clinical characteristics in the 20 patients with CDC.

Case	Hematologic malignancy	Site of involvement	Microbiological findings	Specific diagnostic category
1	NHL	liver	C. Parapsilosis	PROBABLE
2	AML	liver, spleen	C. Krusei	PROBABLE
3	AML	liver	C. Parapsilosis	PROVEN
4	NHL	liver, bowel	C. Albicans	PROBABLE
5	AML	spleen	C. Albicans	PROVEN
6	AML	liver, spleen	C. Krusei	PROBABLE
7	MDS	liver, lung, brain	C. Glabrata	PROBABLE
8	AML	liver, lung	C. Albicans	PROBABLE
9	ALL	liver, kidneys, fundus oculi	C. Tropicalis	PROBABLE
10	MM	liver, lung	C. Krusei	PROBABLE
11	AML	liver	C. Krusei	PROBABLE
12	AML	liver, spleen	C. Albicans	PROVEN
13	ALL	liver, spleen	C. Albicans	PROVEN
14	ALL	liver	C. Albicans	PROVEN
15	AML	liver, spleen	C. Glabrata	PROBABLE
16	CLL	liver	C. Albicans	PROBABLE
17	AML	liver, spleen	C. Albicans	PROVEN
18	ALL	liver, spleen	C.Glabrata and Tropicalis	PROBABLE
19	AML	liver	C. Krusei	PROVEN
20	ALL	liver	C. Albicans	PROBABLE

P029

RESPIRATORY VIRUSES IN HAEMATOLOGICAL PATIENTS: IMPACT ON OUTCOME. RESULTS OF A SINGLE INSTITUTION'S 4-YEAR EXPERIENCE

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Respiratory viruses (RV) may be responsible of life-threatening complications among haematological patients (pts). In order to evaluate the impact of RV infections on outcome (30-day mortality) of haematological pts, we retrospectively analyzed all the cases of RV infections diagnosed from Jan 2011 to Mar 2015 at our Institution. Symptomatic pts were tested for Influenza (Flu), Parainfluenza (PIV), Respiratory Syncytial Virus (RSV) and Coronavirus (CoV). RV infections were extracted from a prospective database where all cases of infections occurred to pts admitted at our Institution are collected. The variables analyzed were: age, gender, underlying haematological disease, phase of disease, treatment, type of RV, neutropenia, lymphopenia, presence of pulmonary infiltrates (PI), respiratory failure, concomitant infections, intensive care unit (ICU) admission. Eighty-five RV infections were detected in 83 pts (M/F: 47/36; median age 59y). Underlying haematological diseases were: acute leukaemia 33, lymphoma 20, myeloma 20, chronic lymphocytic leukaemia 4, myelodysplastic syndromes 2, severe aplastic anemia 2, immune thrombocytopenia 1 and drepanocytosis 1. Fourteeen pts underwent ASCT and 63 received chemotherapy. Flu was isolated in 57 pts (29 H1N1, 18 and 10 not H1N1 Flu A and Flu B, respectively), PIV3 in 17, RSV in 14, CoV in 3. Overall PI were present in 50 (60%) pts, mainly in influenza (56%) and PIV (88%); in 25 cases respiratory failure occurred and 8 pts required admission at ICU. A concomitant mycotic infection was diagnosed in 7 pts. Nine out of 83 pts died (6 H1N1, 1 not H1N1 Flu A, 2 PIV3); only 1 among those receiving ASCT. At univariate analysis, H1N1 infection (p=0.029), respiratory failure and ICU admission (p<0.0001) were associated to an adverse outcome; uncontrolled haematological disease (p=0.099) and PI (p=0.07) showed a trend toward statistically significance. At multivariate analysis only ICU admission was an independent variable associated to death (p=0.01), whereas H1N1 and uncontrolled haematological disease were of borderline significance (p=0.079 and 0.082, respectively). Our study demonstrates that RV infections represent a potentially fatal complication also among non SCT pts. Progression to lower respiratory tract infection is frequent, mainly due to Flu and PIV. H1N1 infection seems to be more severe. Hematologists should be aware of this emerging problem and proper diagnostic and management guidelines should be implemented.

P030

INFECTIOUS COMPLICATIONS IN ELDERLY PATIENTS WITH MULTIPLE MYELOMA: A COMPARISON BETWEEN BORTEZOMIB INCLUDING AUTOLOGOUS STEM CELL TRANSPLANTATION AND CONVENTIONAL AUTOLOGOUS STEM CELL TRANSPLANTATION

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Introduction: Autologous Stem Cell Transplantation (ASCT) is still a mainstream strategy in Multiple Myeloma (MM) patients (pts) <60 years (yrs). However also fit elderly pts can benefit from ASCT including strategies.CY-BOR is a schedule currently used in our centre in fit pts >60 yrs, combining bortezomib(BOR), cyclophosphamide (CY) and dexamethasone (DEX); this schedule has been incorporated in a global strategy, in the context of a clinical prospective multicenter study, including induction and mobilizing therapy with CY-BOR, followed by ASCT with BOR-High-Dose-Melphalan (HD-MEL). As the use of BOR is associated with an increased risk of viral infections, we decided to retrospectively compare data concerning the engraftment (WBC and PLT) and infectious complications after ASCT in 2 groups of pts, treated according to either CY-BOR protocol or other conventional therapies, followed by ASCT with HD-MEL without BOR. *Methods:* The first group includes 34 pts (14M/20F), median age 66 yrs (52-75), who received three courses of CY-BOR as induction therapy, were mobilized with CY3g/m^2 (at day+8) and received ASCT with MEL(140-200mg/m^2 day-1) and BOR(1mg/m^2 days -6,-3,+1,+4). The second group includes 16 pts (12M/4F), median age 65 yrs (range 60-73), who were treated with different induction schedules not including BOR, were mobilized with CY(3-7g/m^2) and underwent ASCT with MEL (140-200mg/m^2) without BOR. *Results:* In the first group a median number of 5.0x10e6 (2,75-7,90) CD34+ cells/kg. Median time for PMN engraftment was 11 days (9-14) and 15 days(10-26) for PLT>20.000/ l. In 14 pts (41%) we did not observe neutropenic fever. We observed FUO in 9 pts and in 9 pts episodes of infections: 2 sepsis by s.bovis and s.mitis, 5 pneumonia, 1 asymptomatic CMV reactivation and 1 CMV related pneumonia. In the second group a median number of 3.79x10e6 CD34+ cells/kg (2.3-7.3) were infused. Median time for PMN engraftment was 11 days (9-13) and 12 days (10-16) for PLT>20.000/ l. We observed: 3 FUO and 2 pneumonia.In 11 pts(69%) we did not observe any infections. *Conclusions:* We did not observe any differences in engraftment time between the 2 groups. Pts treated according to the CY-BOR protocol experienced a slightly higher number of infectious complications (53% *vs* 31%).Although we cannot draw any statistical correlation due to the small sample, we can argue that BOR might induce a deeper immune suppression and consequently expose pts to a higher number of infectious complications.

P031

LOW-RISK MYELODYSPLASTIC PATIENTS SUPPORTED WITH ERYTHROPOIETIN PLUS LIPOSOMIAL IRON SHOWS A REDUCED NUMBER OF FEBRILE EPISODES THAN PATIENTS WITH INTRAVENOUS IRON SUPPORT

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Background: Intravenous iron support simultaneous to erythropoi-

etin administration improve hemoglobin response in myelodysplastic patients. Ther'are many evidences that iron, useful for bacterial growth, might increase risk of infection. *Objectives:* Aim of this study is to verify incidence of number of febrile episodes in low-risk myelodysplastic patients supported with iron. *Methods:* This study is a retrospective,multicentric study. Between july 2008 and december 2014, 107 patients affected by low-risk refractory anemia were studied. Median follow-up was 24 months (R12-60). 20 patients had no support, 27epo support, 30epo+liposomial iron (14 mg 2 tablets orally/day for 3 months), 15epo+iron sulfate (525 mg 2 tablets orally/day for 3 months), 15epo+iv sodium ferrigluconate (62.5 mg iv in NS100 ml in1h/day for 5day/month). Statistical analysis was performed by Chi Squre test and Fisher exact test. *Results:* In group with no support median packed red blood cells unit (PRBCU) transfused was 0.2/month (R0-0.5). Median number of febrile episodes/year was 1.5 (R0-2). In group supported with epo only medianPRBCU transfused was 0.4/month(R0-0.7). Median number of febrile episodes/year was2 (R0-2).In group supported with epo+iron sulfate medianPRBCU transfused was 0.3/month (R0-0.6). Median number of febrile episodes/year was 3(R0-3). In group supported with i.v. sodium ferrigluconate median PRBCU transfused was 1.5/month (R1-3). Median number of febrile episodes/year was 6 (R0-9). In group supported with liposomial iron medianPRBCU transfused was 0.2/month (R0-1). Median number of febrile episodes/year was1 (R0-2). *Conclusions:* Number of febrile episodes seem not related to basal neutrophil count or hemoglobin level reached after 3 month treatment. Number of febrile episodes is higher in group with higher transfusion need and in group treated with i.v. sodium ferrigluconate (p 0.02). Probably liposomial iron support provides a reduced amount of non- transferrin bound iron that might block bacterial growth. These data need confirmation on a larger cohort of patients.

P032

LIPOSOMIAL IRON SUPPORT IMPROVES FATIGUE IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES AS REFRACTORY ANEMIA. MULTICENTRIC STUDY

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Introduction: Fatigue is the most invalidating symptom in neoplastic disease. Fatigue frequently is linked to an iron deficiency. In inflammatory diseases as myelodysplastic syndromes fatigue might be linked to a functional iron deficiency with elevated ferritin level and a saturation of total iron binding capacity <20%. *Objectives:* Aim of this study is to verify if liposomial iron support in myelodysplastic syndromes as refractory anemia improves fatigue perception in patients with a saturation of total iron binding capacity <20%. *Methods:* Between june 2011 and december 2014, 20 patients affected by refractory anemia were studied. Median follow-up was 12 months (R10-24). Patients were randomized 1:1 to receive in groupA α erythropoietin 40000 IU sc/week+calcium levofolinate 7.5 mg/day orally+Vitamin B12:400 mg/day orally. In group B patient received liposomial iron 14mg 1 tablet orally/day+α erythropoietin 40000 IUsc/week+calcium levofolinate 7.5 mg/day orally+Vitamin B12:400 mg/day orally.In group A median age was60 years (R65-70), M/F:8/2. In group B median age was 66 years (R60-75), M/F:6/4. Caryotype was normal in group A and B patients. Median level of haemoglobin was 9 g/dl in group A (R8.5-11) and 8.8g/dl (R8.5-11.5)in group B.Fatigue was measured with Modified Fatigue Impact Scale (FISC - Fisk 1994). *Results:* Patients in group A reached a median hemoglobin level of11.5g/dl after 3 month of therapy and referred a median FISC score of74 (R65-80). Patients in group B reached a median hemoglobin level of12.5g/dl after 3 month of therapy and referred a median FISC score of54 (R42-68). *Conclusions:* Liposomial iron support improves fatigue perception in patients with refractory anemia. This study needs confirmation on a lager cohort of patients.

P033

PEGILATED-FILGRASTIM MAY PREVENT INFECTIONS IN WHIM SYNDROME: A CASE REPORT

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WHIM syndrome is a rare primary immunodeficiency disorder whose name is an acronym derived from its main clinical features, in particular Warts, Hypogammaglobulinemia, Infections, Myelokathexis (retention in the bone marrow of mature neutrophils resulting in severe peripheral neutrophil reduction). Typically, bone marrow analysis shows hyperplastic granulopoesis (GP), with hypergranulated polymorphonucleated cells and excess in GP precursors. Kariotype analysis is usually normal. Molecular studies demonstrated that the majority of WHIM syndrome cases are caused by autosomal dominant mutations truncating the carboxyl terminus of CXCR4, this driving to signalling alteration in the normal adhesion-promoting function of CXCR4; in particular the 5338X mutation on CXCR4 gene is implicated in the pathogenesis of the disease. M.M. is a 41-year-old woman diagnosed with WHIM syndrome in 2010. The patient's clinical history is characterized by recurrent bacterial infections since childhood, in particular cistitis, pneumonia, enteritis, sinusitis, and severe papilloma virus infections with warts and condyloma acuminata. Several expectoration cultural analyses had been positive for Staphylococcus aureus, Candida albicans, Pseudomonas aeruginosa. Moreover, due to immunodeficiency, an anorectal carcinoma was diagnosed. M.M. also underwent isterectomy, vulvectomy and vaginectomy for recurrent epithelial neoplasia. In order to prevent infections, M.M. had been treated with granulocyte colony stimulating factor (G-CSF), but she had to discontinue such treatment due to toxicity. Then we proposed the patient to start treatment with pegilated G-CSF, this allowing a non-daily administration. PEG-Filgrastim treatment was started in August 2010 and it was administered subcutaneously every 10 days with moderate fever and myalgia only on the day of administration. Since the start of this treatment infections became more infrequent, with only one hospitalization because of pneumonia. The monitoring of blood values revealed an absolute neutrophil count (ANC) increase after PEG-Filgrastim administration, with neutrophils stably over 1000/mmc. In this case report we describe, for the first time, a novel use of Peg-Filgrastim in WHIM syndrome. This approach resulted to be effective and safe in reducing infections. With the notable limitation of a single case, our data may be useful to further investigate the role of Peg-Filgrastim in patients with congenital neutropenia.

P034

(1-3)-β-D-GLUCAN DETERMINATION IN ONCO-HEMATOLOGIC PATIENTS AND ITS ROLE IN THERAPEUTIC STRATEGY: A PROSPECTIVE STUDY

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(1-3)-β-D-glucan (BDG) is a cell wall polysaccharide found in most fungi, detectable in the serum. Its use to detect invasive fungal infections (IFI) has a reported sensitivity usually lower than specificity (38-100%, 45-99% respectively); similar ranges are described for positive and negative (30-89% and 73-97%) predictive values. The EORTC-MSG guidelines recently included BDG among criteria for mycological evidence of infection. The aim was to evaluate the role of EORTC-MSG criteria with and without BDG in the diagnostic process that leads to antifungal treatment (AFT) in onco-hematologic patients (pts) with neutropenic fever (NF). We prospectively studied 104 consecutive onco-hematologic pts (August 2013-June 2014). Pts with NF underwent routine IFI diagnostic tests and were classified according to EORTC-MSG criteria as proven, probable or possible IFIs independently of BDG test. We collected samples at admission and sequentially after fever onset; BDG (Fungitell® assay) was determined at the end of the study. NF episodes were 49 (32 acute leukemia, 12 NHL, 3 Multiple Myeloma). Seventeen pts (35%) received AFT. Based on EORTC-MSG criteria independently of BDG test

results, a diagnosis of IFI was made in 12 (24.48%) pts: one proven IFI (8.33%), C. tropicalis in blood sample; 11 (91.66%) probable or possible IFI. Seven pts (14.28%) with NF underwent empirical AFT, not being classifiable as IFI, lacking one or more criteria. BDG assay was positive in 5 (10.2%) cases. BDG assay sensitivity and specificity were 22.22% and 94.28% respectively; PPV and NPV were 50% and 82.5% respectively. IFI classification of pts who developed fever was not modified after BDG inclusion, with the exception of one case upgrading from possible to probable IFI. BDG antigenemia can be a useful diagnostic tool if used in the proper clinical setting (ie, neutropenic pts) and performed by experienced personnel. Our data confirm that BDG has a high specificity and NPV, but low sensitivity and PPV. Seven out of 17 AFT were performed on a pure empirical basis: these cases did not respond to IFI criteria and had a negative BDG. Efforts in increasing BDG sensitivity, strengthening the reliability of its NPV, may contribute to avoid inappropriate AFT: larger studies are needed to confirm this. On the other hand, we observed an unsatisfactory PPV of BDG, that suggest the need of using BDG in combination with clinical, radiological, and microbiological findings.

P035

MYCOPLASMA PNEUMONIAE INFECTION ASSOCIATED WITH HEMOLYTIC ANEMIA IN AN ADULT PATIENT: CASE REPORT AND LITERATURE REVIEW

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Introduction: Mycoplasma Pneumoniae (MP) infections, frequently observed between 5 and 20 years, are rare in older age. MP causes respiratory disorders and, rarely, extrapulmonary manifestations (haematological, dermatological, neurological musculoskeletal, renal, cardiac and gastrointestinal) which can be observed before, during and after involvement of the respiratory tract. We describe a case of a MP infection, in an adult patient, characterized by simoultaneous hemolytic anemia and pneumonia. *Case Report:* A 36 year old man was referred to our clinic because of severe anemia (Hb: 3.9g/dl, MCV: 96fl) and worsening dyspnea (Sat O2: 94% with oxygen therapy 5L/min), developed in the previous 10 days. Anemia was associated with clinical and laboratory findings of haemolysis: jaundice, dark urine, positivity of direct and indirect Coombs test, LDH: 932 U/L; reticulocytes: 8%; haptoglobin: 0.1 g/L; total bilirubin: 4.6 mg/dl with indirect bilirubin: 3.9 mg/dl). CBC also showed thrombocytosis (PLT: $560x10^3/mm^3$) and neutrophilic leukocytosis (WBC: $37.8x10^3/mm^3$; PMN: $30x10^3/mm^3$). Sinus tachycardia (106b/min) and bilateral basal crackles at chest auscultation were reported. Neither hepatosplenomegaly nor lymphadenopaties were present. Chest CT scan showed bilateral micronodular lesions in the middle and basal regions of the lungs, "tree in bud" pattern opacities and a 5 cm lung consolidation at left. Anemia was treated with red blood cells transfusions (RBCs) (3 units), steroids (prednisone 1mg/kg/day) and intravenous immunoglobulins (i.v. Ig, 400 mg/kg/day for 5 days). Pneumonia was treated with empirical antibacterial therapy (piperacillin/tazobactam); after the detection of IgM positivity for MP, levofloxacin (i.v., 500mg/day) was administered. At patient's discharge, two weeks later, clinical, CT scan and laboratory improvement were observed, (Hb: 10.4g/dl, without laboratory findings of haemolysis). *Conclusions:* MP infections with coexistent pneumonia and hemolytic anemia are rare in adults (three cases reported in the literature between 1996 and 2015; PUBMED search: Mycoplasma Pneumoniae, hemolytic anemia, adult, case reports). Haemolytic anemia can be treated with RBCs transfusions (if life-threatening anemia), steroids and i.v. immunoglobulins. Pneumonia treatment consists of macrolide, quinolone or tetracycline. Even in adults, at onset of hemolytic anemia, it is useful to test for MP infection to deliver an adequate therapy.

P036

POSACONAZOLE AS PRIMARY PROPHYLAXIS REDUCES INVASIVE FUNGAL INFECTIONS IN AML PATIENTS: A SINGLE CENTRE MATCHED PAIRED ANALYSIS

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Prevention and prompt treatment of invasive fungal infections (IFI) in acute myeloid leukemia (AML) patients can improve overall survival by reducing infection-related mortality and allowing to receive full planned chemotherapy in a timely manner. Since January 2013, 35 AML patients undergoing intensive chemotherapy in our institute and potentially eligible for bone marrow transplantation received posaconazole (PSZ) as IFI prophylaxis. Non promyelocytic AML patients received a fludarabine, cytarabine and idarubicin containing regimen (FLAI) as first line treatment. M3 AML patients were treated according to GIMEMA AIDA 2000 protocol. PSZ was given at the standard dose of 200 mg for 3 times/day, concurrently with a fat snack or with at least 100 ml of an acidic drink. Because of unpredictable absorption rates PSZ serum levels (TDM) were assessed routinely. To detect factors affecting PSZ exposure we analyzed each period of hospitalization as a single independent event. PSZ showed a good tolerability profile. A median number of 3 TDM for each period of hospitalization was performed (range 2-6). In 39/65 (60%) episodes of prolonged neutropenia, with at least two TDM, the threshold PSZ serum concentration of at least 0,7 mcg/mL was reached, with stable plasmatic levels. Median PSZ plasmatic value at first assessment was 0.73 mcg/mL (range 0.1-3.9). The strongest negative factors affecting PSZ absorption are the temporary discontinuation of prophylaxis and the concomitant assumption of proton pump inhibitors. We retrospectively compared through a matched-paired analysis 22 non M3 AML patients treated with PSZ as IFI prophilaxys to a control historical series of 22 patients who had received fluconazole (FLC). The total number of neutropenic periods was 46 In PSZ cohort and 50 in FLC cohort. Patients median age, sex and days of severe neutropenia were not significantly different between the two cohorts. No proven or probable IFI were observed in the PSZ cohort, compared to 12% (6 IFI episodes over 50 severe neutropenic periods) in FLC cohort ($p<0.05$). Mean days of targeted/empirical intravenous antifungal therapy for single patient in PSZ cohort was 0 days *Vs* 2.2 days in FLC/ITZ cohort ($p<0.05$). Our clinical experience confirms the benefit and potential cost-effectiveness of primary prophylaxis with PSZ in AML patients receiving intensive treatment, as no patients in PSZ cohort experienced IFI nor received empirical intravenous antifungal therapy (Table 1).

Table 1. Historical comparison.

Regimen	Patients treated	Vials used	Vials/patient (range)
CHOP/COMP	38	436	11.5 (3-18)
ABVD	25	442	17.7 (3-40)
Fludarabine-Mitoxantrone	15	184	12.3 (3-18)
VNCOP-B	14	160	11.4 (2-21)
IEV	14	83	5.9 (4-10)
Cladribine	8	150	18.8 (8-42)
Fludarabine-Cyclophosphamide	7	52	7.4 (3-13)
MACOP-B	6	54	9.0 (2-20)

P037

COST-EFFECTIVE ANALYSIS OF PROPHYLAXIS WITH LAMIVUDINE FOR PREVENTION OF REACTIVATION IN OCCULT HEPATITIS B IN PATIENTS WITH NON-HODGKIN LYMPHOMA CD20+ UNDERGOING CHEMOTHERAPY

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Occult HBV infections (OBI) are defined by the persistence of HBV in the liver without serum HBsAg and HBV-DNA. They can represent a life threatening event during immunosuppressive CHT. OBI occur in approximately 18% of HBcAb+ patients. Guidelines suggest surveillance for HBV markers in immunosuppressed patients, in particular in treatment with monoclonal antibodies. In our study, the prevalence of OBI reactivation in NHL, in 498 patients of our centre, was 10.42% in HBcAb+ HBsAb- patients. In this work, a cost-effectiveness analysis regarding the use of Lamivudine for the prevention of reactivation in OBI in patients with NHL undergoing chemotherapy with or without Rituximab was performed. In fact, considering guidelines and literature, universal prophylaxis should have been applied

to all HBcAb+HBsAg - patients. A cost-benefit issue arises: is it more cost-effective to treat all the HBcAb+HBsAg - patietns with Lamivudine to prevent the OBI reactivation occurence in a small quote of them, or may it be more effective a "wait and see" protocol? Our idea was to perform a cost-effectiveness analysis, comparing the costs of prophylaxis of an eventual HBV reactivation and the "monitoring" approach that was used in our patients based on international guidelines. The cost of Lamivudine prophylaxis was calculated in a time interval of 12 months, which encompasses the time of a standard Rituximab-containing regimen and a minimum time of follow-up. It has been noticed that, very often, NHL patients need more than one treatment to obtain remission, and, sometimes, if they do not obtain a CR, undergo to long-term "maintenance" with Rituximab. These patients (HBcAb +) are at high risk of HBV reactivations, due to long periods of immunosuppression. Nevertheless, even if our calculations underestimated the costs of prophylaxis, the "monitoring approach" resulted cost-effective. Moreover, even though in our series no serious events in terms of morbidity and/or mortality occurred, in other papers a monitoring approach did not guarantee patients survival. These detrimental results could be ascribed to the delayed start of lamivudine treatment if the monitoring is not adequately strict. Also, it has been reported that performing only the transaminase monitoring should not be acceptable to prevent severe reactivations. Our monitoring approach resulted efficacious probably because of the monthly ALT assay was strictly observed (Table 1).

Table 1.

	Unitary Cost	n. patients	Total per patient	Duration [days]	Total
Cost of prophylaxis					
Lamivudine	€ 3,18	48	€ 152,64	360	€ 54.950,40
HBV DNA monitoring	€ 130,00	48	€ 6.240,00	6	€ 37.440,00
HBsAg monitoring	€ 17,00	48	€ 816,00	6	€ 4.896,00
AST/ALT monitoring	€ 5,74	48	€ 275,52	12	€ 3.306,24
Total	**€ 155,92**	**48**	**€ 7.484,16**		**€ 100.592,64**
Cost of HBV Reactivation					
HBV DNA monitoring	€ 130,00	48	€ 6.240,00	6	€ 37.440,00
AST/ALT monitoring	€ 5,74	48	€ 275,52	12	€ 3.306,24
Cost of DRG 205 [+24 Groups]	€ 3.709,33	5	-	-	€ 18.843,50
Total	**€ 3.904,84**	-	-	-	**€ 60.746,24**

Drg 205: Liver disease except malignancies, cirrhosis, alcoholic hepatitis with cirrhosis.

P038

PEGFILGRASTIM IN PRIMARY PROPHYLAXIS OF FEBRILE NEUTROPENIA DURING RITUXIMAB-BENDAMUSTINE TREATMENT IN INDOLENT NON HODGKIN LYMPHOMA: A REAL-LIFE EXPERIENCE

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Febrile neutropenia (FN) is a serious side effect of chemotherapy, and even when it does not result in significant morbidity, mortality and costs, it normally leads to a delay in chemotherapy treatments. Pegfilgrastim is a pegylated long-acting recombinant form of G-CSF which extends the half-life, requiring less frequent dosing. The objective was to evaluate the efficacy and safety of pegfilgrastim in newly diagnosed patients with indolent NHL, in treatment with R-Bendamustine (RB), in order to determine whether a single injection of pegfilgrastim is as effective as daily injections of filgrastim, in terms of toxicity, febrile neutropenic episodes, antibiotic usage, and hospitalization duration. 47 patients (25 M/22 F), median age 47.4 years (range 33-87), since first course of treatment performed blood counts twice weekly and received, d8-d19, prophylactic oral quinolones and anti-fungal drugs. During neutropenia, filgrastim (5 µg/kg/d for at least 3 days) was given "on demand" if neutrophils count was <1000 x 10^9 cells/L. Median number of filgrastim administrations was 3.4 (r.3-5); nadir neutropenia was registered after a median of 9.1 d. (r.8-15); median of nadir neutrophil count was 1.22 x 10^9 cells/L (range 0.3-1.7 x 10^9 cells/L), with maximum duration of 11 d. From the second course, all patients switched to pegfilgrastim (6 mg) prophylaxis, injected subcutaneously with a single administration on day+4. During pegfilgrastim, neutropenia was never longer than 7 days, with a consequent reduction of risk of infections. Median nadir neutrophil count, evaluated for at least 3 courses of therapy (r.3-6) registered at d+ 11, was 1.734 (range 0.88-2.11 x 10^9 cells/L); only six patients (12.7%) needed, after pegfilgrastim, a supplement of 3 administrations of filgrastim. During pegfilgrastim, neutropenia, when present, was shorter than during filgrastim treatment (median of 3.7 d, range 3-8). Pegfilgrastim was well tolerated: main side effects were mild fever and bone pain (7/47: 15%). Moreover, no hospitalization was needed during pegfilgrastim, while two hospitalization for pneumonia were needed during filgrastim. During observation, no patient died during filgrastim or pegfilgrastim. In patients affected by newly diagnosed patients indolent NHL, in treatment with RB, pegfilgrastim seems to reduce the incidence of neutropenia, is better tolerated and may increase the possibility to maintain the schedule of treatment.

P039

LOCAL MICROBIAL EPIDEMIOLOGY AND THE EMERGENCE OF MULTI-RESISTANT BACTERIA IN PATIENTS HOSPITALIZED IN SINGLE HEMATOLOGY UNIT

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Febrile neutropenia is associated with high mortality in hematologic patients, expecially for septic shocks by multi-resistant pathogens. We analize 250 consecutive patients admitted to our Haematology ward during 2014 (25% acute leukemia, 20% lymphoma, 12% myelodisplastic syndrome, 28% Multiple myeloma, 15% autologous peripheral steam cell trasplantation) to study the local epidemiology of a peripheral hospital of south Italy. We therefore screened 250 patients with severe haematologic diseases for colonization at admission and during febrile neutropenia and we obteined laboratory-confirmed coltural and bloodstream infections. 25 patients (10%) were positive with colture, but only 12 (4%) subsequently developed a Pseudomonas aeruginosa or Klembsiella pneumoniae multiresistent bloodstream infection during febrile neutropenia with sepsis. Cumulative incidence of developing at least 1 episode of Blood Stream Infexction by Klembsiella Pneumoniae (70%) Escherichia coli (9%) and Pseudomonas sp. (21%) were the most frequently isolated among gram negative rod. Routine screening for P. aeruginosa and Klembsiella P. at admission did not sufficiently predict subsequent bloodstream infections caused by those phatogens, but could be an alarm to monitor better those patients during neutropenia postchemotherapy. The incidence, local epidemiology, and risk factors(the use of central venous catheters, febrile neutropenia post chemotherapy,high risk disease as acute leukemia are the key issues to control local microbial epidemiology and the emergence of multi-resistant bacteria.

P040

HIGH INCIDENCE OF CYTOMEGALOVIRUS REACTIVATION IN PATIENTS WITH LYMPHOMA TREATED WITH BENDAMUSTINE

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Bendamustine is an anticancer drug, which has recently evolved as an important agent for a number of lymphoid malignancies in Europe and the USA. The drug consists of an alkylating nitrogen mustard group bound to a purine-like benzimidazole ring, and because of this unique bifunctional structure the bendamustine activity profile is significantly different from classical alkylators. The clinical studies published so far have reported fairly low or mild toxicity of bendamustine-containing regimens. In general, bendamustine seems to present a favourable toxicity profile.The purpose of our study and evaluate the reactivation of CMV in patients with lymphoma treated with bendamustine. From January 2012 to March 2015 we have treated in our Division with bendamustine in monotherapy or in combination with Rituximab, 72 patients (27F and 45M) with a median age of 71 years (range 50-87 years). 18 CLL; 47 B-NHL; 5 T-NHL and 2 MM. In all patients was evaluated serologically set-up of the CMV and the 80% of the patients tested positive for IgG, no patient tested positive for IgM. Of the 72 patients treated are evaluated, for CMV reactivation, 60 patients (22 F 28 M and with median age of 71 years old; 14 CLL; 41 B-NHL; 3 T-NHL and 2 MM). It is documented reactivation in 22/60 Pts (36%); the reactivation was only of laboratory or even clinically in 10 (16%) and 12 (20%) patients respectively. All patients with clinical reactivation of CMV have stopped chemother-

apy and have started specific antiviral treatment (i.v. ganciclovir or o.s. valganciclovir). 6/12 pts (50%) are deads from complications related to reactivation. No patient who made single agent bendamustine (7 patients) has documented reactivation of CMV. The reactivation of CMV was documented in media after the third treatment cycle. In conclusion the association bendamustine and rituximab shows a high incidence of reactivation of CMV, this reactivation, if not treated quickly, is a serious complication for these patients (50% of deaths CMV related) this can depend, in these patients, from age and from precedent treatments. From these preliminary data suggest a CMV-DNA monitoring in patients with lymphoma treated with Rituximab and Bendamustine.

Chronic Lymphocytic Leukemia and Lymphoproliferative Disorders 1

P041

BENDAMUSTINE MONOTHERAPY IN ELDERLY PATIENTS AFFECTED BY RELAPSED OR REFRACTORY B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Chronic lymphocytic leukemia (CLL) is characterized by a highly variable clinical course. Among the biological features underlying this heterogeneity, genetic lesions and the mutational status of the immunoglobulin heavy chain variable genes (IGHV) are of importance. Therapeutic options in CLL have been considerably expanded during recent years. The combination of fludarabine, cyclophosphamide and rituximab (FCR) has become gold standard in the first-line treatment of physically fit patients. Bendamustine demonstrated clinical activity in pre-treated hematological malignancies due to its unique mechanism of action distinct from standard alkylating agents. Aim. We have assessed the efficacy and safety of bendamustine in elderly patients with pre-treated chronic lymphocytic leukaemia. *Methods:* In the last 48 months we treated 28 elderly patients (12 F and 16 M, median age: 79 years, r.: 75-90 years) with relapsed/refractory CLL who had been heavily pre-treated (more than 4 lines of treatment). Patients received bendamustine 90 mg/m(2) on days 1 and 2 of a 4-week cycle. 18/28 patients received six cycles, while the remaining two received four cycles. *Results:* Overall response rates were 80%, with clinical complete response rates of 50%. At present, 20/28 patients are alive and show no disease progression. One patient died due to respiratory infection six months after the end of therapy. Thrombocytopenia and gastrointestinal toxicities were more frequent adverse events. Grade ≥3 adverse events were infrequent and most commonly included thrombocytopenia (20%), anemia (10%), and infection (10%). *Conclusions:* Our results suggest that the bendamustine monotherapy is effective and well tolerated in the treatment of relapse/refractory CLL in elderly patients. Although further data are required to fully establish the efficacy of intravenous bendamustine in the management of this subset of patients, it appears to be a useful addition to the armamentarium of currently available therapies for this haematological malignancy.

P042

RITUXIMAB PLUS BENDAMUSTINE IN PATIENTS WITH RELAPSED OR REFRACTORY WALDENSTRÖM'S MACROGLOBULINEMIA

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Background: WM is an incurable disease, with an overall medial survival of only 5-6 years. Age, hemoglobin level, platelet count, (2) microglobulin, and monoclonal IgM concentrations are characteristics required for prognosis. First-line therapy of WM has been based on single-agent or combination therapy with alkylator agents (*e.g.* chlorambucil or cyclophasphamide), nucleoside analogues (cladribine or fludarabine), and the monoclonal antibody rituximab. Novel therapeutic agents that have demonstrated efficacy in WM include thalidomide, lenalidomide, bortezomib, everolimus and bendamustine. *Methods:* We report the treatment outcome for 20 (12 male, 8 female; median age: 72y, range: 66-78) relapsed/refractory Waldenström's macroglobulinemia (WM) patients. Treatment consisted of bendamustine (90 mg/m(2) I.V. on days 2, 3) and rituximab (375 mg/m(2) I.V. on day 1) for all patients. Two rituximab-intolerant patients received bendamustine alone. Each cycle was 4 weeks, and median number of treatment cycles was 4. *Results:* The clinical stage (remission, progression or stable disease) was defined with clinical re-evaluation after chemotherapy and re-staging 6 months after end of therapy. At best response, median serum IgM declined from 3500 to 500 mg/dL, and hematocrit rose from 30.1% to 37.2%. Overall response rate (CR+PR) was 84%. Overall therapy was well tolerated. Prolonged myelosuppression was more common in patients who received prior nucleoside analogues. *Conclusions:* Bendamustine in combination with Rituximab demonstrates an excellent effectiveness in previously treated WM patients, with an acceptable tox-

icity profile. These agents, when compared to traditional chemotherapeutic agents, may lead in the future to higher responses, longer remissions and better quality of life for patients with WM.

P043

THE ROLE OF MICROENVIRONMENTAL STIMULI IN INFLUENCING THE EXPRESSION OF ENDOTHELIN 1 IN CHRONIC LYMPHOCYTIC LEUKEMIA CELLS

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Introduction: The axis of endothelin 1 (ET-1) and its receptor A has recently been demonstrated to be up-regulated and to induce a prolonged survival in chronic lymphocytic leukemia (CLL) cells. In order to investigate if extracellular stimuli could influence ET-1 expression in CLL cells, we studied the effect of the activation of CD40, toll-like receptor (TLR) or B cell receptor (BCR) pathway on the regulation of this gene. *Methods:* Leukemic B cells were purified from peripheral blood of untreated CLL patients and cultured in medium alone or stimulated with CpG oligonucleotides+IL-2 (CPG), anti-IgM (IGM) or CD40L+IL-4 (CD40). In some experiments CLL cells were pre-treated with wortmannin for AKT inhibition, PD98059 for MEK1 inhibition or BAY 11-7085 for NF-kB inhibition. We valued ET-1 expression by Real-Time PCR, flow-cytometry and immunofluorescence. IkB α phosphorylation and degradation were analyzed in Western Blot, as sign of NF-kB activation. *Results:* We found a significant increase of ET-1 mRNA expression in CLL cells after 4H of stimulation with CD40 (p=0.028) or CPG (p=0.018) but not with IGM, in comparison to controls. Concordantly, ET-1 immunofluorescent staining was increased in CLL cells stimulated with CD40 and ET-1 flow cytometric amount was significantly higher in leukemic cells stimulated with CPG ($p<0.05$), if compared to unstimulated ones. To identify the signal transducer downstream of CD40 pathway, responsible for ET-1 induction, we pre-treated CLL cells with AKT, MEK1 or NF-kB inhibitor before CD40 stimulation and we found that the ET-1 mRNA augment was strongly abolished by BAY 11-7085 ($p<0.05$) but not by wortmannin or PD98059: this was also confirmed by ET-1 immunofluorescence. Then we wondered if BAY 11-7085 could affect ET-1 expression in not stimulated CLL, since NF-kB is known to be constitutively activated in these cells. After a pre-treatment with the inhibitor and 4H of culture, we observed lower ET-1 mRNA and protein levels in presence of BAY 11-7085 ($p<0.05$ and p=0.012) than in absence. We finally demonstrated that CD40 stimulation increases NF-kB activation in CLL cells whereas BAY 11-7085 inhibits this event. *Conclusions:* These results demonstrate that: i) the activation of CD40 or TLR pathway induces an early augment of ET-1 expression in CLL cells, ii) the ET-1 induction by CD40 stimulation is mainly due to NF-kB activation, iii) NF-kB is important to maintain ET- 1 expression also in not stimulated CLL cells.

P044

EVANS SYNDROME SECONDARY TO CHRONIC LYMPHOCYTIC LEUKAEMIA IS ASSOCIATED WITH ADVERSE BIOLOGICAL FEATURES AND OUTCOME

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Secondary Evans syndrome (ES) is characterised by simultaneous or sequential development of autoimmune haemolytic anaemia (AIHA) and immune thrombocytopenia (ITP) in the presence of an underlying aetiology. As opposite to isolated AIHA and ITP, that can frequently complicate chronic lymphocytic leukaemia (CLL) course, ES secondary to CLL is an extremely rare occurrence, with very few available epidemiological and clinical data. In this study, we retrospectively searched for patients developing ES among consecutive patients with CLL diagnosed and followed up in two major Institutions from Veneto region between 2000 and 2014. Of the 860 patients with CLL that were analysed, 25 (2.9%) developed ES. Additional 32 patients (3.7%) developed isolated ITP, and 63 isolated AIHA (7.3%). Demographic and clinical characteristics at CLL presentation of patients developing either ES, ITP or AIHA were similar, and not different from patients not developing autoimmune cytopenias (AIC). ZAP70 expression was high in 79% of patients with ES, being significantly higher than in patients with no AIC (50%, p=0.01), but similar to patients with isolated AIHA or ITP. Immunoglobulin heavy chain variable region gene (IGHV) was un-mutated in 86% of patients with ES, which was significantly more prevalent than in patients with no AIC (41%, p=0.001), or compared to patients with ITP or AIHA. Furthermore, del(17)(p13) and TP53 gene mutations were significantly more represented in patients with ES (23% and 33% of tested patients, respectively) than in patients with no AIC (5% for both, p=0.003). In terms of overall survival (OS), patients developing ES had a significantly inferior OS than patients with no AIC (Figure 1), but similar to patients with isolated AIHA or ITP. No impact on OS was observed for patients with ES according to time of onset of each AIC (simultaneous ITP and AIHA, ITP before AIHA or vice versa). However, patients with ES diagnosed concurrently to CLL presentation had a significantly inferior OS compared to patients developing ES later in the course of disease (5 years OS 40% *vs* 74%, p=0.006). Treatment of ES was challenging, due to the quite high rate of primary resistant forms and frequent recurrences. In conclusion, ES secondary to CLL is an infrequent but not so rare occurrence, that is significantly associated to unfavourable biological prognostic factors. ES represents a difficult-to-treat complication, which may impair the final outcome of patients with CLL.

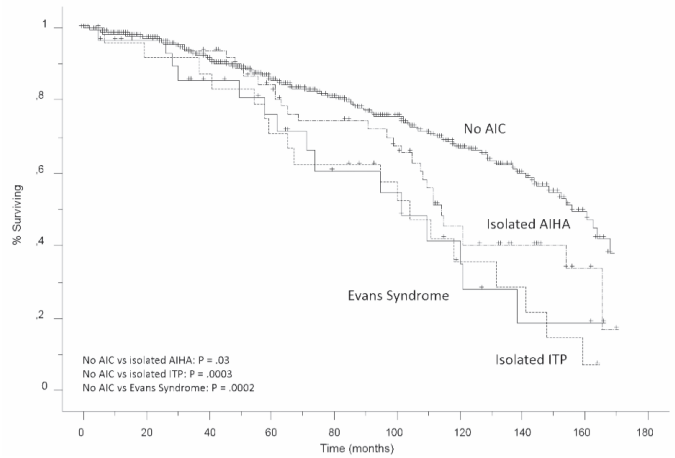

Figure 1. Overall survival in patients with chronic lymphocytic leukaemia non developing autoimmune cytopenias (AIC) (740), compared to patients with isolated AIHA (63), isolated ITP (32) or Evans syndrome (25).

P045

RETROSPECTIVE ANALYSIS OF SECOND CANCERS IN 747 PATIENTS WITH CRONIC LYMPHOCYTIC LEUKEMIA

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Background: Chronic lymphocytic leukemia (CLL) is characterized by a chronic immunodeficiency of innate ad adaptive immunity, which causes an increase risk of infections and second cancers (SC). *Aims:* The aim of this study was to describe the risk of SC in patients with CLL. *Methods:* We retrospectively analyzed clinical and biochemical data of 747 patients. FISH analysis (n=492), CD38 expression (n=612), ZAP70 (n=513), IGHV (n=480), TP53, NOTCH1, BIRC3, SF3B1 (199 tests) mutational status, were evaluated at diagnosis or before starting treatment. We used Mann-Whiney, Fisher exact or Chi-square, Log-rank test, Kaplan-Meier method and Cox model. Time to SC (TTSC) was calculated from the date of initial presentation to the SC (event) or last known fol-

low-up (censored). Overall survival (OS) was calculated from the date of initial presentation to the death (event) or last known follow-up (censored). Combined antibody deficiency (CAD) means low levels of IgG and IgA or IgM. *Results:* 133 (18%) patients experienced 157 SC, of which 64% developed after CLL diagnosis. The list of SC is shown in Table 1. By Kaplan-Meir analysis we observed that the 10-year TTSC was 15% but rose to 37% after 20 years from diagnosis. When patients were stratified by tumor kind the highest risk was observed for non-melanoma skin cancer (TTSC at 20 years was 25% *vs* <10% for all other SC, p<0.001). Some clinical and biological markers were more common in patients with a history of SC (gender [p=0.008], Rai III-IV [p=0.019], Binet C [p=0.041], 17p deletion [p<0.001], need for treatment [p=0.025]) than patients without any SC. No differences were found for ZAP70 expression, TP53, NOTCH1, BIRC3, SF3B1 gene mutations. Patients who need a specific CLL therapy and subjects with CAD had a shorter TTSC with respect to treatment naïve subjects (Figure 1A, 17 years *vs* not reached, p<0.029, hazard ratio [HR] p=0.620) or those without CAD (Figure 1B, 16 years *vs* not reached, p<0.002, HR 2.01, p=0.002). Furthermore, those patients who developed a SC after CLL diagnosis had an adverse outcome and a shorted OS than all other patients (median OS 23 *vs* 39 years, p=0.034). *Discussion:* We provide evidence that previously treated patients with unfavorable prognostic markers and CAD carried the highest risk of developing SC. These data suggest that chronic immunodeficiency of CLL patients could only in part be related to the disease itself but could be worsen by chemo-immunotherapy.

Table 1.

	NMSC	Hem. M.	Gastro.	Genit.	Respi. T.	MSC	Brest C.	Others	Total
BEFORE	10	7	12	6	4	6	3	8	56
AFTER	33	18	17	11	9	3	5	5	101
TOTAL	43	25	29	17	13	9	8	13	157

NMSC = non melanoma skin cancer; Hem.M.= hematological malignancies; Gastro. = gastrointestinal cancers; Genit. = Genitourinary cancers; Resp. T. = respiratory tract cancers; MSC = melanomas skin cancer; Brest C. = brest cancers.

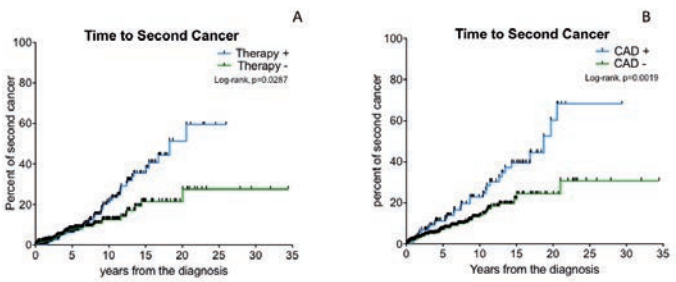

Figure 1.

P046

MICROVESICLES IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Normal and malignant cells secrete extracellular MV, usually defined as shedding membranous vesicles that are produced by budding from the plasma membrane. Elevated levels of circulating MV in CLL are able to activate bone marrow stromal cells and enhance production of VEGF, a pro-survival factor for CLL B-cells. We analyzed serum from untreated CLL pts searching for the number of circulating MV in order to test their possible prognostic role when evaluated at diagnosis. Sera from 101 CLL pts (mean age 70 yrs, range 41 – 89; 60 M, 41 F; 52 patients with A, 36 with B, and 13 with C Binet clinical stage, respectively) were collected at diagnosis. Briefly, 1 ml of serum was processed with serial centrifugations: 2,000 x g for 15 min; 10,000 x g for 30 min; 100,000 x g for 70 min. Resulting pellets were washed with pre-filtered (0.22 m) PBS and centrifuged at 100,000 x g for 70 min. MV enriched pellets were analyzed by a FACSCalibur cytometer and Cell Quest software (Becton Dickinson). Standard micro beads (0.3 – 0.9 – 3 m) were used to define the size limit for MV and their size assessment. Pre-filtered PBS was also used to set the lower limit of MV gate. TruCOUNT beads (BD) were added immediately prior to analyze samples and to determine the number of MV/ l. To identify and characterize MV, CD19-APC was used. A higher mean number of MV was found in CLL pts with respect to 28 healthy subjects (991.8±768.4/ l *vs* 270±325/ l; p <0.001). Of interest, in CLL pts about 15% of MV were found to be CD19+. A higher number of MV was found in pts with advanced clinical stage (Binet A+B vs, p 0.02), while a trend was observed with surface expression of CD38, (p 0.06). No correlation was found according to CD49d and ZAP-70 expression, IgVH mutational status and cytogenetics abnormalities. A correlation was also found with respect to the number of circulating B-lymphocytes, with either total and CD19+ MV (r=0.23 and r=0.25, respectively, p<0.05). Overall, pts who required therapy showed a more elevated number of MV at diagnosis(1,333/ l±941) with respect to those who did not (764/ l±565) (p 0.003). The time to treatment was longer in pts with lower number of both total (p 0.05) and CD19+ MV (p<0.05). Finally, overall survival was shorter in pts with higher levels of total MV (p 0.001), while the number of CD19+ MV was not significant. Our study suggests a possible prognostic relevance of the circulating MV number detected at diagnosis in CLL pts.

P047

EPSTEIN-BARR VIRUS DNA LOAD IN CHRONIC LYMPHOCYTIC LEUKEMIA IS AN INDEPENDENT PREDICTOR OF CLINICAL COURSE AND SURVIVAL

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The relation between Epstein-Barr virus (EBV) DNA load and clinical course of patients with chronic lymphocytic leukemia (CLL) is unknown. We assessed EBV DNA load by quantitative PCR at CLL presentation in mononuclear cells (MNC) of 220 consecutive patients that were enrolled between June 2007 and December 2013 and followed-up in two major Institutions as part of the prospective CLL-Veneto registry (learning set). A subsequent retrospective cohort of 112 patients with CLL was used for independent confirmation (validation set). In all patients biological material was collected at diagnosis, before receiving any cytotoxic treatment. In 20 patients EBV DNA load was also assessed on plasma samples, and in five cases DNA was extracted both from MNC and sorted CD19+ CD5+ B-cells for comparison of EBV DNA load. Forty-one age-matched healthy subjects were tested for EBV DNA load on MNC with the same methods. EBV DNA load was detectable in 59%, and high (≥2000 copies/µg DNA) in 19% of patients. EBV DNA load was significantly higher in CLL patients than in healthy subjects (P <.0001), (Figure 1). Patients tested on sorted B-lymphocytes had similar EBV DNA load compared to MNC, while EBV DNA was consistently undetectable in the plasma of tested patients, irrespectively of EBV DNA load in MNC. No relation was found between high EBV DNA load and clinical stage or biological variables, except for 11q deletion (P=.004), CD38 expression (P=.003), and NOTCH1 mutations (P=.03). High EBV DNA load led to a 3.14-fold increase in the hazard ratio of death and to a shorter overall survival (OS; P=.001), and increasing levels EBV DNA load were directly associated to worse outcome. Poor OS was attributable, at least in part, to shorter time-to-first-treatment (TTFT, P=.0008), with no higher risk of Richter's transformation or second cancer. Multivariate analysis selected high levels of EBV DNA load as independent

predictor of OS after controlling for confounding clinical and biological variables. Either detectable EBV DNA load (55% of patients, ≥2000 copies/μg DNA in 22%), and the predictive value of EBV DNA load was confirmed in the validation set, both in terms of OS and TTFT. EBV DNA load in MNC at presentation is an independent predictor of OS in patients with CLL. Further studies are needed to clarify whether EBV has an active role in enhancing CLL progression or is merely a manifestation of the underlying immunosuppressed state associated with the disease.

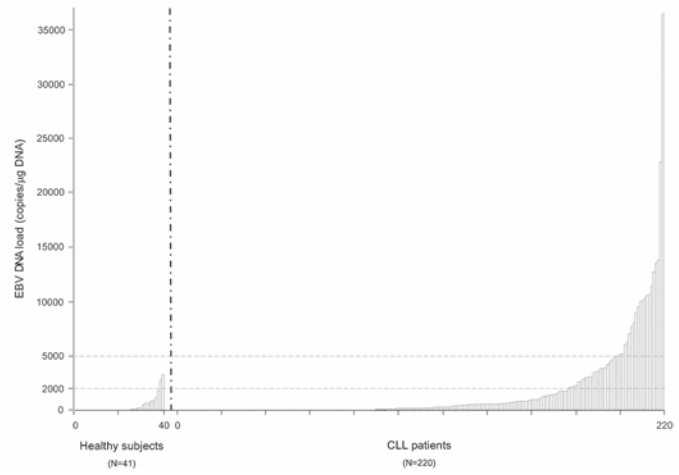

Figure 1.

P048

A GLOBAL PROGRESSION RISK SCORE FOR EARLY STAGE CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS: THE NEVER-ENDING STORY

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Background: In the last two decades several phenotypic, molecular and chromosomal markers have been identified which are significantly associated with prognosis of CLL patients. Therefore, clinicians in charge of CLL patients would benefit from a simplified prognostic index. *Methods:* We analyzed prospectively collected data from 337 Binet A CLL patients enrolled in the O-CLL1-GISL protocol with the aim of developing scores capable of predicting time to first treatment (TTFT). *Results:* We developed two scores based on weighted, multivariate models: the first of these included clinical and laboratory parameters [clinical score (c-score)], while the second one was based on biological markers [biological score (b-score)]. The c-score allowed to predict the TTFT of patients through the combination of Rai stage, 2-microglobulin and absolute lymphocyte count (ALC), while the b-score through IGHV mutational status and CD38 expression (Table 1). The c-score showed a C-statistic of 0.72, while the b-score was 0.67. When the two scores were forced in a multivariate analysis, both showed an independent predictive value on TTFT with a similar HR, demonstrating their complementarity. Thus, we attempted to integrate the two scores performing a further multivariate analysis in which all parameters, significantly associated with TTFT at univariate analysis, were tested. ALC, Rai stage, 2-microglobulin together with IGHV mutational status, resulted independently associated with TTFT. Thus, we constructed a weighted score [global score (g-score)], including all the above 4 variables, which allowed the identification of 3 different risk groups (Table 1). Low risk (3-year TTFT probability 95.3%, HR=1), intermediate risk (3-years TTFT probability 74.5%, HR=5.3, 95% CI 2.9-9.6, P<0.0001), and high risk patients (3-years TTFT probability 28.6%, HR=18.7, 95% CI 9.6–36.6, P<0.0001) had significantly different TTFT. The C-statistic of the g-score was 0.75, showing a better concordance than the other two scores. Moreover, its validity was externally validated in a series of 297 newly diagnosed Binet A CLL patients from the Mayo Clinic. *Conclusions:* Using this multistep process and external validation, we developed a score with high discriminatory power and predictive significance on the individual patient level.

Table 1. Proposed progression risk scores.

Clinical score		
	score 0	score 2
Rai staging system	0	I/II
β2-microglobulin	normal	elevated
ALC (10^9/L)	<10	≥10
score 0= low risk; score 2= intermediate-low risk; score 4= intermediate-high risk; score 6= high-risk		

Biological score		
	score 0	score 2
IGHV	mutated	unmutated
CD38	<30%	≥30%
score 0= low-risk; score 2= intermediate-risk; score 4= high-risk		

Global score			
	score 0	score 1	score 2
Rai stagingsystem	0	I/II	-
β2-microglobulin	normal	-	elevated
ALC (10^9/L)	<10	-	≥10
IGHV	mutated	-	unmutated
score 0-2= low risk; score 3-5= intermediate risk; score 6-7= high risk			

P049

A CASE REPORT OF LYMPHOBLASTIC LEUKEMIA IN YOUNG PATIENT:UNUSUAL CLINICAL PRESENTATION

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Acute lymphoblastic leukemia(ALL) is a clonal haematological disorder which lead to inadequate normal hematopoiesis. A 32-year-old woman

taking isoretinoin for acne, was referred to our clinic with a diffuse bone pain and a presumptive diagnosis of juvenile arthritis. Laboratory test showed ESR of 120 sec, and LDH 3782 UI/L. Approximately at the same time, she developed night-time fever. On initial physical examination she looked healthy with normal vital signs. Initial blood tests and radiological investigations (TC total body) were inconclusive. Initially the presence of immature cells in the peripheral blood smear as interpreted a result of the steroid therapy for bone pain. A subsequent hemogram showed some blasts, and we performed a marrow evaluation that pointed out a diagnosis of B-cell ALL. When fever occurs along with bone pain, the differential diagnosis includes infections, ADR (adverse drug reaction), rheumatic conditions, and malignancies. A high index of suspicion is required in order to reach a correct and timely diagnosis. Age and sex can't help as pointers of juvenile arthritis. In ALL with prominent osteoarticular symptoms corticosteroids administration, for the presumed rheumatic disease, may have serious consequences if prednisone leads to a complete but transient remission of ALL. Physicians who examine patients with musculoskeletal complaints should have a high index of suspicion for underlying malignancies, especially ALL, even in the absence of circulating lymphoblast during steroid therapy.

P050

ACUTE T-LYMPHOBLASTIC LEUKEMIA/LYMPHOBLASTIC T-cell LYMPHOMA. ROLE OF EARLY allo-SCT

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Precursor T cell LBL/ALL is a rare and aggressive neoplastic disorder. Clinical features are bulky mediastinal disease, pleural effusion and bone marrow involvement by blasts oriented *versus* T-lineage, also with acute leukemia clinical presentation. It's matter of debate the role and the correct timing of allogeneic stem cell transplantation (allo-SCT). We report clinical outcome results of 8 newly diagnosed and younger than 60 years T-ALL patients (median age 33 years, range 23-47 years, 5 males, 3 females), from Jan 2010 to Dec 2014, treated in induction with HyperC-VAD and alternated Metotrexate/ARA-C schedule. All patients had bulky mediastinal at diagnosis. Six of eight patients had bone marrow involvement at diagnosis. Five of them had TCR positivity in PCR on bone marrow blood, (3 of them had a bi-clonal peak). Patients were treated with a range from one to four HyperC-VAD/MTX-ARA-C course, on the base of the obtainment of the best response. Two patient presented refractory disease in course of induction, and shifted to a second line therapy; three patients obtained a partial response and 3 obtained a complete response after induction. Responsive patients with HLA-matched donor underwent allo-SCT with conventional conditioning regimen (TBI-Cy). Two patients underwent allo-SCT in first remission (one in CR, by haploidentical donor; one in PR, by sibling). Two patients underwent allo-SCT after a second line therapy, made necessary by a loss of response during the period required to search and mobilization of the donor (one by haploidentical donor, one by MUD, both in PR). At the end of follow-up, the two patients treated with allo-SCT in first remission were alive and in CR. The two patients who underwent allo-SCT in second remission both died by sepsis in aplasia after allo-SCT. All other patients died for progression of disease after second line therapy. Survival analyses on Overall survival (OS), according to the Kaplan-Meier method, showed a median OS of 18,65 months.

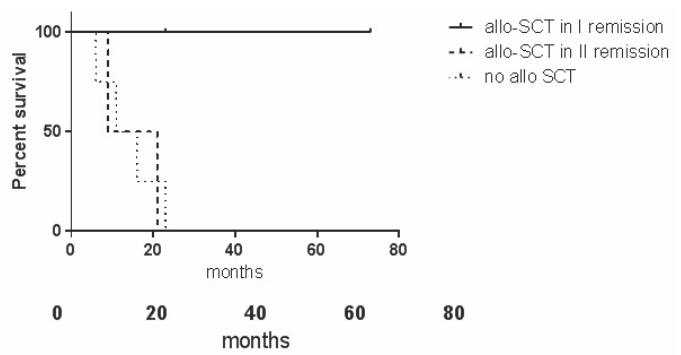

Figure 1. Survival of OS first remission.

Median 5-years OS was 26%. None of clinical features investigated showed significant association with OS. Consistent with the low number of cases, we could note a trend in favour of allo-SCT in first remission (Figure 1) (p 0.059) and TCR negativity on BM (Figure 2) (p 0.11). In conclusion, efforts should be directed towards a the development of new drugs (eg, nelarabine), and the strengthening of clinical trials that help to understand the correct timing for allo-SCT.

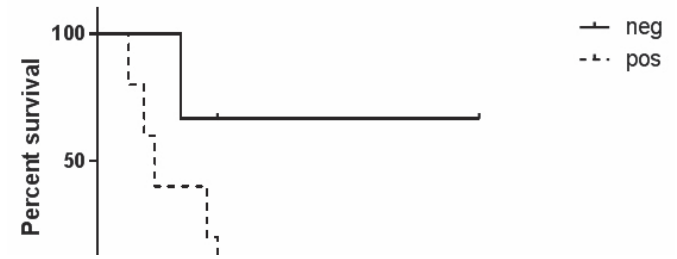

Figure 2. Survival of OS TCR.

P051

SOLUBLE CALRETICULIN PREDICTS FOR TIME TO FIRST TREATMENT OF PATIENTS WITH EARLY CHRONIC LYMPHOCYTIC LEUKEMIA

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Increased circulating levels of soluble calreticulin (sCRT) are found in the sera of patients with autoimmune diseases while in lung cancer a correlation between higher sCRT and hystopathologic subtype has been reported. However, studies addressing the issue in patients with hematologic malignancies are lacking. We analyzed the correlation between well-established clinico-biological parameters of prognostic relevance in chronic lymphocytic leukemia (CLL) and serum levels of CRT by evaluating the impact of these variables on the time to first treatment (TFT) in 70 previously untreated CLL patients in Binet stage A. Peripheral venous blood samples collected at the time of first diagnosis and stored at 70 C were analyzed for the quantitative determination of circulating CRT level using a commercial Human CRT ELISA assay (CUSABIO, Biothech CO, LTD). Levels of sCRT of CLL Binet stage A patients slightly differed (median, 0.55 ng/mL; range, 0.1-2.9) from levels of healthy controls (median, 0.4; range 0.1-1.2)(P=0.09). In CLL patients levels of sCRT did not correlate with age (P=0.25), gender (P=0.70), LDH (P=0.90), ß2-microglobulin (P=0.48), CD38 (P=0.41), ZAP-70 (P=0.50), mutational status of IGHV (P=0.65). In contrast, a positive correlation with PB lymphocytosis (P=0.04) and Rai substages (P=0.03) was observed. After a median follow-up time of 36 months (range, 1-204 months) 19 out of 70 (27.1%) patients needed therapy according to IWCLL criteria. ROC analysis identified a sCRT of 0.8 ng/mL as the best threshold (AUC=0.67; sensitivity 0.58; specificity 0.78) that identified two subsets of patients with different clinical outcomes with respect to TTFT (Hazard ratio [HR], 4.51; P=0.002). In multivariate analysis sCRT (P=0.003), Rai substages (P=0.02) and PB lymphocytosis (P=0.02) entered the Cox model at a significant level. In an independent CLL patient cohort levels of CRT were investigated by gene expression profiling. CRT gene expression was invariably low with no difference between patients with mutated or unmutated IGHV. This is in keeping with a limited role of CLL cells in the production of sCTR levels. In CLL it has been shown that CRT, an antigen highly expressed by stromal cells, may stimulate B-cell antigen receptor (BCR) therefore contributing to the protective effect that stroma exerts on CLL cells [PLoS One. 2010 Dec 30;5(12):e15992]. Our results shed a new light on the role of CRT as predictor of disease-progression in early CLL.

P052

ANALYSIS OF TIME TO FIRST TREATMENT IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Chronic lymphocytic leukemia (CLL) is a heterogeneous disease with a variable outcome. Recently, clinical and biological risk factors have been proposed to evaluate the association between the time before starting treatment and the prognosis in patients with CLL. In this study we investigated time to first treatment (TTFT), measured as the time that elapsed between diagnosis and first treatment, and its relation with clinical and biological parameters and overall survival. We retrospectively evaluated patients (pts) with *de novo* CLL treated at the University Hospital of Bari (Italy) between April 2006 and September 2014. At diagnosis pts were studied for clinical characteristics and biological prognostic factors: age, β-2 microglobulin, absolute lymphocyte count, sex, Rai stage, number of lymph node groups involved, pattern of bone marrow involvement, splenomegaly, mutational status of the immunoglobulin heavy chain gene variable region (IGVH), cytogenetic abnormalities detected by FISH. Median TTFT was calculated for all pts, divided into two groups: the first group included pts treated within 40 months from diagnosis and the second group those treated 40 months or more after diagnosis. Univariate analyses of each group were performed to evaluate correlations between the clinical variables and time to treatment. In addition, overall survival was calculated and compared in the two groups. In total, 74 pts were included; 43 in the group of pts treated within 40 months from diagnosis, and 31 in the group treated after 40 months. Statistically significant differences were seen between the 2 groups regarding the pattern of bone marrow involvement (diffuse *versus* other patterns of marrow involvement; P=0.04), number of lymph node groups involved, (one *versus* more than one; P=.02); mutational status of the immunoglobulin heavy chain gene variable region (IGVH) (mutated *versus* unmutated; P=0.05). No statistically significant difference was seen among the other clinical variables and TTFT. Moreover, comparing the OS of the group with TTFT >40 months *versus* the group with TTFT <40 months, a statistically significant difference was observed (P:0.01) In our study the pattern of bone marrow involvement, the number of lymph node groups involved and the mutational status of the IGVH variable region could help to recognize at diagnosis that subset of patients that may show rapid evolution to progressive disease. Multicentric studies and larger cohorts of patients are warranted to confirm these preliminary data.

P053

BIRC3 MUTATED B-CELL LYMPHOPROLIFERATIVE DISORDERS ARE ADDICTED OF NON CANONONICAL NF-κB SIGNALING

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The BIRC3 gene, a master negative regulator of non-canonical NF-κB signaling, is recurrently mutated in splenic marginal zone lymphoma (SMZL) and chronic lymphocytic leukemia (CLL). Here we aim at assessing the functional relevance of BIRC3 mutations in these B-cell lymphoproliferative disorders. BIRC3-inactivating mutations of SMZL and CLL cause the truncation of the C-terminal RING domain of the protein, whose E3 ubiquitin ligase activity is essential for proteasomal degradation of NIK, the central activating kinase of non-canonical NF-κB signaling. Consistently, BIRC3 mutations lead to constitutive non-canonical NF-κB signaling activation in the BIRC3 mutated VL51 (SMZL) and MEC1 (CLL) cell lines, as documented by the stabilization of NIK, phosphorylation of NF-κB 2, processing from p100 to p52, nuclear localization of p52 and upregulation of NF-κB target genes. Conversely, the BIRC3 wild type SSK41 (SMZL) and KARPAS 17-18 (SMZL) cell lines are devoid of biochemical and molecular clues of non-canonical NF-κB signaling. Western blotting for NIK expression and immunohistochemistry for p52 nuclear localization confirms non-canonical NF-κB signaling activation also in BIRC3 mutated primary SMZL and CLL tumors. Cell lines harboring BIRC3 mutations are resistant to ibrutinib, consistent with the ability of BIRC3 mutations to activate NF-κB independent of BTK. Knockdown of NIK by RNA interference or its pharmacological inhibition with (5[1,3 (2H, 4H)-isoquinolinedione]) turns off non-canonical NF-κB signaling and induced cell death in BIRC3 mutated cell lines, suggesting the addiction of BIRC3 disrupted B-cell tumors to this pathway. These data show that BIRC3 mutated SMZL and CLL are uniquely dependent on non-canonical NF-κB signaling and that non-canonical NF-κB pathway can be a novel therapeutic target in BIRC3 mutated B-cell lymphoproliferative disorders.

P054

FLUDARABINE, CYCLOPHOSPHAMIDE AND LENALIDOMIDE IN RELAPSED/REFRACTORY PATIENTS WITH CLL, RESULTS OF THE PHASE1-2 GIMEMA STUDY LLC606

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The restoration of immuno-mediated functions seems the primary mechanism of action of lenalidomide in chronic lymphocytic leukemia (CLL). The aim of this phase I/II study was to evaluate whether in patients with relapsed/refractory (R/R) CLL lenalidomide combined with fludarabine and cyclophosphamide (FCL) could result in an increased activity preserving the immune function. Forty R/R patients with CLL and a median age of 66 years (range: 47-77) were included in the study. The median number of prior treatments was 1; 65% of patients had been previously treated with FC or FCR and 36% were refractory to the prior treatment. Seventy-seven% of patients were in Binet stage B or C, an unmutated IGVH status was recorded in 66% of cases, del 13q in 32.5%, del11q in 27%, del 17p in 17% and no aberrations in 22.5%. Treatment consisted of 6 monthly courses of FC (F, 30 mg/sqm iv; C, 250 mg/sqm, d1-3) combined with lenalidomide given daily from d1 to d14. In the first phase of the study, the dose-finding phase, lenalidomide was given at the starting dose of 2.5 mg and then escalated to reach the maximum tolerated dose (MTD) that was identified as 5 mg. Supportive treatment included bactrim, valacyclovir, primary prophylaxis of granulocytopenia with G-CSF, allopurinol and low-dose aspirin to prevent thromboembolic events. CLL diagnosis, treatment requirement and response were assessed according to the iwCLL 2008 guidelines. The median number of administered courses was 6. The overall response rate was 62.5%, the complete response rate (CR) 22.5%. The progression-free survival (PFS) at 24 months was 28%, the overall survival (OS) 72.11%. OS was influenced by the IGVH mutational status (p=.09) while the CD38 expression (p<.0001), the IGVH mutational status (p=.007) and the Binet stage (p=.03) showed a significant effect on PFS. OS and PFS were not significantly influenced by the cytogenetic aberrations. The common non-hematologic adverse events of any grade were fatigue (15%), muscle spams/tremors (12.5%) and skin rash (10%). Grade 3/4 neutropenia was recorded in 65% of cases. However, only 3 cases (7.5%) of severe infection were observed. Only a mild case of tumor flare reaction and one of tumor lysis syndrome were recorded. Taken together, the results of this study show that in R/R CLL patients a regimen including lenalidomide at the dose of 5 mg combined with FC is an active salvage strategy associated with acceptable toxicity, particularly in terms of infections.

Acute Myeloid Leukemia 1

P055

ISCHEMIC STROKE AS INITIAL PRESENTATION OF ACUTE PROMYELOCYTIC LEUKEMIA:A CASE REPORT

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Introduction: Compared to bleeding, mayor thrombosis are a less commonly encountered problem in acute promyelocytic leukemia (APL) but thrombotic events appear to be more common in APL than in other acute leukemia,with reported ranging from 2 to 10-15%.Thrombosis may be unrecognized and reflects the complexity of the coagulopathy in APL. We present a case of a patient with ischemic stroke as initial presentation of APL. *Case Report:* A 53 year-old man with history of obesity and hypertension presented to the Emergency Department for hypertensive crisis and hemiparesis. Ischemic stroke we suspected and a CT scan was performed. It showed lesions of the insular cortex and hyperdensity of the middle cerebral artery (MCA) as in stroke. The blood tests showed WBC 830x10^9/L with neutrophils 300x10^9/L, Hb 12,8 gr/L and plt.130.000/L. There were on the blood smear 4% of blasts cells as atypical promyelocytes.The patient was referred to Hematology Department were bone marrow aspirate was performed and showed about 50% of abnormal promyelocytes with Auer roads. The diagnosis of low risk of APL was suspected and ATRA at dosage of 45mg /m^2 must be started. RT PCR analysis confirmed rearrangement PMLRARA (isoform bcr3, FLT3 ITD not mutated) and the diagnosis of APL low risk with ischemic stroke was performed. We immediately start an induction treatment of all trans retinoic acid (ATRA) and arsenic (ATO). Despite the differentiation syndrome and sepsis with hypotension from Escherichia Coli, the patient has brought a positive clinical evolution with stabilization of the neurological clinical picture and the achievement of complete hematological remission after one month of the induction treatment. Now the patient had completed two cycle of consolidation treatment of ATO+ATRA. No sign of recurrence we observed. Conclusions Approach chemotherapy free is one the current goals of the APL treatment.It was shown that a combination of ATRA and ATO is at least not inferior to standard ATRA and anthracycline based chemotherapy for adults with low-intermediate risk APL. More recently, in the extended series of patients the date demonstrate a significantly augmented survival benefit coupled to a higher antileukemic efficacy provided by ATRA +ATO compared to ATRA+CHT. In our case to prefer ATRA+ATO to ATRA+CHT it possible to minimize bleeding and infectious risks in unfit patient (Figure 1).

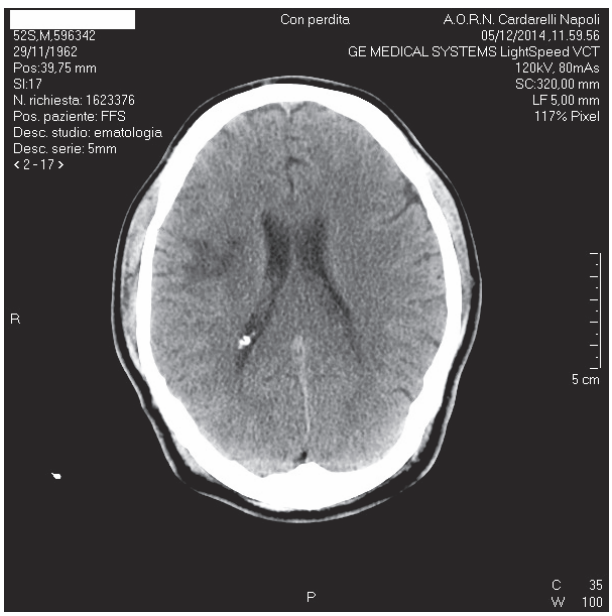

Figure 1.

P056

OCCULT OR MANIFEST INVOLVEMENT OF CENTRAL NERVOUS SYSTEM IMPACTS ON OUTCOME IN ADULT ACUTE MYELOID LEUKEMIA. A MONOCENTRIC EXPERIENCE

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Background: Routine diagnostic lumbar puncture is not recommended in adult patients (pts) with acute myeloid leukemia (AML) without Central Nervous System (CNS) symptoms and little is known about the incidence of CNS involvement and its impact on survival. *Design and Methods:* The aims of our study were 1) to determine the incidence of occult/manifest CNS disease in a series of consecutive AML pts 2) to correlate CNS disease with clinical/biological parameters 3) to examine the impact of CNS involvement on outcome. We collected cerebrospinal fluids (CSF) samples from 90 newly diagnosed AML pts, median age 57 years (range 18-75), median white blood cells count (WBCc) 17x10<sup>9/L (range 0,70-315x10<sup>9/L). Cytogenetic/genetic information was available in 87/90 (96%), 28 (32%) belonged to the category of "good-risk", 31 (36%) and 28 (32%) to the one of "intermediate-" and "poor-risk", respectively. Forty-one pts (45%) had FAB M4/M5 AML. Sixty-four pts received standard (SDARAC) and 21 high-dose-ARA-C-based regimens, 5 supportive care. All CSF samples were examined by conventional cytology (CC) whereas flow cytometry immunophenotype (FCM) was performed in 82/90 (91%) samples. *Results:* Twenty-seven (30%) pts were CNS positive (CNS+): 9 (10%) by CC and FCM (manifest CNS+) and 18 (20%) only by FCM (occult CNS+). Median age and cytogenetic/genetic features did not significant differ between CNS+ and negative (CNS-) pts. A CNS+ status was significantly associated with M4/M5 AML (15/27, p=0.001), WBCc >30x10<sup>9/L (15/27, p=0.03) and high LDH levels (20/26,p=0.007). Two (7%) of CNS+ pts died during the induction course (ID) and 17 (74%) achieved complete remission (CR), similar to the CR (73%) and ID (4%) rates of the entire cohort. Overall survival (OS) of CNS+ pts was significantly shorter than CNS- (18% *vs* 47% at 4 years, p=0.01). In a subset analysis, 4-year OS of manifest CNS+, occult CNS+ and CNS- pts was 13%, 20% and 47%, respectively (p=0.047). CNS involvement identified pts with a significantly shorter OS also in the "good-risk" (p=0.04) and SDARAC(p=0.006) subset. *Conclusions:* Our data suggest that incidence of CNS involvement in newly diagnosed AML pts is higher than currently expected. Manifest/occult CNS positivity should always be investigated at diagnosis, regardless of neurologic symptoms, since it may affect outcome. Further prospective studies on larger series are warranted to confirm this data.

P057

A RETROSPECTIVE MULTICENTRE COMPARISON OF INTENSIVE CHEMOTHERAPY WITH AZACITIDINE FOR THE FRONT LINE TREATMENT OF ELDERLY ACUTE MYELOID LEUKEMIA PATIENTS

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Azacitidine (AZA) has demonstrated encouraging clinical and biolog-

ical activity in AML. Based on this, we evaluated retrospectively: 1) the efficacy of AZA in a large group of elderly patients with untreated AML, diagnosed according to WHO criteria; 2) the OS of these patients as compared to a matched historical group of individuals treated with IC. The AZA group included 103 patients, median age was 75 years (range 61-88). Median white blood cell count (WBCc) was $2.6x10^9$/L (range 0.27-105), median bone marrow (BM) blast count was 30% (range 20-90%) and 56% of patients had BM blasts ≥30%; 49 (48%) had a secondary AML. Karyotype was evaluable in 83 patients and, according to the refined Medical Research Council criteria, 60 (58%) had intermediate-risk abnormalities and 23 (22%) an unfavorable risk karyotype. Absence of uncontrolled infections, adequate renal and epatic function, life expectancy longer than 4 months were the criteria to receive AZA. IC control group consisted in 113 patients aged 61-75 years, enrolled in the randomized AML17 EORTC/GIMEMA trial, who received an induction course consisting in mitoxantrone, cytarabine and etoposide±Gemtuzumab Ozogamicin (GO), followed by 2 consolidation cycles including idarubicin, cytarabine and etoposide±GO. Median age was 68 years (range 61-75). Median WBCc was $5.6x10^9$/L (range 0.5-213), 27 (24%) had a secondary AML. Karyotype was evaluable in 68 patients, 56 (50%) had intermediate-risk and 12 (11%) adverse risk abnormalities. Seventy-nine patients (77%) received AZA at the conventional dose of 75 mg/m^2, the remaining 24 at a flat dose of 100 mg daily; all patients were given a schedule of 7 consecutive days per month. The median number of cycles delivered was 6 (range 1-60). Overall response rate, estimated according to International Working Group in AML, was 43%: complete remission (CR) 23%, partial remission (PR) 20%. Median duration of response was 6 months (range 2-18) and median OS was 298 days. Intermediate karyotype, WBCc <$10x10^9$/L, performance status 0 and achievement of CR/PR were significantly associated with OS duration (p=0.01, 0.009, 0.009 and <0.001, respectively). CR rate was significantly higher in IC *vs* AZA group (51% *vs* 23%, respectively) (p<0.0001), but projected 2-years OS was not significantly different (23% *vs* 17%, respectively). Our analysis indicates similar outcomes with AZA compared to IC. Controlled, randomized clinical trials are warranted to confirm such a conclusion (Figure 1).

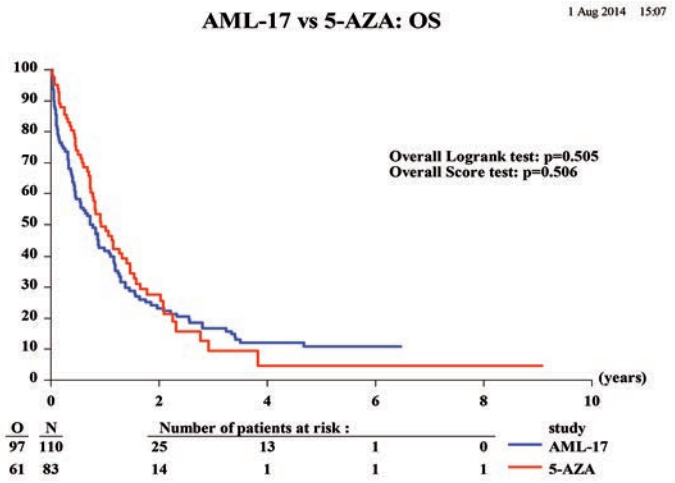

Figure 1.

P058

PERFORMANCE AND TOXICITY OF FLAI (FLUDARABINE, CYTARABINE, IDARUBICIN) AS INDUCTION CHEMOTHERAPY FOR HIGH RISK SECONDARY ACUTE MYELOID LEUKEMIA ARISING FROM PHILADELPHIA- NEGATIVE CHRONIC MYELOPROLIFERATIVE NEOPLASMS OR MYELODYSPLASTIC SYNDROMES

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Introduction: The aim of this study was to evaluate the outcome and the safety profile of FLAI as induction chemotherapy regimen in high risk AML, secondary to Myelodysplastic Syndromes (MDS) or Ph negative Chronic Myeloproliferative Neoplasms (MPN). The rationale of FLAI combination is that Fludarabine is a fluorinated purine analogue that is toxic against Multidrug Resistance (MDR) overexpression cells and enhances Ara-C cytotoxicity by increasing cellular concentration of Ara-C 5triphosphate, thus inhibiting DNA repair. *Patients and Results:* Seventy two high risk AML arising from Ph neg MPN (15/72, 21%) or MDS (57/72, 79%) were treated with FLAI induction regimen at our Dpt in the last 10 years. The M/F ratio was 35/37 with a median age of 63 yrs (range 24-75). The MDR (PGP >6 MFI) phenotype was present in 33/72 cases (46%). The induction regimen (FLAI) included: Fludarabine (25 mg/sqm), Ara-C (2 g/sqm) on days 1-4, Idarubicin (8-10 mg/sqm) on days 1-3. Pts were evaluated for response rate, treatment-related adverse events, Overall Survival (OS) and Disease Free Survival (DFS). After FLAI, complete remission (CR) occurred in 60% of pts (42 of 70 evaluable cases); 8/70 pts (11%) achieved a partial remission and 20/70 (29%) were resistant. There was 2 cases of death during induction (DDI 3%). Proven infections occurred in 31/72 pts (43%), including bacteremia (25 episodes) and/or pneumonia (14 cases). Grade II°-III° WHO oral mucositis was reported in 15 pts (21%). Median time to neutrophil (>1x109/l) and platelet (>50x109/l) recovery was 24 (range 19-42) and 26 (range 20-43) days, respectively. Supportive therapy consisted of a median of 24 RBC units (range 4-42) and 8 platelets units (range 2-49). G-CSF was administered in 20/72 (28%) pts. The biologic and clinical characteristics at diagnosis and their relationship with CR achievement, OS and DFS will be analyzed and reported. Overall, after a median follow-up of 19 months (range 1-101), 30/72 (42%) pts are alive (27/30 in CR). The probability of 1-year OS and DFS were 65 and 59%. *Conclusions:* The FLAI induction regimen, as an example of conventional chemotherapy, is effective in secondary AML arising from MDS or Ph neg MPN with a favorable CR rate, acceptable safety profile and low DDI. Therefore, despite the development of new drugs, FLAI remains a valid option, in clinical practice, such as induction treatment in secondary AML and could be considered a good candidate as a comparator in prospective randomized clinical trials with new drugs.

P059

FLUDARABINE, CYTARABINE AND IDARUBICIN FOR INDUCTION TREATMENT OF POOR-RISK ACUTE MYELOID LEUKEMIA

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Backgroud: In an attempt to improve the outcome of AML, many strategies have been developed and tested. However, despite progressive intensification of the induction-consolidation treatment programs, no significant advantages have been observed in terms of CR rate, disease free-survival (DFS) or overall survival (OS) particularly in poor-risk patients. Poor-risk cases, that are identified mainly by prior history, leukemic cell mass and cytogenetic abnormalities, share multiple mechanisms of drug resistance that are responsible for treatment failure. *Methods:* In our institution we treated in the last 3 years 25 cases of poor-risk AML with fludarabine plus cytarabine and idarubicin regimen (FLAI). The FLAI regimen consisted of fludarabine 25 mg/m^2/d days1–5, Ara-C 2 g/m^2/d days 1–5, idarubicin 10 mg/m^2/d days 1, 3 and 5. *Results:* The complete remission (CR) rate was 85% after the first course and 95% after the second course. Non-hematologic toxicity was very mild, that is very important in elderly patients, but hemopoietic toxicity was substantial, with a time to hematologic recovery of 3 to 4 weeks and one case of death in CR. Peripheral blood stem cells (PBSC) could be mobilized and collected successfully in 19 cases. *Conclusions:* This three-drug combination is effective and has a limited non-hematologic toxicity, but fludarabine may increase the hematologic toxicity and enhance the development of serious opportunistic infections.

P060

LATE MOLECULAR AND EXTRAMEDULLARY RELAPSE OF ACUTE PROMYELOCYTIC LEUKEMIA:A CASE REPORT

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Introduction: Despite the excellent results achieved with the introduction of all-trans retinoic acid (ATRA) in the first line treatment of acute

promyelocytic leukemia (APL) and of maintenance treatment, relapses still occur in 15%to 25%of cases. Most relapsing patients can achieve a second complete remission (CR2) with ATRA, chemotherapy (CT), both treatments, or arsenic trioxide (ATO). However, the most effective post remission therapy in those patients is not defined. Moreover, the incidence, estimated at around 40%, of extra medullary (EM) relapse occurring in APL treated with ATRA and CT. We present a case of a patient with late molecular and extra medullary relapse *Case Report:* A 42 years old female received diagnosis in 2001 of PMLRARA-positive APL in our Institution and treated according to GIMEMA AIDA protocol. Oral maintenance with ATRA and low doses of CT for two years followed induction and consolidation courses based on ATRA+CT. The patient was in molecular CR until 2010 when appeared intense pain in left hip. MRI showed lesion of acetabular region of hip left with reduced signal intensity on T1-weighted sequences.In the same side PETCTSCAN showed high captation. Bone marrow aspirate was performed and showed hematologic remission but RT PCR analysis showed molecular relapse. The diagnosis of extra medullary and molecular relapse was performed. She had a successful re-induction with ATO (15 mg/m^2) for two courses and ATRA (45mg/m^2) for four courses. MRI showed reduction of size lesion confirmed by PET CT imaging. RT PCR analysis became negative. ABMT with high-dose busulphan and melphalan with autologous PML/RARA PCR negative hematopoietic cells rescue followed re induction therapy At restaging MRI showed disappearance of lesion confirmed by PETCT imaging. At 48 months from ABMT, no sign of recurrence we observe. *Conclusions:* Recurrence of APL with extra medullary localization is a highly treatable disease with the introduction of drugs such as ATRA and ATO.Associate ATRA ATO, although we use ATRA in the frontline therapy, potentiates the effect of ATO. While there is no standard for postremissional therapy in relapses, the ABMT in patients fit and in molecular remission is a very effective procedure. In our case study at 48 months after transplantation the patients is alive and in complete constant remission

P061

POST REMISSION SEQUENTIAL MONITORING OF MINIMAL RESIDUAL DISEASE BY WT1 Q-PCR AND MULTIPARAMETRIC FLOW CYTOMETRY PREDICTS RELAPSE AND MAY HELP TO ADDRESS THE RISK ADAPTED THERAPY IN ACUTE MYELOID LEUKEMIA PATIENTS

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Minimal residual disease (MRD) monitoring is crucial for the identification of AML patients at high-risk of relapse. We retrospectively analyzed 104 newly diagnosed AML patients consecutively treated and monitored by Q-PCR on WT1 and by Multiparametric Flow Cytometry (MFC) for Leukemia Associated Immunophenotypes (LAIPs) at baseline, after induction, after the 1st consolidation and after the 1st intensification. By multivariate analysis the factors independently associated with impaired Relapse Free Survival (RFS) were: BM-WT1 ≥121x10^4/ABL (p=0.02) and LAIP ≥0.2% (p=0.0001) after 1st consolidation [RFS at the median follow up of 12.5 months (range 1-47): 82% *vs* 51% (p<0.0001) and 81% *vs* 57%, respectively (p=0.0007)] and PB-WT1 ≥16x10^4/ABL (p=0.0001) after 1st intensification [RFS at the median follow up: 95% *vs* 43% (p<0.0001)]. Within the different ELN risk categories, by multivariate analysis, BM-WT1 ≥121x10^4/ABL after 1st consolidation cycle in the ELN Int-1 [HR 14.4; p=0.01] and Int-2 group [HR 11.7; p=0.04] and LAIP ≥0.2% after 1st consolidation cycle in the ELN Int-2 group [HR 10.1; p=0.04] significantly dissected the patients at high-risk of relapse. Interestingly, in the ELN Int-1 and Int-2 group we observed that 11/20 (55%), 11/15 (73%) and 9/11 (83%) MRD-positive cases after consolidation or intensification or at both time-points relapsed, respectively. In the ELN low-risk group we observed that 3/12 (25%), 4/5 (80%) and 2/2 (100%) MRD-positive patients after consolidation or intensification or at both time points relapsed, respectively. Our data confirm that both methods could be useful for the sequential MRD monitoring of AML patients in order to plan a risk adapted post remission therapy, especially for newly diagnosed patients with low and intermediate ELN risk who are not addressed to allo-SCT front-line, basing on the clinical and biological characteristics of the disease at diagnosis. Indication to an allo-SCT in 1st CR would be supported by the identification of a relapse risk exceeding the 50%, which has been observed in all MRD-positive cases within the ELN Int-1 and 2 risk group and in MRD-positive cases after intensification or both after consolidation and intensification within the ELN low-risk group. Our data confirm the potential usefulness of a sequential MRD monitoring with both Q-PCR and MFC. Further prospective trials on larger numbers of patients are warranted to address the issue of a risk-adapted post-induction therapy of AML.

P062

IMPAIRED NEUTROPHIL FUNCTION IN PATIENTS WITH ACUTE LEUKEMIA

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Background: Neutropenia is recognized as the most important risk factor to bacterial and fungal infections in patients undergoing chemotherapy. However, another risk factor that could play a role in increased susceptibility to infection by these patients is impaired function of neutrophils. In particular, in patients with acute leukemia (AL), few studies to date have shown altered neutrophil function. Therefore, aim of this study was to analyze the function of mature neutrophil in terms of phagocytic activity and capacity to produce oxidative burst in patients with acute leukemia at diagnosis and after induction chemotherapy. *Methods:* We analyzed n.10 patients with acute myeloid leukemia (AML), n.6 with acute B-lymphoblastic leukemia (B-ALL), n. 2 with acute T-lymphoblastic leukemia (T-ALL) and 10 healthy controls (HC). Phagocytic and oxidative burst was detected using the Phagotest Kit and Phagoburst Kit respectively. The cells was analyzed by flow cytometry using a EPICS-XL-MCL cytomiter. The results were expressed as percentage of fluorescent cells in the population studied. *Results:* In patients with acute leukemia at diagnosis we observed both a reduced phagocytic activity and a reduced ability to produce oxidative burst compared to healthy donors (42±19 *vs* 75±4, p=0.0004 and 58±11 *vs* 85±8 p=0.0006 respectively). In particular the reduction is more evident in patients with AML than those with B-ALL or T-ALL (for phagocytic activity:32±14, 46±11, 55±7, for oxidative burst 53±10, 61±18, 65±4 respectively) and such activities are restored partially after induction therapy (for phagocytic activity: 47±26, 59±16, 61±6, for oxidative burst 58±3, 67±10, 68±5 respectively). No correlation was found with the absolute number of neutrophils nor with the percentage of blasts. *Conclusions:* These few preliminary data shows that impaired phagocytic activity and oxidative burst of neutrophils may contribute to increased risk of infections in patients with AL.

P063

THE ROLE OF INDOLEAMINE 2,3-DIOXYGENASE ENZYMES IN REGULATING THE INTERPLAY BETWEEN MESENCHYMAL STROMAL CELLS AND LEUKEMIC CELLS IN ACUTE MYELOID LEUKEMIA

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Mesenchymal stromal cells (MSCs), an essential element of hemopoietic niche, are multipotent cells with a unique immune-modulating ability. Thus, MSCs both play a crucial role in the hemopoietic stem cell (HSC) proliferation and differentiation, and also can generate an immune-tolerant milieu. Indoleamine 2, 3-dioxygenase (IDO1 and IDO2) enzymes catabolize tryptophan to kynurenine and play a key role in the induction of immune tolerance in different settings, including acute myeloid leukemia (AML). Furthermore, IDO1/IDO2 pathway is

a nodal modifier of MSC immunomodulatory properties. We hypothesized that: 1) MSC-dependent mechanisms are involved in leukemia initiation, maintenance and progression; 2) the expression of IDO1 and IDO2 by MSCs is part of a MSC-dependent mechanism able to create a tumor-supportive milieu. We proposed: 1) to investigate if MSCs are altered in AML patients; 2) to study IDO1/IDO2 role in MSC-leukemic milieu interactions. To this aim, we isolated MSCs from the bone marrow of AML patients (AML-MSCs) at diagnosis. We first analyzed their biological properties and we compared them to healthy donor-derived MSCs (HD-MSCs). We found that AML-MSCs showed a reduced proliferative capacity but a normal immunophenotype and immunomodulatory capacity as compared with HD-MSCs. Furthermore, AML-MSCs showed normal karyotype (FISH analysis). Interestingly, AML-MSCs showed an enhanced capacity to differentiate in adipocytes. We next investigated IDO1/2 expression and functions in MSCs. We demonstrated that IDO enzymes are expressed in AML-MSCs as well as in HD-MSCs. IDO1 is efficiently upregulated by different inflammatory stimuli, and IDO1 protein expression parallels mRNA. IDO2 mRNA is upregulated in particular after IFN-γ stimulation, while IDO2 protein is stably expressed in all analysed conditions. When T cell proliferation was tested in MSC co-cultures, we found that MSC immunomodulatory potential is IDO-dependent both in AML- and HD-MSCs. Finally, we found that in co-culture assay with primary blasts, MSCs stimulated blast proliferation and this effect is, at least in part, IDO-mediated. These data suggested that IDO functions could influence MSC/leukemic milieu cross talk. Overall, our results would likely contribute to unravel signalling pathways underlying to MSC–dependent leukemia support. These findings may help to discover novel niche-target prognostic/therapeutic factors and contribute to develop more effective therapies.

P064

CD2/CD25 IMMUNOPHENOTYPICAL PROBE TO DISTINGUISH MINIMAL RESIDUAL DISEASE IN ACUTE MYELOID LEUKEMIA FROM BASOPHIL AND DENDRITIC HEMATOPOIETIC PROGENITORS

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Minimal Residual Disease (MRD) monitoring by flow cytometry (FC) relies on the expression of "leukemia associated immunophenotypes" (LAIPs) defined as the presence of a combination of antigens and/or FC physical abnormalities that are absent or very infrequent in normal bone marrow (BM) cells. LAIPs chosen for MRD monitoring must be evaluated in healthy controls to allow for a correct interpretation of the FC results in patients. Among LAIPs, CD2 is one of the most frequent antigens found in AML blasts and CD25 is expressed in 30% of cases of AML; normally, CD2 is expressed on dendritic cells, NK and T lymphocytes, while CD25 is present on basophils and lymphocytes. In order to distinguish leukemic blast cells from normal CD34+ hematopoietic progenitors, we tested an immunophenotypical "probe" CD2/CD123/CD34/CD25/HLA-DR both in healthy and AML MRD BM, to understand if a possible detection of co-expressing CD34+/CD2+ and/or CD25+ population is aberrant or simply the result of normal hematopoiesis. In healthy controls, among CD34+ compartment, we detected the co-expression of CD34+ and CD2+ cells differentiating towards dendritic cells considering their CD123 and HLA-DR positivity. In the same sample, CD34+ cells showing CD25 positivity differentiated towards basophils in view of their immunophenotype CD123+/HLA-DR-. At diagnosis, in 30% of AML BM, we detected the expression of CD2 and/or CD25 on leukemic cells; in the corresponding MRD, we detected a very high percentage of cases showing the co-expression of CD34+ and CD2 or CD25 that differentiated toward dendritic or basophilic lineage respectively, considering their position in the dot-plot CD123/DR, and, in conclusion, these cells were considered as normal hematopoietic cells. Only in two cases, AML MRD showed CD34+ cells with a different pattern CD123/HLA-DR and were considered aberrant. Detailed knowledge of normal BM immunophenotype is mandatory to identify LAIPs. In fact, CD2 and CD25 expression may represent lineage differentiation of CD34+ progenitors. When the immunophenotypically defined CD34+ cell compartment from MRD samples is projected in dot-plots CD123/DR, templates for the normal CD34+ cell differentiation pathways can be defined and cell cluster located in an "aberrant space" becomes evident. This approach allows discrimination between normal and malignant CD34+ cells and can therefore be used for MRD detection.

P065

DENDRITIC CELLS LEUKEMIA OF DIFFERENT DERIVATION: IMMUNOPHENOTYPE AND ULTRASTRUCTURE HETEROGENEITY

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Among acute myeloid leukemia (AML) and related precursors neoplasms, Blastic Plasmacytoid Dendritic Cell Neoplasm (BPDC) is a rare subtype of acute leukemia characterized by the clonal proliferation of precursors of plasmacytoid dendritic cells (DC). Although most of DC malignancies probably correspond to the neoplastic counterpart of the lympho-plasmacytoid DC, neoplasias of myeloid DC might also exist. Until now, there are very few information on phenotypic markers characteristic of DC leukemia of myeloid derivation and very few is known about DC populations and their differentiation from CD34+ progenitors. In the present study, we have analyzed flow cytometry (FC) and electron microscopy (EM) data resulting from two bone marrow cells from patients with AML. The sample #1 was a BPDC showing CD34-, CD117-, CD123+, HLA-DR++, CD303+, CD304+, CD56++, CD1c+, CD11c-, CD2-, NG2+, CD22+ leukemic cells as estimated by FC. By EM, cells were roundish with one or two nuclei, a thick and dense chromatin rim, a huge nucleolus and few nuclear pores. The cytoplasm contained many inclusions of medium density, variable amount of smooth and rough endoplasmic reticulum, a small Golgi apparatus and many free ribosomes. The sample #2 was from an AML differentiating towards DC and as estimated by FC, pathological cells showed a distinct immunophenotype, with a very representative clonal DC population (about 15% of global cells): CD34+, CD117+, CD123+, HLA-DR++, CD303+, CD304+, CD56-/+, CD1c-/+, CD11c-/+, CD2-, NG2-/+, CD22-/+. By EM, cells were roundish or slightly elongated some with short and wide projections DC-like, with one large nucleus with shallow indentations, a rim of dense chromatin and one or more nucleoli. The cytoplasm contained round electron dense inclusions, some scanty cisternae of rough endoplasmic reticulum, a small Golgi apparatus and many free ribosomes. In order to improve the diagnostic classification of these malignancies, our interest is to correlate the findings about DC AML and DC populations and the phenotypic characteristics of AML to study the clinical behavior and prognosis. In this study, we confronted one case of BPDC and one of AML differentiating towards DC and we found a deep distinction between immunophenotype and ultrastructure of pathological cells. These findings suggest a different origin of these diseases, supporting the conviction that there may be malignancies originating from myeloid-derived DC.

P066

PULMONARY INFECTIONS IN PATIENTS WITH ACUTE MYELOGENOUS LEUKEMIA RECEIVING AZACITIDINE TREATMENT

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Azacitidine (AZA) is approved in the treatment of Acute Myeloid Leukemia (AML) patients with 20–30% of marrow blasts not eligible for intensive chemotherapy. In this subset, pulmonary infections are frequent complications but their incidence, etiology and impact on overall outcome during treatment are still unclear, probably because of the lack of clinical and microbiological documentation, due to the preferential outpatient management of these subjects. The availability of an hematological emergency ward in our Institute made homogeneous the infective diagnostic work-up in presence of infective signs. We retrospectively evaluated 36 AML patients [M/F 25/11, median age 75.5 years, interquartile range (IQR) 70.4 – 79.8] treated with AZA from

04/2009 to 01/2015. All patients received AZA at standard dosage (75 mg/m^2 for 7 days every 28 days) as outpatients. Microbiological work-up included blood cultures, culture from other sites, galactomannan detection in serum and sputum/bronchoalveolar lavage. The total AZA cycles were 342, with a median of 7 per patient (range 1–34). There were 22 episodes of lung infection, documented by chest CT in 16/22 (44.4% of patients, 6.5% of AZA cycles). Based on the diagnostic work-up, pulmonary infiltrates were considered of fungal origin in 4/22 cases (18.1%), associated to bacteremia in 1/22 (4.5%) and of unknown origin in 17/22 (77.4%). As to the time of occurrence of pneumonia, 15 episodes were documented from the 1st to the 4th AZA cycle (11.5% of 130 cycles), while the other 7 episodes occurred after the 5th (3.3% of 212 cycles) (p=0.003). A fungal pneumonia was documented in 2/130 cycles 1-4 (1.5%) and in 2/212 cycles since cycle 5 (0.9%). Ten/16 (62.5%) patients with pneumonia interrupted AZA within 3 months from the infections due to deterioration of clinical conditions, hematologic disease progression and/or death, including 2 out of 4 patients whit fungal pneumonia. In conclusion, pulmonary infections are a common complication in AML patients receiving AZA and are often associated to therapy interruption. Pulmonary fungal infections are more frequently during the first cycles of AZA. It should be defined if the poor outcome of patients with pulmonary infections during AZA is related to a disease related immunologic deterioration or is directly related to the complication. If confirmed in other experiences, the need of an antimicrobial prophylaxis particularly during the first AZA cycles should be considered.

P067

THERAPEUTIC STRATEGY FOR ELDERLY PATIENTS WITH ACUTE MYELOID LEUKEMIA CARRYING THE DNMT3A MUTATION

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Background: 5-Azacytidine (5-AZA) has emerged as a valid substitute of the Conventional Care Regimens (CCR) in a small subset of elderly patient with a bone marrow blast <30% *AIMS:* to assess both safety and efficacy of in-label use of 5-AZA in elderly AML patients who have reached a bone marrow blast count between 5 and 30% after an induction conventional chemotherapy *Methods:* 13 patients (8 males; 5 females) with a median age of 74 years and a newly diagnosed AML have been enrolled. At the diagnosis, the median bone marrow blast count was 45% (range 24%-95%). Cytogenetics showed: normal karyotype in 7 patients, +8 trisomy in 2, complex karyotype in 4. A DNMT3A mutation was documented in 5 cases. Neither FLT3-ITD mutations nor NPM1 mutations were present. All patients received a CCR induction chemotherapy: Low Dose Cytarabine for 5 days in 4 patients, Fludarabine (25mg/sqm intravenously for 5 days) and Cytarabine (2gr/sqm intravenously for 5 days) in 4 and Idarubicine (10mg/sqm intravenously for 3 days), Cytarabine (100mg/sqm intravenously for 5 days) and Etoposide (50mg/sqm intravenously for 3 days) in 5. At the day +31 bone marrow evaluation, no one obtained a CR, in 5 patients blast count ranged from 20% to 30%; in 4 from 15% to 20%; and in 4 from 5% to 10%. All patients were then treated with 5-AZA at 75mg/sqm subcutaneously for 7 days (5-2-2) every 28 days. The median number of cycles was 8 (range 1-15). Adverse hematological events were: grade III-IV neutropenia in 7 patients (54%) and thrombocytopenia in 9 (69%). Fever was the major non-hematological side effect during 5-AZA (77%). *Results:* Among the 12/13 evaluable patients the median survival was 16 months (range 2 – 44). Survival was longer (17 months) in the 5 patients with DNMT3A mutation as compared with those with wild-type DNMT3A (11 months). In addition, transfusion requirements and quality of life improved in all evaluable patients. Therapy with 5-AZA was overall well tolerated with only one patient requiring a long-term hospitalization. In conclusion, a bone marrow blast reduction after conventional induction chemotherapy and a subsequently treatment with 5-AZA can be a valid option in elderly patients with AML and DNMT3A mutation. More patients and longer follow- up are required for confirming these encouraging preliminary results.

P068

MYELOABLATIVE ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN A PATIENT WITH COMPLICATED BILIARY MYELOID SARCOMA AND CONSEQUENT ACUTE MYELOID LEUKEMIA

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Myeloid sarcoma (MS) is an extramedullary localization of immature granulocytic precursor cells, generally characterized by poor prognosis. Gastrointestinal tract involvement is a rare phenomenon. We report the case of a 62 year old man, who was admitted to our department with fever, abdominal pain and jaundice due to a common bile duct mass constricting the choledochus duct. After endoscopic stent implantation, an explorative laparotomy with cholecystectomy was performed. Following histologic analysis allowed the diagnosis of myeloid sarcoma of cholecystis and choledochus. Bone marrow involvement was excluded by bone marrow biopsy. Due to initial febrile cholestasis, subcutaneous low dose Cytarabine (LDAC, 20mg/BID) together with broad spectrum antibiotics were started. Staging after one cycle of LDAC documented a significant tumor mass reduction, thus two intensive chemotherapy cycles with Idarubicin, Cytarabine and Etoposide were performed. Febrile cholestasis occurred in both aplasia periods, required a biliary drainage change and broad antimicrobical therapy. Re-staging documented a complete remission (CR) of myeloid sarcoma, therefore a consolidation radiotherapy with 24 Gy was added. After four months of follow-up (FU) fever and petechial skin due to piastrinopenia and leucocytosis with 74% of myeloid blasts in peripheral blood appeared. Immediate intensive chemotherapy induced a CR of the Acute Myeloid Leukemia (AML), thus a second consolidation course was performed. During the aplasia periods of both cycles febrile cholestasis occurred and required biliary drainage replacement. After two cycles a CR of AML and MS was obtained and a semi-intensive Daunorubicin/Cytarabine cycle given. Consequent myeloablative chemotherapy followed by hematopoietic stem cell transplantation (HCT) with peripheral blood stem cells from a 10/10 matched unrelated donor could be realized. Graft *versus* host disease prophylaxis contained Ciclosporin, Methotrexate and anti-thymoglobulin. The aplasia period was complicated by fever without cholestasis. During the whole hospitalization period, the biliary drainage was not opened and the patient could be discharged after 21 days. The patient is currently at 10 month FU maintaining a CR of AML with 100% chimerism and no signs of MS or infection. The present case highlights the feasible management of a complex case with high risk disease and severe infective complications through an interdisciplinary approach including final HCT.

P069

AZACITIDINE IN ACUTE MYELOID LEUKEMIA: A SINGLE CENTER EXPERIENCE

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Azacitidine is a hypomethylating agent approved for the treatment of acute myeloid leukemia with myelodysplasia related changes (AML-WMRC) with blasts from 20 to 30%. From 2011 to april 2015 we treated with azacitidine (75 mg/mq day 1-7 every 28 days) 23 patients with AMLWMRC with marrow blasts from 20 to 30%, including only the ones that completed at least 6 cycles. All patients were more than 70 years old. Among these patients, 12 had AMLWMRC *de novo*, 9 secondary to myelodysplastic syndrome, 2 secondary to primary myelofibrosis. Only 4 patients were pre-treated with standard chemotherapy (ARA-C low dose as single agent or "3+7"). For hematologic tossicity, 4 changed schedule to 100 mg total dose day 1-5 every 28 days. The treatment was always well tolerated. *Results:* the median number of cycles for patients was 19.34 (range 7-50), the median overall survival was 23,2 months (range 8-50). Actually 7 patients are alive, 6 of them are still in treatment with azacitidine. Azacitidine changes the natural history of AMLWMRC in elderly patients and can be well tolerated after several cycles.

P070

CASE REPORT OF ACUTE MYELOID LEUKEMIA SECONDARY TO PRIMARY MYELOFIBROSIS TREATED WITH AZACITIDINE

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Azacitidine is a hypomethylating agent approved for the treatment of acute myeloid leukemia with myelodysplasia related changes (AML-WMRC) with blasts from 20 to 30%. In literature there are few data about azacitidine for treatment of AMLWMRC secondary to primary myelofibrosis (PM). We report a 75 years old patient treated with azacitidine for AMLWMRC secondary to PM. PM was diagnosed in 2011, with moderate thrombocytosis, mild leukocytosis, mild anemia and splenomegaly (spleen 17 cm). An important constitutional symptom was invalidant pruritus. We started treatment with hydroxyurea with a good control of platlets count but not of pruritus. So we started prednisone without benefit. In february 2014 PM evolved in AMLWMRC with marrow blasts from 20 to 30% and circulating blasts of 10%. Severe anemia (with transfusional dependence), moderate thombocytopenia and leukocytosis appeared. Splenomegaly slightly increased (spleen 19 cm). We started azacitidine (75 mg/mq day 1-7 every 28 days) in association with hydroxyurea to control leucocytosis. The treatment was well tolerated. After 2 cycles pruritus disappeared; after 6 cycles marrow blasts were still 20%. After 8 cycles the transfusional dependence decreased from 4-5 units/month to 1-2 units/month. In our experience, azacitidine is safe and effective in AMLWMRC secondary to PM to control pruritus and transfusional dependence.

Hodgkin Lymphoma

P071

CIRCULATING ARGINASE-1 IN SERUM IS A NOVEL BIO-MARKERIN HODGKIN LYMPHOMA

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Background: Arginase-1 (Arg-1) is a key enzyme contained in neutrophils' granules to mediate immunosuppression in Hodgkin Lymphoma (HL). Our previous work showed that Arg-1 is a potential biomarker in HL.To define its value as a marker to monitor treatment response, we correlated serial Arg- levels with clinical response in newly diagnosed and relapsed classical HL patients. *Materials and Methods:* Serum was collected from 61 (39 early stage and 21 advanced stage) newly diagnosed HL patients before, during, and after treatment, and from 10 refractory/relapsed patients before and after treatment with Brentuximab. s-Arg-1 was determined by enzyme-linked immunosorbent assay and was related to pre-treatmentSUVmax and SULpeak, as measured by quantification of 2-[18F]fluoro-2-deoxyglucosepositron emission tomography (PET) images, and to treatment response. *Results:* Baseline s-Arg-1 correlated with stage of disease and bulky disease, and weakly with SUVmax and SULpeak.s-Arg-1 was positively correlated to the absolute count of Neutrophils (ANC) and Arg-1 detected in neutrophils by RT-PCR. Since neutrophilia could affect the amount of s-Arg-1, we considered the normalised value (norm-s-Arg-1) defined as s-Arg-1/ANC to explore any correlation with semiquantitative parameters of PET at diagnosis. Medians of SUVmax and SULpeak were respectively 12.1 (range 2.7-23.2) and 7.8 (range 1.6-15.1). SUVmax and SULpeak were weakly positively correlated to norm-s-Arg-1 (respectively, r=0.32, p=0.03 and r=0.31, p=0.03). Response to treatment was observed in 17 of 21 early stage and 25 of 39 advanced stage patients. A level of 200ng/mL s-Arg-1 resulted in 68% (95% C.I. 58-87) sensitivity and 61% (95% C.I. 42-86) specificity in predicting response status at 30 months(area under curve, AUC, 0.69, p=0.01). Reduction in s-Arg-1 could be observed as early as after two cycles of chemotherapy in all responsive patients, while s-Arg-1 remained elevated during and after treatment in non-responsive patients. s-Arg-1 was elevated in all relapsed patients at time of relapse, and remained elevated after salvage treatment in the 4 non-responsive patients, while it was suppressed in Brentuximab-responders. *Conclusions:* Baseline s-Arg-1 correlate with classical Hodgkin's lymphoma tumor burden and serial levels correlate with response to treatment.

P072

HODGKIN LYMPHOMA: FROM HITS TO DRUG CANDIDATES?

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Despite the clinical success of anthracycline-based chemotherapy with doxorubicin, bleomycin, vinblastine and dacarbazine (ABVD) for patients with advanced-stage Hodgkin lymphoma (HL) novel therapeutic agents are needed for refractory or relapsed patients. Starting from the synthesis, we identified three compounds (hits) among a larger series of N-biphenylanilides, *i.e.* PTA34, PTA73 and RS35, having a N-biphenyl-nicotinic based moiety with promising ADME (Absorption, Distribution, Metabolism, and Excretion) profiles and interesting activities against Hodgkin lymphoma cell lines. The preclinical characterization was carried out in Hodgkin lymphoma cell lines, HDLM-2, L428,

L540, L1236 and KM-H2 cells. All hits showed a high capability to kill cells by induction of both apoptosis and necrosis in the nanomolar range. The evaluation of cell cycle demonstrated that they blocked cells in G2/M phase in all HL cells but did not in solid tumor models, thus suggesting a selective activity for this hematological disease model. The analysis of effectors involved in the blockage of cells in G2/M phase showed the up-regulation of Myt1 and no activation of Cdk1 confirming the blockage in the early phases of mitosis further suggested by the increased level of cyclin B1. To distinguish among the different apoptosis pathways that mediated the cells death after exposure to our hits, mitochondria involvement was studied by the evaluation of the induction of mitochondrial membrane depolarization and the production of ROS. In addition, the human apoptosis array analysis allow the investigation of the main effectors responsible for apoptosis such as, proteins of Bcl family, TNF receptors, etc. PTA34, PTA73 and RS35 induced a marked mitochondrial membrane depolarization and production of ROS after 3 days exposure. The protein array showed that after only 1 day of drugs exposure, the apoptosis induction was mediated by the activation of the extrinsic pathway of apoptosis more that the intrinsic one which seemed to be a later event. The further *in vivo* validation of results will conclude the preclinical evaluation of the effectiveness of these N-biphenylanilides derivatives as antitumor agents, providing a new promising therapeutic approach against Hodgkin lymphoma.

P073

SECOND MALIGNANCIES IN LYMPHOMA SURVIVORS: A SINGLE CENTRE EXPERIENCE

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Background: Improvements in the treatment of both Hodgkin's and non Hodgkin's Lymphomas (HL and NHL) have resulted in an increasing number of long term survivors. But this patient's population is at high risk of developing serious late therapy related complications that can negatively affect their lives or lead them to an early death. The most common secondary diseases in the people cured from lymphomas are cardiovascular diseases and second malignancies (mainly solid tumours). *Aims and Methods:* In our institution the HL and aggressive NHL long term survivors are followed up in a dedicated clinic since September 2014. Here we report preliminary data on second malignancies. We have collected retrospective data on second tumours in 312 lymphoma survivors. *Results:* We have analyzed data regarding 312 patients coming in our clinic from 15 September 2014 to 23 April 2015, 159 were affected by HL and 153 by NHL. One hundred sixty three are females, 149 males; median age at observation is 53 (range 22-89). All of them are in complete response for lymphoma for at least 5 years from the completions of curative therapy. Thirty nine patients (12.5%) experienced a second cancer, 4 of them had 2 neoplasms, so we documented 42 second tumours. They were: 9 breast, 14 skin basocellular, 5 colon and sigma, 6 thyroid, 4 prostatic, 1 lung, 2 bladder carcinoma, 1 testis and 1 cutaneous appendages cancer. Regarding the previous therapies all but 2 the females with breast cancer had undergone to mediastinal radiotherapy; 3 out of 6 with thyroid cancer had mantel or neck radiotherapy, the one with lung cancer had MOPP chemotherapy and mantle radiotherapy; no one of the intestinal cancers had abdominal radiotherapy, one of the patients with urinary cancer had abdominal radiotherapy and MOPP/ABVD regimen. Median age of breast cancer in our setting is 52 (range 38-70). The median time between diagnosis of lymphoma and diagnosis of second malignancy was 20.5 years (range 2-41). *Conclusions:* In our Department we described a significant number of cases of second neoplasms in the lymphoma survivors population. That outline the importance of a plan for early diagnosis of cancers in this setting of patients.

P074

MULTIFOCAL VERTEBRAL INVOLVEMENT AS UNUSUAL PRESENTATION OF RELAPSING HODGKIN'S LYMPHOMA: CASE REPORT

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Hodgkin's lymphoma usually presents with progressive enlargement of peripheral lymph nodes, while a solitary bone involvement is very rare even in the relapse setting. Here we report a case of a Hodgkin's lymphoma (HL) patient who relapsed with symptomatic multiple vertebral localizations in the absence of any other site of disease. Case report. A 23 year-old boy was referred to our Institution because of cervical adenomegalies and splenomegaly. An excisional biopsy of an enlarged cervical lymph node was taken and the diagnosis of HL classic type – scleronodular -, was made. Bone marrow examination was negative, whereas a whole body positron emission tomography (PET) showed multiple adenomegalies at both sides of diaphragm and an intense hypermetabolism of the spleen. The patient was treated with standard chemotherapy (ABVD). Since after 2 ABVD courses, an interim PET showed a very good partial remission, the patient received 4 additional ABVD courses. One month after the therapy completion a new PET showed a mediastinal progression of the disease: the histological analysis of the mediastinal biopsy confirmed the HL relapse. A second-line treatment with IGEV was then started, allowing an adequate CD34+ cell collection without any objective response in terms of disease. A third-line treatment with brentuximab vedotin was then started (8 infusions) with no results. The patient received salvage mediastinal radiotherapy, achieving a complete metabolic response as demonstrated by PET. After two months, the patient developed a lower back pain: a new PET revealed a FDG-avid focus localized in the body of D7-L1-L3-L5, in the absence of any nodal localization. A MNR showed hyposignals without intervertebral discs involvement. At that time the patient had no fever or signs of inflammation at blood tests. The biopsy of L3 was non-diagnostic. After a short period of watchful waiting, another FDG-PET showed a reduction of the L3 focal uptake and the appearance of new hypermetabolic areas in L5. The patient underwent a L5 biopsy that was diagnostic for relapsing HL. Our case illustrates a localized vertebral relapse of HL. To our knowledge there are only a few reports of exclusive vertebral involvement by relapsed HL. This finding may be due to the abnormal expression, acquired during the course of disease, of chemokine receptors by neoplastic cells. Our experience also outlines the role of FDG-PET in the early identification of HL relapse (Figure 1).

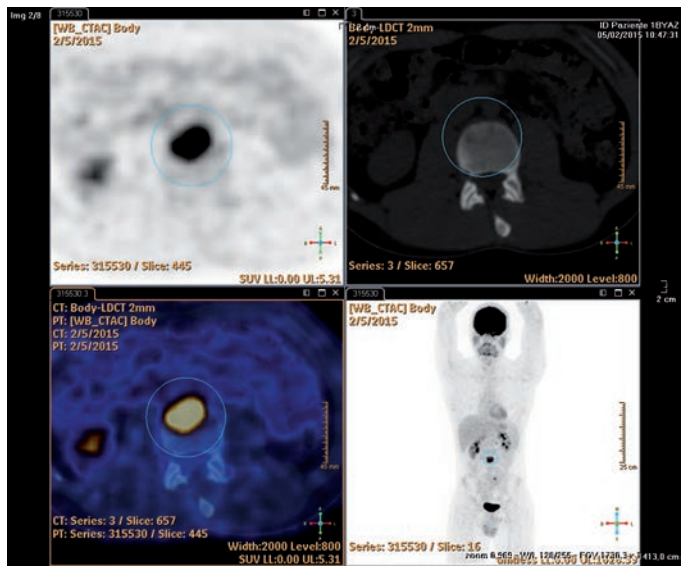

Figure 1.

P075

COMPARISON BETWEEN WHOLE-BODY MRI AND PET/CT IN STAGING NEWLY DIAGNOSED FDG-AVID LYMPHOMAS: OUR EXPERIENCE

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Background: Positron emission tomography/computed tomography (PET/CT) with the use of 2-deoxy-2-[18F]fluoro-D-glucose (18F-FDG)

is an established imaging modality that has been proven to be of benefit in the management of malignant lymphomas. Diffusion-weighted magnetic resonance imaging (DWI-MRI), is a noninvasive technique that allows to provide morphological information, through images with high contrast resolution, and functional information, probing the random motion of water molecules. *Aims:* to compare whole body MRI (WB-MRI) and FDG-PET/CT for staging newly diagnosed FDG-avid lymphoma. *Methods:* Fifty-three patients (28 males; mean age 42 years; range 15-84) with histologically confirmed malignant lymphoma (27 Classical Hodgkin, 12 Diffuse Large B-cell, 10 Follicular, 4 Mantle Cell) underwent both WB-MRI and FDG-PET/CT before treatment. Ann Arbor stages obtained with WB-MRI (DWI, T1w and STIR sequences, without c.e). and FDG-PET/CT findings were compared using Cohen's k statistics. Moreover WB-MRI and FDG-PET/CT stages were compared with pathological staging obtained using bone marrow and other biopsies if clinically indicated. *Results:* Very good agreement between WB-MRI and FDG-PET/CT (k=0.83, p <0.05) was found. WB-MRI stage was equal to those of FDG-PET/CT in 89% (47/53; in particular 25/27 Hodgkin lymphoma, 22/26 Non Hodgkin lymphoma). Very good agreement between WB-MRI stage and pathological stage (k=0.83; sensitivity=90.4%), and between FDG-PET/CT and pathological stage (k=0.89; sensitivity=92.5%) was found. Understaging and overstaging occurred respectively in 9.4% (5/53) and in 1.9% (1/53) with WB-MRI, and in 7.5% (4/53) and in 0% (0/53) with FDG-PET/CT. Differences with pathological stages were caused by missed identification of bone marrow involvement and gastrointestinal lesions identified only with biopsies. *Conclusions:* WB-MRI can be considered a good technique for lymphoma staging, without radiation exposure or contrast administration.

P076

BRENTUXIMAB-EPIRUBICINE-VINBLASTINE AND DACARBAZINE AS SAVAGE THERAPY FOR HODGKIN'S LYMPHOMA IN A PATIENT WITH CYSTIC FIBROSIS: A CASE REPORT.

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Cystic fibrosis (CF) is a life-limiting autosomal recessive disease and it is the most common monogenetic disease in Caucasians. Expected lifetime is about 35 years. CF has various clinical manifestations resulting from the abnormal thick secretions, most common being chronic lung infection and airway obstruction. We present a case of a patient with CF and newly diagnosed Hodgkin's Lymphoma (HL). Because of infectious issues, pulmonary comorbidity and lung toxicity of the most common treatment schedules for HL, the curative treatment of this particular clinical setting of patients is still matter of debate. A 22 years old man, affected by CF, with a history of frequent lung infections and a new diagnosis of HL SN stage IIIs A, IPS 1 (involvement of mediastinic lymphonodes and spleen) was evaluated in our institution in november 2013. Considering the risk of lung complications using containing Bleomycin polichemotherapy (ABVD), as first treatment option, it was chosen IGEV schedule. The interim-PET performed after the first two cycles documented disease progression, therefore we opted for the association of Brentuximab vedotin (antiCD30), epirubicin, and dacarbazine vinbalstina (B-EVD). Two complete cycles were administrated, with a following new early PET evaluation, which documented responsiveness. On this light, the treatment was continued up to a total of six completed cycles. After the last one, the patient developed an infective lung complication resolved completely with broad-spectrum antimicrobic therapy. The final evaluation showed the acheving of a complete remission. To date, the patient has not presented any worsening of his respiratory function. In conclusion, the association Brentuximab vedotin-chemotherapy has been resulted an effective and safe choice of therapy for patients with concomitant severe lung disease.

P077

LYMPHOMA SURVIVORSHIP AND CARDIOVASCULAR DISEASES SURVEILLANCE: A SINGLE CENTER EXPERIENCE

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Background: Improvements in the treatment of Lymphomas have resulted in an increasing number of long term survivors. This patient's population is at risk of developing late therapy related complications as cardiovascular diseases and secondary cancers. *Aims and Methods:* In our institution since September 2014 the Hodgkin's (HL) and aggressive non Hodgkin's Lymphomas (NHL) long term survivors are followed up in a dedicated clinic. This approach allows clinicians to better appreciate and monitor not only signs and symptoms of the known hematologic disease but also of other medical problems that can affect people undergone previous radiation therapy and/or chemotherapy (survivorship). An additional target of this project is to define a program of early detection of otherwise asymptomatic cardiovascular, endocrine, respiratory, neurologic and oncologic disease. In this work we focus on the preliminary results of screening of asymptomatic cardiovascular disturbances. *Results:* We analyzed data regarding 312 patients coming in our clinic from 15 September 2014 to 23 April 2015, 159 were affected by HL and 153 by NHL. One hundred sixty three are females, 149 males; median age at observation is 53 (range 22-89). All of them are in complete response for lymphoma for at least 5 years from the completion of curative therapy. Electrocardiographic and echocardiografic evaluation has been performed in 112 patients without known cardiological problems, selected mainly according to the age, cardiovascular risk factor, time from therapies, type and amount of antiblastic (antracyclines) and mediastinal radiotherapy received. Sixty four out of 112 (57%) showed previous unknown cardiac disturbance: particularly 36 presented diastolic relaxation abnormality, 31 valvulopaties of different type and grade, 2 has low grade myocardic thickness, 7 arterial hypertension, 2 tachycardia and 1 low grade pulmonary hypertension (some patients had more than one alteration). The median age of patients with cardiovascular abnormalities discovered during the screening is 51.5 (range 32-75), 40 of them had received therapy for HL and the other 24 for NHL, 40 were women and 24 men. *Conclusions:* Our analysis confirms that an high percentage of patient survived to lymphomas can develop cardiovascular diseases and outline the importance of cardiac surveillance. Their monitoring can detect asymptomatic structural and functional anomalies as diastolic relaxation abnormality considered an early sign of cardiomyopathies.

P078

EXTRALYMPHATIC INVOLVEMENT IN PATIENTS WITH HODGKIN'S LYMPHOMA: CLINICAL FEATURES AND OUTCOME

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Hodgkin's lymphoma (HL) is a highly curable disease and 5-year survival is improving, being currently 86%. Cure rates of more than 90% are expected for early HL and more than 70% for those with advanced HL. At presentation, HL is usually supradiaphragmatic, with contiguous spread often occurring predictably from one nodal group to the next along the lymphatic pathways. HL is usually almost entirely confined to the lymph nodes. Extralymphatic involvement is much less common in HL than in non-Hodgkin lymphoma. Extralymphatic invasion of adjacent tissue is seen in up to 15% of cases. *Aims and Methods:* To further assess the presenting features and the prognostic significance of extralymphatic disease in HL we performed a retrospective single institution study of 178 cases with a median follow-up of 54 months. Median age was 35 years (range, 15–83 years); 133 pts (75%) were younger than 45 years of age. Stages III-IV were present in 106 pts (60%), bulky disease in 82 pts (46%), extralymphatic involvement in 32 (18%); 103 pts (58%) had an IPS score 0-2 (low-risk) and 72 (40%) had a score >2 (intermediate-high-risk). Combined radio-chemotherapy was administered in 115 pts (65%) and chemotherapy alone in 62 (35%). *Results:* Extralymphatic disease was documented in 32 pts (18%) with a median age of 35 yrs (16-82 yrs), 24 (75%) had B symptoms, 16 (50%) had bulky disease, 28 (87%) had stage IV disease. Extralymphatic sites included the lung in 15 pts (71%), bone in 12 (38%); liver in 6 (19%), kidneys in 1 (3%), pleura in 2 (6%), pancreas in 1 (3%). The 32 patients with extralymphatic disease had poor prognosis compared with the nodal group (5 year progression-free survival [PFS], 73% *versus* 82%; p=0.010). Compared with the nodal subset, the extralymphatic subset presented more frequently with advanced stage disease

(79% *vs* 21% p<0.001), a significantly higher (>2) HD-score (35.5% *vs* 10.3% p<0.001), B symptoms (63% *vs* 46% p=0.020), and a higher ESV level (64% *vs* 37% p=0.001). Complete remission (CR) rates in the extralymphatic and the nodal subsets of patients were 56% *vs* 71% (p=0.003), respectively. *Conclusions:* In our study extralymphatic disease in pts with HL is a rare occurrence (18%) associated with a poor clinical outcome. Further multicentric studies are needed to confirm our data.

P079

PROGNOSTIC IMPACT OF AN UNBALANCED IMMUNE SYSTEM IN PATIENTS WITH CLASSICAL HODGKIN'S LYMPHOMAS

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The clinical and pathologic features of classic Hodgkin's lymphoma (cHL) reflect an abnormal immune response that is thought to be due to the elaboration of a variety of cytokines by the malignant Reed-Sternberg (RS) cells or surrounding tissues. Most cHL cases are characterized by the expression of tumor necrosis factor receptor (TNFR) family members and their ligands, as well as an unbalanced production of Th2 cytokines and chemokines. Activation of TNFR members results in constitutive activation of nuclear factor-kappa B (NF-kappa B), a transcription factor shown to be important for *in vitro* and *in vivo* growth of RS cell lines. The expression of Th2 cytokines and chemokines leads to the reactive infiltrate of eosinophils, Th2 cells, and fibroblasts characteristic of cHL, and can also contribute to a local suppression of the Th1 cell-mediated cellular immune response. In our study we evaluated the prognostic significance of peripheral blood B,T, NK cells at diagnosis in 178 immunocompetent pts with cHL treated at our institution between January 2006 and December 2013. Median age was 35 years (range, 15–83 years); 133 pts (75%) were younger than 45 years of age. Stages III-IV were present in 106 pts (60%), bulky disease in 82 pts (46%), extralymphatic involvement in 32 (18%), 103 pts (58%) had an IPS score 0-2 (low-risk) and 72 (40%) had a score >2 (intermediate-high-risk). Combined radio-chemotherapy was administered in 115 pts (65%) and chemotherapy alone in 62 (35%). *Results:* At the end of treatment, 144 patients (81%) were in complete remission (CR) and 32 (18%) in partial remission. After a median follow-up of 54 months, 41 patients (23%) had relapsed. The variables that had a negative impact on PFS at univariate analysis were advanced stage, bone marrow involvement, IPI score 3-5, PET2 positive, NK cells<200/mcl, CD19<85/mcl, CD3/CD19 ratio>13, CD4/CD19 ratio>10; at multivariate analysis, advanced stage, PET2 positive, CD4/CD19>10 were independent prognostic factors of PFS. *Conclusions:* New biological markers taking into account the surrounding microenvironment could be predictive of response to treatment and survival in cHL patients. These results warrant further studies in a larger group of cHL.

P080

BRENTUXIMAB VEDOTIN IN RELAPSED/REFRACTORY HODGKIN'S DISEASE AND ALCL: A MULTICENTRIC EXPERIENCE IN STANDARD CLINICAL PRACTICE

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Brentuximab vedotin (BV) is a novel antibody-drug conjugate comprising an antiCD30 antibody conjugated to the antitubulin agent monomethyl auristatin E. Clinical trials indicate that BV obtains promising results for the management of relapsed/refractory (R/R) HD and ALCL. We performed a retrospective multicenter study on 47 pts with HD and ALCL treated with BV in Piemonte region between February 2011 and March 2015 to determine the safety, OS and PFS. Planned clinical characteristics were: R/R HD and ALCL pts, treated with BV before autologous stem cell transplant (ASCT) or after ASCT as a bridge to allogeneic stem cell transplant (SCT) or in relapse after SCT or if not feasible for any transplant. We enrolled 41 pts with HD and 6 pts with ALCL. Male were 21 (45%); median age of HD patients was 38 ys, 19 (48%) in stage III-IV, 17 (42%) with B symptoms and 8 (20%) with PS above normal. Median age for ALCL group was 77 ys, 4 (67%) stage III-IV, 4 (67%) with B symptoms and 4 (67%) with PS above normal. Median number of prior therapy was 2.5 (range 1-10) for HD and 1 (range 1-3) for ALCL. BV was used before ASCT in 9 pts, in 19 pts planned as bridge to SCT (SCT not done in 3 cases for PD), in 5 pts after SCT and in 13 pts was used because of unfit for any transplant. Pts received a median of 4.5 cycles of BV (range 1-16): 5 for HD and 3 for ALCL. All pts were evaluable for interim PET (after 3-4 cycles); ORR (CR+PR) was achieved in 24 pts (51%): 21 (52%) pts with HD and 3 (50%) pts in ALCL group. CR was observed in 13 (32%) HD and 1 (17%) ALCL. At the end of treatment in 41 pts evaluable at the time of present analysis the ORR was documented in 18 (44%) pts (17 HD and 1 ALCL). CR was observed in 10 pts with HD and 1 ALCL. Globally, haematological toxicity was mild with only 2 cases (4%) grade 3-4; grade 3-4 neurotoxicity was documented in 2 pts (4%), gastrointestinal in 4 pts (8%). With a median follow up of 7 months from BV start, 1y PFS and OS rates were 7 months and 21 months for the entire cohort; 1y PFS for HD group was 7 months while 3 months in ALCL group. Eight of 11 pts in CR at the end of BV are still alive and in persistent CR; three pts died after SCT due to GVHD, EBV re-activation and Pneumonia. 1y PFS in pts treated with bridge to alloSCT intent was 38%,1y OS was 69%. This retrospective report confirm that single agent BV can be used outside clinical trials without significant toxicity and is effective with best ORR after 3-4 cycles (Figure 1).

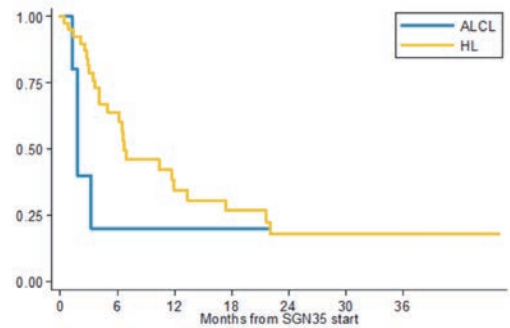

Figure 1. PFS for histology.

P081

FAVORABLE EARLY STAGE HODGKIN LYMPHOMA TREATED WITH 2 CYCLES OF ABVD PLUS INVOLVED-SITE RADIOTERAPY 20 GY: INITIAL VERY GOOD RESULTS IN "REAL-LIFE"

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Background: Favorable early stage Hodgkin lymphoma (HL) is not frequent among all HL; cooperative GOELAMS and GHSG group trials demonstrated that lower doses of involved field radiotherapy (IFRT) are as effective for disease control as higher doses and extended fields, with lower acute toxicity with achievement of 5-years PFS and OS rate of 90% and 95% respectively. *Aims:* To evaluate in favorable early stage HL treated with 2 cycles of ABVD plus reduced volume radiotherapy, according to current definition of "involved-site" RT (ISRT), at dose of 20 Gy. *Patients and Methods:* We retrospectively analyzed 16 newly diagnosed HL patients in stage I or IIA treated, from 2013 to 2015, in two Hematology and one Radiation Oncology Departments in Turin. All patients were staged with a CT scan and baseline 18 FDG/CT-PET. Favorable stage I-IIA was defined as without risk factors according to the GHSG criteria. All patients underwent in first line to 2 courses of ABVD (Doxorubicin 25 mg/m^2, Bleomycin 10.000 u/m^2, Vinblastine 6 mg/m^2, Dacarbazine 375 mg/m^2) followed by ISRT 20 Gy in 10 fractions, planned on initial involved sites at diagnosis. At the end of treatment

were re-assessed with CT and 18 FDG/CT-PET scan one and three months after ISRT respectively. Response was evaluated by international criteria for response evaluation in malignant lymphoma. *Results:* Median age was 40 (range 20-72). 8 (50%) male, 8(50%) female. All patients were classified in favorable stage according to the GHSG criteria. At the diagnosis all patients had a PS 0, normal levels of albumin, Hb and WBC count. Hasenclever score: 4(25%) patients had score 0, 8(50%) score 1, 4 (25%) score 2 respectively. All patients completed the planned therapy with 2 cycles of ABVD followed by ISRT 20 Gy. At the end of therapy all patients achieved a Complete Response. According to WHO no had Toxicity Grade 4, 4 Neutropenia Grade 3.With a median follow-up of 16 months all patients were alive and none had progression or recurrence of disease or experience others events. *Conclusions:* Our experience, applying the "involved site" concept to early stage favorable HL, showed excellent result in term of toxicity and disease control, in line with French and German studies, stressing like the first line therapy for favorable early –stage HL may be 2 cycles of ABVD plus limited dose of ISRT like 20 Gy. The extension to clinical routine of this approach could validate the excellent results obtained in the GHSG study.

P082

BRENTUXIMAB VEDOTIN IN THE MANAGEMENT OF RELAPSED/REFRACTORY LYMPHOMA IN YOUNG PATIENTS: OUR REAL-LIFE EXPERIENCE

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Brentuximab Vedotin is a CD30-directed antibody-drug conjugate (ADC), currently approved for treatment of relapsed Hodgkin lymphoma (HL) and relapsed anaplastic large-cell lymphoma after front-line chemotherapy. A successful experience with brentuximab vedotin as single agent (1.8 mg/kg) in the management of relapsed and refractory lymphoma is described. *Case 1:* N.A., 21 y.o. male, diagnosis in 2011 of ALCL, IIIB, non B non T, ALK-positive, CD30+, underwent to CHOP/14 x 6, achieving a CR. Then, he underwent to IEV, but, after 2 courses, PET/CT documented relapse. So, he was switched to DHAP, with an improvement, but, 1 month after first course, for progressive disease, there was a switch to Brentuximab. After first course, the patient went to Emergency Medicine for respiratory problems, and, after improvement of conditions, he continued. Treatment was well tolerated, and, after 6 courses, the patient is in CR and he undergoes, in 2013, to AlloSCT. Now, after 12 month, he has no signs of disease. *Case 2:* T.A., 35 y.o. female, diagnosis in 2011 of classic nodular sclerosis HL, IIB, with mediastinal bulky disease. ABVD was started, with PR and then radiotherapy on mediastinal mass, without other results. After 3 months, after a relapse, IGEV x 3 courses, after which she underwent to AuSCT in 2012. PET/CT documented progressive disease: Brentuximab was started and, even if after 3 courses the patient was in SD, after 6 courses a PR was achieved, with a CR after 12 courses. The treatment was well tolerated, the only documented side effect was alopecia. *Case 3:* G.R., a female 30 y.o, diagnosis in 2004 of HL, IIB with splenic localization. VEBEP was started, with PR. Then, during radiotherapy, a relapse of disease was documented. IGEV was started, 3 courses, and then she underwent to AutoBMT with a CR. In 2006, PET/CT documented second relapse: MOPP was started (12 courses), until 2007, with PR. In 2007, a third relapse treated with Gemcitabine, with a SD. The patient underwent to DHAP-R x 4, achieving a PR. So, Rituximab maintenance was started, until progression (2012). In that moment, she was switched to Brentuximab and, after 3 courses, a nCR was achieved, which became CR after 6 courses. The only side effect was a Grade 1 Neuropathy. Brentuximab Vedotin has been proved to be effective either in relapsed/refractory HL and NHL as single agent, and it's an excellent option as bridge to au/alloSCT in young patients.

P083

CAN THE RATIO BETWEEN LESION AND LIVER SUVMAX IMPROVE OUTCOME PREDICTION IN HODGKIN LYMPHOMA WITH RESPECT TO THE 5-POINT DEAUVILLE SCORE?

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Interim PET (iPET) evaluated according to the 5-point Deauville score (5p-DS) can identify Hodgkin lymphoma (HL) patients with insufficient response to standard ABVD treatment. In PET-guided treatment approaches, iPET-positive patients are candidates for more intensive and potentially more toxic treatments. To avoid risks due to unnecessary treatment intensification, identification of patients at risk for treatment failure has to be optimized. Recent studies demonstrated that interim target lesion SUVmax has a prognostic significance in patients with lymphoma. Ratio between target lesion and liver SUV has been proposed, to convert visual qualitative scale as 5p-DS in a continuous semi-quantitative scale. Aim of this retrospective study was to evaluate the ratio between lesion and liver SUVmax as prognostic factor in patients with HL undergoing iPET during first-line chemotherapy with ABVD when compared to the 5p-DS. We studied 68 patients with HL (median age 39 years, range 16-72; 30 females, 38 males) diagnosed at our Institution between 2007 and 2014. Stage was limited in 38 patients and advanced in 30 patients. iPET was performed after 2 cycles, was considered positive when scored 4 or 5 on the 5-pDS. We also evaluated interim target lesion SUVmax, liver SUVmax and rPET as semi-quantitative parameters. rPET was defined as ratio between target lesion SUVmax and liver SUVmax in each iPET. rPET ratios of higher than 1 correspond to uptakes in the lesion higher than the uptake in liver therefore considered as positive on the 5p-DS. iPET was scored positive in 11/67 patients (16%) according to the 5p-DS, 1 patient was not evaluable. Median target lesion SUVmax was 1.9 (range 0-15.7), median liver SUVmax 2.7 (range 1.4-4.2), median rPET 0.66 (range 0-6.54). We used ROC analysis to determine the best cut-point for rPET to identify treatment failures. ROC analysis showed an AUC of 0.81, with an optimal rPET cut-point of 1.14 (specificity 94.6%, sensitivity 53.3%). The 2-year progression-free survival was 27.3% in patients with a positive iPET according to the 5p-DS, while it was only 15.3% in patients with rPET>1.14. We conclude that rPET is a strong prognostic factor in HL patients. The cut-point of 1.14 has a high specificity in predicting PFS. It is independent from administered activity and body weight. rPET may help to even better define the highest risk group of HL patients that are candidates for treatment change during first-line chemotherapy.

Non-Hodgkin Lymphoma 1

P084

MONITORING STRATEGY OF ANTHRACYCLINES CARDOTOXICITIES BY BIOMARKERS AND ECOCARDIOGRAPHY IN 104 PRESPECTIVES PATIENTS AFFECTED BY LYMPHOMA

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Anthracyclines (AC) still constitute the mainstay of the 1st line treatment in lymphoma: their use, however, is limited by the occurrence of Cardiac Toxicity (CT). *Patients and Methods:* We started a prospective observational trial in lymphoma patients undergoing treatment with conventional or liposomal AC. We used a comprehensive approach to monitor for AC CT, using a telemedicine(TM) system integrating echocardiography, ECG and biomarkers (Troponin I-TnI). In the period 2012-2014 we enrolled 104 patients in our study, 98 (104) completed the planned treatment (55 males and 44 females). Median age was 60.31 years (range 18,4 to 85,1 years), and 39 patients were>65 years. 25 were HL and 79 NHL (DLBCL was the most represented subtype with 57 cases). Remaining cases were FCL, MCL, TCL, and MZL. Liposomal AC was used in 39 patients while classical AC in 59, with mean cumulative doses of 283.33 and 272.76 mg/sqm, respectively. *Results:* With a median time of 22 months from enrollment, 78/104 patients were recalled in order to follow up with echocardiography and biomarker dosage with Troponin-I (TnI) and BNP. 17/104 patients deceased (cause of death was acute coronary syndrome in 2 cases, disease progression in the others); 7/104 patients refused the follow up. In 7 patients out 78(8,9%) confirmed an elevated a TnI>0,015 and 1/78 revealed TnI>0,055. 7/78 patients developed a BNP rise>100(8,9%). Thanks to this monitoring system we noticed 1 patient in the group of AC who developed a chronic cardiac toxicity events (mild degree ventricular dysfunction and mitral valve insufficiency).While patients underwent to liposomal doxorubicin although an elevated biomarker results, did not show clinically evident heart disease. *Conclusions:* In a low-risk setting for AC CT, a monitoring strategy combining clinical, imaging, instrumental and biomarker data seems to enhance the sensitivity of separate methods. Even with low cumulative doses, with a median follow up of 22 months, subclinical signs of AC CT were found in at least 11% of patients. The use of liposomal AC allow the safe treatment of patients with a previous heart disease diagnosis rather it seem protective in the higher cumulative doses. A longer follow up will be able to clarify the impact of arising of TnI more than 0.03 and 0.08 on the developing of AC CT in all our series. On the basis of our experience a multicentric trial has begun on behalf Italian Lymphoma Foundation (FIL) (Figure 1).

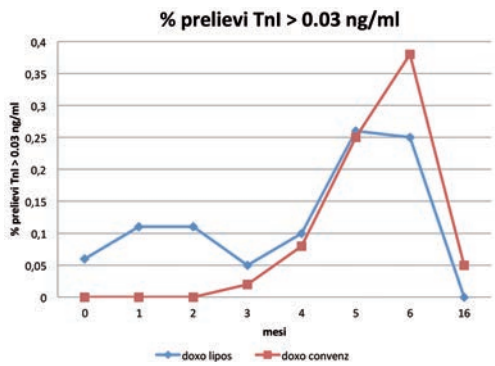

Figure 1.

P085

BENDA-BEAM HIGH-DOSE THERAPY PRIOR TO AUTO-SCT IS EFFECTIVE IN RESISTANT/RELAPSED DLBCL: A PHASE II MULTICENTER STUDY

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Clinical trials of high-dose therapy (HDT) followed by autologous stem cell transplant (ASCT) in lymphomas are often not conclusive because of histological heterogeneity and limited statistical power. We previously demonstrated (Visani Blood 2011) the safety of Benda-BeEAM therapy prior to ASCT in resistant/relapsed (R/R) HD and NHL patients. The regimen showed long-lasting significant anti-lymphoma activity with a 3-yr PFS of 75%. We designed a phase II study to evaluate the efficacy of the BeEAM conditioning in R/R diffuse large B-cell non-Hodgkin lymphoma (DLBCL) patients (pts). The study was registered at European Union Drug Regulating Authorities Clinical Trials (EudraCT) N. 2011-001246-14. 57 pts (median age 54 yrs, range 19-69) were enrolled. 44 pts with R/R DLBCL are evaluable. The primary end-point is to evaluate the 1-yr complete remission (CR) rate. 33/44 pts had advanced stage disease (III-IV), 14 were primary refractory and 30 had relapsed after a median number of 2 lines of therapy (range 2-3). 11 pts had 1 or more relevant comorbidities (range 1- 5). 22 pts were in II or subsequent CR, 19 were in partial remission and 3 had progressive disease. A median number of 5.9x10^6 CD34+/kg cells (range 2.8-9.42) collected from peripheral blood was reinfused to patients. All patients engrafted with a median time to ANC>0.5x10^9/L of 10 days. Median times to achieve a platelet count >20x10^9/L and >50x10^9/L were 12 and 16 days respectively. 10/44 pts presented a fever of unknown origin (23%), whereas 21 pts (47%) presented a clinically documented infection. All pts received G-CSF after transplant for a median time of 8 days (range 8-13). The overall transplant related mortality was 2.7% (1 patient died of incomplete hematological recovery). 38/44 pts are evaluable up to now for response to treatment. 31/38 (81.5%) obtained a CR, 3/38 a PR, 4/38 did not respond to therapy. After a median follow-up of 12 months after transplant (range 2-30), 4/38 pts were refractory, 7/38 relapsed and 27/38 (71%) are still alive, in continuous CR. The stringent inclusion criteria at enrollment allow to precisely evaluate the impact of HDT with Bendamustine followed by ASCT in a highly selected population of patients with DLBCL. Our data preliminary provide the evidence that the Benda-BEAM regimen is safe and has promising high efficacy in R/R DLBCL. *Acknowledgements:* AIL Pesaro Onlus supported the study. Mundipharma Italy provided Bendamustine free of charge.

P086

THE ROLE OF 18F-FLUOROTHYMIDINE PET/CT IN PATIENTS WITH SUSPECT LYMPHOMA RELAPSE

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A monocentric prospective study was designed to evaluate the role of F-18-fluoro-3-deoxy-3-L-fluorothymidine (FLT) PET/CT in lymphoma patients presenting with positive or equivocal F-18-deoxyglucose (FDG) PET/CT at end-treatment or follow-up evaluation. From May 2010 to March 2015, 40 patients were enrolled in the study, all undergoing FLT

PET/CT within 3 weeks from a previous positive FDG scan and, when possible, biopsy confirmation as standard of reference to define PET results (true positive-TP; true negative-TN; false positive-FP; false negative-FN). The highest lesion SUVmax was measured with both tracers. Median age was 55 (range 20-76); 24 patients were male and 16 female. Thirty patients had non-Hodgkin lymphoma and 10 Hodgkin lymphoma (HL). At diagnosis, 8 patients had stage II, 8 stage III and 24 stage IV. Six patients were evaluated at the end of their treatment and 34 during follow-up. FDG-PET was judged positive in 36 out of 40 cases (SUVmax range 3-31; mean 10.1); 28/36 resulted also FLT positive (SUVmax range 3-16.8; mean 7.8) and 16/28 patients had the most active lesion biopsied, demonstrating 15 TP but 1 FP (cervical node, patient with a history of HL, SUVmax 6.9 and 6.8 for FDG and FLT respectively: reactive follicular hyperplasia). The remaining 12 patients could not be biopsied: however, clinical judgment and subsequent follow-up data confirmed 11 TP cases and 1 FP (reactive node). Eight FLT scans resulted inconclusive because of the low SUVmax values (range: 1.7-11.7; mean 4.8); 5 were judged as TP at biopsy (2) or clinical evaluation (3), whereas 3 represented reactive-inflammatory tissue. Among the 4 FDG-PET considered inconclusive (SUVmax range 4.9-8.8; mean 7.2), 3 out of 4 also resulted FLT inconclusive (SUVmax range 2.6-4.9; mean 4), but the final clinical evaluation excluded lymphoma (1 reactive cervical node, 1 nodal sarcoid-like disease, 1 aspecific pericecal finding). The remaining 1/4 resulted FLT positive (SUVmax 6.9 and 6.5 for FDG and FLT, respectively) and was clinically confirmed to be a TP case. In the particular setting of residual/recurrent lymphoma, our preliminary data suggest that FLT can be complementary to FDG, although the elimination of inconclusive PET findings remains a controversial step. Further lesion-based, semiquantitative analyses (*i.e.* target to background/mediastinal blood pool/liver ratio) and correlation with Ki67 proliferation index are ongoing. Updates will be presented at the meeting.

P087

COMPARISON OF TOTAL BODY ULTRASONOGRAPHY WITH TOTAL BODY COMPUTERIZED TOMOGRAPHY AND 18-FLUORO-DEOXYGLUCOSE POSITRON EMISSION TOMOGRAPHY TO DETECT RECURRENCE OF DISEASE IN PATIENTS HODGKIN AND NON-HODGKIN LPH

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Introduction: Total body CT (TB-CT) scan is the NCCN recommended imaging technique with a biannual basis frequency for the first 5 years. PET/CT is strongly recommended for 18 fluorodeoxyglucose (18FDG) avid Lph (Lph). Ultrasound (US) may help in the follow up of Lph patients (pts), with less radiation exposure and less expenses. *Methods:* we retrospectively evaluated our Lph data-base. In the last 4 years we selected 150 Lph pts who relapsed after chemotherapy. N=50 pts were identified who were investigated with both (US) and TB-CT (N=43) or PET/CT (N=7). Pts were assessed on a 3 monthly bases. US assessed: superficial nodes (S): laterocervical, supra and sub clavicular, axillary, and abdominal (A) and, if possible, anterior mediastinal (M) nodes stations. As a control group N=50 randomly chosen pts out of 150 complete remission (CR) pts were analyzed. *Results:* histology of the 50 pts were as follows: HD N=10, Follicular (Fol) NHL N=9, Marginal (MZL) N=3, MALT N=3, Mantle cell (MCL) N=11; not specified N=2, DLBCL N=9, Burkitt N=1; T-NH N=2. In 32 cases there was a concordance between US and CT or PET/CT regarding S and A Ly. In one case CT scan revealed M relapse in respect to US. In 9 cases of contemporaneous M+A+S involvement CT was superior to detect only M involvement. In 3 cases CT or PET/CT revealed lung and or bone involvement with concordance with US in S and A ly. Out of 3 cases of negative US 2 positive cases of CT scan regarded M involvement and 1 A Ly. Nevertheless, overall, there was not a statistically (ST) significant difference (SD) in detection of Lph relapse between US and CT or PET/CT (P=0.186). Pts have been stratified according to BMI (BMI>=25 and <25): BMI does not influence concordance in relapse detection between US and CT or PET/CT (P=0.8). There was not a ST SD in relapse detection according to Histologies. In the CR control group: N=44/50 there was concordance US *vs* CT or PET/CT. In 3 pts PET/CT was a false positive and biopsy confirmed the US negativity. In 1pts/50 US was positive *vs* neg CT and the pts relapsed 3 months later. N=2 pts US revealed increased and dishomogeneous spleen *vs* normal CT and both relapsed 2.8-3.2 months later. In CR pts US specificity is 100%. *Conclusions:* US detected Lph relapses without a St SD in respect to CT and PET/CT. US allows a close follow up, it is radiation-free, and is less expensive. Further prospective studies are warranted in order to assess its usefulness in Lph patients.

P088

SHORT-COURSE R-CHOP FOLLOWED BY (90)Y-IBRITUMOMAB TIUXETAN IN PREVIOUSLY UNTREATED HIGH-RISK ELDERLY DIFFUSE LARGE B-CELL LYMPHOMA PATIENTS: 7-YEARS LONG-TERM RESULTS

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Introduction: High-risk elderly patients with diffuse large B-cell lymphoma(DLBCL) generally display a poor prognosis and markedly soffer from chemotherapy-related toxic effect when undergoing standard regimens.Sequential combined strategies made up of a short courses of chemoimmunotherapy followed by radioimmunotherapy(RIT)are aimed at enhancing the global efficacy of the treatment itself, along with a decreased exposure to extensive amounts of cytotoxic drugs. Long-term follow-up analysis are required to confirm the efficacy and safety of such approaches in this particular setting of DLBCL patients. *Methods:*From December 2006 to October 2008, 55 high-risk previously untreated DLBCL patients,aged 61 to 83 years,were treated in seven Italian institution within a non randomized multicenter phase II trial of four cycles of rituximab,cyclophosphamide, doxorubicin,vincristine and prednisone (R-CHOP21) followed by a single administration of (90)Y-Ibritumomab tiuxetan 6 to 10 weeks later (Zinzani *et al.* Clin Cancer Res; 2010). Response to treatment was evaluated after 4 cycles of R-CHOP21 and after RIT through physical examination, computed tomography (CT) scan of neck, chest, abdomen and pelvis and positron emission tomography (PET) scan. Thereafter a CT and PET scan were performed every 6 months during the first 2 years, then annually for 5 more years. *Results:*The overall response rate to the entire regimen was 80%, including 40 of 55 (73%) patients achieving a complete response (CR) and 4/55 (7%) a partial response(PR). At time of writing, 22/55 (40%) patients experienced a progression disease(PD)and 21 of 40(52,5%) patients who obtained a CR are still alive in continuous CR.With a median follow-up of 7 years, the disease free survival was 42.6% with a progression free survival of 36.1%. The overall survival at 7.9 years was 38.9%.Death occurred in 27 patients and was mostly related to lymphoma progression. Two patients developed a secondary hematological malignancies: one had an acute myeloid leukemia and the other a myelodisplastic syndrome, at 4 and 3 years from diagnosis of lymphoma respectively. *Conclusions:* Our data confirm the feasibility, efficacy and safety of 4 cycles of RCHOP21 followed by RIT consolidation treatment.This results are comparable with those reported using 6 cycles of R-CHOP21.Not the least, this combination allows to despense less chemotherapy,reducing short and long term toxicity in such a frail group of patients.

P089

BENDAMUSTINE COMBINED WITH RITUXIMAB IN RELAPSED OR REFRACTORY DIFFUSE LARGE B-CELL LYMPHOMA PATIENTS: A RETROSPECTIVE MONOCENTRIC ANALYSIS

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Introduction: Relapsed and primary refractory diffuse large B cell lymphoma (DLBCL) shows poor outcome, with 3-years overall survival less than 50%. We have retrospectively analyzed our experience in order to assess the efficacy of the combination of rituximab and bendamustine (BR) as a salvage therapy. *Patients and Methods:* Thirty-two patients with relapsed/refractory DLBCL were treated at our Institution between July 2008 and November 2014. All patients received rituximab 375 mg/mq on day 1 and bendamustine 90 mg/mq for 2 consecutive days every 28 days for up to 6 cycles. Patients' median age was 70 (range 47-85) years; there were 17 males and 15 females. According to histology, all patients had DLBCL, 5 of them transformed from follicular lymphoma. Median number of prior therapies was 2 (range 1-8); 13 patients (40.6%) had relapsed after chemo-immunotherapy. Nine patients (28.1%) had failed a prior stem cell transplant and 19 (59.3%) had a primary refractory disease. Twenty-four (75.0%) patients presented with advanced stage and 9 (28.1%) displayed an intermediate-high/high international prognostic risk (IPI>2) at the time of BR treatment. *Results:* The median number of cycles each patient received was 4 (range 1-6); 13 patients (40.3%) completed all 6 cycles. The overall response rate was 43.8% with a complete response rate of 31.3%. Three patients were able to undergo subsequent autologous stem cell transplantation (ASCT) as a consolidation for their response. Overall survival was 25% at 4 years, and median progression-free survival was 6.2 months. Seven patients are still in continuous complete response, 2 of them after ASCT, with a median duration of response of 11,2 months (range 7-57). The most frequent grade 3 or 4 hematologic toxicity consisted of neutropenia in 11 patients (34.3%) and thrombocytopenia in 1 patient (3.0%). Extrahematologic toxicity was generally mild, with just one case of grade 3 hypercalcemia. *Conclusions:* Combination therapy with BR can be used as an effective salvage regimen in patients with relapsed/refractory DLBCL lacking a valid therapeutic option, showing a good toxicity profile.

P090

DROPLET DIGITAL PCR REPRESENTS A NEW SENSITIVE METHOD FOR DETECTING B-RAFV600E MUTATION AT DIAGNOSIS AND DURING THE FOLLOW-UP OF PATIENTS AFFECTED BY HAIRY CELL LEUKEMIA

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The BRAFV600E mutation has been found in up to 100% of patients affected by hairy cell leukemia (HCL). The aim of this study was the comparison of the real-time PCR (RQ-PCR) and the droplet digital PCR (DD-PCR) in the detecting the BRAFV600E mutation in patients affected by HCL. We assessed 20 cases with splenic marginal lymphoma and 28 HCL patients by a specific real-time PCR for the BRAFV600E mutation and a new PCR technique: the digital droplet PCR (PrimePCR™ DD-PCR™ Mutation Assay: JAK2 WT for p.V617F, Human- Biorad). At diagnosis, 44 cases were assessed: all patients showed the IgH clonality; when they were tested for the BRAFV600E mutation by QT-PCR, all marginal cases resulted wild-type, whereas the mutation was detected in 23 out 24 of the HCL patients; the wild-type patient was affected by a "variant" HCL. Same results were obtained by the DD-PCR. Other 4 cases were assessed as the first determination when in complete remission; all these patients resulted BRAF wild-type. Twenty-eight HCL cases have been monitored during the follow-up by QT-PCR (62 samples) and DD-PCR (118 samples): a significant reduction of "mutational burden" was observed after 2CdA, but even more significant it appeared after rituximab. Interestingly, patients concomitantly receiving rituximab and 2CdA showed a more rapid clearance of disease. At the last follow-up, 19/28 (68%) did not show anymore BRAF mutation; other 2 cases presented the mutation, still detectable but not quantifiable (<10 copies), and 3 patients showed a very low "mutational burden" (0.12). All these patients were in CR. The 4 remaining cases, positive after molecular assessment, were in partial response. Three patients relapsed: in all the BRAF mutation was still detectable at significant levels (more than 2) by real-time and DD-PCR. Sensitivity tests have been performed for both techniques, diluting a mutated DNA with a pool of wild-type DNAs, from 1x10 to 5x10-4. The sensitivity of the real-time PCR was 1x10-4, whereas that of the digital PCR was 5x10-5. When real-time and DD-PCR were applied to the same samples, only one case resulted wild-type by real-time PCR but mutated by DD-PCR, even if at low levels. In one third of cases values measured by DD-PCR were higher than those measured by real-time, whereas in 6% DD-PCR values were lower. In the remaining 65% of tests, both techniques gave the same values. In conclusion, the DD-PCR appeared as a promising molecular technique in HCL.

P091

HIGH EFFICACY OF THE MACOP-B REGIMEN IN THE TREATMENT OF ADULT LANGERHANS CELL HISTIOCYTOSIS. A 20-YEAR EXPERIENCE

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Adult langherans cell histiocytosis (LCH) is an orphan disease. Chemotherapy is usually reserved to patients presenting with single system multifocal (SS-m) or multisystem (MS) disease but due to the lack of randomized studies no standard first line therapy has been defined yet. Pediatric regimens based on the vinblastine/prednisone backbone are not well tolerated in adults and probably less effective. We previously demonstrated high efficacy of the dose dense polichemotherapy regimen MACOP-B (Doxorubicin, Methotrexate, Cyclophosphamide, Vincristine, Bleomycin, Prednisone) in 7 adult patients with SS-m or MS-LCH, in terms of high response rate and durable responses. Here we report an update of this study with long term follow-up data. A total of 11 patients (4 Male, 7 Female) (6 with SS-m and 5 with MS-LCH) were treated with 12 cycles of MACOP-B from 1995 to 2014. Median follow-up was 6.7 years. All 11 patients completed the planned treatment and were assessable for response after 6 and 12 weeks. After 6 weeks overall response rate (ORR) was 100% with 6 complete responses (CR) (55%) and 5 partial responses (PR) (45%). After 12 weeks, at the final evaluation, ORR was 100% with 8 CR (73%) and 3 PR (27%). Four patients (36%) (2 with MS, 2 with SS-m disease) relapsed or progressed after the achievement of initial response (1 after CR, 3 after PR), and overall progression free survival (PFS) was 64%, with 7 of 11 patients who are in first continuous CR after MACOP-B. Notably after a median follow up of 6.7 years, of the 8 patients who initially obtained a CR only 1 patient relapsed after 62 months, leading to a disease free survival (DFS) rate of 87.5%. Overall survival rate was 82% (2 deaths). Since one patient is in second CR after salvage therapy with autologous bone marrow transplantation, 8 of 9 alive patients are disease free at the last follow up (after 228, 216, 144, 96, 66, 47, 32, 24 months of follow-up). Detailed characteristics of single patients are described in Table 1.

Table 1.

Patient N°	Age, Gender	Disease type (SS-m *vs* MS)	Response after MACOP-B	Relapse/ Progression	DFS/PFS	Status
1	23/F	SS-m	CR	No	144+	Alive (CR)
2	62/M	MS	PR	Yes	8	Dead (PD)
3	40/F	SS-m	CR	Yes	62	Dead (PD)
4	27/M	MS	CR	No	228+	Alive (CR)
5	18/F	SS-m	CR	No	216+	Alive (CR)
6	23/M	MS	PR	Yes	5	Alive (II CR, +66m after ASCT)
7	31/F	SS-m	CR	No	96+	Alive (CR)
8	43/F	MS	CR	No	47+	Alive (CR)
9	43/M	MS	CR	No	32+	Alive (CR)
10	62/F	SS-m	CR	No	24+	Alive (CR)
11	59/F	SS-m	PR	Yes	6	Alive (PD, ASCT ongoing)

At the latest follow-up there was no difference in outcome (in terms of OS and PFS) between SS-m (n=6) and MS-LCH patients (n=5). These data confirm high activity of MACOP-B in adult LCH, indicating that a substantial fraction of patients achieve long lasting responses and can be cured with this therapeutic approach.

P092

LENALIDOMIDE MONOTHERAPY OR IN COMBINATION WITH RITUXIMAB OR STEROIDS IN RELAPSED/REFRACTORY DIFFUSE LARGE B-CELL LYMPHOMAS: RESULTS OF A RETROSPECTIVE ANALYSIS

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Patients with RR DLBCL not eligible to high dose therapy had poor prognosis. Lenalidomide showed activity in heavily pretreated DLBCL. We conducted a retrospective study to investigate lenalidomide alone or in combination with rituximab or steroids in RR DLBCL. Inclusion criteria were RR DLBCL patients treated between 2007 to 2013 with: single agent lenalidomide (Len) 25 mg for 21/28 days, lenalidomide 25 mg for 21/28 days with dexamethasone 20 mg/week (Len-Dex); lenalidomide 20 mg for 21/28 days plus rituximab 375 mg/sqm/weekly for 4 courses and then monthly (R-Len). Primary end point was efficacy, in terms of overall response (ORR: complete,CR, +partial, PR), CR and duration of response (DOR); secondary end points were: clinical benefit (CR+PR+stable disease,SD), feasibility and safety. Fifty-two patients were included, 38 (73%) were given Len, 8 (16%) R-Len and 6 (11%) Len-Dex. Clinical characteristics at the start of Lenalidomide were: median age 66 years, stage III-IV 34 (65%), IPI>2 42 (81%), bone marrow involvement 5 (10%). Median number of previous therapies was 2 (1-6). Previous transplant was done in 10 cases (20%), 16 (31%) had refractory disease. Median time from last previous therapy and lenalidomide was 4 months (0.4-23.8). ORR was 22 (42%), with 14 CRs (27%). Median DOR was 11.4 months (0-38.6). ORR and CR rates by treatment were: ORR 45%, CR 29% for Len, ORR 17%, CR 17% for Len-Dex and ORR 38%, CR 25% for R-Len. Clinical benefit calculated for all patients was 26 (50%). Median number of cycles performed in patients who achieved an ORR was 19, while in patients who had a progression was 3; 96% of patients had at least one interruption in treatment; 84% of the expected dose was delivered. Grade 3-4 hematological toxicities were: 23% neutropenia, 18% thrombocytopenia; grade 3-4 non-hematological toxicities were uncommon, but 13% of infections and 1 toxic death, due to pneumonia, were recorded. At a median follow up of 19 months, 14 (27%) patients maintained CR, 7 (13%) responders were on treatment, 8 (15%) were alive with active disease and 23 (44%) died. 2yPFS was 44%. In conclusion, with the limit of a retrospective analysis, the treatment with single agent lenalidomide or in combination with rituximab or steroids was well tolerated and showed promising results in heavily pretreated RR DLBCL. The introduction of biological agents is a potential effective salvage treatment and can ameliorate the prognosis of these patients.

P093

MULTICENTRE ITALIAN STUDY ON GENE-ENVIRONMENT INTERACTION IN LYMPHOMA AETIOLOGY. TRANSLATIONAL ASPECTS: STUDY DESIGN, CURRENT STATUS AND PRELIMINARY RESULTS

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Introduction: A multicentre case-control study on lymphoma aetiology is currently in progress in six Italian areas. The study has been funded by the Italian Ministry of Education, University and Research (MIUR) with the 2007 and 2009 PRIN program, and by the Italian Association for Research on Cancer (AIRC, 2010 Investigator Grant program), and it is part of the International InterLymph Consortium (http://epi.grants.cancer.gov/InterLymph/). We plan to recruit 1000 incident lymphoma cases and an equal number of controls. *Methods:* Cases are identified at the Haematology and Oncology hospital departments in Cagliari, Nuoro, Bari, Taranto, Florence, Perugia, Novara and Verona, among subjects aged 20-74 years, resident in the study areas, first diagnosed with lymphoma (all subtypes according to the 2008 WHO Classification of Lymphoma). Controls are randomly selected from population lists or from among patients admitted to the local hospitals for selected non cancer diagnoses. After signing an informed consent form, each participant undergoes an in person interview and donates a blood sample for the analysis of viral biomarkers, organochlorines, dioxins and dibenzofurans, as well as for genome wide scan within the InterLymph Genome Wide Association Study (GWAS) project. Gene polymorphisms will be subsequently analysed in relation to their interaction with lifestyle factors, occupational and environmental exposures, and health history, based on questionnaire information, and with clinical data, in affecting risk of and survival from the major lymphoma subtypes. *Results:* Thus far, we recruited 688 cases and 495 controls. Refusal rate is 6.5% among cases and 26.6% among the controls. Blood samples are available for 688 cases and 428 controls. Preliminary analyses show a significant interaction between the NAT1 fast acetylator phenotype and dietary intake of heterocyclic amines (p=0.024), and between the NAT2 slow acetylator phenotype and use of permanent hair dyes (p=0.005) in respect to risk of B-cell lymphoma. *Conclusions:* The good level of co-operation within the participating centres has allowed to recruit cases at diagnosis, to collect a blood sample for the majority of controls, and for all the cases before starting chemotherapy, and to identify the controls very close in time to case recruitment. Our study might prompt future collaborative projects combining haematological and epidemiological expertise at the highest possible level.

P094

HIGH-DOSE CLARITHROMYCIN IS A FEASIBLE AND ACTIVE MONOTHERAPY IN PATIENTS WITH RELAPSED/REFRACTORY EXTRANODAL MARGINAL ZONE LYMPHOMA: FINAL RESULTS OF A SINGLE-ARM PHASE II TRIAL

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Background: Clarithromycin displays immunomodulatory and antineoplastic properties. As single agent, this macrolide is associated with tumour responses in patients with relapsed/refractory extranodal marginal zone lymphoma (rrEMZL), with a putative dose-dependent effect. Tolerability and activity of high-dose clarithromycin (HD-K) in patients with rrEMZL were addressed in a phase II trial (clinicaltrials.gov NCT01516606). *Methods:* HIV-negative adults with rrEMZL and at least one measurable/parametrable lesion were enrolled and treated with 4 courses of oral clarithromycin 2 g/day, days 1-14, every 21 days. Activity (ORR) was the primary endpoint, with an estimated sample size of 21 pts (P0=40%, P1 ≥70%; 5%, 80%). HD-K would be considered active if ≥12 responses were recorded. *Results:* 23 pts were registered (median age 70 ys, range 47-88; M:F ratio: 0.27). HD-K was given at ≥2nd relapse in 11 pts. Six pts had multiorgan disease; ocular adnexae was the most commonly involved organ. No pt had ECOG-PS >1 or increased serum LDH levels; only one pt had B symptoms. Five pts had concomitant HBV/HCV infections. H. pylori and C. psittaci infections were excluded at the time of patient registration. The treatment was completed in 18 pts; 79 (86%) of 92 planned courses were actually delivered. Tolerability was excellent, even among HBV/HCV-positive pts; grade 1-2 nausea was the commonest side effect, but it was manageable and did not require dose reduction; only two pts had grade >2 toxicity (grade-3 nausea). Six pts achieved a complete remission and six a partial response (ORR=52%; 95%CI=32-72%). Age, number of previous lines of treatment, site of disease, and number of involved organs did not influence response. The four pts with HBV/HCV infections completed the planned treatment without relevant side effects; three of them achieved an objective response lasting 17+, 18+ and 21+ months. At a median follow-up of 24 (16-33) months, only two pts with responsive (1) or stable (1) disease experienced relapse, with a 2-year PFS of 60±10%; all patients are alive.

Conclusions: HD-K is a safe and active salvage treatment in EMZL pts, even among pts with concomitant HBV/HCV infection. HD-K appears more active than conventional-dose clarithromycin and deserves to be further investigated in EMZL and other lymphoma categories, mainly in combination with immunomodulators.

P095

CONTRIBUTION OF FLOW CYTOMETRY TO THE DIAGNOSIS AND FOLLOW UP OF GASTRIC LNH

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The diagnosis of lymphoma is based on morphology,but the diagnostic accuracy is greatly increased by the use of ancillary techniques. *Aims:* To evaluate the role of flow cytometry in the study of gastric biopsies with suspect of NHL.We have assessed the validity of integration of histological and cytometric assays to optimize diagnosis of NHL and to lower the number of inconclusive cases at histological exam.The aim of flow cytometry was to define lineage and clonality of lymphoid proliferation, thus giving a support to final assessment. *Methods:* 27 patients with primary gastric lymphoma underwent, from January 2007 to June 2010,to double gastric biopsy at diagnosis or during follow up. Diagnosis was MALT in 15/27(56%), DLBCL in 8/27(30%), other histological type in 4/27(14%). Every patient underwent complete staging and the stage was I/E in 12/27 and II/E in 15/27. Therapy was antibiotic eradication of HP in 5/27 and chemo-immunotherapy in accordance with guidelines. Samples for histology were fixed with formalin and stained with HE,then tested by antibodies for CD3, CD5, CD10, CD20, CD21, CD23, CD30, CD38, CD43, CD45RO, CD79a, CiclinaD1, BCL2, BCL6 ,Ki67, EMA, κ e λ . Samples for flow cytometry were put in saline, sent to laboratory within 30 minutes and processed with standard methods. A cytometric pattern was defined as pathological if there was an evident atypical assembly of B or T lymphoid antigens along with clonal restriction for Ig light chains or TCRV repertoire. *Results:* We have carried out 36 biopsies in a population of 27 patients and we obtained a final diagnosis in all cases. Diagnosis was: NHL in 9/36(8 MALT and 1 follicular lymphoma), plasmacytoma in 1/36, and benign conditions in 26/36.In all cases there was strict concordance between the two methods,but in one single cases positivity of flow cytometry led to histological revision and,finally,to full concordance. *Summary and Conclusions:* This study demonstrated that flow cytometry is an easy and reliable diagnostic tool for gastric lymphoma: assumed that its goal is to assess lineage and clonality, it is fast, sensitive and specific. It is synergistic to histology, especially in difficult cases like biopsy performed during a revaluation after chemotherapy or antibiotic therapy, or reactive conditions where lymphoid infiltrates can mimic lymphoma. Histological diagnosis certainly remains the gold standard for lymphoma diagnosis but flow cytometric typing can usefully support histology to reach a correct diagnosis in 100% of cases.

P096

SPLENECTOMY IN SPLENIC MARGINAL ZONE LYMPHOMA: A SINGLE CENTER EXPERIENCE

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Background: Splenic marginal zone lymphoma (SMZL) is a rare chronic B-cell lymphoproliferative disorder and its treatment is not yet standardized. Front line therapy options include splenectomy as well as rituximab (RTX) single-agent or in combination with chemotherapy. *Aims:* To analyze the efficacy of splenectomy in a single centre SMZL series, in comparison with the results obtained by systemic therapy (ST), and to identify predictive factors affecting survival of splenectomized patients (pts). *Methods:* We reviewed all consecutive pts diagnosed with SMZL and treated at our Institution. Response assessment was made as recommended by International Workshop criteria. Survival analysis was performed with Kaplan-Meier method and univariate analysis with Log-Rank test with a significance at 0.05. *Results:* From May 1997 to November 2014, 91 pts with SMZL were treated. Front line therapies included: 54 splenectomy (cohort 1) and 37 ST (cohort 2) including IFN-ribavirine (1 HCV+ patient(pt)), chlorambucil (CHL) based therapies (9 pts), 2CDA (1 pt), RTX (1 pt), RTX-fludarabine based regimens (7 pts), RTX-CHL based regimens (13 pts), RTX-bendamustine (4 pts) and R-CHOP (1 pt). Patients characteristics are shown in Table 1. Only leucocyte count and bone marrow (BM) involvement at diagnosis significantly differ in the two cohorts. Response, evaluable in 89 pts, was as follows: in cohort 1, 59 pts obtained a response (91%), 2 pts were in stable disease (4%), 2 had a progression (4%); in cohort 2, 28 pts (76%) obtained an objective response (9 pts (24%) complete remission, 19 (51%) partial remission), 7 stable disease (19%) and 1 had progression (3%). Haematologic and non haematologic side effects were acceptable and no toxic death occurred. After a median follow up of 49 months (ms)(range 1,2-188), median PFS was 45 and 23 ms in cohort 1 and 2 respectively; median TTNT was significantly longer in splenectomized pts (83 *vs* 25 ms in cohort 1 and 2 respectively, p 0,03). In splenectomized pts ECOG-PS>2 and B symptoms adversely affected PFS, while BM involvement less than 30% and HCV negativity were related to a better TTNT (Figure 1). Overall survival at 3 years was 87% and 89% in cohort 1 and 2 respectively. Of note 30% of pts died for causes not related to lymphoma. *Conclusions:* In our experience splenectomy is a valid front line therapy option for SMZL with higher TTNT compared to systemic treatment, especially in pts with HCV negative and low BM burden (involvement<30%).

Table 1.

		Splenectomy n=54 (59,3%)		Systemic therapy n=37 (40,7%)		p
		N.	%	N.	%	
Median age, years (range)		64,8 (37,8-85,4)		67,5 (37,3-84)		ns
STAGE	I	2	3,7%	0	0,0%	ns
	II	1	1,9%	0	0,0%	
	IV	51	94,4%	37	100,0%	
B SYMPTOMS		11	20,4%	11	29,7%	ns
ECOG>=2		6	11,1%	5	13,5%	ns
HCV positivity		5	9,3%	5	13,5%	ns
Leukocyte Count > 20,000/µL mean leucocyte count (n/µL)		8 10400	14,8% -	11 21900	29,7% -	0,006
Lymphocytosis > 4000/µL mean lymphocyte count (n/ µL)		24 6800	44,4% -	24 17050	64,9% -	0,004
Bone marrow involvment present mean %		49 23	90,7% -	37 37	100% -	0,000

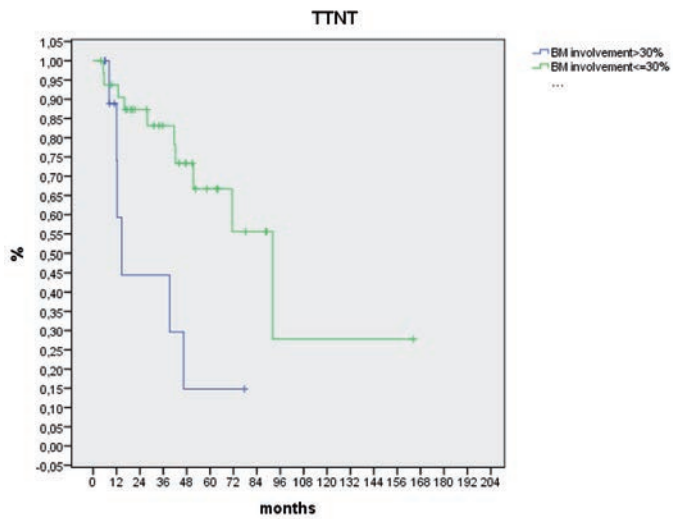

Figure 1.

P097

ORAL CHEMOTHERAPY: AN INNOVATIVE CHOICE

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Despite improvement of lymphoma treatments, many patients still relapse, the majority of whom being elderly and reluctant or unable to receive iv chemotherapy. It is known that treatment with all-oral protocols lowered management costs, without impairing efficacy. In our study, outpatient oral chemotherapy schemes were specifically designed to offer a well tolerated and easy to administer therapeutic option. This program was planned by clinicians, psycho-oncologists and hospital pharmacists, the latter providing detailed information on drugs management. Molecules of widespread use and moderate cost were employed: NIET (Idarubucin30mg/sqm d1, Procarbazine 100mg/sqm d1-4, Etoposide100mg/sqm d1-4, Dex 20 mg d1-4), FC (Fludarabine 25 mg/sqm, Cyclophosphamide 150 mg/sqm d1-4), CD (Cyclophosphamide 100 mg/sqmx2d1-5 Dex 20 mg d1) and Chlorambucil 10mg d1-10. A total of 100 patients were evaluated: median age at treatment start was 77 y (33 – 95), the majority of patients were unfit (37) or frail (46) according to multiparametric geriatric evaluation. Fifty-five patients had indolent lymphoma (Chronic Lymphocytic Leukemia, CLL 29; Small Lymphocytic Lymphoma, SLL 12; others 14), 45 had aggressive lymphoma (Diffuse Large B cell Lymphoma, DBLCL 28, Mantle Cell Lymphoma,MCL 6 and T-cell lymphoma 6, others 5). Twenty-nine patients had no prior treatment, 49 underwent 1-4 previous lines of therapy, 11 patients 5-6 lines and 11 patients more than 6 lines. Efficacy and toxicity were evaluated on 62 patients receiving at least 3 cycles of therapy: 74% had an objective benefit, 14% had stable disease, 12% had a progressive disease. G3-4 haematological toxicity was detected in 12 cases and non-haematological G3-4 toxicity in 2. Psycho-oncological tests revealed that 72% of the patients had a HADS (Hospital Anxiety and Depression Scale) score below the cut-off, both at T0 and at T1, suggesting low psychological distress. What is more, 88% of the patients judged positively the information about the treatment and the presence of the pharmacist. These data showed that chemotherapy does not negatively impact the psychological status. Our all-oral approach is an efficient alternative to traditional iv chemotherapy for elderly and frail patients with no cure expectation. The therapy shows some efficacy, improves the comfort from symptoms, is well tolerated and has restrained costs.

P098

VITAMIN D DEFICIENCY AND SUPPLEMENTATION IN ITALIAN PATIENTS WITH B CELL LYMPHOMAS TREATED WITH RITUXIMAB-CONTAINING CHEMOTHERAPY

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Data from the German RICOVER-60 study indicate that Vitamin D deficiency is a risk factor in elderly patients with diffuse large B-cell lymphomas (DLBCL) treated with Rituximab-containing chemotherapy (R-CHOP) (Bittenbring *et al.*, J Clin Oncol 2014). A retrospective analysis of cohorts of follicular lymphoma (FL) patients showed an inferior survival also for FL patients (Kelly *et al.*, J Clin Oncol 2015). *In vitro* data suggest that Vitamin D supplementation could enhance rituximab-mediated cytotoxicity. We measured prospectively Vitamin D levels in an Italian cohort of 87 newly diagnosed patients with B cell lymphomas (62 patients with DLBCL, 25 patients with FL) who were candidates for Rituximab-containing chemotherapy. Vitamin D levels were considered deficient (<10 ng/ml) in 37 patients (43%), insufficient (10 to 30 ng/ml) in 44 patients (51%), and normal (>30 to 100 ng/ml) in 6 patients (7%). There was no difference between FL and DLBCL patients. Looking at patient characteristics we found a trend for lower Vitamin D levels with older age (p=0.06). In addition, there was a significant seasonal variation with highest Vitamin D levels in the third trimester (p=0.006). As normalization of Vitamin D levels have been shown to improve *in vitro* rituximab-mediated cellular cytoxicity by NK cells, we implemented a substitution regimen to achieve Vitamin D levels early during treatment. We supplemented Vitamin D (cholecalciferole) in a daily dose of 25000 U for a period that varied according to the initial Vitamin D levels and subsequently continued maintenance with cholecalciferole 25000 U once a week. A second determination of Vitamin D levels after a median of 1.1 month in 24 patients showed a significant increase of Vitamin D levels from 14+1.4 ng/ml to 27+1.9 (mean+SEM, p=0.001). Supplementation resulted in rapid normalization of Vitamin D levels in 10/24 patients (42%) No episodes of hypervitaminosis or hypercalcemia were observed. In 13 patients without supplementation, Vitamin D levels showed no significant variation (15+3 ng/ml at diagnosis *vs* 12+1.6 ng/ml during therapy). Studies of Vitamin D levels on NK cell population are ongoing. We conclude that Vitamin D deficiency is frequent in patients with aggressive and indolent B cell lymphomas also in Central Italy. Vitamin D levels can be rapidly normalized using a daily substitution regimen. This may help to improve efficiency of rituximab-containing treatment regimens.

P099

NEWLY DIAGNOSED DIFFUSE LARGE B-CELL LYMPHOMAS TREATED WITH RITUXIMAB - CHOP: DEFINITION AND VALIDATION OF A PROGNOSTIC SCORE MODEL BASED ON MYC, BCL2 AND BCL6 EXPRESSION IN IMMUNOHISTOCHEMISTRY

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Overexpression of MYC and BCL2 and low expression of BCL6 assessed by HIC have been reported as negative prognostic factors in DLBCL. We recently presented (Botto B, ASH 2014) a pilot study, that suggested a prognostic score based on overexpression of MYC, BCL2 and low expression of BLC6, assessed by HIC, in a retrospective cohort of 69 *de novo* DLBCL, with an high proliferation index (MIB1 >70%), treated with R-CHOP between 2010 and 2013. The aim of the present study was to expand the analysis and validate this prognostic score in a larger retrospective cohort of DLBCL, without restrictions of MIB1. *de novo* DLBCL patients, treated with R-CHOP between 2003 and 2013 were included. Cases enrolled in the previous pilot study were considered as control group. Samples were investigated using TMA sections for MYC, while BCL2 and BCL6 staining had been evaluated at diagnosis. Positivity was defined for MYC and BCL2/BCL6 expression by immunostain if >40%, >40% and 25% of cells showed positive expression, respectively. FISH is ongoing. Among 119 patients eligible, 99 were evaluable, 20 were not due to missing clinical data. Clinical characteristics were: median age 63.5 years (IQR 52;73), 66 (68%) stage III-IV, 27 (28%) with LDH upper normal and 30 (30%) with IPI >2. These characteristics are superimposable to those of the pilot study, a part for a lower rate of IPI>2 (30% *vs* 67%). Median MIB1 at diagnosis was 70% (IQR 60-85). At the moment of the present analysis, MYC was investigated in 45/99 cases and an overespression was detected in 10 (22%). BCL2 and BCL6 were analyzed in 77 and 74 cases, with BCL2 overexpression in 64 (83%) and BCL6 low expression in 25 (34%). Median 2y-PFS and OS were 71% and 84% respectively. Applying the prognostic score defined in the previous pilot study (risk of 2 points for MYC or BCL2 positivity and 1 point for BCL6 negativity, pooled scores 0-1, 2 and >3) 2y-PFS were different across the 3 groups: 100% *vs* 83% *vs* 64% (p 0.002). While in the pilot study no differences in OS were showed, in this expanded cohort OS were different across the groups: 100% *vs* 89% *vs* 82% (p 0.07). Our data showed that the HIC prognostic score based on MYC, BCL2 and BCL6 expression, outlined in the previous study, was applicable, reproducible and valid in a larger cohort of DLBCL, regardless of MIB1, forecasting significant different PFS and OS rates. This study provides to extend HIC and FISH analysis in a total sample of above 200 cases.

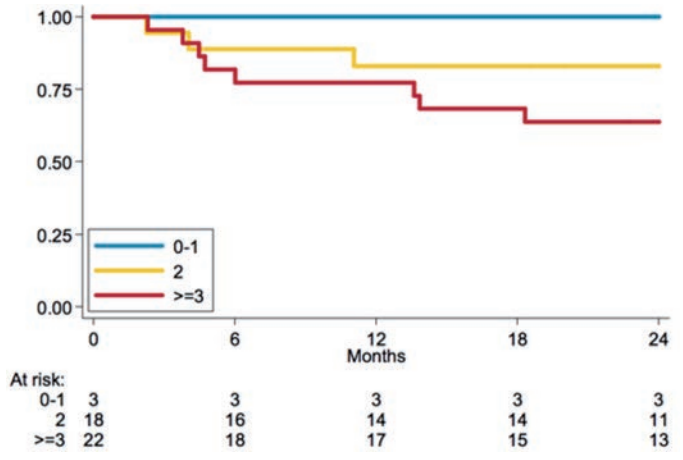

Figure 1. Progression-free survival according to risk score.

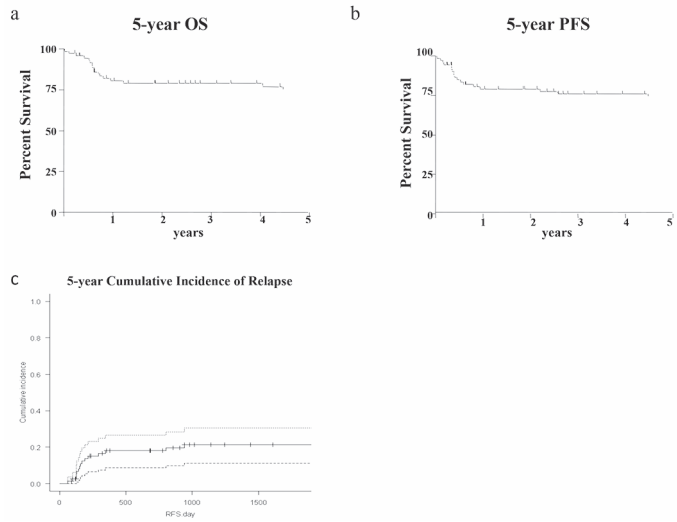

Figure 1. 5-year OS, PFS and CI of relapse of patients with Burkitt lymphoma treated with GITIL a short-intensive chemotherapy regimen.

P100

HIGH CURE RATES OF THE SHORT-TERM GRUPPO ITALIANO TERAPIE INNOVATIVE NEI LINFOMI CHEMOTHERAPY PROGRAM FOR ADULT PATIENTS WITH BURKITT LYMPHOMA

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Burkitt lymphoma (BL) is a rare disease that account for 2% of all cases of non-Hodgkin lymphoma in adults. We have previously published the results of a short and intensive chemotherapy regimen translated from the pediatric patients to adults. Here we update and extend our previous results to a cohort of 82 patients with confirmed diagnosis of BL. From December 1991 to January 2014, we enrolled 82 patients with Burkitt lymphoma. The original regimen was applied to the first 22 patients who were treated at the Fondazione IRCCS Istituto Nazionale dei Tumori and was described on Br. J. Hematol, 2004;126:815-20. Patients not achieving complete remission (CR) before consolidation, were shifted to sequential high-dose chemotherapy (HDS) comprising final infusion of autologous stem cell transplant (ASCT). Rituximab was included in R-HDS only for *in vivo* purging of collected stem cells. An updated version of the protocol was introduced from 2003 and was applied to a set of 60 patients enrolled by the GITIL in five different centers. The new regimen was augmented with rituximab both in the induction phase and after the consolidation phase. Also for R-HDS salvage regimen, more rituximab infusions were added for a total of 6 administrations. Patients characteristics were as follow: M/F 56/26, median age 43 years (range 18-90 years), stage I-II/III-IV 32/49, IPI H or HI in 36, bulky disease in 40, CNS involvement in 5, high LDH levels in 47, ECOG performance status ≥2 in 26, BM involvement in 15 patients, respectively. Toxicity was manageable with only 2 died of early regimen-related toxicity. CR was obtained after consolidation or by R-HDS salvage regimen in 88% of the subjects. With a median follow-up of 1994 days, KM estimates of 5-year OS and PFS were 75% and 74% respectively, and cumulative incidence of relapse, with Gray test, was 21% (95% CI, 12.6-31.7) (Figure 1). We did not observe any difference in terms OS (p=0.6) and DFS (p=0.7) between patients treated before 2003 by a single center or after 2003 in a multicenter setting. Univariate analysis showed that advanced stage, high risk IPI, poor performance status, high LDH levels, SNC and BM involvement were all associated with poor OS, PFS and DFS (p≤0.001). In this study we confirmed that the short intensive chemotherapy program achieves high percentages of CR in patients with BL and demonstrated that it is easily exportable to other hospitals with reproducible results in a multicenter setting.

Myeloma and Monoclonal Gammopathies 1

P101

CHRONIC MYELOMONOCYTIC LEUKEMIA WITH COEXISTING MONOCLONAL GAMMOPATHY: CONCOMITANT COMPLETE REMISSION WITH AZACITIDINE

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Introduction: The coexistence of MGUS with CMML is sporadically observed. Although azacitidine showed significant *in vitro* cytotoxicity against plasma cell (PC) malignancies, its clinical effects in such setting has never been evaluated. *Aims and Methods:* Herein, we report the disappearance of IgA-lambda paraprotein, as incidental finding, observed in a CMML-2 patient treated with azacitidine. A 72-year-old woman was diagnosed in June 2011 as having asymptomatic CMML-1 in association with an IgA-lambda (2.0 g/L) MGUS. The patient was followed–up without any medication until June 2014 when she presented with a life-threatening bronchopneumonia associated with remarkable leukocytosis, transfusion-dependent anemia and thrombocytopenia. A diagnosis of CMML-2 was made; wide spectrum antibiotics and life supports were given as required. Peripheral leukocytosis (WBC=65.000/uL with 5% monoblasts) was controlled by hydroxiurea. A 25% of CD33, CD69RPGM1 and CD68KP1 positive monoblastic precursors along 8% of IgA-lambda PCs were seen in the bone marrow (BM). The karyotype was normal. Serum paraprotein was high as 3, 2 g/L; Bence-Jones proteinuria was absent. No skeletal lesions were present. So that, the patient was started (September 2014) on azacitidine (75 mg/m^2, schedule 5 +2 +2), achieving soon the transfusion independence and the normalization of the peripheral blood counts; the complete remission (CR) of CMLL-2 was demonstrated after six cycles by a comprehensive BM evaluation that showed, at the same time, the full disappearance of plasma cells. Concomitantly, the disappearance of the serum monoclonal peak and a normal pattern of electrophoresis were observed, although the paraprotein was yet detectable on immunoelectrophoresis. Today, the patient is in CR and is receiving the eighth azacitidine course. *Conclusions:* Our patient presented a severe and life threatening bronchopneumonia at the transformation of her primary CMML-1 in a more aggressive form 3 years following the primary diagnosis; at that time a slight progression of her MGUS was also observed. Azacitidine allowed for the CR of CMML-2 and, at the same time, for the disappearance of IgA-lambda paraprotein. So that, our incidental and anecdotal observation showed that azacitidine is provided of clinical activity on malignant plasma cells so that this agent could be candidate, alone or more properly in combination to other anti-myeloma compounds, to clinical evaluation in this setting.

P102

COMBINED USE OF HLC AND FLC IN MULTIPLE MYELOMA AND MONOCLONAL GAMMOPATHIES PATIENTS

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Introduction: International Myeloma Working Group guidelines recommend serum protein electrophoresis (SPEP) and serum free light chain (FLC) immunoassays with derived kappa/lambda ratios for diagnosis, prognosis and monitoring in multiple myeloma and monoclonal gammopathies. Heavy light chain (HLC) immunoassay can be used in screening, monitoring and risk stratifying of multiple myeloma and monoclonal gammopathies patients. *Materials and Methods:* Beginning from July 2012 we have observed 150 patients in our Haematology Unit selected by the presence of monoclonal component at SPEP. We have effected FLC plus HLC assays at diagnosis and after at regular intervals for monitoring of multiple myeloma disease or monoclonal gammopathies. We have evaluated HLC and FLC ratios on the basis of patients disease (Multiple Myeloma (MM), smouldering myeloma, Monoclonal Gammopathy (MGUS), Waldenström disease (MW). *Results:* In many patients combined evaluation of HLC and FLC is useful for identification of CR an MDR in course of multiple myeloma monitoring. In several cases both tests are needed to exclude presence of minimal amounts of M-Ig. Persistent disease was indicated by an abnormal HLC ratio in 10/43 patients who achieved CR with normal FLC ratios. Somewhat contradictory is the observation of a normal HLC ratio in 15/48 IFE-positive patients achieving nCR or VGPR. Also we have observed several cases of double M-component in course of therapy for multiple myeloma with normal FLC ratio. *Conclusions:* Combined evaluaton of HLC and FLC is useful for diagnosis, prognosis and monitoring of Multiple Myeloma and Monoclonal Gammopathies.

P103

NEUTROPHIL TO LYMPHOCYTE RATIO IMPROVES THE RISK ASSESSMENT OF ISS STAGING IN NEWLY DIAGNOSED MM PATIENTS TREATED UPFRONT WITH NOVEL AGENTS

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Background: Recent reports identify the ratio between absolute neutrophils count (ANC) and absolute lymphocyte count (ALC), called NLR,as predictor of progression free survival (PFS) and overall survival (OS) in cancer patients. We retrospectively tested NLR in a cohort of 309 newly diagnosed MM patients treated upfront with novel agents. *Methods:* NLR was calculated using data obtained from the complete blood count (CBC).PFS and OS were evaluated. *Results:* Median NLR was 1.9 (range 0.4-15.9). Higher NLR was not due to ISS stage, plasma cell infiltration or cytogenetics. The 5-year PFS and OS estimates were, respectively, 18.2% and 36.4% for patients with NLR ≥2 *versus* 25.5% and 66.6% in patients with NLR <2. NLR ≥2 reduced PFS for ISS stage I and OS significantly for stages I and III, but not stage II. Among younger patients (age <65 years, N=179), NLR ≥2 had a negative prognostic impact on both PFS and OS, in all ISS stages. By combining ISS stage and NLR in a model limited to young patients, we found that 19% of the patients were classified as very-low risk group, and 70% and 11% were in standard-risk and very-high risk groups, respectively. The 5-year estimates were 39.3%, 19.4% and 10.9% for PFS and 95.8%, 50.9% and 23.6% for OS for low, standard and high risk groups, respectively. *Conclusions:* We found NLR as predictor of PFS and OS in MM patients treated upfront with novel agents. NLR can be combined with ISS staging system allowing a better identification of patients with dismal outcome.

P104

EFFICACY OF "POMALIDOMIDE AND DEXAMETHASONE" IN SECONDARY PLASMA CELL LEUKAEMIA

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Introduction: sPCL corresponds to leukaemic transformation of a previously diagnosed Multiple Myeloma (MM) and it represents terminal event with a median survival of a few weeks. Bortezomib and old generations IMIDs demonstrated variable degrees of efficacy in PCL. Pomalidomide as single agent or as combination in PCL has not been investigated. *Case Report:* A 72-year-old woman was diagnosed with IgGk (ISS II) MM. The bone marrow aspirate was extensively infiltrated by plasma cells. The peripheral blood smear did not reveal circulating plasma cells. FISH analysis revealed a t(4;14). A complete bone survey showed several lytic lesions. Lenalidomide together with dexamethasone was started (CC-5013-MM-020 protocol). The patient did not respond to the induction therapy and, therefore, nine 6-week cycles of VMP regimen (bortezomib-melphalan-prednisone) was instituted. The patient was completely clear of the disease. After 11 months, in the bone marrow an extensive infiltration of the immature plasma cells was observed. Salvage protocol with bendamustine plus dexamethasone and lenalidomide was given (RV-MM-GIMEMA/GISL 430). After 3 BdL cycles the patient showed deep vein thrombosis of the left lower limb although thromboprophylaxis therapy. Therapy was then modified to bendamustine plus

dexamethasone, but with this combination there was no decrease of the M-protein. The therapy was, therefore, switched to bortezomib plus pegylated liposomal doxorubicin (Caelyx)and dexamethasone. A partial response was documented after 6 cycles. Two months after the end of the PAD regimen, progression into PCL was observed. Pomalidomide (4 mg on days 1-21 of repeated 28-day cycles) together with dexamethasone (40 mg on days 1, 8, 15, 22) was started as a final attempt. After the fourth course, the patient achieved an interesting response (disappearance of circulating plasma cells, significant decrease of M-component). The main grade 3-4 adverse events of clinical interest were neutropenia and fatigue. Grade 4 neutropenia has been managed with dose interruptions and prophylactic use of G-CSF and anti-infective drugs. Grade 3/4 fatigue caused a delay in the start of sixth cycle and a consequent reduction in the dose of pomalidomide from 4 mg to 2 mg/d. At 6 months from diagnosis, the patients still alive receiving pomalidomide plus dexamethasone at the best-tolerated doses. *Conclusions:* Our encouraging responses suggest that the combination of pomalidomide plus dexamethasone is feasible, generally well-tolerated and shows promising results also in PCL.

P105

EVALUATION OF HEVYLITE® ASSAYS FOR THE QUANTIFICATION OF IGA MONOCLONAL COMPONENTS

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Background: In patients with Monoclonal Gammopathies (MG), Monoclonal Component (MC) quantification is necessary for diagnosis, risk stratification and assessment of response to therapy. Serum Protein Electrophoresis (SPE) is the recommended technique to identify and measure MCs. When a MC is not quantifiable by densitometry, nephelometric assays for intact immunoglobulins may be used. However, such assays can be limited by measurement of background polyclonal immunoglobulins, lack of antigen recognition and lack of parallelism with polyclonal calibrators. Hevylite® immunoassays (Binding Site,UK) allow to quantify immunoglobulin Heavy-Light Chain (HLC) pairs, providing also an index of clonality by means of Ig'k/Ig' ratios. Objective of the study: To evaluate HLC assay for the quantification of MC in patients with IgA MG. *Methods:* 132 sera from 122 patients, selected on the basis of IgA positive serum immunofixation (sIFE), were analysed. Total IgA values were compared with the sum of HLC IgAk and IgA. In 34 (26%) samples the MC was quantifiable by densitometry and results were compared with the involved HLC value. *Results:* A good correlation between total IgA obtained by nephelometry and the sum of IgA and IgA obtained by HLC assay was found (y=1.067x - 0.406; R^2=0.95; p<0.0001). The Bland-Altman analysis did not show statistically significant or systematic differences between the two methods (mean: 0.12 g/L, CI 95%: -4.37; +4.60), even if some outliers are present. The correlation between densitometric MC measurement and involved HLC value was acceptable (n=34; y=1.162x - 0.519; R^2=0.98; p<0.0001), but Bland-Altman analysis showed a higher mean difference (mean: 1.04 g/L, CI 95%: -4.69; +6.78), probably due to the different analytical principles. It is worth noting that in 26 samples (20%) in which a small IgA MC was identified by sIFE, IgA HLC ratios were within the normal range. *Conclusions:* Our results suggests that HLC measurement can be useful to support the quantification of IgA MC not quantifiable by SPE, due to its ability to monitor the patient irrespective of the migration of the MC by SPE. The HLC measurement provides a quantitative evaluation of IgA MCs, probably less affected by the interference due to residual polyclonal IgA compared to immunochemical measurement of total IgA.

P106

UNUSUAL DIAGNOSIS OF SYSTEMIC AMYLOIDOSIS FROM UNKNOW MULTIPLE MYELOMA IN SMALL BOWEL PSEUDO-OBSTRUCTION: CASE REPORT

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A 78-year-old woman was admitted to our hospital on September 2014 with a history of abdominal pain and clinical signs of intestinal occlusion. Laboratory tests showed a mild normochromic normocytic anaemia (Hb 11.6 g/dl, range 12-17), a reduction of total proteins (5.4 g/dl, range 6,4-8,3) and pcholinesterases (3890 UI/L, range 4700-14000). Electrocardiography showed voltage reduction in all derivations; CT scan abdomen showed dilatation of small bowel with mesenteric edema and the patient was taken to the operating room. Laparotomy exploratory revealed a dilatation of small bowel with mesenteric edema by pseudo-obstruction and gastric mass suspected to be of neoplastic etiology. Partial gastrectomy was performed and patient was referred in chirurgic division. Histological examination showed deposition of eosinophilic material in gastric mucosa, submucosa and muscolaris mucosae. The deposit had an apple-green birefringence by Congo red stain under polarized light. The diagnosis of amyloidosis was performed. Periumbilical fat biopsy confirmed systemic amyloidosis diagnosis. Hematology was consulted and various examinations were executed: presence of Bence Jones protein–k chains in urine, IgG-K/urine 2570 mg/L (range 0-10), IgG-L/urine 10.4 mg/L (range 0-5) ratio K/L 247.1, serum protein electrophoresis showing hypogammaglobulinaemia with gammaglobulin 0.22 g/dl (range 0.80-1.35), IgG 3 g/L (range 7-16), IgA 0.5 g/L (range 0.7-4), IgM 4.0 g/L (range 0.4-2.3), β2-microglobulin 2.4 mg/L (range 0.2-2.3), bone marrow fine-needle aspiration showing plasmacellular involvement rate in 40% of total cells. We concluded for a multiple myeloma (MM) and systemic primary amyloidosis (AL). Was programmed chemotherapy protocol VMP (bortezomib, melphalan, prednisone) but patient died before to start therapy with cardiac failure, few days after MM diagnosis. AL amyloidosis is often associated with MM, smoldering multiple myeloma, MGUS. About 10% of AL patients may have MM at the time of diagnosis and 30% of MM patients may have amyloid deposition at the time of diagnosis. In this case report the patient received occasionally diagnosis of amyloidosis and after haematological examinations was diagnosed a MM unknown. In conclusion this case report and literature data suggest to make a hematological examination in patients with amyloidosis diagnosis to exclude a MM or others plasma cell disorders.

P107

CANCER ASSOCIATED FIBROBLAST IN MULTIPLE MYELOMA: THE UROKINASE RECEPTOR SYSTEM IN TUMOR GROWTH REGULATION

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Background: Multiple myeloma is a B-cell malignancy with terminally differentiated plasma cells (PC). It is known that tumor progression is allowed by a favorable tumor microenvironment (TME) and fibroblasts represent the principal cellular component in TME. Recent findings indicate that the urokinase plasminogen activator (uPA), and its receptor (uPAR) are critical in tumor progression. *Methods:* BM mononuclear cells (BMMCs) were isolated from 12 patients with relapse/refractory MM, 10 patients with asymptomatic MM, 10 with remission MM, 15 with MGUS. Cancer Associated Fibroblasts (CAFs) were purified through anti-fibroblasts-microbeads and they were analyzed and identified by FSP1 and -smooth muscle actin (α-SMA) expression on gated CD45-population. Expression of α-SMA in BM CAFs was also demonstrated by immunofluorescence staining. CAFs were cultured in DMEM medium, fixed and permeabilized according to routine methods. The primary antibodies were anti–uPAR, and anti–α-SMA. Fibroblast nuclei were stained with DAPI. The relative quantity of uPA, uPAR, MMP-2 and α-SMA messenger RNA were determined by the comparative Ct method using 18S ribosomal RNA as the normalization gene. *Results:* Flow cytometry analysis showed that CAFs were increased in patients with relapse-MM compared to patients with asymptomatic, remission MM and MGUS suggesting that CAFs expansion is involved in MM progression. The increased frequency of α-SMA in CAFs of relapsed MM patients was demonstrated by the immunofluorescence analysis. Over-

all, these results suggest that MM activation is associated with the overexpression of uPAR. CAFs activation was also demonstrated by Real Time PCR and the figure shows the overexpression of activation molecules as well as proinvasive systems in CAF of relapsed MM in comparison of MGUS and asymptomatic MM. *Conclusions:* In MM developement and progression the BM niche appears to play an important role in differentiation, migration, proliferation, and drug resistance of the malignant PCs. The main goal of this proposal was to globally approach the expression of CAFs' activation and proinvasive systems in the initiation and progression of MM. For the first time, we demonstrate that an activation of the fibrinolytic system occurs in relapsed/refractory MM compared to less active stages of the disease. Modulating uPAR expression on CAF could be an important key to understand the mechanisms involved in the progression of MM.

P108

RETREATMENT WITH DUPLET OR TRIPLET-COMBINATION IN PAZIENTS WITH MULTIPLE MYELOMA RELAPSED AFTER LENALIDOMIDE- OR BORTEZOMIB-BASED REGIMENS

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No treatment can be currently considered standard in relapsed MM and the choice of appropriate therapy is based on several factors among which the outcome of prior treatment plays an essential role. First consideration in front of a patient with progressive MM is whether a re-treatment with the same agent and in which combinations, can be a valid therapeutic option. This retrospective study aimed to compare the efficacy of a re-treatment with duplets- (DC) or triplet-combinations (TC), containing lenalidomide or bortezomib. All patients, enrolled in first line in 3 prospective controlled trials, who were in first relapse and re-treated with the same novel agent were included in this study. Sixty-three patients met these criteria, 25 received DC [7 were re-treated with Lenalidomide plus dexamethasone (Rd) and 18 with Bortezomib plus Dexamethasone (VD) whereas 38 received TC as per Bendamustine, Bortezomib and Dexamethasone (BVD)] regimen. The two treatment groups were similar as regard median age [69 years (range 40-79) and 68.5 (48-85); p=0.911], median KPS [90% (range 60-100) and 90% (70-100) p=0.427], IgA myeloma rate (24 *vs* 27%; p=0.590). More than half of patients of both groups had ISS stage II-III (60% in DC and 75% in TC; p=0,787) whereas adverse cytogenetic features were documented in 5/25 patients (20%) treated with DC *vs* 4/23 (17%) receiving TC (p=0.326). Similar was also the number of patients with renal failure, being 2 (9%) in DC and 4 (10.5%) in TC group (p=0.858) and the median of PFS1 (33 *vs* 31 months; p=0.875). With a median follow-up of 53 months (11.5-90), median TTP was 7 months in DC group and 19.2 months in TC group (p=0.007); median second PFS was 7 months in DC group *vs* 17 months in TC group (p=0.031) No statistically differences were found in term of OS (median 71 months in DC group and 68 months in TC) and post-relapse survival (median 31.7 months in DC group *vs* 38.8 months in TC group; p=0.982) probably due to effectiveness of the changed of subsequent therapy. In conclusion, in patients with MM in first relapse, re-treatment with a triplet combination such as BVD seems to be more effective compared with duplet combinations containing both lenalidomide or bortezomib suggesting that TC could overcome resistance and reduce the impact of clonal evolution.

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ROLE OF HEVYLITE® EVALUATION IN MULTIPLE MYELOMA PATIENTS ACHIEVING COMPLETE RESPONSE AFTER TREATMENT

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Multiple myeloma (MM) is characterized by a clonal proliferation of neoplastic plasma cells in bone marrow, which produce variable amounts of a monoclonal immunoglobulin (M-component). The International Myeloma Working Group (IMWG) guidelines recommend the use of protein electrophoresis, serum/urine immunofixation and free light chain (FLC) assay for assessing the quality of response to treatment in MM, a parameter which may assumes a relevant prognostic value. In this setting, flow cytometry (FC) is emerging as a useful tool to detect minimal residual disease (MRD). More recently, the detection of the heavy/light chains (HLC) in serum has allowed a more accurate measurement of IgG and, particularly, IgA M-components. In order to evaluate the effect of HLC ratio, FLC ratio and MRD on clinical outcome of MM patients achieving immunofixation negative complete response (CR) after first line therapy (IMWG criteria), we followed a prospective cohort of 25 patients with MM, who had achieved CR after initial treatments including novel agents, with or without autologous stem cell transplantation (AuSCT). Sera samples were tested for HLC and FLC ratios by immunonephelometry, while bone marrow samples were analyzed by home-made multiparametric FC for assessing MRD. The Kaplan-Meier method was used to plot and calculate data on progression free survival (PFS) at 18 months. Variables were analyzed by log-rank test and p-value <0.05 was considered statistically significant. At CR detection time, 18 patients showed normal HLC ratio, 11 had normal FLC ratio (thus they were in "stringent" CR), and 13 had negative MRD. After a median follow-up of 42 months, 12 patients remain in CR and 13 have relapsed, 11 of whom are deceased. Overall, though neither HLC or FLC assays, nor MRD evaluation, alone or in different combinations, showed a statistically significant influence on the clinical outcome, a trend toward a better PFS (81% *vs* 50%) was observed in patients with sCR, particularly in those with IgA subtype (100% *vs* 35%), where normal HLC also identified a subgroup with a more favorable outcome (PFS 67% *vs* 41%). Interestingly, PFS was lower in patients with both abnormal HLC and FLC ratios than in those with only abnormal FLC (40% *vs* 55%, respectively). Our preliminary and still numerically limited experience suggests that abnormal HLC might have a prognostic role in patients with IgA MM in CR and might enhance the negative effect of abnormal FLC on PFS estimate.

P110

REAL CLINICAL PATHWAYS OF MULTIPLE MYELOMA

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A clinical pathway defines the optimal care process, sequencing and timing of interventions. In 2014 the health regional service of Regione Lombardia attempted to define an optimal care process in performing diagnostic procedures in Multiple Myeloma (MM) as follows: "Performing bone marrow aspiration or biopsy, x-ray skeletal survey, magnetic resonance imaging (MRI) of the spine, positron emission tomography (PET) during the 3 months preceding the first hospital admission for MM in 70% of cases". We analized 14 MM real clinical pathways in our institution from 01/01/2014 to 12/31/2014. In this period 6 patients (42.9%) had their first admission to hospital and 8 (57.1%) were followed as outpatient. In the hospitalized patients the following procedures were performed: 6 bone marrow aspiration/biopsies: 3 (50%) in the 3 months prior admission (1 were performed during hospitalization in another clinic) and 3 (50%) during hospitalization 6 x-ray skeletal survey: 3 (50%) in the 3 months prior admission and 3 (50%) during hospitalization 1 MRI of the spine performed during first hospitalization. No PET were performed in our hospitalized patients. Our data shows that the majority of patients affected by multiple myeloma has their normal clinical pathway in an outpatient setting. We think that this analysis can help to better define the optimal clinical pathways for NHL patients.

P111

REDUCED INTENSITY ALLOGENEIC STEM CELL TRANSPLANTATION IN MULTIPLE MYELOMA: LONG TERM RESULTS AND ROLE OF SUBSEQUENT TREATMENTS

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The role of allogenic stem cell transplantation (alloHCT) in multiple myeloma (MM) is currently a debatable issue. Besides its toxicity and the availability of safer and effective new drugs, alloHCT remains the only potentially curative therapy. We analyzed survival outcomes of 67 consecutive MM patients treated with reduced intensity (RIC) alloHCT at our institution from 2000 to 2014. Median age was 55 years (range 31 – 65); 27 patients (40%) received alloHCT as first line consolidation, 40 (60%) received it during subsequent lines of therapy. Fifty-four patients (81%) were chemosensitive at time of alloHCT. Pre-alloHCT treatments with new drugs were: lenalidomide (27%), bortezomib (52%), both drugs (52%), none (48%). All but two patients received autologous HCT before alloHCT. Donor-type was an HLA compatible (HLA>8/10) sibling in 40 cases (60%) and a matched unrelated donor in 27 (40%). Non relapse mortality and progression of disease at 5 years were 6% (95% confidence interval [CI]:2-14%) and 35% (95% CI:23-47%). Cumulative incidence of grade II-IV acute graft *versus* host disease at days +100 was 14% (95% CI:7-23%), and chronic GVHD at 5 years was 35% (95% CI:23-47%). With a median follow-up of 84 (range 1-175) months, the 5-year overall survival (OS) and progression-free survival (PFS) were 69% (95% CI:6-55%) and 35% (95% CI:6-22%), respectively. On multivariate analysis, chemorefractory disease at alloHCT was associated with a significantly inferior PFS and OS (Hazard risk [HR] 3.55 [95% CI:1.48-8.47] and 7.29 [95% CI:2.36-22.5]). Performing alloHCT at first line was associated with a better PFS (HR 0.34 [95%CI:0.13-0.91]) but not with a prolonged OS. At disease relapse/progression patients received: thalidomide (23%), bortezomib (58%), lenalidomide (59%), pomalidomide (16%), bendamustine (16%), donor lymphocyte infusion (36%). Three-year OS after post-alloHCT first relapse was 69% (95%CI:6-55%). Our retrospective data show that alloHCT is a feasible treatment with an encouraging PFS and OS. Chemosensitive disease and alloHCT as first line consolidation are associated with better survival outcomes. Despite a high incidence of relapse/progression after alloHCT, responses to subsequent treatments were common, thus leading to a prolonged OS.

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RELATIONSHIP BETWEEN IgA AND IgG MGUS, IMMUNOPARESIS AND κ/λ RATIO

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Backgrounds: In patients (pts) with MGUS, κ/λratio and Immunoparesis (IP) are related and have a poor prognostic significance. The importance of IP is greater if it involves two classes of immunoglobulins (Igs). *Methods:* Since 2009 to today, we evaluated the type of decreased polyclonal Igs and the relationship with κ/λratio, in 157 pts with MGUS (135 with IgG and 22 with IgA). *Results and Discussion:* 88 of 157 pts have abnormal κ/λratio (56%) and 80 have IP (51%). In pts with Normal κ/λratio 24 had decreased 1 class of Igs and 5 had decreased 2 classes of Igs. In pts with abnormal κ/λratio 34 had 1 decreased Ig, and 20 had decreased 2 Igs (p=0,03). The pts with 2 decreased Igs had abnormal κ/λratio in 94,5% of the cases. In 22 pts with IgA MGUS, 16 had IP (72%): 11 showed only IgM decrease (69%), 1 showed only IgG (6%) and 4 showed the decrease of both (25%). In 135 pts with IgG MGUS, 64 had IP (47%): 40 showed only IgM decrease (63%), 7 showed only IgA (11%) and 17 showed decrease of both (27%). Therefore in both type of MGUS, IgM was the Ig more often decreased. The IgA and IgG MGUS seem to have a different incidence of IP (p=0,06). In pts with IgA MGUS, 8 had normal κ/λratio (in 3 was decreased IgM and in 1 IgM and IgG); 14 have abnormal κ/λratio (in 7 was decreased IgM, in 1 IgG and in 4 both). In pts with IgG MGUS, 66 had normal κ/λratio (of them 21 had decreased IgM, 1 IgA and 1 both Igs); 71 pts had abnormal κ/λratio (of them 19 had decreased IgM, 6 IgA and 16 both Igs). Therefore in both type of MGUS, non-IgM Igs resulted more often decreased in pts with abnormal κ/λratio. If we consider all the 148 pts, IgM were decreased in 26 with normal κ/λratio and in 42 with abnormal κ/λratio. The other Igs (IgA in IgG MGUS and IgG in IgA MGUS) were decreased in 3 pts with normal κ/λratio and in 28 with an abnormal κ/λratio (p=0,009). In pts with κ/λratio between 0,13 and 0,25 and between 1,66 and 3,31 21 had IP (47%). In the pts with κ/λratio >3,31 or <0,13 38 had IP (74%) (p=0,05). *Conclusions:* IgM was the most frequently involved Igs in IP, but in pts with abnormal κ/λratio non-IgM Igs were often decreased too and 2 types of Igs were often contemporary involved. Pts with IgA MGUS seem to have a higher frequency of IP and κ/λratio abnormal. Pts with a more severe alteration of k/ had more frequently IP. As a consequence, the κ/λratio correlate with immunological impairment in pts with MGUS.

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DIFFERENT BEHAVIOUR OF MONOCLONAL AND POLYCLONAL LIGHT CHAINS IN PATIENTS WITH MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE

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Backgrounds: In our patients with MGUS we previously observed a significant correlation between abnormal K/L ratio and immunoparesis: K/L ratio is abnormal in 63% of patients with immunoparesis and in 36% of patients without immunoparesis (p=0,0016). Now we would investigate separately the behaviour of monoclonal and polyclonal light chains. *Methods:* To evaluate polyclonal component, we stratified our 188 patients with MGUS in two groups referring to the medium value of the normal range. We also evaluated the number of patients with a monoclonal light chain value beyond the upper limit of the normal range. Then we evaluated the correlation of policlonal and monoclonal chains value with immunoparesis and with K/L ratio. *Results and Discussion:* Immunoparesis was present in 95 pts. The Polyclonal chains levels are low in 76% of patients with immunoparesis and in 55% of those without immunoparesis (p=0,03). They are low in 67% of patients with an abnormal K/L ratio and in 31% of those with a normal one, too (p<0,001) Monoclonal light chain levels are increased in 92% of patients with abnormal K/L ratio and in 37% of those with a normal K/L ratio (p<0,0001). As a result, alteration of K/L ratio could be related to a polyclonal light chains decrease as well as to a monoclonal light chain increase. In patients with immunoparesis, the cases with monoclonal chain increased are slightly more numerous (p=ns), but considering separately the pts based on K/L ratio, the presence of immunoparesis not affected on increasing of monoclonal chain neither for the group with normal K/L ratio, nor for the group with abnormal K/L. *Conclusions:* The decrease of Polyclonal chains is related with abnormal K/L ratio and immunoparesis, the increase of Monoclonal chain is related only with abnormal K/L ratio. Therefore, the increase of monoclonal chain and immunoparesis could be 2 independent paramaters. Naturally to clarify the prognostic role of the alteration of different type of light chain levels. we need a larger sample of patients.

P114

FEASIBILITY OF LENALIDOMIDE MAINTENANCE THERAPY IN FRAIL PATIENTS WITH MULTIPLE MYELOMA: SUBGROUP ANALYSIS OF A RANDOMIZED PHASE 3 TRIAL

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In the EMN01 phase 3 study, newly diagnosed multiple myeloma (NDMM) patients ≥65 years or not eligible for transplantation were randomized to receive induction with lenalidomide-dexamethasone or melphalan-prednisone-lenalidomide or cyclophosphamide-prednisone-lenalidomide. After induction, patients were randomized to receive maintenance with lenalidomide alone (10 mg on days 1-21 in 28-day cycles) or plus prednisone (25 mg every other day in 28-day cycles), until disease progression or intolerance. In this post-hoc analysis, we performed the geriatric assessment including age, comorbidities (according to Charlson Comorbidity index), Activity of daily living and Instrumental Activity of Daily Living scores. Patients were classified as fit, unfit and frail, and

safety and efficacy of lenalidomide-containing maintenance was evaluated A total of 643 patients started induction treatment, including 280 fit, 202 unfit and 161 frail patients. During induction, 30 fit (11%) *vs* 38 frail patients (23%; p=0.0006) discontinued treatment due to adverse events (AEs). Deaths not related to progressive disease were 4 in the fit group (mainly due to stroke [n=2]) and 17 in the frail group (mainly due to cardiologic AEs [n=7] and infections [n=3]). A total of 402 patients started maintenance treatment, including 192 fit, 121 unfit and 89 frail patients. During maintenance, 24 fit (13%) *vs* 13 frail patients (15%) reported at least one grade ≥3 hematologic AE, while 12 fit (6%) *vs* 12 frail patients (13%) had at least one grade ≥3 non-hematologic AE (p=0.04), mainly cardiologic events and infections. After a median follow-up of 30 months from start of maintenance, median PFS was 24 months in fit *vs* 27 months in frail patients (HR=1.136, p=0.464). Median overall survival (OS) was not reached; 2-year OS was 90% in fit *vs* 76% in frail patients (HR 2.804, p=0.0001). Among the frail patients who started maintenance, 49 did not reduce any drug doses during induction, while 40 patients did (p=0.02). Frail patients who reduced doses at induction tolerated therapy better and had a higher probability to start maintenance. Thus, an induction treatment tailored to the frailty status allows a higher number of patients to complete induction, and to start and benefit from lenalidomide maintenance.

P115

THE ROLE OF IRON ASSESSMENT IN MULTIPLE MYELOMA PATIENTS UNDERGOING AUTOLOGOUS STEM CELL TRANSPLANTATION

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After the observation of an unexpected high incidence of low ferritin levels in multiple myeloma (MM) patients undergoing autologous bone marrow transplant (aPBSCT), we collected data on iron assessment in 23 patients affected by MM undergoing aPBSCT (12 females, 11 males; median age of 57 years). We used T-test for continuous variables, chi square for categorical factors and Pearson r for correlation; p value 0,05 was considered significant. We confirm our clinical observation finding a statistical significant difference between ferritin levels at diagnosis and post induction (170 ng/ml *vs* 51 ng/ml p 0.0039); the same finding was observed using hepcidin levels (9.75 nmol/L *vs* 4.31 nmol/L p 0.0058). We found a direct correlation between ferritin and hepcidin levels at time of aPBSCT (r2 0.63, p 0.002). This observations confirm the important role of hepcidin in iron balance and its strict link with ferritin allowing us to use the latter molecule as iron balance marker in a larger cohort of MM patients. Thus we report data about 96 patients affected by MM undergoing to aPBSCT (43 females, 53 males; median age of 57 years). We found a significant difference between ferritin levels at diagnosis and post induction (185 ng/ml *vs* 58 ng/ml p <0.0001). In our series GI endoscopy was carried out in case of iron depletion and no concurrent disease was detected. No patients received erythropoietin. So we investigated the possible role of new drugs focusing on Bortezomib. Moreover Campanella *et al.* observed how Bortezomib prevents upregulation of ferritin enhancing toxic activity of iron. We found a significant difference in incidence of high ferritin levels between patients receiving Bortezomib and patients not receiving it (9% *vs* 27%); interestingly no patient showing high ferritin levels at post induction evaluation achieved complete response compared to 24.3% of patients showing low or normal ferritin levels (Figure 1).

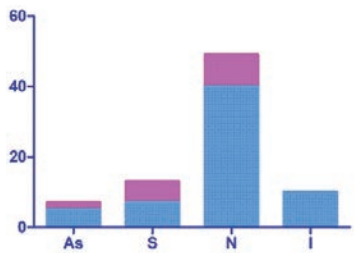

Figure 1. Patients distribution according to iron levels: as=iron deficiency anemia; s=iron deficiency; n=normal iron storage; i=increased iron storage; in the upper part of the columns patients achieving complete remission.

In conclusion iron balance in MM patients undergoing aPBSCT could be a response marker and should be duly investigated in order to correct iron deficiency. We found that Bortezomib play a central role in decreasing ferritin levels by two mechanism: interrupting paracrine loop by curing disease and preventing the ferritin upregulation. The low complete response rate in patients with high ferritin levels need to be investigated in further studies in order to clarify the relationship between Bortezomib administration, iron balance and response to therapy.

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SERUM FREE LIGHT CHAIN EVALUATIONS IN MULTIPLE MYELOMA PATIENTS TREATED WITH NOVEL AGENTS: ROLE IN DETECTING EARLY RELAPSE AND DEFINIG THE PROGNOSIS ON AFTER-RELAPSE OUTCOMES

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Quantification of sFLC has been introduced in the diagnostic workup of MM, despite that the value of this biomarker in the monitoring of the disease still remains an open issue. We analyzed a large series of MM patients relapsed after a first line novel agents-containing regimen with the aim to evaluate the role of serial sFLC assay (Freelite; The Binding Site, Birmingham, UK) measurements for the detection of early biochemical relapse and as prognosticator on post-relapse outcomes. One-hundred patients were analyzed: 81 had Ig secreting MM, 17 light chain MM, and 2 non-secretory disease. All patients received first-line novel agents-based treatments, including immunomodulators in 25, proteasome-inhibitors in 45, and both in the last 30 patients. In all, 50 patients were treated with a program of autologous stem cell transplantation. All patients had a progressive disease defined by conventional IMWG criteria. An abnormal sFLC ratio (sFLCR) at relapse was detected in 78 patients, and the median concentrations of kappa and lambda sFLCs were 88.5 and 94.7 mg/L, respectively. In 15 patients who were classified as having secretory-MM at diagnosis, sFLC escape, defined as an increase in sFLC without a concomitant increase in M-protein, preceded disease progression by a median time of 3.5 months. Notably, 11 out of these 15 patients (73%) had an organ damage as first manifestation of progressive disease according IMWG criteria, with (4 patients) or without (7 patients) a concomitant rise in M-protein. Overall, the median time from sFLC escape to the start of salvage therapy was 4 months. At relapse, an involved/uninvolved sFLCR ≥120 (high sFLCR) was the most powerful cut-off value for discriminating post-relapse outcomes and was observed in 15% of patients. A high sFLCR, compared to sFLCR <120, was associated with shorter time to second progression (5 *vs* 20 months, p=0.002) and overall survival after relapse (12 *vs* 41 months, p=0.004). By multivariate analysis, a sFLCR ≥120 was an independent factor predicting for earlier start of salvage therapy (p=0.028) and shorter time to second progression (p=0.050). In conclusion, the present analysis support the usefulness of integrating sFLC assay into the algorithm of MM monitoring. Serial measurements of sFLC assay during post treatment follow-up allowed to anticipate the detection of relapse in 15% of patients. Moreover, the sFLCR at relapse was a significant prognosticator for post-relapse outcomes.

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GRANULOCYTE-LIKE MYELOID DERIVED SUPPRESSOR CELLS ARE INCREASED IN MULTIPLE MYELOMA DUE TO IMMUNOLOGICAL DYSREGULATION OF MESENCHYMAL STEM CELLS

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Granulocytic-Myeloid-derived suppressor cells (G-MDSCs), a heterogeneous population of myeloid cells with peculiar immunosuppressive properties against T-cells, are increased in Multiple Myeloma (MM) patients, as recently described by our and other groups. MM plasma-cells depend on the bone marrow microenvironment for the growth and survival; important in this context is the role of mesenchymal stem cells

(MSCs), stromal adult stem cells with immunomodulatory properties. The aim of this study was to investigate the role of MSCs on expansion and activation of G-MDSCs. Using flow cytometric analysis, we observed that G-MDSC (CD11b+CD33+CD14-HLADR-) percentage in MM was greater than MGUS and healthy donors (HD) (61.2±1.6% *versus* 53.7±2.2% and 49.7±1.4% respectively, p=0.026 and p<0.001). We found a T-cell anergy driven by MM-G-MDSCs. Subsequently, we investigated the capacity of MSCs from MGUS and MM patients and HD to generate MDSCs. Briefly, human peripheral blood mononucleated cells isolated from HD were cultured alone, with HD (n=6), MGUS (n=6) or MM MSCs (n=7) (1:100 ratio). After one week, PBMCs were collected and G-MDSCs isolated using anti-CD66b magnetic microbeads. The phenotype of G-MDSC was confirmed by cytofluorimetric analysis. Their immunosuppressive capacity was analyzed evaluating T-cell proliferation when co-cultured with autologous CFSE-labeled T cells stimulated by phytohaemagglutinin (PHA). Only MM MSCs-educated G-MDSC exhibited suppressor effect with a reduction of T cell proliferation of about 34±9.6% (p<0.01) compared to G-MDSC control (isolated after culture in medium alone). Notably, neither MDSCs control nor myeloid cells co-cultured with HD or MGUS MSCs showed suppressive activity. Using real time PCR, we analyzed the expression of immune modulatory factors (Arg1, NOS2, COX2, TNF, TGF, IL6, IL10, IL1) by MSCs after 48h of co-culture with PBMCs. MM MSCs showed an increase of COX2, IL6, IL10, IL1, TGF and NOS2 expression (p<0.05) compared to HD MSCs, suggesting that multiple mechanisms are involved in MDSCs induction by MM MSCs. The same immune modulatory factors were investigated in MDSCs before incubation with T cells. MM MSCs-educated MDSCs expressed higher levels of Arg1, NOS2 and IL6 (p<0.05) compared to MDSCs control. In conclusion, MSCs from MM but not MGUS patients are able to activate G-MDSCs with potential implication in immune escape that favours plasma-cells growth, survival and resistance to drugs.

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BORTEZOMIB INHIBITS OSTEOCLASTOGENESIS AND BONE RESORPTION THROUGH MODULATION OF CHIT1 AND YKL40 EXPRESSION

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Osteolytic bone disease is a common manifestation of multiple myeloma (MM) that leads to progressive skeleton destruction and is the most severe cause of morbidity in MM patients. CHIT1 and YKL40 are mammalian chitinases evolved to hydrolyze chitin. Recently, our group has demonstrated that chitinases are important during human OC differentiation and silencing CHIT1 and YKL40 with siRNAs in mature osteoclasts (OCs) results in decreased bone resorptive activity *in vitro*, suggesting a key role for chitinases in OC function. The aim of this work was to investigate the inhibition of osteoclastogenesis by bortezomib (BO) analyzing its regulation of chitinase expression and explore the role of chitinases in osteolytic activity of plasma cells (PCs). First, we confirmed that BO exposure during OC differentiation led to the inhibition of osteoclastogenesis reducing expression of OC markers (MMP9, RANK, CTSK and TRAP; p<0.0001) and reducing bone digestion ability in a dose-dependent manner (2,5 and 5 nM). We further showed that drug treatment down-regulated expression of CHIT1 and YKL40 in a dose-dependent manner (p<0.0001). In particular, BO affected both the component (cytoplasmic and secretory) of CHIT1, YKL40 and MMP9, whose expression is closely associated with chitinases production. Measuring CHIT1 enzyme activity in cell-free supernatants, it also resulted affected by BO during all differentiation process (70±5%, 62±1%, 65±4% and 68±4% respectively at 5, 7, 15 and 21 days; p<0.002). Moreover, immunofluorescence evaluation of OCs showed that BO was able to translocate YKL40 into the nucleus, while CHIT1 remained into the cytoplasm. Subsequently, we chose to investigate if chitinases are involved in PC ability to participate in bone resorption. U266 expressed higher CHIT1 activity (466±111.29 *vs* 216±19.07 and 98±41.44) and showed higher levels of CHIT1 and YKL40 mRNA than SKM-M1 and MM1 MM cell lines (p<0.001). A significant reduction of MM cell digestion activity was observed silencing CHIT1 and YKL40 alone (21.98±5.2% and 25.08±3% *vs* negative control; p<0.003) or in combination (82.85±7%; p<0.0001). Interestingly, exposure of U266 to BO was able to decrease CHIT1 and YKL40 expression in a dose-dependent manner (p<0.0001). In conclusion, we demonstrate that BO treatment inhibits osteoclastogenesis and bone resorption through downregulation of CHIT1 and YKL40, not only in OCs but also in PCs. All these BO effects contribute to its antimyeloma efficacy.

P119

AZACITIDINE IMPROVES THE T-CELL REPERTOIRE IN PATIENTS WITH MYELODYPSLASTIC SYNDROMES AND ACUTE MYELOID LEUKEMIA WITH MULTILINEAGE DYSPLASIA

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Patients with myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML) with multilineage dysplasia show several immunological abnormalities. Azacitidine represents a therapeutic option for these disorders and has been demonstrated to potentially influence T-cell polarization. The aim of this study is to monitor the kinetic of the T-cell receptor (TCR) repertoire during Azacitidine treatment in order to explore its potential ability to reverse the immune derangement typical of these patients. Our study consisted in a flow cytometric and spectratyping analysis performed on the peripheral blood of 11 patients and 30 normal controls. The flow cytometry analysis was based on a panel of 24 β variable (BV) family-specific antibodies. The profile of the third complementarity-determining-region (CDR3) in separated CD4+ and CD8+ T-cells was then analyzed by spectratyping. By flow cytometry, we first demonstrated an improvement from baseline to the following evaluations in the frequency of CD3+ (mean 63.00% *vs* 68.24%, p=0.03), CD4+ (40.09% *vs* 42.05%, p=0.04) and CD8+ cells (20.27% *vs* 25.10%, p=0.02), while the frequency of regulatory T-cells (0.53% *vs* 0.45%, p=0.84) and BV expansions was stable in both CD4+ (mean 1.60% *vs* 2.53%, p=0.36) and CD8+ cells (5.20% *vs* 5.60%, p=0.79). Noteworthy, when monitored by spectratyping during their treatment our patients showed significant changes in their CDR3 profiles, which were much more evident in CD4+ T-cells. In fact, the frequency of skewed B*Vs* was significantly decreased from baseline to the following evaluations in the CD4+ subset (mean 81.45% *vs* 70.17%, p=0.004). This pattern was even more pronounced in patients responding to Azacitidine (89.60% *vs* 61.47%, p=0.002). Also in the CD8+ subset a slight but statistically significant trend towards a reduction in the frequency of skewed CDR3 profiles (mean 99.27% *vs* 98.74%, p=0.01) was demonstrated. Our findings firstly confirmed in our patients an overall derangement of the TCR repertoire. However this pattern seems to gradually improve during Azacitidine treatment, as witnessed by the improvement in T-cell counts observed on flow cytometry but much more by the progressive restoration of the CDR3 diversity detected by spectratyping, especially within the CD4+ subset. Therefore our data suggest that Azacitidine could be potentially able to reverse the immune derangement typical of patients with MDS and AML with multilineage dysplasia.

Myelodysplastic Syndromes 1

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SUCCESSFUL RETREATMENT WITH AZACITIDINE OF A PATIENT WITH ACUTE MYELOID LEUKEMIA TRANSFORMED FROM MYELODYSPLASTIC SYNDROME AFTER THE SUSPENSION OF THIS AGENT

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The outcome of AML transformed from azacitidine-responsive MDS following the discontinuation of this agent is poor. Herein, we report the case of a 63-old woman diagnosed on April 2007 as having RAEB-2 (IPSS: Int– 2) unsuitable for intensive chemotherapy (IC) due to multiple (cardiac, pulmonary and gastrointestinal) comorbidities (MDS-CI=3, high risk). So that, she was started on azacitidine (75 mg/m^2, schedule 5 +2 +2), achieving the complete remission (CR) after eight cycles. She continued azacitidine until March 2011 (28 cycles), when a febrile diverticulitis, an infected perianal fistula and, soon after, a pneumonia were observed. These clinical complications occurred having the patient normal neutrophils count and were considered as due to pre-existing comorbidities, rather than associated to the well tolerated azacitidine therapy that was stopped, requiring the patient complex surgery and antibiotics for long time. Thus far, azacitidine discontinuation was followed by progressive pancytopenia until the progression (4 months later) to low blast count (25%) AML with multilineage dysplasia;cytogenetic and molecular studies showed no abnormalities. Therefore, despite the full resolution of the above described clinical complications, the patient was qualified, once again, as unsuitable for IC. So that, as salvage therapy, azacitidine was resumed (September 2011). The response to azacitidine was initially suboptimal until the fourth course, after which a significant improvement of PB counts, a reduction of the transfusion requirement and, ultimately, the achievement of a partial response (8% blasts) were observed. At present, 3.8 and 8.1 years from the AML and RAEB-2 diagnoses, having the patient received 50 cycles of azacitidine, she is in discrete general conditions with moderate transfusion requirement and a limited, although persistent, disease (10% BM blasts). We interpreted these findings as possibly related to the expansion of a relatively indolent blast population while the patient was off-treatment; however the malignant cells retained, at least in part, the original responsiveness to azacitidine once this agent was restarted. Therefore, our case suggested the possibility of retreatment of previously azacitidine-responsive patients if the evolutions in low blasts count AML was related to a therapy interruption for reasons other than hematologic progression.

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SUCCESSFUL RETREATMENT WITH ERYTHROPOIETIN IN AZACITIDINE-RESPONDING CHRONIC MYELOMONOCYTIC LEUKEMIA PREVIOUSLY EVOLVED FROM REFRACTORY ANEMIA

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The occurrence of CMML transformed from RA is likely uncommon. Herein, we report a RA patient presenting the loss of response to epoetin after seven years of successful treatment, along the need of red blood cells (RBC) transfusions, thrombocytopenia, and prominent absolute monocytosis at CMML transformation. The patient received azacitidine regaining the transfusion – independence (TI) and achieving the complete remission (CR) of CMML-2 after two and six courses respectively. Therefore, he continued to receive the azacitidine as maintenance. However, after the fourteenth course, progressive anemia needing RBC transfusions occurred. The examination of the bone marrow showed the disappearance of blast cells and a marked reduction of monocytes, although in a MDS framework, which was characterized by trilineage dysplasia and erythroid predominance with the features of ineffective erythropoiesis, as observed at the beginning of the RA about eight years before. So that, according to its disease-modifying effects in MDS, we suppose that azacitidine may have been "down-staged" the patient's malignancy from the transformed CMML-2 to a framework interpretable such as refractory cytopenia with multilineage dysplasia (low risk according R-IPSS) showing a similar clinical anemic phenotype compared to that due to primary RA. Therefore, while the patient continued to receive azacitidine, he was restarted on epoetin. A rapid response with the achievement of TI was soon recorded. Therefore, the patient continued to receive concomitant treatments with azacitidine and epoetin, maintaining the CR of CMML-2 and the TI respectively. These findings may reflect a synergistic effects exerted by two agents or, rather than, their separate actions on distinct mechanisms and/or different malignant clones underlying the MDS framework. Our case showed that MDS can display over its course different clinical phenotypes, combining a mixture of aspects associated with high and low risk MDS forms respectively, reflecting an underlying biological heterogeneity over time in the context of a continuum clonal evolution. This process may be influenced and at least partially reverted by the disease- modifying treatments, such as azacitidine and epoetin, which combinations should be explored in prospective studies.

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AZACITIDINE FOR CHRONIC MYELOMONOCYTIC LEUKEMIA PATIENTS IN A REAL-LIFE TREATMENT SCENARIO

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Introduction: Azacitidine is provided of significant clinical activity in patients with chronic myelomonocytic leukemia (CMML). *Aims:* To report the outcome of 17 (10 male) CMML patients who received azacitidine (75 mg/m^2, 5-2-2) during the last 5 years in our center. *Methods:* At the start of therapy, median age was of 75 (60-85) years; CMML-2, CMML-1 with severe and symptomatic cytopenias and CMML-related AML with <30% bone marrow (BM) were recorded in 13, 2 and 2 patients respectively. The median PS was (50- 90) 70%. Seven patients had proliferative CMML, 9 were transfusion-dependent at the start of hypomethylating therapy and only two had abnormal karyotype. Previous therapies were: intensive chemotherapy (1), ESA (7) and hydroxyurea (9); 5 patients were treatment-naïve. The median number of azacitidine courses was 14 (1-32). Treatment was well-tolerated and no remarkable side effects were recorded. *Results:* Out of 17 patients, 14 have completed almost the fourth cycle of azacitidine and were submitted to BM evaluation. one patient presented a frank AML progression after the second azacidine courses. So that, 15 patients were evaluable for response. Out of them, 6 (40%) achieved complete remission (CR) and 6 (40%) a partial remissions (PR) with an overall response rate (ORR) of 80%; three (20%) patients presented a primary failure to azacitidine (2 stable disease and 1 AML progression). After a median follow-up of 21 (1-48) months 9/17 (53%) patients (4 in CR, 4 in PR and 1 with active AML respectively) are still alive: overall survivals (OS) from the CMML diagnosis and from the start of azacitidine were of 27 and 17 months respectively; among responding patients, 2 was successfully transplanted by an unrelated donor after six azacitidine courses. Overall, 6 patients progressed to AML; 1 being primarily unresponsive to azacitidine and 5 having obtained a previous response to treatment (secondary failure). Among the 8 deceased patients, 5 died from AML progression and 3 because of medical complications which were deemed by us unrelated to CMML as well as to hypomethylating therapy but rather due to pre-existing cardiac comorbidities (2 patients) and a secondary lung cancer in the remaining case. *Conclusions:* Azacitidine was safe and effective allowing for an ORR of 80% and a long term OS from the start of treatment without any serious side effects in a real-life treatment scenario of elderly and medically complex CMML patients

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IRON CHELATION THERAPY IMPROVES HAEMATOLOGICAL RESPONSE IN HIGH-RISK MYELODYSPLASTIC PATIENTS TREATED WITH AZACITIDINE

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Azacitidine (AZA) improves long-term outcomes of higher-risk MDS patients and is now the reference frontline therapy of higher-risk MDS not eligible for allogeneic stem cell transplant. Anaemia is the most common symptom of MDS and most patients become transfusion-dependent with the risk of iron overload. Deferasirox is an orally available iron chelator administered once-daily in transfusion-dependent patients with iron overload. We report our experience on using the azacitidine in patients with high-risk MDS, evaluating the efficacy and safety. Concomitant treatment with deferasirox was performed in a routine clinical setting following Consensus Guidelines on Iron Chelation Therapy. In our Institution from October 2009 to December 2014 we have treated 29 elderly patients (19 male and 10 female, median age 76 years, r. 72-88) affected by HIGH-RISK MDS (IPSS INT-2/HIGH). Patients received subcutaneous azacitidine at 75mg/m(2) daily for 7 days every 4 weeks. All patients completed at least 6 cycles of therapy. 11/29 (38%) patients underwent more than 8 cycles of therapy. 17/29 patients underwent as well iron chelation therapy with deferasirox receiving a starting dosage of 10 mg/kg/day, subsequently titrated according to serum ferritin (SF) measured monthly. Complete response (CR), partial response (PR), and hematologic improvement (HI) were observed in 2 (7%), 5 (21%), and 11 (38%) patients, respectively. The median number of cycles to clinical response was 4 (range 4-8). The 2-year rate of acute myeloid leukemia-free survival was 48%. Five serious adverse events occurred in five patients with one fatal outcome. 15 out of 18 patients who showed any hematologic response (CR+PR+HI) meeting International Working Group 2006 criteria had also performed deferasirox therapy. No increased toxicity was noted when deferasirox was used concomitantly with azacitidine. Our results confirm the effectiveness of the therapy with azacitidine in HIGH-RISK MDS elderly patients with acceptable toxicity profile. Peripheral cytopenias were the most commonly occurring adverse event, with gastrointestinal adverse events and injection-site reactions among the most commonly occurring non-haematological adverse events. In conclusion, azacitidine is an important agent for use in the treatment of elderly patients with MDS. Furthermore concurrent use of deferasirox in patients with iron overload seems to significantly improve the hematologic response by reducing transfusion requirement.

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DOES G6PD-DEFICIENCY RELATED OXYDATIVE STRESS AND HEMOLYSIS AFFECT ERYTHROID RESPONSE TO ERYTHROPOIETIN STIMULATING AGENTS IN MYELODYSPLASTIC PATIENTS?

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Background: Anemia is the most frequent cytopenia in Myelodysplastic Syndrome (MDS). Epoetin α, Epoetin β and Darbepoetin α (ESA) have been investigated in several studies as useful to treat anemia in this category of patients.Available pre-clinical data support oxidative stress and hemolysis contributing to ESA resistance but not clinical data is today available. G6PD deficiency is an X-linked condition characterized by a markedly reduced capability to protect red blood cells from oxidative stresses. In the island of Sardinia the prevalence is reported to be as high as 12%. *Patients and Methods:* We retrospectively analyzed all MDS patients who had received ESA in our centre. Diagnosis of MDS was made according to WHO criteria. Patients were stratified based on International Prognostic Scoring System (IPSS). At diagnosis baseline EPO level and G6PD quantitative estimation were detected. Red blood cell (RBC) transfusions requirement before starting treatment was evaluated. Erythroid hematologic improvement (HI-E) was evaluated according to the International Working Group (IWG) response criteria (Cheson *et al.* JCO 2006). *Results:* Thirty patients met the above specified criteria. Of them 7 were G6PD-defiecient and 23 had normal G6PD level values. Seventeen were male and 13 female, median age was 71 years (range 51-96). Twenty patients presented with refractory anemia (RA), 8 refractory anemia with ringed sideroblasts (RARS), 2 refractory cytopenias with multilineage dysplasia (RMCD). Twenty-four patients were IPSS low- risk and 6 Intermediate I. At baseline serum EPO level was less then 200 IU/L in all patients. Forty percent required RBC transfusion before starting ESA treatments. Twenty patients (80%) achieved an HI-E (14 major and 10 minor). In the G6PD-deficient group HI-E was observed in 7 over 7 patients (major in 4 and minor in 3). In the control group HI-E was observed in 17 over 23 patients (major in 10 minor in 7). (P=0.29). *Conclusions:* We evaluated 30 MDS low- risk and Int I IPSS patients who received ESA in the last 20 years in our centre. Despite the common belief that oxidative stress and hemolysis may contribute to ESA resistance, no statistically significant difference to potentially resistance to ESA treatment in G6PD deficiency have been observed. We conclude that G6PD-deficiency does not contraindicate the use of ESA in this setting of patients.

Table 1. Patients' characteristics.

	G6PD deficient patients	G6PD normal patients
Number	7	23
Sex	2 F 5 M	11 F 12M
Age (years)	70 (62-93)	70,5 (51-96)
Diagnosis	3 AR, 3 RARS, 1 RCMD	17AR, 5 RARS, 1 RCMD
G6PD level (UI/g Hb)	Median 0,4 (range 0,04-0,89)	Median 1,19 (range 1,02-1,44)
ESA type	5α-epo, 1β-epo, 1 DAR	19α-epo, 3β-epo, 1 DAR
Serum EPO level < 200 UI/L	7	23
RBC requirement before treatment	7 no, 0 yes	11 no, 12 yes

Abbreviation:
F = female; M = male; G6PD = glucose-6-phosphate dehydrogenase; ESA=erythropoietin stimulating agent; Hi= Hematologic improvement ; MDS= myelodysplastic syndrome; RA= Refractory Anemia; RARS = Refractory Anemia with ringed sideroblasts : RCMD =refractory cytopenias with multilineage dysplasia; RBC = red blood cell

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TFR2 AND EpoR EXPRESSION AT DIAGNOSIS AS POSSIBLE PREDICTORS OF ERYTHROPOIETIN TREATMENT RESPONSE IN MDS PATIENTS

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The myelodysplastic syndromes (MDS) are heterogeneous hematopoietic diseases associated with bone marrow failure, peripheral cytopenias, and a tendency to progress to acute myeloid leukemia. Anemia and transfusion dependency constitute major problems for MDS patients. Treatment with erythropoiesis stimulating agents is a first-line treatment for the anemia of most patients with MDS. However not all MDS respond and a majority of patients eventually relapse with transfusion dependent anemia. Erythropoietin (Epo) is the principal regulator of red blood cell production. Upon Epo binding to its cognate receptor (R), the Epo-R promote the activation of JAK2 and Lyn, which in turn phosphorylate the signal transducer and activator of transcription 5 (STAT5). Dimerization of phospho (P)-STAT5 enables its translocation to the nucleus and binding to target gene promoters, ultimately promoting the expansion, differentiation, and survival of red blood cell precursors. Transferrin receptor 2 (TFR2) is a second transferrin (Tf) receptor that binds to Tf with a 25-fold lower affinity but acts as an iron sensor regulating hepatic hepcidin production. At least 2 alternatively spliced forms of transcripts, α and β, are transcribed from the TFR2 gene. Several recent findings (Forejtnikova *et al.*, Blood 2010; Wallace *et al.*, Br J Haematol. 2015; Nai *et al.*, Blood 2015) highlight the interconnection between the hepatic and erythroid functions of TFR2 in regulating iron metabolism and erythropoiesis. Our monocentric and retrospective study aimed to identify the possible impact of TFR2 and EpoR mRNA expression at diagnosis in MDS patients. We report the expression pattern of TFR2 α, β and EpoR in various subtype of MDS in comparison to normal controls and we found heterogeneous expression with a tendency of TFR2 being less expressed in RAEB-2. Moreover a positive and significant correlation between TFR2 and EpoR expression was seen. Finally, to further assess the clinical implication of the correlation observed, we analysed the erythroid response in the cohort of patients that underwent to Epo treatment and we noticed that only patients with TFR2 and EpoR levels comparable to normal controls reached an increase in hemoglobin level of ≥1.5 g/dl after 12 weeks of treatment. Taken together, our results provide evidence suggesting TFR2 and EpoR as potential molecular markers in predicting at diagnosis the response of Epo treatment in MDS patients.

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REPOPULATING PROGENITOR CELLS IN PRIMARY MDS BONE MARROW CELL CULTURE IN SEVERE HYPOXIC CONDITIONS

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We evaluated the repopulating ability and stem cell potential of hypoxia maintained primary bone marrow (BM) progenitors derived from Myelodysplastic Syndromes (MDS). Thirty seven BM samples were obtained at diagnosis from MDS patients (WHO: 9 RA, 13 RCMD, 7 RAEB, 8 AL/post MDS). Mononuclear BM cells were isolated and cultured with TPO, FLT3-L, SCF, IL-3 in severe hypoxic conditions (0,3%O2, 5%CO2, 95%N2), for 10-13 days (LC1). The stem cell potential of these cultures was explored by transferring cells to growth-permissive secondary cultures in normoxia (LC2), in the presence of SCF, G-CSF, IL-6, IL-3, and according to the Culture-Repopulating Ability (CRA) assay methodology, cell proliferation was evaluated daily. Expression of CD34, CD38, CD117, CD133 was determined and when present, the persistence of chromosomal aberrations was analyzed by FISH at various stages of cell culture. MDS mononuclear BM cells were intravenously injected in sublethally irradiated NOD-SCID mice before hypoxic culture and after LC1. The presence of CD45+ human cells in the peripheral blood of injected mice was evaluated by flow cytometry analysis every two weeks, and quantitated to evaluate the repopulating ability and the different engraftment capacity of cells before hypoxic culture and after hypoxic selection. Mice were sacrificed at day 56 after graft; marrow trephines and spleen biopsies were evaluated morphologically to test the engraftment of human MDS cells. In all cases, cultured cell number decreased of one log or more after 10-13 days of culture in hypoxic conditions. Only 12/37 MDS cases showed a significant repopulating ability at day 17 of LC2, all classified as Low/Int-1 IPSS risk category. In IPSS High and Int-2 risk cases, repopulating ability according to CRA was absent. CD34+ cells were always decreased after hypoxia and did not coexpress CD38. We demonstrated that CD34+/CD38- cells after hypoxic culture in 5 MDS cases, maintained chromosomal aberrations (del5q, +8, del20q, -Y, complex karyotype). Sublethally irradiated NOD-SCID mice transplanted with hypoxia cultured cells showed an higher percentage of CD45+ human cell than mice transplanted with non hypoxia selected cells (5,5% *vs* 0,56%). Severe hypoxic culture conditions are maintaining a sub-population of MDS cells endowed with increased repopulating capacity *in vitro* and *in vivo* in sublethally irradiated NOD-SCID mice; repopulating ability was observed exclusively in IPSS lower risk MDS cases.

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ROLE OF THE TRANSCRIPTION FACTOR CTCF IN THE PATHOGENESIS OF MYELODYSPLASTIC SYNDROMES

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Myelodysplastic syndromes (MDS) are hematologic disorders characterized by ineffective hematopoiesis. Cytopenia can be observed in all three myeloid lineages suggesting the involvement of the multipotent, immature, hematopoietic cell in disease pathogenesis. MDS is considered a "preleukemic" status due to a significantly elevated risk of developing an overt acute leukemia. Genetic and epigenetic abnormalities, critically altering cell proliferation and differentiation of stem and progenitor cells, have been found to be associated with MDS. In the attempt of unravelling a maturation defect driver event linked to MDS epigenetics, we studied CCCTC-binding factor (CTCF), a master gene in the expression regulation, already known to be involved in hematopoiesis. CTCF binds to non-methylated DNA, and can both activate and inhibit transcription by forming chromatin loops between regulatory regions and gene promoters. We investigated CTCF genomic binding landscape in bone marrow-derived CD34^(+) cells from two high-risk MDS patients and one healthy donor (HD) by chromatin immunoprecipitation followed by deep-sequencing (ChIP-Seq). After sequence mapping and peak calling, differentially bound sites between patients and control were annotated and associated to putative target genes. In particular, differential binding analysis (DiffBind R package) returned 398 and 130 enriched regions in HD and MDS, respectively (|fold-change|≥2, p≤0,01). Gene ontology was performed by two bioinformatic approaches, namely GREAT tool (Cory *et al.*, 2010) and ChIPeakAnno (R package). Overall, the obtained gene lists strongly correlated with kinase/phosphatase activity in MDS and oxidative stress response in HD. Publically available microarray (GSE19429) and RNA-Seq (GSE63569) datasets were used to identify a subgroup of CTCF-target genes also differentially expressed in MDS patients (fold-change≥|1.2|, FDR≤0.25). These genes were further studied for functional network and pathway analyses with IPA (www.ingenuity.com). Overall, we found that the most differentially enriched/expressed genes were related to myeloid differentiation and cell death. These results suggest an active role for CTCF in MDS pathogenesis and further studies are underway in our laboratory to better elucidate the mechanisms by which CTCF loses the control of specific target genes.

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HEMATOLOGICAL RESPONSE AND IMPROVEMENT IN LIVER DAMAGE PARAMETERS IN A PATIENT WITH REFRACTORY ANEMIA AND LIVER CIRRHOSIS

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This is a case of a 78 years old woman who came to our observation with the diagnosis of REFRACTORY ANEMIA (IPSS low-risk). She also suffered from HcV-related liver cirrhosis. The patients was already on erythropoietin α treatment at standard doses; as no response was observed she went on a chronic trasfusional support (3 RBCs units per month). After seven month the patients showed datas consistent with a significant iron overload accompanied by a further increase of AST and ALT serum levels.In detail: Hb:7.8 g/dl, serum iron: 228 mcg/dl, transferrin saturation: nd, serum ferritin: 3450 ng/ml, AST: 118 IU/L, ALT: 158 IU/L.At this point, according to current guidelines concerning iron transfusional overload and iron chelating therapy, we decided to start Deferasirox therapy at a daily dose of 15 mg/kg. A few months later the beginning of Deferasirox, without modifing any previous therapy, the patients experienced a progressive improvement of iron overload parameters, of AST and ALT levels and of hemoglobin levels. Consequently we have observed a reduction of the patient transfusional need that now, one year since the beginning of iron chelating therapy is of one RBCs unit per month and an improvement in her quality of life. These are the last patient parameters(one year after starting Deferasirox) Hb: 9.6 g/dl, serum iron: 312 mcg/dl, serum ferritin: 1872 ng/ml, AST: 74 IU/L, ALT: 91 IU/L. The patient carries on with Deferasirox assumption. This case is the expression of how transfusional iron overload, promoting oxidative stress in hematopoietic and in liver cells, may contribute to alter hematopoiesis in bone marrow disorders as myelodysplastic syndromes and to get worse a liver function already compromised in patients who suffer from chronic hepatitis. We are strongly convinced that Deferasirox treatment may contribute to improve hematological and liver function parameters probably by reducing (as demonstrated in several "*in vitro*" models)oxidative stress in erythroid bone marrow precursors and in liver cells, ROS generation, lipid peroxidation and free iron levels.

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IRON CHELATION THERAPY WITH THE DEFERIPRONE PLUS DEFEROXAMINE REGIMEN USED IN PATIENTS WITH THALASSEMIA SAFELY AND EFFECTIVELY REVERSED IRON OVERLOAD-RELATED HEART FAILURE IN A PATIENT WITH MYELODYSPLASTIC SYNDROME

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Here we report the use of intensive ICT in a transfusion-dependent MDS patient who had developed heart failure due to iron overload. A

78-year-old man with refractory anemia with ring sideroblasts presented in our clinic. He had a intermediate risk category (IPSS-R), He was regularly transfused. After receiving 25 units of red blood cells, the patient's serum ferritin value was 1214 g/L and a liver iron concentration12 (LIC) by MRI of 6.5 mg/Fe/g liver (dw). The patient was started on subcutaneus deferoxamine (DFO; 30 mg/Kg/day five times per week), but itch limited compliance. The patient was switched to deferasirox (DFX; 15 mg/Kg/day). DFX was well tolerated initialy, but later the patient experienced headache and systemic skin reactions. When the patient was subsequently twice rechallenged at a lower dose of DFX plus a low dose of steroid, the skin reaction reappeared. DFO was restarted (at 40 mg/kg daily) but compliance was still poor. After 10 months, effort dyspnea appeared (NYHA class III) and transthoracic echocardiography showed a reduction in LVEF (to 40%). The median serum ferritin value increased to 2118 g/L, MRI-T2star showed that LIC had increased to 16.5 mg/Fe/g liver/dw and cardiac MRI indicated a myocardial T2* of 7.9 +/-1.33 msec without alterations of delayed enhancement. Holter EkG recording was normal, and ergometric stress test and angioTC were negative. After having obtained off-label consent from our Ethical Committee and written informed consent from the patient –combined ICT with deferiprone (DFP) and DFO was prescribed. at the dose of 75 mg/Kg after 2 weeks, and DFO 30 mg/kg daily. Hematological parameters were monitored weekly. After 6 months of therapy, transthoracic echocardiography and MRI indicated a recovery in LVEF to 55% (Figure 1). During 12 months of combined ICT, the patient experienced a stable improvement in both cardiac and hepatic condition. Median serum ferritin was 1186 g/L and MRI evaluation showed cardiac T2star of 13.3 +/-1.37 msec and LIC of 8.1 mg/Fe/g liver/dw. The efficacy and safety of combined ICT in reducing severe IO is well known in patients affected by Talassemia, but to our knowledge, this is the first report showing its use in a transfusion dependent MDS patient.

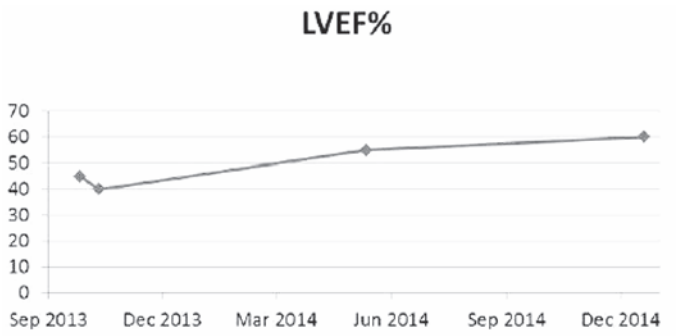

Figure 1.

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IMPACT OF EPOETIN ZETA (BIOSIMILAR EPO α) IN LOW-RISK MDS: ITT ANALYSIS OF A MULTICENTRIC PHASE 2 STUDY

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Background: Erythropoietin (EPO) treatment has been indicated as a safe and effective therapy for low-risk MDS. However, EPO α patent expiration has opened the road to biosimilar epoetins as a valid option, but their clinical efficacy and safety has not been demonstrated in myelodisplasic syndromes (MDS). *Aims:* To verify the efficacy of EPO zeta (retacrit, Hospira, biosimilar EPO α) in the treatment of patients affected by MDS with low/int-1 risk according to IPSS score. *Methods:* In this study, we collected data from 35 MDS patients (male/female ratio 1,5/1), from 4 different Sicilian Centers, aged between 58-84 (median 76.5), treated with EPO zeta. According to IPSS score and WHO classification:13 were diagnosed as with low risk Refractory anemia (RA); 5 with Intermediate-1(Int-1) RA; 4 with low and 11 Int-1 Refractory Cytopenia with Multilineage Dysplasia (RCMD); 2 Int-1 Refractory anemia with excess of blasts (RAEB-1, bone marrow blasts <10%). Inclusion criteria were: 1) Haemoglobin (Hb) below 10 g/dl; 2) Iron, folate and B12 vitamin deficiency correction before therapy; 3) transfusional need of maximum 3 units of packed red blood cells (PRBC) per month in the 90 preceding days; 4) serum EPO levels below 500 UI/ml. EPO was started at 40.000 U s.c./week. Response was defined according to IWG Response Criteria. After 8 weeks, whether there was no response, EPO dose was raised to 40.000 U s.c./twice per week. Data were analysed as Intention to treat (ITT). *Results:* Overall, after 8 weeks, response was achieved in 23 patients, 65% of total, (10 low risk RA; 4 int-1 RA; 3 low RCMD; 5 Int-1 RCMD; 1 Int-1 RAEB-1). In all cases, response was given by the Hb raise. Refractoriness was documented in 12 patients, 35% of total, (3 low RA; 1 Int-1 RA; 1 low RCMD; 6 Int-1 RCMD; 1 Int-1 RAEB-1). At 16 weeks, 7 refractory patients were evaluable. Of them, 2 (28%) patients responded to the doubling of EPOz (1 with low and 1 with Int-1RA), while the remaining 5 (72%) were still refractor to the treatment (4 RCMD Int-1 and 1 RAEB Int-1). No side effects ≥grade 2 were registered. We were able to identify RCMD and the IPSS Int-1 as risk factors for refractoriness (p=0,015 and p=0,03 respectively). No differences were noted regarding basal Hb and serum EPO levels between the two groups *Conclusions:* Compared with data from literature (Park *et al.*, Blood 2008), our ITT analysis revealed that EPOz is a safe and effective therapeutic option in low-risk MDS patients.

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β FIBRINOGEN G-455-A GENE POLYMORPHISM: IS IT A FACTOR FOR ASPIRIN PLATELET INSENSITIVITY IN PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA?

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Essential thrombocythemia (ET) is a myeloid neoplasm characterized by platelet activation and thrombotic risk. Aspirin (ASA) is the standard therapy to normal platelet hyperaggregation and to prevent the thrombosis. It is reported that thrombocythaemic patients are ASA insensitive. It is debated if inherited thrombophilia increases the thrombocythemic platelet activation and, hence, the ASA platelet insensitivity. Therefore, we evaluated Fibrinogen G-455-A gene polymorphism, as thrombophilic molecular mutation associated with increased platelet aggregation, platelet count, β-thromboglobulin (β-TG) and platelet factor 4(PF4) as markers of platelet activation, fibrinogen (Fg), platelet functional activity (PFA) as indicator of ASA platelet sensitivity and clot formation time (CFT), as indicator of aspirinated platelets contribution to clot firmness. We studied 40 patients (24 men, 16 women; mean age 56 years, range 37-77) with ET according to WHO criteria. Fifty subjects served as controls. The mean duration of disease was 11 years. All patients were on ASA 100 mg once daily. The Fibrinogen G455-A genotype was determined using a commercialized polymerase chain reaction kit with sequence-specific primers. Platelets were measured by automated analyzer. β-TG and PF4 were determined by ELISA. PFA and CFT were measured by Platelet Function Analyzer (PFA-100) and by ROTEM δ, respectively. All patients had heterozygous Fibrinogen G455-A. The mean platelet count was 441±72x109/L. All patients had normal Fg (244±47 mg/dl), high β-TG and PF4 (244±15 IU/ml *vs* 20±11 IU/ml and 162±56 IU/ml *vs* 6±2 IU/ml, respectively) ($p<.0001$ and $p<.0001$, respectively), prolonged C/EPI closure time (CT, unit: s, n.v. 84-160 s) (252±48 s) and normal CFT (CFT, unit: s, n.v. 30-110 s)(50±7s). These findings suggest that Fibrinogen G-455-A gene polymorphism is not associated with ASA platelet insensitivity in patients with ET.

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RUXOLITINIB IN PATIENS WITH MYELOFIBROSIS, ROMAGNA EXPERIENCE

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Background: Ruxolitinib is an inhibitor of Janus Kinases, approved for the treatment of myeelofibrosis, a Ph negative disease characterized by progressive inefficacy haematopoiesis and bone marrow fibrosis. The clinical trials (CONFORT-I and II) demonstrated durable clinical benefit in patients with this neoplasm, reducing splenomegaly and improving the disease related symptoms (pain, night sweats worst fatigue). *Materials and Methods:* We describe our experience in nine patients with myelofibrosis treated with Ruxolitinib. All patients were currently diagnosed according WHO criteria including molecular analysis of JAK2 mutations We have stratified all patients for IPSS risk and summarized demographic and base-line patient characteristics in (Table 1A). *Results:* Eigh patients achieved their best response in terms of spleen reduction within twenty-five weeks of treatment; patient Ra8 in one week, patient Ra1 and Ra5 in four weeks, patient Ra3 and Ra6 in eight weeks, patient Ra4 and IRST1 in ten weeks, patient Ra7 in twenty-five weeks, patient Ra2 achieved his best response after forty-seven weeks because a bad drug compliance and a very low Ruxolitinib dose intake. Patient Ra7 developed a pulmonary solid tumor and discontinued treatment after forty-seven weeks (Table 1B). *Conclusions:* Ruxolitinib il well tollerated. Haematological toxicity is limitated (thrombocytopenia and anemia), and controlled by dose modulation, transfusion support, epoetine administration. Ruxolitinib in our experience, has shown success in alleviating the syntomatic burden, reducing splenomegaly and improving the quality of life in patients with myelofibrosis. *Acknowledgements:* MPDs-CML group of Romagna.

Reference

1. Pasamonti F., Maffioli M., Cervantes F., Vannucchi A.M., Morra E., Barbui T., Caramazza D., Pieri L., Rumi e., Gisslinger H., Knoops L., Kiladjian J.J., Mora B, N. Hollaender, C. Pascutto, C. Harrison and M. Cazzola. Impact of ruxolitinib on the natural history of primary myelofibrosis: a comparison of the DIPSS and the CONFORT-2 cohorts. Blood 2014 123: 1833-1835.
2. Ruxolitinib: long-term management of patients with Myelofibrosis and future directions in the treatment of Myeloproliferative Neoplasms. A. Ycoub, O. Odenike, S. Verstovsek Cur. Hematol Malig Rep 2014 9:350-359 3 Ruxolitinib: A review of its use in patients with Myelofibrosis. Greg L. Plosker Drugs (2015) 75:297-308.

Table 1.

A. DEMOGRAPHIC AND BASE-LINE CHARACTERISTIC OF PATIENTS AT DIAGNOSIS

Number all patients	9	
Gender M/F	6/3	
Age median - min/max	68 – 56/77	
Elderly >65years	5	56%
Young <65years	4	44%
Primary MF	4	44%
Secondary MF	5	56%
Constitutionals Symptoms yes/no	8/1	
Palpable splenomegaly yes/no	9/0	
HB median - min/max	10 – 8.6/14.3	
WBC median - min/max	16200 – 4360/ 49470	
Plateles median - min/max	261000 – 73000/778000	
IPSS		
Low	0	0%
Int-1	1	11%
Int-2	5	56%
High	3	33%
JAK2 Pos/Neg	8/1	
JAK2V617F mutated alleles		
UPN Patients	JAK2V617F	%
Ra1	Pos	85.1
Ra2	Pos	86.1
Ra3	Neg	-
Ra4	Pos	97.31
Ra5	Pos	97,5
Ra6	Pos	65.9
Ra7	Pos	22.9
R8	Pos	73.9
IRST1	Pos	97

B. REDUCING SPLENOMEGALY

UPN	At diagnosis (cm)	Min (cm)	At Week	Last (cm)	Time treatment (W)
Ra1	10	5	4	7	66
Ra2 *	3	0	47	0	71
Ra3	4	0	8	0	43
Ra4	12	0	10	8	117
Ra5	18	15	4	15	7
Ra6	12	10	8	10	8
Ra7**	15	6	25	6	45
Ra8***	5	0	1	5	17
IRST1	17	12	10	12	40

* very low compliance; ** suspended therapy for solid tumor; *** evolution AML; W=week

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JAK2V617F CLONAL ARCHITECTURE IN MPNs DURING JAK2 INHIBITOR TREATMENT

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The Myeloproliferative Neoplasms (MPNs) are characterized by a recurrent point mutation of JAK2 gene (JAK2V617F). This mutation, which usually affects only one of the JAK2 gene alleles in Essential Thrombocythemia (ET), frequently becomes homozygous in Polycythemia Vera (PV) and Myelofibrosis (MF) due to mitotic homologous recombination. The germline JAK2 46/1 haplotype (GGCC) is strongly associated with JAK2V617F acquisition with complete linkage disequilibrium, although the risk of developing MPNs is independent of its presence. These observations suggest that it could be

possible to determine the JAK2V617F clonal architecture starting from 46/1 SNP allele burden and the homologous recombination frequency. Recently, Hasan and colleagues described a fast method combining allele burden evaluations of both 46/1 and JAK2 and the frequency of mitotic recombination to derive the percentages JAK2 V617F clones in PV at least in 46/1 heterozygous patients for rs12343867 polymorphism (C/T). Based on these results we evaluated changes in the JAK2V617F clonal structure in ten 46/1 C/T haplotype patients who presented a reduction of at least 10% of JAK2V617F allele burden values after treatment with ruxolitinib, a JAK1 and JAK2 inhibitor, over 1 to 5 years of followup (FU). For all patients after two 2 years of FU we calculated a median reduction of JAK2 V617/V617F and JAK2V617/wt clones of 32.31% and 8.82% in PV (n.4); 12.22% and 17.86% in PMF (n.5); 35.99% and 100% in ET (n.1), respectively. Furthermore, an almost complete molecular remission (CMR) was seen in two PV patients with 4 and 5 yr of FU and in one ET patient after 5 years of treatment. In these patients we obtained an homozygous clones reduction of 99.60%, 86.91% and 100% respectively (Figure 1). Future analysis will be performed to study the clonal architecture in an increased number of patients treated with JAK inhibitors as single agent or in combination with other drugs. In conclusion, our results showed that ruxolitinib may preferentially target the homozygous clones inducing in some cases an almost complete molecular remission with prolonged treatment. Furthermore, this method described by Hasan *et al.* is a simple and rapid method to determine the clonal status of JAK2V617F mutated subjects who are heterozygous for the 46/1 haplotype and it can be useful to monitor changes in clonal architecture induced by drugs without the need of single cell colony analysis.

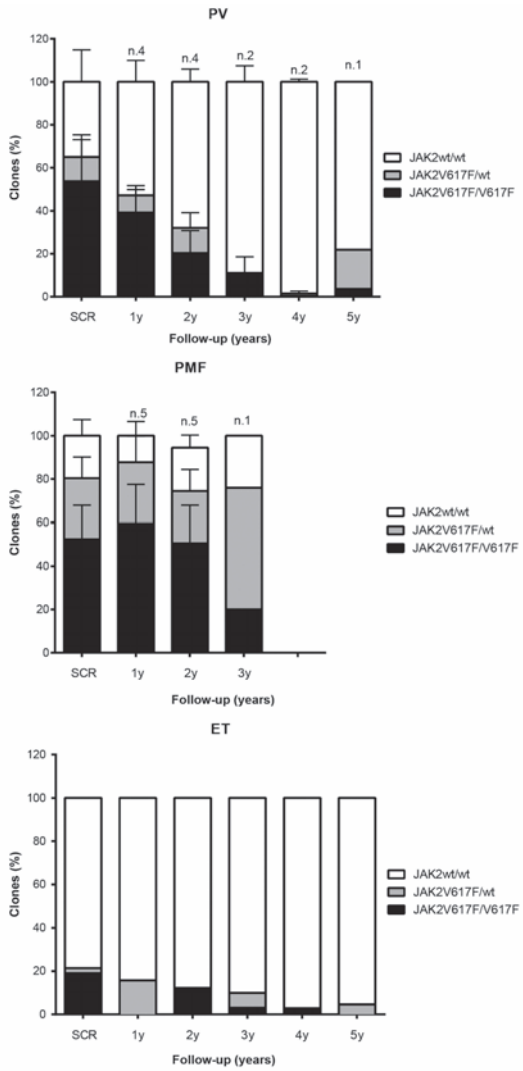

Figure 1.

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HISTONE-DEACETYLASES INHIBITORS: PRE-CLINICAL STUDIES IN CHRONIC MYELOPROLIFERATIVE NEOPLASMS

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Philadelphia–negatives chronic myeloproliferative neoplasms (MPNs) include Polycythaemia Vera, Essential Thrombocythaemia and Primary Myelofibrosis. Additional mutations, discovered in MPN, cause epigenetic modifications and may cooperate with aberrant JAK/STAT pathway. Histone deacetylases (HDACs) are involved in many biological processes and are commonly deregulated in a variety of tumors and HDAC inhibitors are recently under development as anticancer drugs and in clinical trials for hematological and solid neoplasms. The aim of this study was to evaluate HDAC involvement through two specific inhibitors, Givinostat and Panobinostat (Selleckchem), used individually and in combination with PI3K/mTOR inhibitors in the functional deregulation of hematologic progenitors in patients with MPN. First, we evaluated the inhibition of proliferation in cell lines (WST1 assay). Panobinostat was effective at nanomolar concentrations. Human cell line SET2 was more sensitive to Givinostat (145±30) than other cell lines, especially K562 (864±42). In murine cell lines there weren't significant differences of sensitivity to the drugs. Then, SET2 cell line was treated with Panobinostat and RAD001 (mTOR inhibitor) or BEZ235 (mTOR/PI3K inhibitor) and the combination resulted synergic (CI=0.88 and 0.6, respectively, Calcusyn). About the apoptosis study (FACS analysis), these drugs increased Annexin V positive cells dose-dependent. By Western blotting analysis, it was observed a decrease of anti-apoptotic protein Bclxl and total JAK2. The increase of P27 (pro-apoptotic protein) and the decrease of 4EBP1 (mTOR effector) were detected only with Panobinostat. Moreover, a greater inhibition of clonogenic growth with Panobinostat was observed in patients than in controls (IC50 3 times lower in erythroid and myeloid colonies). Givinostat was more effective in inhibiting the growth of erythroid colonies of patients compared to control subjects (IC50 2 times lower), but no difference for myeloid colonies. Finally we have studied *in vivo* the effect of Givinostat on mouse model BaF3 JAK2V617F-Luc and the data suggested the efficacy of the treatment with 80mpk and 2x40mpk on the survival. These results support further studies using HDACi in combination with other drugs in order to identify one or more strongly synergic combinations to test in mouse model and eventually in clinical trials for MPN patients.

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THE PI3K/mTOR INHIBITORS BKM120 AND RAD001 ARE EFFECTIVE AGAINST JAK2V617F MUTATED CELLS AND SYNERGIZE WITH RUXOLITINIB IN *IN VITRO* AND *IN VIVO* PRECLINICAL MODELS

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Activation of pathways such as ERK and PI3K/mTOR has been documented in myeloproliferative neoplasms (MPN) beyond the key JAK/STAT pathway characterized by the Janus kinase2 mutation (JAK2V617F). We explored both *in vitro* and *in vivo* the potential relevance of targeting PI3K/mTOR pathway with the PI3K inhibitor BKM120 alone and in combination with mTORC1/2 (RAD001 and PP242) and JAK2 (ruxolitinib) inhibitors. We found that BKM120 preferential inhibited the murine BaF3 and BaF3-EpoR cells expressing JAK2V617F (IC50: 364±200nM and 1100±207nM respectively) compared to their wild type (WT) counterpart (5300±800nM and 3122±1000nM: p<.05). Human JAK2V617F mutated HEL and SET2 cells resulted also sensitive to BKM120 (2000±500nM and 1000±300nM). BKM120 significantly increased G2/M phase, decreased S phase of cell cycle (p<.01) and induced apoptosis (IC50, SET2=10μM, BaF3-EpoR VF=1.8μM). As well as a reduction of phospho-mTOR and -4EBP1, we notice by western blot that BKM120 treatment induced a differential downregulation of the two isoforms of phospho-STAT5a and b and we are investigating on the effects of triple combinations to elucidate the mechanism of action. BKM120 impaired colony formation from myelofibrosis (MF) and poly-

cythemia vera (PV) CD34+ cells at doses 2 to 8-fold lower than healthy controls (p<.01). BKM120 strongly inhibited erythroid endogenous colonies growth from PV patients (IC50, 9±4nM). Effect of drugs combination was analyzed according to Chou and Talalay calculating the combination index (CI), a CI<1 indicates synergistic activity. Triple combinations including BKM120/ruxo plus either RAD001 (Torc1 inhibitor) or PP242 (Torc1/2 inhibitor) resulted highly synergistic (median CI=0.27 and 0.52) to indicate the importance of complete mTOR inhibition. *in vivo* studies performed with the heterozygous JAK2V617F knock-in mouse showed that co-treatment with 30mpk BKM120+ 30mpk ruxo+1.5mpk RAD001 resulted in reduction of leukocytosis, reticulocyte count and improvement of splenomegaly: median spleen index (spleen and body weights ratio) was 1.1 in triple-treated cohort *versus* 2, 2.3, 3.4 and 3.6 in 60mpk BKM120, 60 ruxo, 3 RAD001 and Vehicle respectively. The level of phosho-STAT5 and -4EBP1 in spleen of triple-treated mice was significantly reduced compared to single drug treatment. Concurrent targeting of PI3K/mTOR and JAK/STAT pathway resulted in marked antitumor activity in preclinical models and might represent a new therapeutic strategy for MPN.

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PATIENTS WITH TRIPLE NEGATIVE OR CALR-MUTATED ESSENTIAL THROMBOCYTHEMIA HAVE A LOW RISK OF THROMBOSIS

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Patients with essential thrombocythemia (ET) may carry JAK2V617F (60%), MPL (5%) or calreticulin gene (CALR) (25%) mutations which are mainly mutually exclusive. The remaining 10% of patients are triple negative (3NEG) for these mutations. JAK2V617F patients, as well as those with MPL mutations, have a high thrombotic risk. Preliminary data suggest that, in contrast, CALR and 3NEG patients have an indolent form of ET with a low risk of thrombosis. In this study, we evaluate the thrombo-hemorrhagic risk of 3NEG and CALR mutated ET patients. This study involves 66 ET patients regularly followed in our Department, strictly fulfilling the 2008 WHO criteria. All these patients were JAK2 and MPL wild type. CALR mutations were searched with Sanger sequencing method. Patients were stratified according to mutational status: 44 carried CALR mutation (25 type 1 and 19 type 2) and 22 were 3NEG. Their main laboratory and clinical data are summarized in Table 1.

Table 1.

	3NEG	CALR	*P 3NEG vs CALR*
N (%)	22 (12%)	44 (76.7%)	-
Mean age at diagnosis (y)	41.2 ± 16.2	51.1 ± 15.5	**0.023**
Male/Female	4/18	14/30	n.s.
Mean WBC at diagnosis (x10^9/L)	7.9 ± 2.9	7.7 ± 3.0	n.s.
Mean hemoglobin at diagnosis (g/L)	133 ± 14	131.6 ± 13.0	n.s.
Mean platelets at diagnosis (x10^9/L)	751 ± 236	887.7 ± 349.5	n.s.
Presence of splenomegaly (%)	9 (40.9%)	13 (48.1%)	n.s.
Plts > 1500 x10^9/L at diagnosis	1 (4.5%)	3 (6.8%)	n.s.
IPSET-T score			
low-risk	18 (81.8%)	35 (79.5%)	
intermediate risk	3 (13.6%)	5 (11.4%)	n.s.
high risk	1 (4.5%)	4 (9.2%)	
Patients with at least a thrombotic event (%)	2 (0.9%)	8 (18.2%)	n.s.
Patients with at least a bleeding event (%)	3 (13.6%)	10 (22.7%)	n.s.

The thrombotic risk assessment was performed according to IPSET-thrombosis (IPSET-T) score. Thrombotic events during follow-up (median 12.1 y range 0.2-35) were recorded. Nominal variables were compared with the X2 test and continuous variables with Mann-Whitney test. P values less than 0.05 were considered significant. 3NEG patients were significantly younger at diagnosis compared to CALR patients (p=0.023). Extreme thrombocytosis (platelet count >1500 x109/L) was observed in 1 3NEG and in 3 patients with CALR type 2 mutation and in none with type 1 mutation (p=0.04). Stratifying patients according to IPSET-T score, 79.5% CALR patients and 81.8% 3NEG patients harbored in low-risk category. Thrombotic complications during follow-up occurred in 6 patients (13.6%) with CALR mutations and none in 3NEG group. Hemorrhagic event occurred in 13.6% of 3NEG and 22.7% of CALR patients (p=NS). 3NEG and CALR mutated ET patients have similar phenotype at diagnosis: only younger age at diagnosis and extreme thrombocytosis in 3NEG and CALR type 2 respectively have been observed. In our cohort, both CALR and 3NEG patients segregate mainly in low thrombotic risk category evaluated with IPSET-T score and have a congruously low rate of thrombosis during follow-up. Interestingly, CALR patients seem to be more prone than 3NEG to hemorrhagic events even if we did not obtain a significant difference. Our data suggest that CALR and 3NEG ET patients have similar phenotypes and suffer for indolent form with a low cardiovascular risk.

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PEGYLATED INTERFERON-A PROLONGS SURVIVAL IN PATIENTS WITH POLYCYTHEMIA VERA: THE TURIN EXPERIENCE

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Polycythemia vera (PV) is a myeloproliferative disease with a median survival of 14-15 years. We aimed to evaluate the outcome of a large series of PV patients, with a focus on patients treated with pegylated interferon-α (peg-IFN-α). We included 227 patients consecutively diagnosed with PV since 1995. Since 2006, we treated with peg-IFN-α patients <65 years at high thrombotic risk or intolerant/resistant to phlebotomy. The use of alkylating agents was limited to the oldest patients who were refractory/resistant to hydroxyurea (HU). Median age at diagnosis was 66 years old and median follow-up was 8 years (range 1-20). Sixty-nine% of patients were at high thrombotic risk for age or history of thrombosis, however only 13.3% of the whole cohort developed a major thrombotic event. Major and minor thrombosis were associated with age and thrombotic history (p=0.034) but not with other cardiovascular (CV) risk factors or treatment. Median overall survival (OS) was not reached, with 90% and 58% of patients still alive at the median follow-up of 8 years and at the maximum follow-up of 17 years, respectively. Fifteen% of patients developed myelofibrosis and 6% only acute myeloid leukemia. Regarding treatment, 97% of patients received anti-platelet (89%) or anticoagulant agents (8%) and 86% of patients received cytoreductive therapy, 86% with HU. Twenty-four patients were treated with peg-IFN-α for at least 3 months (range 3-110) and their OS was longer than patients treated with HU (p=0.034) or with alkylating agents (p=0.002) (100% alive at the last follow-up). Even if peg-IFN-α was available only from 2006, patients treated with peg-IFN-α had a median follow-up from diagnosis similar to patients treated with HU (86 *vs* 97 mo). Median age in the peg-IFN-α group was 53 and only 11% of the patients had previous thrombosis. However, at multivariate analysis peg-IFN-α positively impacted on survival regardless of the age and the thrombotic history. Moreover, a reduction of JAK2 burden was observed in 58% of the patients treated with peg-IFN-α, an encouraging data considering the still relatively short median treatment duration of 26 months. In conclusions our study confirmed some known thrombotic prognostic factors (age and thrombotic history) but no other CV risk factors, probably due to the positive role of anti-platelet therapy. Peg-IFN-α revealed to be a promising therapy in PV, not only to control symptoms but also to reduce JAK2 burden and improve OS.

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LIVER TRANSPLANTATION RATE FOR BUDD-CHIARI SYNDROME IN MYELOPROLIFERATIVE NEOPLASMS

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Budd-Chiari syndrome (BCS) is frequent presenting complication of myeloproliferative neoplasms (MPN) both yet known or undiagnosed. Therapeutic options for BCS include medical therapy (anticoagulants, cytoreduction, diuretics), transjugular intrahepatic porto-systemic shunting (TIPS) and orthotopic liver transplantation (OLT). TIPS and OLT are effective and rescue therapies in patients with BCS, both soon after diagnosis and later during follow-up, and seem to have similar long-term outcome. OLT can be the first invasive therapy in the presence of a worse hepatic disease at presentation or may be delayed when liver fails or hepatic cell carcinoma (HCC) arises. Over the last 20 years, we studied 25 MPN patients with BCS (5 males, 20 females, median age 33.7 y): 14 Essential Thrombocythemia (ET) 11 Polycythemia Vera (PV)(at diagnosis: platelets 430.5±210.7 x109/L, WBC 11.6±5.6 x109/L and hemoglobin 149±34 g/L). In 16 cases BCS occurred at diagnosis while in 8 during follow up (median 6.2 y). Twenty patients carried JAK2V617F mutation (10 PV and 10 ET) while in 5 old ET cases JAK2 was not available. The patients received a step-wise treatment approach comprehending medical therapy (MT) and invasive procedures as TIPS and OLT. Ten patients (40%) received MT; in 6 patients (24%) TIPS was used and in 1 case was shifted to OLT because HCC; 9 other patients underwent OLT as first invasive procedure. Therefore a total of 10 patients (40%) underwent OLT (5 acute liver failure, 4 cirrhosis and 1 HCC). Within the 14 ET patients, 5 (35.7%) underwent OLT in all cases delayed from thrombosis. In contrast, 5 (45.4%) PV patients had acute liver failure, needing urgent OLT (p=0.026). Our data show that OLT has been performed mainly in PV patients while ET subjects have been often treated with MT or TIPS. When ET needed OLT it was due to cirrhotic evolution or the arise of HCC, while all PV transplanted cases had an acute liver failure. It seems therefore that BCS in PV patients has a more aggressive presentation respect to ET. Considering that JAK2 is an established prothrombotic risk and it is present in both ET and PV, some other features can contribute to the worse picture of liver dysfunction of PV patients. We can speculate that the altered blood viscosity in erythrocytotic patients plays a significant role. Even if MPN did not result to be related to a higher need of invasive intervention for BCS, we suggest that PV are more prone to aggressive procedures that ET

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CRUCIAL PRO-INFLAMMATORY CYTOKINES STRONGLY PROMOTE *IN VITRO* MIGRATION OF SELECTED CD34+ STEM/PROGENITOR CELLS WITH ABNORMAL CLONOGENIC POTENTIAL IN MYELOFIBROSIS

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Introduction: Myelofibrosis (MF) is characterized by constitutive mobilization of CD34+ cells into the peripheral blood. MF is also characterized by a state of chronic inflammation and it is argued that the up-regulated production of pro-inflammatory cytokines selects for the malignant clone. However, the key players linking inflammation and cancer in MF are still poorly known. Here we investigated whether *in vitro* migration of CD34+ cells may be abnormally modulated by selected pro-inflammatory cytokines (Interleukin-1 (IL-1), Tumor Necrosis Factor (TNF)-, Tissue Inhibitor of Metalloproteinases-1 (TIMP-1) and Adenosine-triphosphate (ATP)). *Methods:* Circulating CD34+ HSPCs from MF patients (27 cases: 17 JAK2V617F+, 7 CALR+, 3 MPL+) or cord blood (CB) were firstly phenotypically characterized by flow cytometry for the expression of CXCR4, CD63 (TIMP-1 receptor) and CD38. Purified CD34+ cells were assayed towards a CXCL12 gradient in the presence or absence of the inflammatory cytokines. Migrated cells were counted and characterized for CD38 and CD63 expression; in addition, their clonogenic potential was studied (CFU-C assay). *Results:* We found that the absolute number of circulating CD34+CXCR4+ and CD34+CD63+ was significantly increased in patients with MF as compared with CB-derived CD34+ cells. Accordingly, we observed higher spontaneous and CXCL12-driven migration of CD34+ cells in MF patients. The CXCL12-driven migration rate of MF CD34+ cells towards ATP, TNF-, TIMP-1 and IL-1 gradient (in various combinations) was significantly increased as compared with both MF untreated and CB CD34+ treated cells. Specifically, the MF CD34+ cells migration was highly promoted by the chemo-attractive network of IL-1 plus TNF-. In addition, inflammatory cytokines-driven gradient selectively enhanced CD34+CD38-CD63+ cells migration. Interestingly, at variance with CB 34+ cells, the migration rate of MF CD34+ cells was only slightly increased in the presence of TIMP-1 or TIMP-1/ATP as compared with untreated cells. Finally, the clonogenic potential of migrated MF CD34+ was higher when cells were treated with pro-inflammatory cytokines in comparison with both untreated cells and CB treated cells. Of note, BFU-E growth of MF patients was significantly enhanced. *Conclusions:* Here we demonstrated that in MF crucial pro-inflammatory cytokines strongly promote the *in vitro* migration of selected CD34+ cells with abnormal clonogenic potential.

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RECURRENT LOSS OF FUNCTION OF THE SETD2 GENE IN AGGRESSIVE SYSTEMIC MASTOCYTOSIS AND MAST CELL LEUKEMIA

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Whole exome sequencing (WES) of a case of mast cell leukemia (MCL) negative for any KIT gene mutation allowed us to identify a frameshift insertion of a C in exon 20 and a nonsense mutation in exon 15 of the SETD2 gene. The two mutations were found to hit distinct alleles, pointing to a loss-of-function event. Western Blotting (WB) confirmed the presence of a SETD2 truncated isoform losing the highly conserved WW and SRI functional domains. The SETD2 gene encodes a histone methyltransferase responsible for trimethylation of K36 of histone H3 (H3K36Me3), a key hystone mark associated with active chromatin, transcriptional elongation, alternative splicing, DNA replication and repair. SETD2 has a key role in: i) preventing aberrant transcription through binding to the C-terminal domain of the RNA polymerase II (PolII); ii) regulating splicing through association with the large subunit of the heterogeneous nuclear ribonucleoprotein complex (hnRNP-L); iii) participating both in Homologous Recombination repair after a double strand break has occurred as well as in Mismatch Repair during DNA replication vi) activating p53-dependent cell cycle checkpoint. The truncated SETD2 isoform was actually found to lose the ability to bind histone H3, RNAPolII, and hnRNP-L as shown by co-immunoprecipitation. More importantly, WB confirmed that H3K36Me3 was completely abrogated. Dowmodulation of SETD2 protein, and corresponding reduction of H3K36Me3, could also be observed in the HMC1.2 MCL cell line, that, however, was found to be negative for SETD2 gene mutations. Bortezomib and 5-azacytidine were found to synergize in restoring SETD2 protein expression in the cell line – suggesting that both promoter hypermethylation and enhanced protein degradation cooperate in downmodulating SETD2 in the HMC1.2 cells. Downmodulation/absence of the SETD2 protein and reduced H3K36Me3 levels were also detected in 3 out of 4 additional MCL cases and in 8 out of 10 additional ASM cases (positive for the D816V KIT mutation), further hinting at an important cooperative role of SETD2 loss in aggressive forms of the disease. We hypothesize that loss of SETD2 in advanced SM might afford an alternative mechanism for the inactivation of the p53-mediated checkpoint without the need for TP53 gene mutations (indeed not reported in SM). We are currently investigating whether p53-reactivating

drugs (nutlin, kevetrin) may have a role, at least *in vitro*, in advanced SM.
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MYSEC PROGNOSTIC MODEL APPLICATION TO SECONDARY MYELOFIBROSIS: THE EXPERIENCE OF LATIUM COOPERATIVE GROUP FOR CHRONIC MYELOPROLIFERATIVE NEOPLASM PH NEGATIVE

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Polycythemia Vera (PV) and Essential Trombocythemia (TE) can progress to secondary myelofibrosis (SMF), named post PV (PPV-MF) and post ET (PET-MF). IPSS score reliably predicts outcome in primary MF but it lacks accuracy in SMF. The objective of this study is to apply the Mysec Prognostic Model (MYSEC-PM) proposed by Passamonti *et al.* based on age >65 years, time to SMF >15 years, previous thrombosis, constitutional symptoms, hemoglobin <10 g/dL and circulating blast >1%, to predict survival of our SMF group of patients. *Materials and Methods:* A total of 108 SMF patients from 11 hematology departments of Latium region were included in this study, 51 PPV-MF and 57 PET-MF. Sixty-two were females (57.4%) and 46 males 42.6%. Median age was 68.7 years (range 61.6-74.6). Median time from PV/ET diagnosis to SMF diagnosis was 124 months (range 70.8 – 204.6). JAK2 V617F was positive in 51.8% of patients. Median values of hemoglobin was 10 g/dL (range 6.0-15.4). Constitutional symptoms were present in 20% of patients, history of thrombosis was present in 32%. Diagnosis of PPV-MF and PET-MF was according to the IWGMRT criteria. Survival was calculated utilizing Kaplan-Meier analysis. We selected 95 patients with all available data to calculate MYSEC-PM. *Results:* The Mysec model assigned 3 points to age >65 years, 2 points to time to SMF >15 years, 2 points to history of thrombosis, 2 points to constitutional symptoms, 1 point to hemoglobin <10 g/dL and 2 points to circulating blast >=1%. Patients were divided into three categories: low-risk (score, 0 to 2), intermediate-risk (score 3 to 6) and high-risk (score >6). According to MYSEC-PM in our group of evaluable patients survival was statistically different among low risk and intermediate/high risk (p<0.004) group, but not between intermediate and high risk group (Figure 1).

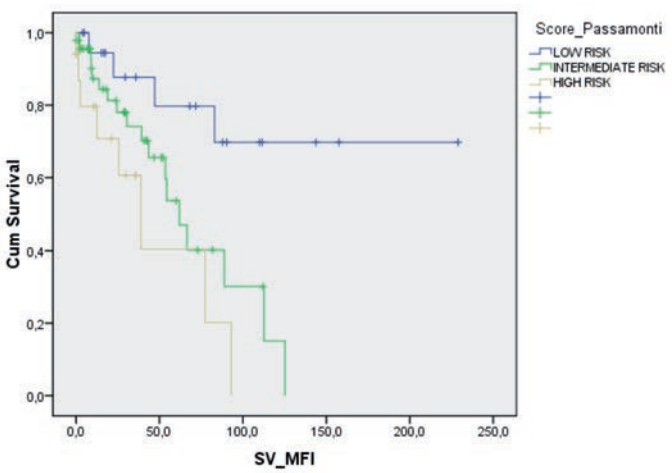

Figure 1. Survival functions.

Conclusions: The predictive value of MYSEC-PM was confirmed in our SMF patients especially for low risk scores. The outcome of intermediate and high risk categories showed a not significant difference suggesting that the clinical course of intermediate and high risk patients could be similar. This consideration can be taken into account to choose personalized "risk based" treatment of SMF patients.

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SAFETY AND EFFICACY OF RUXOLITINIB IN AN ELDERLY PATIENT WITH PRIMARY MYELOFIBROSIS AND ACTIVE HCV AND HBV RELATED-LIVER DISEASE

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The myeloproliferative neoplasms (MPN) are stem cell–derived monoclonal (or oligoclonal) hemopathies. The disease-initiating mutation in PMF is not known but the majority of patients harbor Janus-Kinase 2 (JAK-2)V617F mutation. Activating JAK2 mutations result in constitutive Janus kinase–signal transducer, JAK-STAT activation and consequent MPN-like disease. PMF with high- or intermediate 2-risk disease can be managed by conventional drugs, splenectomy, radiotherapy, allo-SCT or experimental drugs. The primary goal is palliation of anemia, symptomatic splenomegaly, constitutional symptoms or disease complications. JAK2 inhibitors show efficacy in both mutated and wild-type (WT)JAK-2 PMF. Because of the exclusion of patients with active liver disease from the clinical trials, we treat with ruxolitinib, for compassionate use, a 73 years old man with active HCV and HBV-related liver disease, affected by high-risk, WT-JAK-2 PMF. From 2011, patient had hydroxyurea (1 g/day) with haematological toxicities (needing support with erythropoietin and G-CSF) and progressive worsening of PMF. In august 2014: massive splenomegaly (300 mm), abdominal pain, early satiety, constitutional symptoms (fatigue, weight loss and night sweats), severe anemia (hgb:8.9 g/dl), thrombocytopenia (platelets:121.000/mm3) and neutropenia (neutrophils:930/mm3). Bone marrow biopsy: grade II/III fibrosis. HCV-RNA analysis: 5.606.704 copies/ml, with hepatic impairment (Child B, score: 7) according to Child-Turcotte-Pugh. Ruxolitinib was administered at a starting dose of 5 mg twice daily with improvement in abdominal pain and constitutional symptoms within 1 week and progressive weight gain. Improvement of haematological parameters reached with fast discontinuation of G-CSF and erythropoietin. Extra-haematological toxicities didn't occur. After 8 weeks, splenomegaly reduced to 280 mm, ascites disappeared, hepatic impairment improved (Child A:score 6). Peripheral blood counts (PBC): hgb 10.9g/dl, neutrophils 1570/mm3, platelets 178.000/mm3. For the excellent tolerability, the dose of ruxolitinib increased to 10 mg twice daily. After 24 weeks, splenomegaly reduced to 260 mm, liver function and PBC were stable. Bone marrow biopsy: grade II/III fibrosis. The patient achieved a significant clinical benefit and improvement in quality of life. Ruxolitinib had unquestionable palliative value, was well tolerated and safe in elderly patients with active liver disease.

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ROLE OF TREATMENT ON THE DEVELOPMENT OF SECONDARY MALIGNANCIES IN PATIENTS WITH ESSENTIAL THROMBOCYTEMIA

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Introduction: The specific role of different therapies on the development of secondary malignancies (SM) in ET patients is still under investigation. Aims. To retrospectively evaluate the role of different treatments on the development of SM in a large cohort of ET patients followed over a 30-year period. *Patients and Methods:* 1026 ET patients were diagnosed between 1980 and 2000. Median age at ET diagnosis: 62 years (range 17-93); 392 were males, and 634 females; median follow-up: 6.2 years (range 0.1–32); 39% of patients were JAK2V617F positive, 27% wild type; for 34% of cases, this datum is missing. With regard to smoke, diabetes, hypertension, dyslipidemia, 64% of patients presented at least one risk factor (42%, 1; 22% >1), while 28% did not; for 8% of cases this datum is missing. After a median time of 50 months (range 2-158) from ET diagnosis, 63/1026 patients (6%) developed 64 SM: genito-urinary 15, breast 14, non-melanoma skin cancer 5, lung 11, gastro-intestinal 8, hematologic 5, thyroid 3, central nervous system 1, soft tissue 1 and unknown 1. With regard to treatment approaches, we divided our population into 5 different groups: group 0, untreated patients; group 1, patients treated with hydroxiurea (HU) alone or in combination/sequentially with interpheron (IFN)/anagrelide (ANA); group 2, patients treated with alkylating agents (ALK) alone or in combination/sequentially with IFN/ANA; group 3, patients treated with ALK+HU sequentially; group 4, patients treated with ANA and/or IFN only. *Results:* With regard to the exposure time to drugs, a statistically significant difference was found between the groups HU *vs* ALK (p 0.036), HU *vs* ALK+HU (p 0.006) and ALK+HU *vs* ANA/IFN (p 0.006). No differences were found for the groups HU *vs* ANA/IFN, ALK *vs* ALK+HU, ALK *vs* ANA/IFN. In univariate analysis, gender, age, different therapeutic approaches, exposure time, presence of adjunctive risk factors were considered. A statistically significant difference was found only for gender (M *vs* F: p 0.035) and age (>60 years *vs* <60years: p 0.0001). In multivariate analysis, a statistically significant difference was maintained for both gender and age [gender HR1.7 (CI 95% 1.037-2.818) p 0.035; age HR 4.190 (CI 95% 2.308-7.607) p 0.0001)]. *Conclusions:* Different therapies administered, do not appear to have impacted on the development of SM. A similar prevalence of SM was observed also in untreated patients. The only two variables which showed a statistical significance were male gender and age >60 years.

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INFECTIOUS RISK IN MYELOFIBROSIS: EVALUATION ON 426 PATIENTS

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Infectious complications represent one of the main causes of morbidity and mortality in patients (pts) with Primary Myelofibrosis (PMF), Post-Essential Thrombocythemia and post-Polycythemia Vera MF (PET/PPV-MF). However, very few data are available on outcome and risk factors of this potentially fatal complication. To evaluate risk factors for severe infections in MF, clinical and laboratory data of pts with MF were retrospectively collected from the database of 3 Italian Hematology Centers in University Hospitals. Severity of infections were defined according to the CTCAE. Between Jan 1980 and Aug 2014, 426 pts with PMF (319 pts, 75%), or PET-MF (13%) or PPV-MF were diagnosed and followed for a median follow-up of 4 yr (0.5-30.1). Baseline characteristics were (median): age, 67 y (range, 26-87); ≥65 y, 55%; male, 57%; hemoglobin (Hb), 12 g/dL (4-17.9); Hb <10 g/dL, 24%; PLT, 372 109/L (15-2513); PLT <100 109/L, 8.0%; spleen enlargement, 69% (spleen length ≥5cm: 32.4%); constitutional symptoms, 15%. International Prognostic Score System (IPSS) was low (11%), intermediate-1 (intm-1, 21%), intermediate-2 (intm-2, 43%), high (24%). Molecular analysis was performed on 253 pts (59%) and was positive in 84% (JAK2V617F), 14% (CALR), 3% (MPLW515K/L); 6 pts (2%) were triple negative. Karyotype was abnormal in 34 (16.6%) out of 204 evaluable pts (unfavorable in 10 pts; 5%). Overall, 81 pts (19%) experienced 112 grade 2-4 infectious events, for an incidence rate of 3.3% pt-y. Infections were: bacterial (101 events, 90.2%; pneumonia: 54 cases, 53.4%); VZV reactivations (7 events, 6.2%), nodal TBC (2 events, 1.8%), fungal lung infections (2 events, 1.8%). Infectious complications represented the causes of death in 9 (8%) out of 126 deceased pt. Among baseline features, age≥65 y (p=0.004), leukocyte ≥25x10^9/l (p=0.02), spleen length >5 cm (p=0.03), high/intm-2 IPSS (p=0.01), unfavorable karyotype (p<0.001) and JAK2/CALR/MPL negativity (p=0.002) significantly correlated with higher infectious risk; in multivariate analysis, unfavorable karyotype and triple molecular negativity confirmed their negative impact (p=0.008 and p=0.03, respectively). Severe infections were frequent events involving 19% of MF pts and representing the ultimate cause of death in 8% of the cases. Molecular/cytogenetic data identify a subset of patients at higher infectious risk (Figure 1).

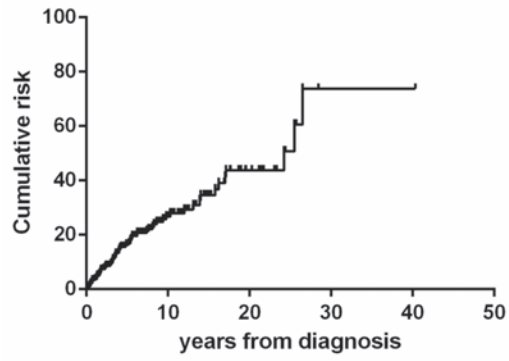

Figure 1. Cumulative risk of infections. The cumulative incidence was 16%, 26.8% and 43.6% at 5, 10 and 20 y, respectively.

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RISK FACTORS FOR INFECTIONS IN RUXOLITINIB-TREATED MYELOFIBROSIS PATIENTS

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A higher infectious risk has been reported in myelofibrosis (MF) patients treated with Ruxolitinib (RUX), the only commercially available JAK1/2 inhibitor. Up-to-date, no predictive factors for infectious complications have been investigated in this setting. Herein, we report a series of 70 MF patients treated with ruxolitinib in two Italian Hematology Centers for a median time of 23 mos (range, 1-41). Infections were defined according to the CTCAE grading. The study was approved by the Ethic Committee of each participating Centers. Between June 2011 and March 2015, 70 MF patients were treated with RUX. Median age at diagnosis was 62 years (range, 26-86); ≥65 y, 29 (41%). Other clinical characteristics were: male sex, 33 (54%); hemoglobin (Hb), 11,6 g/dL (7,1-17,1); Hb <10 g/dL, 18 (26%); PLT, 357 10^9/L (range, 51-979); PLT <150 10^9/L, 8 (11%); circulating blastic cells, 22 (31%); spleen enlargement, 47 (67%). Spleen length palpable ≥10cm below left costal margin (LCM) was present at diagnosis in 31 (44%). Twenty-three patients (33%) complained of constitutional symptoms. The International Prognostic Score System (IPSS) was low in 12 (17%), intermediate-1 in 25 (36%), intermediate-2 in 23 (33%), high in 10 (14%). Fifty-four patients (71%) carried JAK2V617F mutation, 13 (19%) CALR mutations, 1 (1%), while 6 (11%) were triple negative for all tested mutations. Cytogenetic analysis was available in 50 patients (71%) and was abnormal in 17 (34%) and unfavorable in 7 (14%). After RUX start, 42 infectious complications were recorded in 30 patients, with an incidence rate of 38% pt-y (Table 1). The cumulative incidence was 25% and 56% at 12 and 24 mos of therapy. Notably in 3 cases the infectious complication was the ultimate cause of death. In univariate analysis, age ≥65 years (p=0.04), WHO diagnosis of primary MF (p=0.001), platelet count ≤150.000/mmc (p=0.024), splenomegaly ≥10cm below LCM (p=0.015) and no spleen lenght reduction (>25%) during RUX therapy (p=0.03) correlated with an increased infectious risk; in multivariate analysis, PMF

diagnosis, low platelet count and splenomegaly confirmed their adverse prognostic significance. This experience confirms infections as frequent and potentially fatal complications of RUX treatment. Patients with thrombocytopenia and massive splenomegaly seems to be at higher infectious risk.

Table 1. Characteristics and outcome of infections complications in Ruxolitinib-treated patients.

ID PAT	Type of infection	CTCAE	Therapy	Outcome
BMS3543	Sepsis	Gr 5	pip/tazobactam	death
TMG1936	Sepsis	Gr 5	pip/tazobactam	death
BSG20871	Bronchopneumonia	Gr 5	pip/tazobactam, meropenem, linezolid	death
BMB1253	Sepsis	Gr 4	pip/tazobactam + ciprofloxacin	resolved
TGA1935	Pneumonia (K. Pneumoniae)	Gr 3	pip/tazobactam, meropenem	resolved
BGG19292	Hemorrhagic cystitis	Gr 3	plurifloxacin	resolved
BGR24765	Hemorrhagic cystitis	Gr 3	ciprofloxacin	resolved
TCG1948	Dyverticulitis	Gr 3	metronidazole	resolved
BLM25453	Hemorrhagic cystitis	Gr 3	ciprofloxacin	resolved
BDDR24288	Bronchopneumonia	Gr 3	Cefotaxime + clarithromycin	resolved
BRC17075	Bronchopneumonia	Gr 3	ciprofloxacin + azythromycin	resolved
BPZ24363	Bronchopneumonia	Gr 3	sulbactam + clarithromycin	resolved
TPG1946	Bronchopneumonia	Gr 3	Ceftriaxone	resolved
TPG1946	Bronchopneumonia	Gr 3	Ceftriaxone	resolved
BGG19292	Bronchitis	Gr 3	sulbactam + steroids	resolved
BEL29134	Bronchitis	Gr 3	Ceftriaxone	resolved
BRC17075	Bronchitis	Gr 3	Ceftriaxone	resolved
BLF24876	Submandibular abscess	Gr 2	azythromycin	resolved
TVA1924	Skin infection	Gr 2	Amox/clav	resolved
BDP25281	Sialadenitis	Gr 2	Amox/clav	resolved
BRB14362	Otitis media	Gr 2	Topical antibiotics	resolved
BPM10416	Hemorrhagic cystitis	Gr 2	nitrofurantoin	resolved
BLB27530	Bronchopneumonia	Gr 2	Levofloxacin, Amox/clav	resolved
BMAT18375	Bronchitis	Gr 2	Amox/clav+azytromycyn	resolved
BGB29136	Bronchitis	Gr 2	Amox	resolved
BEL29134	Bronchitis	Gr 2	Amox/clav	resolved
BGG22957	Bronchitis	Gr 2	Amox	resolved
BGG22957	Bronchitis	Gr 2	Amox/clav	resolved
BGG22957	Bronchitis	Gr 2	Amox/clav	resolved
BMT3445	Bronchitis	Gr 2	Amox/clav	resolved
BMA13690	Bronchitis	Gr 2	Amox/clav	resolved
TCG1948	Bronchitis	Gr 2	Amox/clav	resolved
TSR1944	Bronchitis	Gr 2	Amox/clav	resolved
TVA1924	Bronchitis	Gr 2	Levofloxacin	resolved
BAD22681	Zoster reactivation	Gr 3	acyclovir	resolved with sequelae
TFF1985	Zoster reactivation	Gr 3	acyclovir	resolved
BMS3543	Viral gastroenteritis	Gr 3	I.V. rehydratrion	resolved
BEL29135	pleuropericarditis	Gr 3	Amox/clav+clarithr	resolved
BDP25281	Viral gastroenteritis	Gr 2	Rehydration	resolved
BAD22681	Polmunary TBC	Gr 3	Rifam+ison+ethamb+pyraz+levo	resolved
BMA13690	Nodal tubercolosis	Gr 3	Rifam+ison+ethamb+pyraz+levo	resolved
BMGM27828	Fungal pneumonia	Gr 4	Voriconazole	resolved with sequelae

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PREVALENCE AND CHARACTERIZATION OF EOSINOPHILIAS, IDIOPATIC HYPEREOSINOPHILIA AND HYPEREOSINOPHILIC SYNDROME IN A LARGE (ONE MILLION) POPULATION IN NORTH ITALY (THE ROMAGNA GREATER AREA, AVR)

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The Eosinophilias (Eos) encompass a broad range of nonhematologic (secondary or reactive) and hematologic (primary, clonal) disorders. Disease prognosis relies on identifying the origin of Eos. The 2008 WHO establishes a semimolecular classification of eosinophilic disorders (ED), and HES is a diagnosis of exclusion. The incidence and prevalence of ED and HES is not well characterized, and also the frequency of PDGFRA rearrangement is really unknown. The principal aim of this study was to investigate the prevalence of ED in the AVR, an homogeneous large geographic area. Second aims were to characterize all ED. *Methods:* The Hub Laboratory (HL) of AVR serves more than one million (1.124.866 in 2012) inhabitants and provides Laboratory Medicine service for all AVR, including hematologic Units. The results of all the tests since 2009 are stored in the LIS database. We downloaded the differential cell counts (DIFF) results obtained in 2012 and selected the cases with ≥1.5 x109/L Eo among the 574.380 unique individuals with at least one DIFF. In order to verify the first Chusid's criteria we searched for other DIFF results with Eo ≥1.5 x109/L in the semesters before and after. For this total cohort of possible HES patients, we performed further data extraction from LIS with the intent to exclude the more frequent causes of secondary Eos (*e.g.* total IgE, fecal parasites, autoimmunity markers, BM smears and cytogenetic). Moreover we matched the clinical records of the uncertain cases for final diagnosis. *Results:* Of 574.380 unique individuals with one DIFF in 2012, 452 satisfied the first Chusid's criteria, and on these cohort we will performed our investigations. Here we report the preliminary results on 157 patients (33% of the total cohort). We excluded 94 patients with secondary Eos (63,7%), and 49 asymptomatic patients no further investigated despite a persistent Eos, and finally we identified 8 patients with sign and sympthoms of HES, with a prevalence in the AVR of 0.14%. Of note, 7 of 8 patients were male, and 5 patients died after several months of observation. The PDGFRA status was investigated in 6 of 8 cases. *Conclusions:* The low prevalence of HES is confirmed by these preliminary data. With the completion of this analysis we will achieve a better characterization of ED and, since we are investigating half of the resident population of AVR, also the real prevalence of these rare diseases will be known, including PDGFRA positive clonal disease.

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DEVELOPMENT OF CHRONIC MYELOMONOCYTIC LEUKEMIA DURING TPO-RECEPTOR AGONIST TREATMENT FOR IMMUNE PRIMARY THROMBOCYTOPENIA: A CASE REPORT

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Introduction: The development and progression of myeloproliferative neoplasms is a discussed safety issue during treatment with TPO-receptor agonists (TRAs). Here we report a case of chronic myelomonocytic leukemia (CMML) observed after repeated treatment with TRAs for immune primary thrombocytopenia (ITP). *Case description:* A 64-year old woman with history of ITP presented with marked leukocytosis, monocytosis, increased LDH levels and worsening anemia (Table 1). She was being treated with romiplostim at the maximum weekly dose of 10 mcg/kg for 8 months after previous transient responses to eltrombopag, romiplostim itself, and corticosteroids. A diagnostic procedure was performed. Peripheral blood smear revealed marked myeloid dysplasia (Figure 1 A). Bone marrow aspiration and biopsy showed increased cellularity with markedly reduced erythropoiesis and enhanced megakaryocyte proliferation, trilinear dysplasia with blast count less than 10% (including monoblasts and promonocytes) and no significant fibrosis (Figure1 B,C). Both peripheral and bone marrow specimens obtained at first diagnosis of ITP were instead negative for any of these myeloproliferative/dysplastic features. Type 1 CMML was diagnosed and cytoreductive treatment with hydroxyurea was started. Cytogenetic analysis revealed no abnormalities and molecular testing for Bcr/ABL-1, JAK2-V617F and PDGFR-A/B mutations were negative. Given her young age and CMML-specific cytogenetic risk, the patient was referred for AML-like chemotherapy induction and allogenic HSCT. Discussion. TRAs are increasingly used as second-line treatment in ITP. In addition, the safety and efficacy data for use of these agents in myelodysplastic neoplasms is growing. Reversible bone marrow fibrosis have been reported with use of either romiplostim or eltrombopag. Moreover, a theoretical risk exists that these agents may promote the progression of usually pre-existing myeloid malignancies through TPO-receptor signalling. Here we reported a case of CMML observed during repeated treatment with TRAs for refractory ITP. Although a causal relationship between TRA treatment and CMML development could not be established, our report support the need for close clinical surveillance for detection of myeloproliferative/myelodysplastic disease in TRA-treated patients.

Table 1.

	ITP diagnosis	After 3-mo TRA-treatment	CMML diagnosis	After 2-mo follow-up
Hb, g/dL	11.7	11	9.2	9
WBC, x 10^9/L	4.04	3.91	21.0	69.0
Neutrophils, x 10^9/L	2.42	1.96	15.1	58.7
Monocytes, x 10^9/L	-	-	2.31	3.45
Platelets, x 10^9/L	19	35	23	10
LDH, U/L	390	-	1400	1002

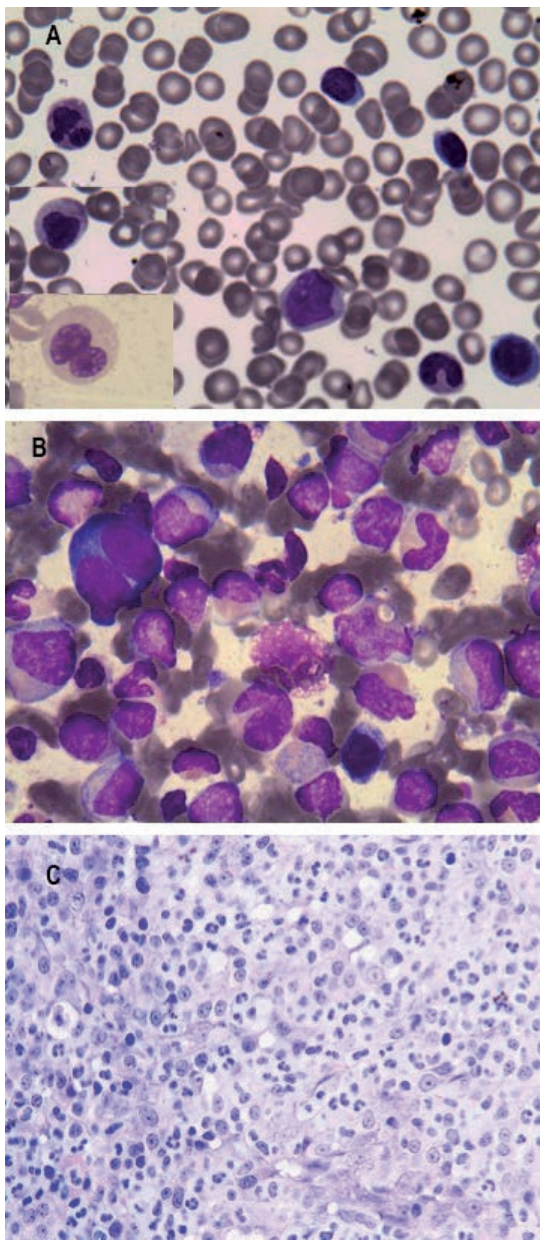

Figure 1.

Allogeneic and Autologous Transplantation 1

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CHANGES IN CIRCULATING ENDOTHELIAL CELLS COUNT COULD BECOME A VALUABLE TOOL IN THE DIAGNOSTIC DEFINITION OF ACUTE GRAFT-*VERSUS*-HOST DISEASE

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Allo-HSCT is burdened by life-threatening complications, being GvHD the major cause of morbility and mortality. Recently, clinical and physio-pathological evidences showed that vascular endothelium can be a target of GvHD in very early phase and circulating endothelial cells (CEC) represent surrogate markers of endothelial damage. We conducted a study with primary endpoint to identify and count CEC in peripheral blood of patients undergoing allo-HSCT as a function of endothelial damage. Using the CellSearch System®, CEC (CD146+/CD105+/ DAPI+/CD45-) were counted before (T1), after conditioning regimen (T2), at engraftment (T3), at GvHD onset (T4), after steroid treatment (T5) in 40 patients undergoing allo-HSCT for their hematologic diseases. Ten healthy subjects served as controls. Acute GVHD (grade I-IV) manifested in 19/39 patients. No clinical and transplant characteristics differences were present between patients with and without GVHD. The median CEC/ml at T1 was 20 (n=33, range 4-718), in comparison to a value of 2 (range 1-14) in controls (p<0.001). At T3 CEC/ml were 47 (range 16-148) in GvHD patients and 92 (range 23-276) in patients without GvHD (p=0.006). This difference remained significant in multivariate analysis (OR 0.97, 95% C.I. 0.96-0.99; p=0.02). At GvHD onset, the relative increase of CEC counts from time of engraftment (T4 *vs* T3) was 44% (range, -43 to 569%) in GvHD patients *versus* 0% (range, -49 to 2%) in patients without GvHD (p=0.003), being confirmed as significant in multivariate analysis (OR 1.04, 95% C.I. 1.0-1.08; p=0.04). Furthermore, we are actually conducting a comparison study in which CEC are counted in parallel with the CellSearch System® and a flowcytometry-based methodology using a dedicated BD Lyotube® for CEC evaluation (CD146+, CD34bright, CD45-, Syto-16+, 7-AAD-). At moment 35/50 patients have been already enrolled and have completed the study, confirming the valuable role of CEC count changes in supporting GvHD diagnosis. Changes in CEC count can represent a promising marker to monitor endothelial damage in patients undergoing allo-HSCT and are a valuable tool in the diagnostic definition of GvHD. Moreover CEC count could be a valid complement in the prognostic stratification of patients candidates to allo-HSCT.

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THE EARLY ASSESSMENT OF THE SYSTEMIC INFLAMMATORY RESPONSE SYNDROME SCORE IS ABLE TO PREDICT ACUTE GRAFT-*VERSUS* HOST DISEASE AND NON-RELAPSE MORTALITY IN PATIENTS AFFECTED BY HAEMATOLOGICAL DISEASES UNDERGOING AN ALLOGENEIC STEM CELL TRANSPLANTATION

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Systemic inflammatory response syndrome (SIRS) is an multifactorial inflammatory state diagnosed when 2 or more of the following criteria are met: body temperature <36 or >38°C, heart rate >90 beats/minute, tachypnea >20 breaths/minute, leukocytes <4000 or >12000 cells/µl or presence of >10% immature neutrophils. The goal of the study was to assess the incidence of SIRS after an allogeneic stem cell transplantation (alloSCT) (from day 0 to 15) and evaluate whether the onset of acute graft-*versus*-host disease (aGVHD) and non-relapse mortality (NRM) are influenced by its occurrence. Data from 256 patients who underwent an alloSCT from 2003 to 2012 were retrospectively collected. The me-

dian age was 48 (range,15-68): 48% were in complete remission (CR) at alloSCT, 29% in partial remission (PR) and 23% in stable/progressive disease (SD/PD). The median number of previous therapies was 3 (range,0-8), and 71% had failed an autologous SCT. The HCT comorbidity score was 0-1 in 71% and ≥2 in 29% of patients. Patients received stem cells from a matched sibling (40%), haploidentical (18%) or unrelated (42%) donors. Myeloablative or RIC regimens were used in 25% and 75% of alloSCT, respectively. At least one SIRS episode (score 2 n=98, score 3-4 n=91) was observed in 73% of patients. The causes of SIRS were: infectious (61%), toxic (10%) and 29% were judged as not clinically relevant. The aGVHD rate at day 100 was 38% and 18% developed grade ≥2 aGVHD. NRM rate was 5.5% at 100-days and 10% at 1-year. Patients who had SIRS experienced aGVHD more frequently (44% *vs* 21%, p<0.001); aGVHD was correlated with infectious and toxic SIRS (p<0.001). The occurrence of SIRS≥3 predicted a worse NRM: 100-days and 1-year NRM was 9% *vs* 4% and 15% *vs* 7%, respectively (p=0.004). In multivariate analysis, taking into account age, previous autologous SCT, previous therapies, disease status, HCT score, donor type and conditioning regimen, only haploidentical donor was correlated with the onset of SIRS (p<0.01). In multivariate analysis that took into account patients characteristics as above and SIRS score, SIRS score ≥2 was an independent variable impacting on the NRM and every increase in SIRS score was correlated with 1.55 fold increase in NRM (p=0.04). In our analysis we observed a significant correlation between the early development of SIRS and the occurrence of aGVHD and NRM after alloSCT. Thus therapies lowering cytokine storm maybe a rational area of investigation.

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EFFICIENT *EX VIVO* EXPANSION OF DONOR NATURAL REGULATORY T CELLS USING GMP- COMPLIANT REAGENTS FOR USE IN ADOPTIVE IMMUNOTHERAPY

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Introduction: Natural CD4+CD25+ regulatory T cells (Tregs) are promising candidate for immunoregulatory cell therapy. It has been previously shown that co-transplantation of freshly isolated or *ex vivo* expanded donor Tregs can prevent GVHD after HSCT. However, due to their low numbers in peripheral blood a dose of freshly isolated Tregs sufficient for therapeutic purposes may be obtained in only a fraction of donors. *Aims:* To overcome this limitation, in this study we aimed to develop of an efficient *ex vivo* Tregs expansion protocol that would be suitable for adoptive immunotherapy trials. *Methods:* Tregs were purified from standard buffy coats (N=3) by immunomagnetic isolation (CD8 depletion and CD25 enrichment). Tregs were activated with anti-CD3 and anti-CD28 coated-beads at a bead-to-cell ratio 4:1 in the presence of Rapamycin 100 nM. IL-2 was added 2 days after activation. Cells were then restimulated every 7 days and *ex vivo* expanded for 21 days. All reagents were GMP (provided by Miltenyi Biotec). Phenotypical and functional characterization of expanded Tregs have been performed at specific time points and after cryopreservation/thawing. Results The mean absolute number of isolated CD25+ T cells was 4.6x10e6±3.8 and the mean percentage of CD4+CD25+FOXP3+ cells was 65±13%. Cell contaminants were: CD8+ T cells <1%, Th17 cells <5%, B lymphocytes <17%,T NK cells <5% and monocytes <6%. Cell expansion reached a peak between the second and third re-stimulation and after 21 days the mean fold increase was 559±245. After 21 days of *ex vivo* expansion, the cells were almost totally CD4+CD25+FOXP3+. Consistently, the percentages of Th17 cells, CD8+ T cells, monocytes, B lymphocytes and T NK cells progressively decreased, reaching values below 1% at the end of culture. Moreover, 21 days-expanded Tregs more efficiently reduced T effectors (Teff) proliferation than the freshly isolated counterpart (80 *vs* 40% inhibition at a Tregs-Teff ratio 1:2). After thawing, the mean percentage of viable expanded Tregs was 75±5% with >95% CD4+CD25+Foxp3+ cells. Thawed expanded Tregs also efficiently reduced Teff proliferation. *Conclusions:* We successfully set up an *ex vivo* expansion protocol, using GMP reagents, which allows the recovery of a high number of functionally suppressive Tregs. Expanded T regs will be employed in a clinical trial for the treatment of patients with steroid-refractory chronic GVHD.

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PARAMETERS OF PROTEIN METABOLISM AND THYROID FUNCTION AS PREDICTORS OF A SCORING SYSTEM FOR ACUTE AND CHRONIC GRAFT-*VERSUS*-HOST DISEASE

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Some "classical" patient- and transplant characteristics, such as age, gender disparity, donor type, HLA-match, and source of stem cells, have been reported as predictors for acute and chronic GVHD. However, no studies analysed these "classical" variables together with parameters of metabolic and endocrine functions that may potentially influence the immune system. Thus, patient-and transplant variables together with index of liver and thyroid function, and some parameters of protein and lipid metabolism were retrospectively analysed in 161 patients at different time points after transplantation in relation to the onset of GVHD. A 2-step multivariate analysis was performed using principal component analysis and Cox regression analysis. Based on the regression coefficient of Cox analysis for each significant predictor, a scoring system for acute and chronic GVHD was calculated. In multivariate analysis, diagnosis of Myelodisplastic Syndrome or Chronic Myeloid Leukemia (p=0.0004), conditioning regimen including Total Body Irradiation (p=0.0003), and pre transplantation urea >34 mg/dl with +21 day urea >54 mg/dl (p=0.0008) were predictors for acute GVHD. The system-score had a range between 0 and 4. The probabilities of acute GVHD according to the sum scores ranged from 8% (score 0) to 98% (score 4). Female donor (p=0.0008), pre-SCT TSH values ≥2 mU/L with +28 day urea ≥39 mg/dl (p=0.02), +6 month total protein <5,5 g/dl with γ-GT ≥347 U/L (p=0.0001) resulted predictors for moderate/severe chronic GVHD. Risk of chronic GVHD at +6,5 month ranged from 3% (score 0) to 97% (score 4). Factors other than those "classical" ones may be associated to GVHD. The scoring system included routine-parameters, which are easily available in clinical practice. Urea levels depend on the balance between protein intake, endogenous catabolism and urinary excretion. The inflammatory microenvironment of GVHD promotes muscle catabolism and hence, increased urea levels. Increased urea levels could be indirect index of increased uremic toxins as well, which may stimulate the production of pro-inflammatory cytokines and the activation of leukocytes. Increased urea levels and uremic toxins could also derive from a dysregulated metabolism of the gut microbiome that may influence immune system. Our findings suggest the usefulness to study in deep the complex network between metabolic/endocrine functions and immune system for a holistic approach of the transplant management.

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MicroRNAs AND PiRNAs FROM EXTRACELLULAR VESICLES OF BONE MARROW MESENCHYMAL STEM CELL MODULATE CELL SURVIVAL AND INHIBITION OF DIFFERENTIATION OF CORD BLOOD HEMATOPOIETIC STEM CELLS

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Umbilical cord blood stem cells (UCB-SC) are hematopoietic stem cells (HSC) frequently employed for transplant procedures. HSC fate is bound to different factors and, among these, the cross-talk with the bone marrow microenvironment represents an important regulatory mechanism of aging, self-renewal, stemness and/or differentiation. Cells communicate by both direct interaction and through the secretion of soluble factors and extracellular vesicles (EVs). Recent studies suggest that the release of E*Vs* is a highly regulated and important process involved in

surface membrane traffic and horizontal transfer of RNAs, protein and DNA. Bone marrow mesenchymal stem cells (BM-MSC) are a component of hematopoietic microenvironment and support hematopoiesis by the constitutive ability to secrete soluble factors and E*vs* We isolated, by ultracentrifugation, BM-MSC-derived E*Vs* (BM-MSC-EVs) and analyzed their interaction with CD34+UCB-SC after 24h of co-colture. Using Illumina platform, we sequenced Small-RNAs from E*Vs* and we identified 87 miRNAs and 5 piRNAs potentially involved in modifying UCB-SC activities. In fact, gene expression profile (GEP) analysis of UCB-SC treated with BM-MSC-E*Vs vs* not treated, detected about 100 down-regulated genes likely targeted by E*Vs* miRNAs and piRNAs. Gene ontology of miRNAs and piRNAs targeted genes, evaluated by Ingenuity pathway analysis, identified different down-regulated biological function, in particular cell death and cellular development. To confirm these data, we evaluated by FACS the apoptosis of UCB-SC treated with BM-MSC-E*Vs vs* not treated, and we observed a significant reduction by 42% (p value <0.05). By contrast, a decrease of cellular maturation was confirmed evaluating the expression of different hematological markers, such as CD19+, CD33+ and CD38+, which resulted to be lower in UCB-SC treated with BM-MSC-EVs, respectively by 45%, 13% (both p values <0.05), and 3% (p value <0.01), with respect to controls. GEP also identified 103 up-regulated genes, most of which codifying for chemokines and cytokines involved in the chemotaxis process of different BM cell types. In conclusion, this study indicates, for the first time, the existence of a communication between BM-MSC and UCB-SC mediated by E*vs* In particular, GEP modifications of UCB-SC induced by miRNA and piRNAs contained in BM-MSC-E*Vs* provide new insights in the biology of cord blood transplantation, potentially useful for future clinical applications.

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CARBAPENEM-RESISTANT ENTEROBACTERIACEAE AND THEIR IMPACT ON STEM CELL TRANSPLANTATION: A SINGLE CENTER EXPERIENCE

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The increasing incidence of CRE, particularly Klebsiella Pneumoniae (KPC), is one of the most important issue since they are an important threat to public health. Patients (pts) with hematological malignancies are therefore the most vulnerable. We analyzed the impact of CRE-KPC colonization and infection in our center focusing on patients submitted to SCT in our division of Hematology from 2010 to 2015. Surveillance was adopted sistematically in 2010, when the first case of KPC was discovered, using peri-rectal swab at admission and weekly thereafter on all pts admitted to the division of Hematology. Epidemiology control was reinforced on stem cell transplant setting according to our local policies, included contact precautions and empirically oriented antibiotics. 254 consecutive pts submitted to autoSCT and 131 submitted to alloSCT were analyzed. Colonization prior to autoSCT was not observed until 2012 when 2 pts presented KPC colonization. Three pts had KPC infection prior to auto.Thirteen pts before autoSCT presented KPC colonization and 9 of them developed microbiologically documented KPC infection during the phase of neutropenia (1 sepsis, 4 urinary tract infections 2 pneumonia; 2 gut infection). All of them received CRE-oriented empiric. Colonization with CRE-KPC after auto-SCT was 7.% No deaths were observed. One hundred forty alloHSCT procedures were performed in 134 pts. KPc colonization/infection prior to HSCT had occurred in 17 cases (12%). Twenty-three pts (16%) developed microbiologically documented KPC infection during different phases after transplantation. Infections included 14 sepsis,4 urinary tract infections and 4 pneumonia and 1 gut infection. Thirteeen cases occurred within 100th day from transplant and were defined as "early" infections. Remaining cases were classified as "late" (5 cases, occurred <6th month) and "very late" (5 cases, >6th month). All colonized patients received KPc-oriented empiric antibiotics in case of fever. Thirteen deaths were registered among cases (13/23, 56%). Of them, 4 deaths were considered due to KPc only (4/13, 30%), while 6 pts died with KPc infection. Remaining 3 deaths were due to other causes. Colonization rate and incidence of CRE-KPC was significantly different between autologous and allogeneic SCT recipients (13/241 *vs* 17/123, p=0.01); 3.8% *vs* 16.4%,p<0.0001). Systematic surveillance and prompt combined antibiotic therapy may be a possible explanation. The incidence of colonization is still rising and all possible efforts should made in order to reduce diffusion of CRE-KPC and this should be priority

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BIOSIMILAR G-CSF VS CLASSICAL G-CSF IN POST ALLOTRANSPLANT RECOVERY. A CASE CONTROL STUDY

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Introduction: The use of biosimilar G-CSF was licensed by the European Medicines Agency only on the basis of extrapolation of data. The setting of allogeneic transplantation is unexplored. We started a case-control clinical study in patients undergoing allotransplant to verify biosimilars safety and efficacy in the post-transplantation hematopoietic recovery. *Materials and Methods:* We analyzed two cohorts comprising 86 patients treated in our HSCT Centre for various hematological diseases. The first cohort included 43 consecutive patients undergoing allogeneic HCST between February 2013 and October 2014 who were treated with G-CSF biosimilars (Tevagrastim® or Zarzio®). The second cohort (control group) included 43 consecutive patients who underwent allogeneic HSCT between January 2011 and December 2012 and received originator G-CSF (Granulokine®). The injection of G-CSF (5 µg/kg/day) began at day 7 after HLA identical sibling or MUD and at day 6 after haploidentical stem cell infusion. We evaluated white blood cell, platelet and red blood cell engraftment, the incidence of graft failure, CMV infection, early infections, aGvHD, six-month relapse and death in the two cohorts. *Results:* Study and control groups were matched for sex, type of disease, Karnofsky score, HCT-Comorbidity Index, status of disease at transplant, conditioning regimen, type of donor, HLA-matching, stem cell source, number of stem cells injected (P not significant). Difference in age was statistically significant but in favor of originator group. White blood cell engraftment took place in median time of 13 (10-28) days in the study group and in 13.5 (10-22) days in the control group (p=0.266). Red blood cell engraftment occurred in median time of 15 (0-72) days in the study group and in 16.5 (0-69) days in the control group (p=0.933), while platelet engraftment in median time of 16 (9-34) in the study group and 16.5 (10-40) in the control group (p=0.386). There was not statistically significant difference in terms of incidence of graft failure, CMV infection, early infections, aGvHD, six-month relapse and death between the two groups (p not siginifcant). The differences remained unmodified even after that logistic regression analysis was used to correct age difference. *Discussion:* This study shows that biosimilars of G-CSF are equivalent to classical products in terms of safety and efficacy when used for count recovery after allogeneic hemopoietic stem cell transplantation.

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MONITORING OF CHIMERISM ON SORTED PERIPHERAL CD34+ CELLS IN PATIENTS WITH ACUTE LEUKEMIA RECEIVING ALLOGENEIC HEMATOPOIETIC BONE MARROW TRANSPLANT

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Although allogeneic hematopoietic stem cells transplantation (HSCT) is the most powerful consolidation therapy for acute leukemia, a relapse can still occur. An early and frequent follow-up after HSCT is the key to prevent an hematologic relapse. Albeit monitoring of minimal residual disease (MRD) by flow cytometry (FC) and polymerase-chain-reaction (PCR)-based techniques are routinely used in the follow-up of transplanted patients, restricting chimerism analysis to sorted circulating CD34+ cells could be an early predictor of relapse after HSCT in adult patients affected by acute leukemia. At diagnosis, for each patient the most useful leukemia-associated aberrant immuno-phenotype (LAIP)

was established to be investigated during follow-up in order to reveal LAIP-positive cells. DNA was extracted from peripheral blood (PB) of donor and recipient before HSCT and chimerism assessment was performed on whole PB and sorted CD34+ cells of recipient after HSCT at specific time points: day 30, 60, 100, 180, 365 and 540 from HSCT. From January 2012 to March 2015, we enrolled 31 patients: a preliminary analysis revealed that results were concordant with MRD-negative status and 100% donor chimerism both in the whole PB and in the CD34+ sorted fraction. Two cases deserve to be reported in details. The patient #2 was enrolled in the project at April 2012. At day 30 from HSCT (matched unrelated donor), chimerism on whole PB was 100% donor with FC-MRD negative; of note, chimerism on sorted CD34+ cells was 60% donor. At day 60 from HSCT, FC-MRD converted to positive (0.23% of global cells) whereas chimerism on whole PB was still 100% donor. At day +100, chimerism on whole PB decreased and the patient experienced bone marrow relapse. The patient #7 was enrolled at October 2012 and underwent HSCT from cord blood. Until day +180, results were concordant: FC-MRD negative status and 100% donor chimerism both in the whole PB and in sorted CD34+. At day 365, chimerism on whole PB was 100% donor with FC-MRD negative while chimerism on sorted CD34+ cells was 86% donor; after two months, a bone marrow relapse occurred. Chimerism analysis on sorted CD34+ cells allowed an early diagnosis of disease recurrence by underlining the presence of patient's cells with concomitant "negative signals" from FC-MRD and whole PB chimerism and can further intensify the predictive potential of the technique. *The project is funded by Ministero della Salute and Regione Toscana (CUP D11J09000190003).*

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PRESERVATION OF GRAFT QUALITY IN CRYOPRESERVED BM AND PBSC UNITS STORED FOR MORE THAN A DECADE

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PBSC or BM are increasingly harvested in remission for possible use in the event of relapse. Although the value of this approach has not been demonstrated, the long-term storage of progenitor cells has become commonplace in many facilities. Flow cytometry assessment of CD34+ cells is generally accepted as a surrogate of the HSC content of the graft and therefore as an engraftment predictor. However, the viability of CD34+ cells at thawing is extremely variable and factors that could impact on this has been poorly reported. We report an analysis of 12 BM and 13 PBSC units cryopreserved in the period 1987-2004 that were thawed and analyzed in reason of the disposal authorized by Local Ethical Committee. Each unit was thawed, diluted and manipulated according to the current SOPs. The following parameters were evaluated: TNC, CD34+, MNC and PMN count and viability, clonogenic potential (CFU) assay in terms of ClonE index (CD34+/CFU). Moreover, sterility was randomly tested in some samples. 1 out of 12 BM bags thawed went off during thawing procedure. Median values for parameters analyzed were: age of cryopreservation of 16.0 (10.5-25.7) years, TNC 5.9x10^9 (2.1-13.9 x10^9), CD34+ 15.2x10^6 (5.0-58.0 x10^6), TNC viability 41.3% (24.9-53.0%), CD34+ viability 67.05% (42.1-84.6%). All samples were able to generate colony forming units with a median ClonE index of 6.9 (4.6-15.1). The strongest relationship observed was the inverse correlation between PMN% and MNC viability (R2=0.3). All the 4 units tested for sterility were negative. Median values of 13 PBSC thawed were: age of cryopreservation of 17.4 (9.9-22.0) years; TNC 11.8 x10^9 (3.7-17.6); CD34+ 49.0x10^6 (8.4-518); TNC viability 85.9% (48.8-94.0%); CD34+ viability 72.7% (58.7-94.1%). All samples generated CFU with a median ClonE 4.9 (1.3-15.8). A strong correlation was observed between PMN% and TNC viability (R2=0.92) with the latter dramatically decreasing as PMN% increased (Fig.1). Moreover, ClonE index positively correlated with CD34+ count (R2=0.56) and CD34+ viability (R2=0.41) as well. Overall, cells viability were not dependent on years of cryopreservation. All the 7 units tested for sterility were negative. The results indicate that BM and PBSC can be cryopreserved for more than a decade without consistent loss of progenitor viability and efficiency. More interestingly, PMN content strongly affects PBSC cells viability.

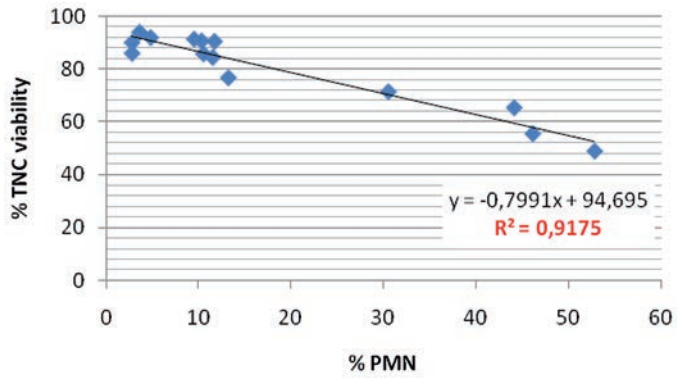

Figure 1. % PMN *vs* TNC viability in thawed PBSC.

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HAPLOIDENTICAL STEM CELL TRANSPLANTATION FOLLOWED BY HIGH-DOSE CYCLOPHOSPHAMIDE POST TRANSPLANT AS GVHD PROPHYLAXIS IN HIGH-RISK HAEMATOLOGIC MALIGNANCIES: A RETROSPECTIVE MULTICENTRIC EXPERIENCE FROM THE REP (RETE EMATOLOGICA PUGLIESE)

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Allogeneic stem cell transplantation is the only curative option for high-risk haematologic malignancies. Haploidentical HSCT offers an opportunity of transplant in 95% of all these patients, in a simple, immediate manner. GVHD prophylaxis performed with post transplant high-dose cyclophosphamide (PT-CY), according to the Baltimore experience, has become a new standard in the Haplo-HSCT setting. To reduce the high rate of relapse reported in the Baltimore experience, in accordance with the Bacigalupo group we adopted a myeloablative conditioning regimen (MAC) in all patients. *Methods:* From April 2011 to February 2015, we retrospectively evaluated 43 patients (M 24, F 19), median age 37 years (range 14-69), with high-risk haematologic malignancies who underwent Haplo-HSCT followed by PT-CY, in 3 different Institutions. Diagnoses were AML (n=29), ALL (n=9), CMML (n=1), NHL (n=1), cHL (n=3); 12 patients had received a previous SCT: 10 autologous SCT, 2 URD. All patients received a MAC, 39 TBF (thiotepa, blusulfan, fludarabine), 4 TBI, fludarabine. At HSCT, 22 patients were in CR (11 in 1rst CR, 11 in 2nd or >2nd CR) while 21 presented active disease. Unmanipulated bone marrow was the stem cell source for all patients. GVHD prophylaxis was performed in all patients with PT-CY (d+3,+4 in 35 patients; d+3,+5 in 8 patients), cyclosporin from d 0, mycophenolate from d+1. *Results:* PMN leukocyte engraftment was reached at a median of 20 days (range 14-29). The cumulative incidence of grade II-III acute GVHD was 11.6% and moderate chronic GVHD 9%. Cumulative TRM at d+100 was 23% and the relapse rate was 20.9%.At d+30, 34 patients (79%) had full-donor chimerism. At d+90, a good immune reconstitution was present in 92% of evaluated patients. The actuarial 34 month DSF rate was 73% for patients in remission at HSCT and 18% for patients with active disease at HSCT. Causes of death were: relapse (n=8), infections (n=8), GVHD (n=3), MOF (n=2). *Conclusions:* Haplo-HSCT with PT-CY offers a useful treatment tool in selected patients with high-risk haematologic malignancies. In this multicentric study, we confirmed that this procedure offers long-lasting remission, with limited toxicity, a low-grade aGVHD incidence and early immune reconstitution. New therapeutic strategies are needed to further reduce the relapse rate. A careful selection of alloreactive donors, HSCT performed in the early stage of disease, and an adequate adoptive immunotherapy are the future challenges.

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TEPADINA®) PLUS BUSULPHAN I.V AS CONDITIONING REGIMEN BEFORE AUTOLOGOUS STEM CELLS TRANSPLANTATION IN PATIENTS WITH ACUTE MYELOID LEUKAEMIA

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From June 2010, 25 acute myeloid leukemia (AML) patients (pts) in first CR were included in a prospective multicenter trial to evaluate the efficacy and toxicity of a new preparative conditioning regimen based on TEPADINA and intravenous (iv) Busulphan for ASCT. This new conditioning regimen consisted of Tepadina 10 mg/kg (5 mg twice every 12 hours) with an intravenous infusion of 4 hours on day -7 and Busulfan (i.v.) 3.2 mg/kg given intravenously on day -6 to-3 (cumulative dosage: 12,8 mg/kg). Patients older than 65 years (n=6) received Tepadine at the dose of 8 mg/kg (4 mg twice every 12 hour) on day -6 and Busulfan on day-5 to 3 at cumulative dosage of 9 mg/kg. Most patients were female (n=15, 63%), and the median age at transplantation was 58 years. All patients received peripheral blood stem cells (PBSC), the median CD34+ cells doses were 5 x106/kg (range: 2,6-9,0). Twenty pts showed normal kariotype, the other 4 pts showed unfavourable cytogenetics. Two pts had mutation of NPM1 gene, none pts had mutation of FLT3 gene. All pts achieved leukocyte and platelets recovery after a median time of 9 (range: 9-14) and 12 (range: 9-30) days, respectively. Grade III-IV mucositis occurred in 6 (25%) pts. Only one patient died due to transplant-related causes after transplantation; in particular this patient died at day+26 because of a gram-negative sepsis worsened by kidney and liver failure. The relapse rate was 32% (8 pts relapsed between 3 and 31 months after autoSCT) and the cumulative treatment-related mortality was less than 1%. The average number of days of hospital stay was 20 (range: 19-35). Febrile neutropenia occurred in 14 patients (59%) with a median duration of 2 days (range: 0-10); out of these patients,11 experienced a grade I-II fever of unknown origin (FUO). Two patients developed a fever caused by gram-positive infection (n=1), while 1 had gram-negative fever. Only 1 patient developed a cytomegalovirus infection. To date 16 pts (64%) are in CCR with a median follow-up of 28 months (range 3-53). Two patients died of progressive disease between 16 and 53 months after autografting. Twenty-two (87%) pts are alive. In conclusion our experience with this new conditioning regimen has shown a significant efficacy and a low toxicity profile. Only minor grades of treatment related nausea and vomiting were observed, also gastro intestinal and liver dysfunction were minor events in our study.

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TOTAL BODY IRRADIATION-BASED *VERSUS* CHEMOTHERAPY-BASED MYELOABLATIVE CONDITIONING BEFORE ALLOGENEIC STEM CELL TRANSPLANTATION FOR ACUTE MYELOID LEUKEMIA. A SINGLE CENTRE EXPERIENCE

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Cyclophosphamide combined with TBI or busulfan are the most widely used myeloablative conditioning regimen in patients with AML. Recent data regarding their comparative effectiveness are lacking. In this study we retrospectively assessed the outcome of 103 patients with AML in first or second complete remission (n=70) and refractory disease (n=33) who received myeloablative conditioning either with a TBI-based (n=47) or chemotherapy-based (56) regimen for ASCT from an HLA-matched sibling (n=56) or an unrelated donor (n=28) or a haploidentical donor (n=19). The conditioning regimen sequence was compared according to overall survival (OS), leukemia-free survival (LFS), TRM, leukemia relapse and GvHD. The Overall survival of all patients were 50% at 3 years. According to disease status, 3 years probabilities of OS were 64% in CR and 22% in refractory disease at time of transplant ($p<0.0001$). Three-year probabilities of survival were 59% and 43% for TBI and chemotherapy ($p=0.1$). Three-year probabilities of DFS were 39.5% and 20.5% for TBI and chemotherapy ($p=0.3$). In multivariate analysis for OS, the indipendent factors statistically significant were disease status at transplant and GvHD grade III-IV. The cumulative incidence of acute GvHD were 38% in TBI arm and 61% in chemotherapy arm ($p=0.02$). Corresponding incidences of transplant-related mortality (TRM) were 10% and 16% respectively in patients receiving TBI compared chemotherapy. In conclusion in our experience TBI is associated with superior outcomes compared with chemotherapy in patients with AML, an important result with the use of TBI-based conditioning regimen is the less development of acute GVHD (38%) respect to chemotherapy.

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WILMS' TUMOR GENE 1 EXPRESSION LEVELS IN ACUTE PROMYELOCYTIC LEUKEMIA COMPARED TO OTHER ACUTE MYELOID LEUKEMIA SUBTYPES

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Introduction: WT1 gene is known as a transcription factor over expressed in the majority of AML and, in this setting, WT1 is recognised as a panleukemic marker. Recently, an anti-apoptotic effect of WT1 has been demonstrated in HLA-60 cell line of Acute Promyelocytic Leukemia (APL), but there are very few data regarding WT1 expression levels in APL patients. *Methods and Results:* We evaluated the expression of WT1 in a large group of 170 AML at diagnosis (Dx), to compare WT1 expression in APL with other WHO-AML subtypes. According to WHO classification we observed: 50(29%) AMLs with MDS-related changes (Msec), 27(16%) AMLs with minimal differentiation (M0-1), 15(9%) AMLs with maturation (M2), 22(13%) Acute Myelomonocytic leukemia (M4), 35(21%) Acute Monocytic leukemias (M5) and 21(12%) APL (M3). The Dx of APL was confirmed with molecular and karyotype analysis and all cases were PML/RARα and t(15;17) pos. To evaluate the WT1 expression we performed a RQ-PCR assay on BM samples using the ELN ProfileQuanKit (Ipsogen, Marseille, Fr), designed on exon 1 and 2 of WT1, following the ELNet protocol; normal expression cut-off was 250 WT1 copies/104ABL, as previously reported. There were only 10(6%) AML cases without WT1 over expression at Dx, whereas 160(94%) cases over expressed WT1, with a median value of 6776 copies WT1/104ABL (range 255-62567). All cases of APL(21/21) over expressed WT1 at Dx. As documented in Table 1 the allelic burden of WT1 in APL was significantly higher than in all other AML subtypes (P<0.001). Moreover, the majority of APL patients (81%,17/21 pts) expressed more than 20x104WT1 copies/104ABL at Dx, compared to only 10/139 (7%) cases in other AML subtypes (P<0,001). In our 21 APL patients we have not found a relationship between the PMLRARα breakpoint type (bcr1, bcr2, bcr3) and the amount of WT1 copy number. All these 21 APL cases achieved a CR after induction therapy with Atra+Idarubicin, with concomitant normalization of WT1 levels. *Conclusions:* Our data underline that the expression of WT1 at Dx is significantly higher in APL compared to other AML subtypes. We do not know the causes of this higher expression of WT1 in APL, but we can assume that it could be related with the remarkable homogeneity of the APL blasts and with the well known proliferative and anti-apoptotic effect of WT1. In conclusion, APL subtype could be an *in vivo* useful and omogeneous model to increase our knowledge regarding the function of WT1 through the study of the interactions between this gene, PML/RARα and drugs with a pro apoptotic and differentiating effects.

Table 1. Number of cases and median and range of *WT1* gene transcript quantification in the overexpressing group at Diagnosis (160 AML) according to cytologic subtypes. *Of note, 17/21 (81%) cases of APL expressed more than 20x10⁴ *WT1* copies/10⁴ *ABL* at Diagnosis, compared to 10/139 (7%) in other AML subtypes (P < 0,001).

AML subtypes	N° of *WT1* overexpressing cases	*WT1* copies/10⁴ *ABL* Median	*WT1* copies/10⁴ *ABL* Range
M0-M1	26/27	8669	1368-30529
M2	15/15	4995	1008-25585
M3 (APL)	**21/21**	**30110***	**2069-62567**
M4	21/22	8111	255-41148
M5	31/35	5968	416-15661
Msec	46/49	4353	353-24205

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FOLLOW UP OF A CHRONIC MYELOID LEUKEMIA PATIENT WITH ATYPICAL KARYOTYPE AND A BCR-ABL FUSION VARIANT AT ONSET OF PATHOLOGY

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Introduction: Chronic myeloid leukemia (CML) is a hematopoietic stem cell cancer driven by a BCR-ABL fusion protein that arises from translocation of chromosomes 9 and 22. In this work we want to evaluate the presence of a rare BCR-ABL fusion variant caused by a three way chromosome translocation in a CML patient positive for t(9;22) but negative for common major and minor breakpoint cluster regions found at the onset of CML a year ago, now on treatment with BCR-ABL inhibitors (Nilotinib). *Methods:* Chromosome preparations were made by using 24-h and 48-h cultured bone marrow cells. FISH technique was performed using both BCR/ABL dual color dual fusion and dual color ES probes. G-banding was also used for karyotyping. *Results:* The presence of Philadelphia chromosome was not detected by classical cytogenetics in 20 analyzed cells, and karyotype was completed as 46,XY. FISH with BCR/ABL dual color dual fusion probe generated a '1O1G1F'signal pattern within the interphase cells in about 8% of cells. BRC/ABL ES dual color translocation probe showed the same pattern in 3% of cells. Although we had used different probes, we obtained comparable results. Probably the only one fusion signal resulted from an extensive deletion adjacent to the breakpoints on derivative chromosome (9) including 5' ABL and 3' BCR sequences. *Conclusions:* After one year of treatment with BCR-ABL inhibitors, CML patient showed a change in his atypical karyotype. About 5-10% of CML patients lack its cytogenetic evidence, however, showed BCR/ABL fusion by molecular methods like statistical analysis suggests. The submicroscopic deletion found in this patient may determine a poor prognosis and can be detected only by a FISH probe. FISH analysis of interphase, that is more sensitive than classical cytogenetics, is able to detect cryptic Ph translocation and is more helpful in the follow up of CML patients. Furthermore FISH leads to a more precise cytogenetical characterization and also evaluates the outcome of the CML as well as detection of minimal residual disease (MRD). If in this patient CML progression had been followed only by molecular biology, we would have had a false negative because commercial kits are not able to assess uncommon rearrangements; for this reason, despite the progress of molecular diagnosis, cytogenetic techniques are still a great tool in the hands of hematologists in the follow up of hematopoietic stem cell cancer driven by BCR-ABL fusion protein.

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QUALITY ANALYSIS OF BLOOD SAMPLES SHOWS DIFFERENCIES ACCORDING TO THERAPY IN CHRONIC MYELOID LEUKEMIA PATIENTS

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Introduction: The European Leukemia Net defined response criteria to monitor CML patients. Sometimes it is difficult to respect these criteria, especially for MR4.5 and MR5 response because of low number of abl copies. We analyzed quality of the samples and investigated if there is a possible correlation with the different TKIs employed. *Patients and Methods:* Blood samples, collected from 22 CML patients (14 male, 8 female), were analyzed at baseline, 3, 6, 9 and 12 months. Median age was 49 (range 24-79); 13 patients received Imatinib, 8 Nilotinib and 1 Dasatinib. Buffy coat, RNA extraction and RQ-PCR was performed at diagnosis and during treatment. Samples for RNA extraction were stored at a concentration of 10x106 cells/vial according to blood cells count performed with Coulter LH500. Flow cytometry evaluation of viability was performed. *Results:* We observed a different distribution of RNA concentration and abl copies between onset and follow up (box plot 1) in all patients. When we checked for differences according to therapy, we observed that Imatinib seems to interfere on sample quality more than

other TKIs (box plot 2). Median viability of 6 fresh samples at follow up was 85.4% (76.5-88.6). Unfortunally, we did not test for viability fresh samples at onset. However, reduced viability could be one more reason of reduced concentration of RNA extracted and number of abl copies. *Conclusions:* Therapy of CML could reduce quality of cells. For this reason, in order to correctly analyze CML samples, especially when deep response is supposed, a larger amount of cells should be collected and stored.

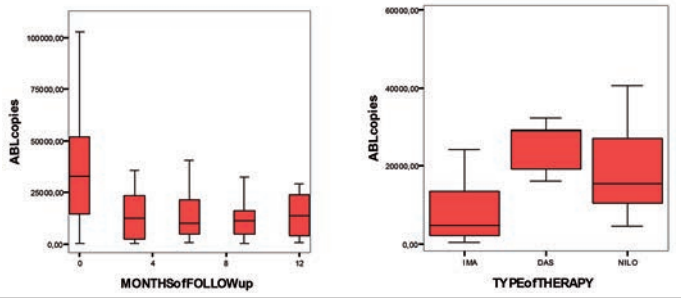

Figure 1.

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CYTOPENIA AND BONE MARROW METASTASIS: A MONOCENTER HEMATOPATHOLOGY STUDY

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BM involvement by non-hematopoietic neoplasms usually occurs as a late event during the course of oncologic diseases. Most patients with BM metastases have hematologic abnormalities, such as one or more cytopenias. A total of 10736 BM trephine biopsies were examined from January 2004 to December 2014. All samples were stained with Hematoxylin-Eosin, Giemsa and Silver impregnation. After morphologic evaluation, an immunohistochemical panel, including cytokeratin (CK) mix (AE1-AE3), was performed. Further characterizations of non hematopoietic neoplasms were subsequently carried out. Solid cancer metastasis was documented in 107 of all 10736 BM biopsy samples (0.99%). Concomitant BM aspirates and touch imprints were evaluable in 92 (86%) cases. Median patient age was 68 years (range 7-88 years). Males and females were 49 (46%) and 58 (54%) of the 107 cases, respectively. The most frequent metastatic tumors were, in order of frequency, as follows: breast (39.25%), gastrointestinal (16.82%), lung (13.08%) and prostate (12.15%) carcinomas. Among them, a previous diagnosis of solid cancer was missing in 25 cases (23.36%). Primary tumor site was determined in 16 out of these latter 25 cases (64%), namely breast 5 (20%), lung 4 (16%), kidney 2 (8%), prostate 1 (4%), neuroendocrine 2 (8%), gastrointestinal 1 (4%) and cervix 1 (4%). In the remaining 9 cases (36%), the rapid deterioration of clinical conditions contraindicated further investigations. However, epithelial origin was documented according to CK mix positive staining, without other specific markers. Cytopenias were the most common hematologic finding, namely anemia (75.7%), thrombocytopenia (64.5%), and leukopenia (23.4%). Of note, pancytopenia was present in 13.1% of patients only. The finding of BM metastases predicted unfavorable outcome, with a median survival from observation of 7 weeks (range 0-361 weeks). This wide range was conditioned by the presence of few breast cancer patients still responsive to anti-hormone therapies (15 women (14%) surviving more than one year). Involvement of the BM by non-hematopoietic lesions can incidentally be observed. Our case series highlighted that persistent unexplained cytopenias in solid cancer patients can be the first indicator of BM metastases. Although rarely, the finding of BM metastasis can lead to the first diagnosis of solid organ malignancies. However, these patients have a poor outcome and, for most of them, only supportive care can be considered.

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SUPERVISED COMPUTER AIDED DIAGNOSIS TO SUPPORT TELEMEDICINE IN HEMATOLOGY

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Observation under the microscope of Peripheral Blood Smears (PBS) is of great importance in hematology. The aim of our study is the development of a CAD system, able to support hematologists in the analysis of PBS, providing more standardized and less subjective results. This study was carried out as part of the Smart Health 2.0 project (funded by the Italian Ministry of University and Research). CAD system consists of 4 steps: image acquisition of the PBS, leukocyte segmentation, feature extraction and leucocyte classification. PBS preparation and digital acquisition were carried out at IRCCS, Istituto Tumori "G. PAOLO II",Bari. Once the PBS was ready, it was digitally acquired using the D-Sight 200 microscope featuring a 40x optical zoom. Leucocyte segmentation is able to extract the portion of image that contains only the leucocyte, and consists of 3 main steps able to detect leucocyte position, plasma and the leucocyte edge, respectively. For the feature selection, we used the Information Gain Ranking algorithm of Weka, able to rank a list of features according to the contained amount of knowledge. The classification step was performed with two different techniques: Back Propagation Neural Network (BPNN) and a Decision Tree (DT). Both classifiers were trained using the same data set used for the feature selection process. The classification performance of the validation set showed that both the BPNN and DT are able to classify Neutrophils, Lymphocytes and Eosinophils with a precision greater than 96%. Regarding the monocytes, the BPNN obtained a precision equal to 100% whereas the DT reached a precision of 77.8%. The major difference between BPNN and DT is revealed when analyzing the precisions of the Basophils: 100% and 50% respectively. Hence, comparing the validation results of the two classifiers, it is evident that the DT performed worse than the BPNN. Finally, the NN classifier was evaluated with a test set composed of 1274 leucocytes, obtaining good results in terms of precision (87.9%) and sensitivity (97.4%). The performance results obtained show that the implemented CAD system could be able to support hematologists in the analysis of PBS, eventually generating an alarm in the presence of abnormal blood cells. Moreover, thanks to a cloud platform, it could allow PBS to be acquired in one place (where there is no hematologist) and observed in a reference hematological centre, improving the quality of life of patients and reducing health costs.

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ARRAY CGH+SNP TECHNOLOGY IN ROUTINE CYTOGENETIC DIAGNOSTIC PROCESS IN ONCOHEMATOLOGY

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A 28 years old Chinese patient referred in January 2015 to our Hospital for fatigue, epistaxis, fever and arthralgia. Blood counts and coagulation tests performed revealed anemia, thrombocytopenia, severe leukocytosis and disseminated intravascular coagulation (Hb 8.4 g/dl, GB 154.110/mmc, PLT 43.000/mmc, INR 1.28 aPTT ratio 0.91, fibrino-

gen 128 mg/dl, D-Dimer 2331 mg/dl). Morfologic and immunophenotypic bone marrow analyses were consistent with T lymphoblastic leukemia. p190 and p210 BCR-ABL fusion transcripts were negative. Total body CT scan evidenced hepatosplenomegaly and enlarged lymph nodes in the neck and abdomen. Metaphase karyotypic analysis of bone marrow and peripheral blood cultured cell showed a normal male karyotype (BM 46,XY[3]; PB 46,XY[7]). As the number and resolution of the analyzed metaphases were unsatisfactory, we performed a CGH+SNP array molecular karyotype (SurePrint G3 Cancer CGH+SNP, 180k, Agilent Technologies) using DNA extracted from bone marrow diagnostic samples. Data analysis (Cytogenomics 3.0 software) revealed a 30 Mb deletion of the long arm of chromosome 6 (6q12-q16.3) in 85% of the whole cell population analyzed. A second 40 Mb deletion, encompassing the former, was present in 70% of the cells analyzed. These large deletions could be identified also by standard karyotyping. Nevertheless, the occurrence of poor quality metaphases may represent a not negligible issue in specific cases, such as acute lymphoblastic leukemia specimens. Two more deletions, undetectable with classical cytogenetic method, were detected: a 1,45 Mb deletion of the short arm of chromosome 9 (9p21.3), a common deleted region in acute lymphoblastic leukemia containing several genes and oncosopressor genes, as MTAP, CDKN2A, CDKN2B, and a second one, a 117Kb deletion of the short arm of chromosome 10 (10q23.31), encompassing the PTEN oncosopressor gene locus. In addition, two copy-neutral loss-of-heterozigosity regions were identified: the first on chromosome 8 (7,35 Mb) and the other on chromosome 10 (7,42 Mb). The case described represent an example of array-based molecular karyotype technology applied during routine cytogenetic diagnostic process. Arrays are emerging as a powerful tool for magnifying resolution in the identification of chromosomal lesions undetected or undetectable by metaphase cytogenetics in hematologic cancers, with possible future implications for karyotyping diagnostic accuracy improvement.

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DIFFERENT WT1 GENE MUTATIONS IN TWO COHORTS OF AML PATIENTS ARE CONGRUENT WITH THE GEOGRAPHICAL DISTRIBUTION

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Introduction and Aims: The present study aimed to assess the mutational state of Wilm's tumor 1 (WT1) gene in two cohorts of acute myeloid (AML) patients. The mutational state of WT1 is a recent marker of AML: acquired mutations of WT1 gene have been reported in approximately 10% of cytogenetically normal-AML (CN-AML) patients and have been related with poor prognosis in both adult and pediatric CN-AML patients. *Patients and Methods:* We studied 13 AML patients recruited from two different Italian geographic areas: Marche (Clinic of Hematology, Hospital-University Company "Ospedali Riuniti", Ancona - group1) and Basilicata (Hematology Division, San Carlo Hospital, Potenza - group2). Patient genomic DNA was isolated at AML onset. WT1 gene was entirely screened for mutations by *in vitro* PCR and direct sequencing. Mutation Surveyor software was applied for the variation analysis. The cluster analysis was performed by means of PopGene32 software starting from the mutation presence/absence matrix. *Results:* We identified 21 WT1 variations, of which 10 were intronic substitution (2 splice variations) and 11 were exonic substitution (10 missense and 1 synonymous mutations). We notified a peculiar geographical distribution of our variants: the variations that we recognized in the first group are absent within the second group; the absence of Basilicata variants was also observed in Marche region. This aspect was clearly highlighted by applying statistical methods (cluster analysis): the dendrogram underlined that the patients of our different groups were stratified in two different dendrogram branches, according to the geographical distribution of the patients. *Discussion:* Here we molecularly analyzed WT1 gene observing for the first time, a peculiar geographical distribution of identified gene variants. The dendrogram distribution overlapped the geographical distribution and stressed the genetic distance between our groups. This aspect suggests that these mutations are related to the specific geographical area (Marche and Basilicata) and could be utilized for a more detailed stratification of the AML patients. Our results also showed that a detailed characterization of the patient genomic structure may be useful for better clarify the contribution of each gene variations to the clinical phenotype. A larger group of AML patients, hopefully including more than two Italian regions, should be analyzed in order to confirm these preliminary observations.

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FLUORESCENT *IN SITU* HYBRIDIZATION (FISH) ON PERIPHERAL BLOOD AND BONE MARROW SMARS: 120 MINUTES FOR DETECTION OF PML/RARα FUSION GENE

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Acute promyelocytic leukemia (APL) is a clonal hematopoietic stem cell disorder characterized by a chromosomal translocation involving the retinoic acid receptor-α gene on chromosome 17 (RARA). In 95% of cases of APL, RARA gene is involved in a balanced reciprocal translocation with the promyelocytic leukemia gene (PML) on chromosome 15, a translocation denoted as t(15;17)(q22;q12). The long-term outlook is now favorable for the majority of APL patients, when treatment is instituted promptly, due to the availability of therapies such as all-trans retinoic acid (ATRA) with anthracycline-based chemotherapy (idarubicin) and arsenic trioxide (ATO). Therefore, rapid diagnosis of APL contributes to a highly effective therapy. The conventional cytogenetic analysis is an excellent method for detecting the t(15;17)(q22;q12) in APL but it is subject to limitations. Since only dividing cells can be analyzed, sometimes there is no useful results for some patients, because of poor chromosome morphology and/or an insufficient amounts of assessable metaphases; furthermore the conventional chromosome analysis is labour-intensive and time consuming. Fluorescence *in situ* hybridization (FISH) overcomes some of these limitations and enables the detection of chromosomal rearrangements even on interphase cells, avoiding the requirement of metaphase obtention. Typically, the technique involves multiple step of sample preparation and hybridization of the sample and the probe so taking about 24 hours to analyze the data. Given the need for a rapid diagnosis in patients with APL, we investigated the usefulness and the accuracy of FISH, performed with a commercial probe, to identify the PML-RAR fusion gene, using a quick method applied on peripheral blood and bone marrow smears. Using this 120 minutes lasting procedure, we obtained bright, distinct, compact and easily evaluable hybridization signals with low background and we were able to clearly and unambiguously detect the fusion gene PML/ RARα in 90% of the cases, due to the variable quality of the material available. This study suggests that FISH performed on peripheral blood and bone marrow smears is a reliable method for the rapid detection of PML/RARα rearrangement.

Platelet Disorders

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SAFETY AND EFFICACY OF ELTROMBOPAG IN ADULT WOMAN WITH REFRACTORY IMMUNE THROMBOCYTOPENIA AND GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY

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Introduction: Eltrombopag provided of excellent clinical efficacy in refractory patients with immune thrombocytopenia (ITP). Eltrombopag is generally well tolerated, although its use in the particular setting of G6PD deficiency has not been described so far. *Aims:* To report the safety and efficacy of eltrombopag in an ITP patient with G6PD deficiency and history of acute hemolytic anemia (AHA). Case report. A 51-year old female was diagnosed as having ITP in September 2014. The patient was a G6PD deficiency carrier with a history of not otherwise specified episodes of AHA, particularly severe during her second pregnancy. Apart the G6PD deficiency, her past history was negative and she was not taking any medication. The patient was considered at high risk of drug-induced hemolytic complications and carefully monitored. So, oral prednisone (1 mg/kg) was given with only a transient benefit until she became fully refractory to steroid treatments. So that, the patient was candidate to splenectomy. However, given the extremely low platelet count, she was started (October 2014) on eltrombopag (50 mg/day) as a bridge to splenectomy. Given that, at the best of our knowledge, the use of this drug has never been reported in the particular setting of G6PD deficiency, the patient was constantly monitored for the possible occurrence of hemolytic complications. A prompt platelet increasing (178.000/uL) was observed 1 week after the start of treatment. The patient achieved the target platelet count; therefore, eltrombopag was tapered to the lowest effective dose. So that, the patient's response was stably maintained while the patient remaining on a dose of eltrombopag between 25 and 50 mg/day without any adverse events; in particular, no variations of hemolytic parameters were observed. Today, after six months of continuous eltombopag administration, the patient constantly maintained the target platelet counts and is waiting to elective splenectomy. *Conclusions:* G6PD deficiency may be associated with development of AHA induced by several drugs; the use of eltrombopag has never been reported in this particular setting. Thus far, we reported for the first time at the best of our knowledge, the evidence of the safety of use of this thrombomimetic agent which provided an excellent treatment outcome without no adverse effects in our steroid refractory adult ITP patient at risk of AHA given her previous past history of hemolytic complications.

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ROLE OF ASHWELL-MORELL RECEPTOR MEDIATED HEPATIC CLEARANCE OF PLATELET IN IMMUNE THROMBOCYTOPENIA: PLATELET KINETIC STUDIES AND AUTOANTIBODY TYPE DATA RESULTS IN CLINICAL PRACTICE

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Background: Detection of antibodies (Abs) against platelet (plt) GPIIb-IIIa and/or GPIb-IX is a characteristic feature of ITP. Fc-mediated clearance of plt by splenic macrophages has so far been addressed as the main mechanism of plt destruction. Recently, an Fc-independent mechanism was proposed involving anti-GPIb Abs (1) which induce plt activation leading to GPIb desialyation. In turn, desialylated plt are removed by the hepatic Ashwell-Morell receptor (AMR). In order to test the clinical relevance of this novel mechanism of plt clearance, plt kinetic studies and Ab type data were retrospectively reviewed. *Patients:* Charts of ITP patients (pts) with data on both plt kinetic studies and Ab testing were reviewed. Pts are enrolled in a local Italian data base (REL-ITP data base), and informed consent to clinical data use is given at enrollment. All pts underwent or are candidate to splenectomy for steroid-dependent ITP. *Results:* 62 pts were identified: 18 evaluated for and 44 already splenectomized; 38/62 (61%) of pts tested positive to one or more than one anti GP Abs. Plt kinetic studies and Ab testing results are shown in Table 1. No correlation was found between outcome and Ab type or site of plt uptake; a trend toward predominant splenic uptake was found in Ab negative pts (Fisher's exact test p=0.064). *Conclusions:* Recent data suggest that Ab type determines plt's fate by activating either Fc- or non Fc-mediated plt clearance resulting in splenic or hepatic uptake respectively. However, our data do not support such a mechanism being operative in ITP pts: no correlation was found between Ab type, number (single *vs* multiple antiGP) of Ab positive test and site of plt clearance. On the other hand, it is well known that plt desialylate as they circulate, thereby becoming the primary AMR ligand. Desialylation also occurs when plt are activated by a number of physiological stimuli. It may well be that AMR-mediated hepatic plt clearance rather represents a physiological mechanism involved in clearance of activated plt and in the regulation of TPO production by hepatocytes (2). References. 1- Li J *et al.* Platelet Desialylation: A Novel Mechanism of Fc-Independent Platelet Clearance and a Potential Diagnostic Biomarker and Therapeutic Target in immune thrombocytopenia. ASH 2014 abs#467. 2- Grozovsky R *et al.* The Ashwell-Morell receptor regulates hepatic thrombopoietin production via JAK2-STAT3 signaling *in vivo* and *in vitro*. ASH 2014, abs #2.

Table 1.

Ab pattern	n(%)	site of plt clearance (111-Indium labeled plt)		
		splenic	hepatic	mixed
All patients (M/F)	62 (27/35)			
Ab negative	24 (38.7)	19 (79%)	0 (0%)	5 (21%)
Ab positive	38 (61.3)	16 (51.6%)	4 (13%)	11 (35.4%)
one positive Ab	13/38			
GpIIb-IIIa	9	5	2	2
GpIb-IX	4	2	1	1
two positive Abs	9/38			
GpIIb-IIIa & GpIb-IX	2	1	0	1
GpIIb-IIIa & GpIa-IIa	7	5	0	2
three positive Abs	16/38			
GpIIb-IIIa & GpIb-IX & GpIa-IIa	16	8	1	7

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DEVELOPMENT OF NEUTRALIZING ANTIBODIES DURING LONG-TEM TREATMENT WITH ROMIPLOSTIM IN PATIENTS WITH IMMUNE THROMBOCYTOPENIA

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Background: ITP is a disorder of increased platelet (plt) clearance coupled with suboptimal plt production. For ITP patients (pts) failing immunosuppressants, an alternative approach is to stimulate thrombopoiesis. Development of 1st generation thrombopoietic agents (TPO) was halted due to induction of neutralizing antibodies (Abs) against both recombinant and endogenous TPO. Romiplostim (R) is a 2nd generation TPO receptor agonist (TPO-RA) with no sequence homology to TPO, thus avoiding the risk of eliciting cross-reacting Abs. However, neutralizing Abs to R have been described (Kuter BJH 2013). We report on prevalence of response loss to R and results of neutralizing Abs testing in ITP pts treated at 2 Centers. *Patients:* 44 pts received R between Sept 2009 and Dec 2014. Pts are enrolled in a local Italian data base (REL-ITP data base), and informed consent to clinical data use is given at enrollment. Whenever feasible, blood samples of pts losing response to R were collected and sent for R neutralizing Ab testing, according to R manufacturer instruction. *Results:* A total of 25/44 pts (56.8%) are evaluable for loss of response, *i.e.* desired platelet count achieved but not sustained. Of these, 11 (44%) discontinued R after achieving a stable response and are in follow up, off any therapy; 8 (32%) are in response on R; 6 (24%) have lost response while on therapy. Outcome of these 6 pts is shown in Table 1. Neutralizing R Abs testing was carried out in 4/6 pts (#1 to 4) and 3/4 (# 1,2,3) tested positive. These Abs did not cross react with eTPO. 4/6 pts went into long-term remission when switched to a different therapy. Pts #2 and #3 were re-tested for Abs, at 9 and 7 mos from first detection respectively and both tested negative. *Conclusions:* The 2nd generation TPO-RA romiplostim is an effective and well tolerated therapy for ITP pts not responding to immunosuppressants. Pts seldom discontinue treatment due to adverse events but over time a fraction of responding pts are unable to maintain a sustained response. Response loss doesn't seem to be so rare an event accounting for 24% of pts in our series. Similarly to Kuter's data (1), in 3 of our pts abrupt

loss of response was associated with development of neutralizing Abs to R, not cross-reacting with endogenous TPO and resolving after R withdrawal. 1-Kuter DJ *et al.* Long term treatment with romiplostim in patients with chronic immune thrombocytopenia: safety and efficacy. DOI: 10.1111/BJH.12260.

Table 1.

n	Sex, yr	Previous ITP treatment	Duration of Rom before LR	Type of response before LR	Ongoing dose of Rom before LR	Clinic at LR	anti-Rom Ab	Ab re-test	Action taken at LR	Outcome
1	F, 38	IVIG, DEX, P	11 months	CR	2 mcg/kg	Sudden drop plt count, no resposne with higher Rom doses	Positive	n.a	RTX	CR, off therapy
2	F, 38	IVIG, DEX, P, Elt	12 months	CR	8 mcg/kg	Sudden drop plt count, no respose with higher Rom doses	Positive	Negative (after 9 months)	IVIG, spl	CR
3	M, 19	IVIG, DEX, P	11 months	CR	9 mcg/kg	Sudden drop plt count, no respose with higher Rom doses; sudden diffuse skin reaction after Rom injection	Positive	Negative (after 7 months)	Elt as "bridge" to spl	CR
4	M, 42	Spl, P, DEX,	4 months	R	10 mcg/kg	sudden drop plt count	negative	n.a	Elt	NR
5	F, 56	IVIG, DEX	5 months	unstable R	6 mcg/kg	Sudden drop plt count, no respose with higher Rom doses	n.a	n.a	RTX, Elt	CR in Elt
6	M, 61	IVIG, P, VCR, RTX, spl	12 months	unstable R	10 mcg/kg	Drop, no more resp	n.a	n.a	Elt	NR

IVIG: intravenous immunoglobulin; DEX: dexamethason; RTX: rituximab; Spl: splenectomy; P: prednisone; VCR: vincristine; R: response; NR: no response; CR: complete response; LR: loss of response; Ab: antibodies; Rom: Romiplostim; Elt: Eltrombopag; n.a: not available

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THE USE OF TPO-RAs IN ITP; ONGOING EXPERIENCE OF REP (RETE EMATOLOGICA PUGLIESE)

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ITP is a disease with one third of pts failing to first and following approaches of therapy including splenectomy. Thrombopoietin receptor agonists (TPO-RAs) are recommended for adults who relapse after splenectomy or who have contraindications for splenectomy. A total of 124 pts: 69 (56%) treated by Eltrombopag and 55 (44%) by Romiplostim. Mean age, number of younger patients (<60 years) and time of primary diagnosis of ITP were similar in both groups: instead 65% of pts were treated by Eltrombopag in the last 2 years. Pts received 2.5 lines of therapy median. The first therapy, except for severe diabetes was a glucorticoid±IgV. Rituximab was considered a line of therapy and it was given in 31% of pts treated by Romiplostim and 20% by Eltrombopag. Splenectomy was applied in 7/69 (10%) pts treated by Eltrombopag, 7/31 (20%) with the age under 60 years and 11/55 (20%) in the Romiplostim group, 11/23 (47%) of those under sixty. The overall response including CR and PR was 44/55 (80%) in the Romiplostim group and 65/69 (94%) in the Eltrombopag group. CR were 24/55 (44%) on Romiplostim and 33/69 (48%) on Eltrombopag group. According to splenectomy pts treated by TPO-RA after splenectomy had an overall response of 8/11 (72%) in the Romiplostim group and 5/7 (71%) in the Eltrombopag group, respectively. The response in pts not splenectomized was 36/44 (82%) in the Romiplostim group and 59/62 (95%) in the Eltrombopag group. The mean duration of response was 30 months for pts treated by Romiplostim and 15 months in the Eltrombopag group; the loss of response was recorded in 4/44 (10%) in patients treated by Romiplostim and 3/64 (5%) treated by Eltrombopag. Failure was the most frequent cause of stop therapy and was 20% in Romiplostim and 7% in Eltrombopag group. Thrombotic events were recorded in 2 and 3% of pts treated by Romiplostim and Eltrombopag. In conclusion Romiplostim and Eltrombopag are effective in enhancing platelet count in the majority of pts with chronic ITP who failed to several lines of therapy; whether TPO-RAs are substitutive of splenectomy is under discussion and studies are warranted.

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STEROID-REFRACTORY IDIOPATHIC THROMBOCYTOPENIC PURPURA IN THE ERA OF THROMBOMIMETIC DRUGS: WHAT ROLE FOR RITUXIMAB?

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Introduction: The role of for rituximab in the setting of steroid-refractory ITP in the era of thrombomimetic drugs represents an open question. *Aims and Methods:* To report the indications and outcome of seven (4 female) steroid – refractory/relapsed ITP patients with median age of 38 (19-43) years. Rituximab (4 weekly infusions of 375 mg/m^2) was given after 50 (6 – 84) months from the ITP diagnosis. One patient with active bleeding requiring platelet transfusions and refractoriness to all measures, including thrombomimetic agents (eltrombopag and then romiplostin), once a complete response (CR) by rituximab has been achieved, was soon after splenectomized, having taken into account the high risk of recurrence and the severe bleeding tendency. Splenectomy was offered to four patients who refused the surgical intervention; in the remaining two patients, splenectomy as well as thrombomimetic agents were not indicated given suspected underlying autoimmune disorders and thrombophilic states. Patients who refused splenectomy the option of a potentially life-long treatment with thrombopoietin agonists, such as eltombopag or romiplostim, was offered after a thorough explanation of the expected benefits and possible disadvantages from the therapy. The long lasting therapy, virtually to be administered for life, was the main reason for which patients asked an alternative treatment able to induce a prolonged remission of the disease without the need to depend for life from the therapy. *Results:* All 7 patients achieved a CR; 6 did not necessitate further therapy whereas one was splenectomized In the 7 patients, CR was maintained after a median of 19 (6-64) months; no patients relapsed. *Conclusions:* Rituximab therapy may achieve long-lasting remission in patients with relapsed or refractory ITP, with a good safety profile. Although thrombopoietin agonists, in the light of their high efficacy, have substantially changed the clinical scenario and the management of steroid refractory ITP, this treatment option involves the need for prolonged administration, also for the whole life and without a predictable suspension. This concern, in the setting of patients who refuse or are unsuitable for splenectomy, can lead to choose a therapy whose administration is predictable and limited in time, such as rituximab, which role is to be yet considered for selected indications even in the era of the new thrombomimetic medications

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COMPLETE REMISSION OF THROMBOTIC THROMBOCYTOPENIC PURPURA SECONDARY TO A TESTICULAR CANCER WITH RITUXIMAB IN YOUNG PATIENTS AFTER FAILURE OF PLASMA EXCHANGE

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Introduction: Thrombotic thrombocytopenic purpura (TTP) is characterized by microangiopathic hemolytic anemia and thrombocytopenia and the association with adenocarcinomas is extremely infrequent. *Case Report:* The young patient after surgery for tumor of the testis with multiple metastases had severe thrombocytopenia, haemolityc anaemia, neurologic abnormalities and skin hemorrhages suggested the diagnosis of TTP. Rapidly the patient went into a coma and immediately begin plasmapheresis with steroid therapy and continuous infusions of fresh frozen plasma. After two sessions he had a severe convulsion and was transferred to intensive care unit and after nine sessions no response on platelets or neurological status was observed. He began therapy with rituximab weekly for 4 doses. After the second administration of rituximab there was a rapid increase of platelets, hemolysis reduction and improvement of the state of consciousness. This case is particularly because the improvements occurred only after rituximab despite the TTP was caused by neoplasia. In the literature aren't described cases of TTP patients with testicular cancer treated with rituximab. At this writing, his TTP is in complete remission after two monthly infusion of rituximab as maintenance. *Discussion:* TTP is caused by congenital or inherited disorders involving the processing of the ultra-large forms of Von Willebrand factor. It is a clinically heterogeneous syndrome associated with thrombocytopenia, Coombs-negative hemolytic anemia, neurologic changes, renal impairment, and fever. The idiopathic autoimmune form of TTP is the

most common but there are various subgroups of acquired TTP. If not promptly treated, TTP is associated with high mortality, making it a true medical emergency. Relapse of TTP also occurs frequently. Assays of ADAMTS13 activity and titration of acquired antibodies against this enzyme are indicated in the follow-up of disease and as prognostic indicators. Treatment centers around daily plasma exchange associated with immunosuppressant drug therapy, particularly steroids and rituximab. *Conclusions:* TTP is a rare, but life-threatening disorder. Prognosis is good with immediate diagnosis and adequate treatment with plasma exchange. To the author's knowledge, this the first report in the literature describing in a patient with TTP during testicular cancer treated with successfully with rituximab after failure of plasma exchange and cortisone therapy.

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ELTROMBOPAG IN ITP PATIENT WITH ACUTE RENAL FAILURE RELATED TO INTRAVENOUS IMMUNOGLOBULIN THERAPY: A CASE REPORT

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Intravenous immunoglobulin (IVIG) is used to treat various immune-mediated diseases, including Immune Primary Thrombocytopenia (ITP). It is generally well tolerated, but it may induce acute renal failure (ARF). The cause of IVIG-associated ARF is unknown. Eltrombopag is an oral, non-peptide thrombopoietin receptor agonist, that has shown efficacy and safety in ITP patients not responding to previous therapy. We report a case of ARF associated with IVIG therapy in ITP patient, that was successfully treated with eltrombopag. A 30 years old man was hospitalized with severe thrombocytopenia (Plt 1000/mmc) associated with ecchymoses, epistaxis and hematuria. At the hospitalization renal function was normal. Serological testing for C3 and C4, ANCA, ANA, anti-DNA, anticardiolipin antibodies, lupus anticoagulant, hepatitis B and C, human immunodeficiency virus, Helicobacter pylori was unremarkable. Direct Coombs test was negative and schistocytes were not detected in peripheral blood smear. Bone marrow aspirate showed a normocellular pattern with an increased number of megakaryocytes. It was diagnosed ITP and was started treatment with prednisone (1 mg/kg/day) and IVIG (0,4 g/kg/day for 5 consecutive day). After fourth IVIG administration the patient became anuric and creatinine increased from baseline of 0,8 mg/dl to 6 mg/dl. Patient required hemodialysis. Ultrasonography revealed absence of hydronephrosis and normal-sized kidneys with increased echogenicity consistent with acute renal disease. After twenty days of steroid therapy, severe thrombocytopenia persisted and renal function did not improve. Therefore he was initiated on eltrombopag 50 mg daily with rapid increased platelet count. Prednisone was slowly tapered up to suspend; eltrombopag was stopped after twenty days of therapy when platelet count was 321000/mmc. Patient required sixteen dialysis to recover renal function completely. This report underlines that ARF could be immune-mediated as ITP or associated with infusion of IVIG. However, before IVIG therapy, it requires a careful assessment of renal function and comorbidities to identify patients at risk of developing ARF. Eltrombopag appears to be a valid and safe option for treatment of ITP in patients with renal impairment.

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PERSISTENT REMISSION OF CHRONIC IMMUNE THROMBOCYTOPENIA AFTER THROMBOPOIETIN MIMETICS DISCONTINUATION

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Primary immune thrombocytopenia (ITP) is an immune-mediated condition characterized by isolated thrombocytopenia, with peripheral blood platelet count of <100.000/µl in the absence of an identifiable underlying cause of thrombocytopenia. Clinical studies in patients with ITP demonstrated that thrombopoietin (TPO) mimetics increase platelet production and can outpace platelet destruction. TPO-receptor agonists are currently used for patients at risk of bleeding, who relapse after splenectomy or who have a contraindication to splenectomy and who have already failed at least one other therapy. As far as we know an interruption of treatment with TPO mimetics is not feasible. In our study we evaluated the feasibility of stopping treatment with Romiplostim and Eltrombopag. We evaluated 54 patients (29 M; 25 F), with chronic ITP referred to our institution between 2008 and 2015, 38 patients received Romiplostim and 16 patients Eltrombopag. Median age was 71 years (range 39-94 years). Romiplostim was started at 1 g/kg per week and the dose was adjusted to a maximum of 10 g/kg per week; Eltrombopag was started at dose of 50 mg/die. With a median follow up of 20 months (range 2-48),11 of 54 (20%) patients were able to maintain a median platelet count of 160.000/µl (range 50-390.000) when TPO-mimetics was stopped, 7 after Romiplostim discontinuation and 4 after Eltrombopag discontinuation. Three patients discontinued Romiplostim without no dose reduction and at time of interruption of Romiplostim one patient was currently treated with 1 g/kg and two with 3 g/kg per week; four patients discontinued Romiplostim after a progressive dose reduction. Four patients discontinued Eltrombopag, of these two patients are out of treatment with a stable platelet count >100.000/µl, one patient, that maintained a stable platelet count >150.000/µl for 12 months out of treatment, relapsed and received Eltrombopag again, obtaining a complete remission, and is now out of treatment with good response. Last patient is out of treatment with two months of follow up with a stable platelet count of 50.000/µl. In our limited experience we were able to discontinue TPO mimetics in selected patients observing stable platelet counts >50.000/µl during the period of observation. TPO mimetics seem a very promising therapy for the treatment of refractory forms of ITP; further investigations are warranted to explore the feasibility of stopping treatment.

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SAFETY AND EFFICACY OF RITUXIMAB IN ADULT WITH CHRONIC IMMUNE THROMBOCYTOPENIA

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Immune thrombocytopenia is characterized by immune-mediated destruction and suboptimum production of platelets. Despite the absence of supporting evidence, the anti-CD20 chimeric monoclonal antibody rituximab has been effectively used off-label in the treatment of patients with primary immune thrombocytopenia (pITP). In this monocentric analysis we retrospectively evaluated 43 adult patients affected by chronic ITP resistant to 2 or more lines of therapy that were treated with four weekly infusions of 375 mg/m^2 rituximab to asses safety and efficacy. 39 were retrospectively evaluated. 20 F, 19 M. Median age was 60 (range 29-91 years) and median platelets value at start treatment was 16.000/µl (range 5.000-40.000). 24/39 (62%) showed an intial response, 17/39 (44%) patients obtained complete response (CR) and 7/39 (18%) showed partial response (R). Of those achieving an overall response 4 (17%) patients relapsed, median time to relapse was 30 months (range 8-45). Of these 4 patients relapsed, 3 received re-treatment with four weekly infusions of 375 mg/m^2 rituximab and 2 patients achieved a complete response, one was no-responder. With a median follow-up of 22 months (range 2-95 months), 17/24 patients (71%) showed a lasting response out of treatment, of these 15 patients maintained a complete response with a median platelets count of 156.000/µl (100.000-362.000/µl) and 2 patients was in partial response. 7 patients underwent further line of therapy (6 treated with TPO-mimetics and 1 with splenectomy). During the follow-up, no opportunistic or severe infectious complications were observed. In our limited experience these data confirm, over a long period of observation, the efficacy and safety of rituximab treatment in the management of patients with resistant ITP and rituximab used off-label may remain a valid option for treating persistent or chronic ITP in adults. Further investigations and specific clinical trials are warranted.

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SWITCH OF TPO-MIMETICS IN PATIENTS WITH CHRONIC IMMUNE THROMBOCYTOPENIA: FLORENCE MONOCENTRIC EXPERIENCE.

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Primary immune thrombocytopenia (ITP) is an immune-mediated condition characterized by isolated thrombocytopenia, with peripheral blood platelet count of <100.000/µl in the absence of an identifiable underlying

cause of thrombocytopenia. Clinical studies in patients with ITP demonstrated that thrombopoietin (TPO) mimetics increase platelet production and can outpace platelet destruction. From November 2008 and April 2015 54 patients were treated with TPO-mimetics: 38 patients underwent therapy with Romiplostim and 16 to Eltrombopag. In our study we evaluated 8 patients who received both of therapies: among patients treated with Romiplostim 5 patients (4 F; 1 M) switched to Eltrombopag and 3 patients (3 M)switched from Eltrombopag to Romiplostim. In the group of 5 patients treated initially with Romiplostim 2 for loss of response started Eltrombopag, obtaining a good response. 2 patients were no responder to both therapies and 1 patient, after transient response to Romiplostim switched to Eltrombopag, obtaining a partial response. In the group of 3 patients treated initially with Eltrombopag, 1 was no responder to both TPO-mimetics, 1 patient had a complete remission with Eltrombopag, but switched to Romiplostim because of adverse events, obtaining a complete remission. The last patient was no responder to Eltrombopag, but had a partial remission with Romiplostim. Romiplostim and Eltrombopag stimulate the TPO-R but have different mechanisms of action, therefore, in our limited experience switching from one thrombopoietic receptor-agonist to the other could be beneficial in clinical practice for patients with severe chronic immune thrombocytopenia who failed to respond or experienced adverse events to the first.

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THE USE OF PLASMASAFE IN THERAPEUTIC APHERESIS: CASE REPORT OF RELAPSE THROMBOTIC THROMBOCYTOPENIC PURPURA IN ALLERGIC PATIENT TO FRESH FROZEN PLASMA AND RITUXIMAB

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TTP is a rare e severe multisystemic disorder characterized by thrombocytopenia, microangiopathic hemolytic anemia, renal abnormalities, fluctating neurological signs and fever. The regulation of von Willebrand factor (VWF) multimer size is essential in preventing microvascular platelet clumping, a central pathophysiologic finding in TTP. In the majority of TTP patients, ADAMTS 13, a plasma metalloprotease cleaving VWF, is deficient. The acquired form is caused by antoantibodies inibiting ADAMTS 13 activity or increasing ADAMTS 13 clearance. Congenital ADAMTS 13 deficiency is the result of homozygous or heterozygous ADAMTS 13 gene mutations. Plasma exchange (PEX) therapy represents the frontline treatment of TTP. Case. On the december 2014 a 62 -year-old female was admitted to our department presenting the sixth relapse of TTP. To admission the patient presented malaise and epigastric pain. Blood test were as follows: platelet count 9000 x 103ul, hemoglobin 7 g/dl, LDH 1506 U/L, bilirubin 3,3 mg/dl (indirect 2,7 mg/dl). On 2006 the diagnosis of TTP was made and antoantibodies inibiting ADAMTS 13 was determined. The patients was treated with PEX with infusion of fresh frozen plasma, rituximab and corticosteroids. On 2010, during third relapse, she developed a severe allergic reaction to FFP and rituximab. Therefore it was necessary to continue treatment with S/D plasma as replacement fluid for PEX. The patient archieved without showing any immunological reaction. Also in this circumtance treatment was promtly instituted with PEX, methyprednisolone, packet red bloods cells (4 units). PEX (23 sessions) was carried out: S/D plasma was main fluid replacement (average volume: 30 ml/kg body weight for each session). One and three months after discharge ADAMTS13 activity is 85% and 89% respectively. Conclusion.TTP is an impostant diagnosis to make because the untreated mortality is 90% which can be reduced with the prompt delivery of PEX. The indications for clinical use of S/D plasma are the same as those of FFP. However, PEX with solvent/detergent - treat plasma allows to lack the unsually large VWF multimers, mantaining unchanged ADAMTS 13 activity, to decrease infection as well as immunological risks. Indeed the industrial treatment let to removal of cell, cell fragment, to neutralize immune antibodies, to dilute antigens/allergens that are the basis anaphylatic reations to FFP.

Chronic Myeloid Leukemia

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CARDIOVASCULAR SCREENING AND MONITORING OF PATIENTS WITH CML-CP TREATED WITH NILOTINIB, AN OUTPATIENT AND NON-INVASIVE MODEL

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Introduction: In our Institute, we organized an outpatient Cardio-Vascular-Oncology Unit dedicated to Chronic Myeloid Leukemia (CML) patients receiving any TKI in order to detect and prevent cardiovascular (CV) events. From 1 October 2013 to 31 January 2015, we followed 50 CML-CP patients with timetabled visits and examinations according to TKI profile and patients' comorbidities. In particular, we followed prospectively 10 newly diagnosed CML patients treated with nilotinib 600mg. *Materials and Methods:* Patients underwent baseline(BL) ambulatory visit: screening of CV risk factors according to European Society of Cardiology (ESC) Score risk charts, physical examination, electrocardiogram (ECG), echocardiography, vascular evaluation (ankle-brachial index (ABI), carotid and lower limbs ultrasonography, evaluation of carotid media-intima thickness (cIMT)). We collected data on lipid profile (LDL-HDL cholesterol, triglycerides-TAG), serum glucose and glycosylated hemoglobin, serum creatinine and ionogram. Follow up (f-up) visits were scheduled every 6months (Table 1).

Table 1.

Evaluation	BASELINE	3 months	6 months	12 months	Every 6 months	Every 12 months
Glucose and HbA1c	X	X	X	X	X	X
LDL, HDL, TAG	X		X	X		X
Uric acid, creatinine, K, Mg	X	X	X	X	X	X
BP	X	X	X	X	X	X
ECG	X	X*	X	X	X	X
Echocardiogram	X		X	X		X
Edinburgh Claudication Questionnaire	X			X		X
ABI measure	X		X	X	X	X
Carotid ultrasound (Doppler) scan	X			X		X
Lower limbs artherial ultrasound (doppler) scan	X			X		X

*To evaluate QTc

We also considered drug interactions. *Results:* We enrolled 4male and 6female (median age 59) with ESC score risk stratification: 6low, 3moderate, 1high. No CV events were recorded. We noticed statistically significant increase in serum cholesterol (medium total cholesterol: BL 173.5mg/dL, at 12 months 237mg/dL, $p<0.01$; medium LDL: BL 89 mg/dL, at 12 months 146 mg/dL, $p<0.01$); no difference in serum TAG. We observed an increase in fasting serum glucose (BL 98 mg/dL, at 12 months 108 mg/dL, $p<0,05$). Only 1 patient had a significant prolongation of QT tract at ECG in first 6months, then returned normal during f-up. No abnormalities in echocardiograms were recorded. No statistically significant variations in cIMT or ABI were observed at 12months

f-up; in 1 patient, an atherosclerotic carotid plaque with 30% of stenosis was observed at 6months. Hygienic and alimentary recommendations were given to patients, low dose anti-hypertensive drug and statin were started (in 3 and 1 patients respectively).*Conclusions:* We systematically followed patients through non-invasive evaluations and we introduced therapies to control CV risk factors present at baseline or with new onset, preventing atherosclerotic disease and optimizing TKI therapy. We do not experienced any major CV events. Our study is based on few patients; larger prospective studies will define whether this method is efficacy to prevent any CV events.

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SEX CORRELATED WITH DIFFERENCES IN LONG-TERM OUTCOME IN CHRONIC MYELOID LEUKEMIA PATIENTS TREATED WITH IMATINIB

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Recently, it has been reported that female sex is associated with increased rate of stable deep molecular response in the long-term for unknown reasons. Aim of our study was to detect possible sex differences in terms of response rates and long-term outcome in a large series of 208 chronic phase chronic myeloid leukemia (CP-CML) patients treated with imatinib at a single center with a median follow-up of 7 years. In this series, there were 114 males, median age 55.7 years, stratified according to Sokal score in low risk (54 patients), intermediate risk (45 patients) and high risk (15 patients). After 3 months of treatment, 68 (59.6%) achieved a complete cytogenetic response (CCyR) and 8 (7%) a major molecular response (MMR or MR3), whereas 6% did not reach early molecular response (EMR) and had a BCR-ABL/ABL ratio >10%. Overall, 32 patients (28%) developed resistance to imatinib, of them 13 with primary resistance and 19 with secondary resistance. Three patients (2.6%) experienced progression to blast crisis. Overall survival (OS) and event-free survival (EFS) estimated at 7 years were 79% and 56.7%, respectively. Ninety-four females completed the whole cohort, median age 59.7 years, stratified according to Sokal score in low risk (46 patients), intermediate risk (38 patients) and high risk (10 patients), without significant differences compared to male patients. After 3 months of treatment, 69 (73%) achieved a CCyR (p=0.02 compared to male patients) and 7 (7%) a MR3 (p=ns), whereas only 1% did not reach EMR (p=0.01 compared to male patients). After a median follow-up of 7 years, in cumulative incidence, 71% of female patients achieved MR3 (as compared to 69% of male patients, p=0.05), 54% an MR4 (as compared to 49% of male, p=0.03) and 38% an MR4.5 (as compared to 31% of male, p=0.02), confirming that female sex is a favourable prognostic factor to achieve deep molecular response. Overall, 21 female patients (22%) developed resistance to imatinib, with 13 having primary resistance and 8 secondary resistance. Again, 3 patients (3%) experienced a progression to blast crisis. OS and EFS at 7 years were 84% (p=0.03 compared to male population) and 73% (p=0.001 compared to male), respectively. Our results definitely suggested that female sex is a strong predictive factor of a good long-term outcome with imatinib.

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PROGNOSTIC FACTORS ASSOCIATED TO ACHIEVEMENT OF STABLE MR4.5 IN CHRONIC PHASE CHRONIC MYELOID LEUKEMIA TREATED WITH IMATINIB

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Achievement of a deep molecular response in chronic phase chronic myeloid leukemia (CP-CML) patients treated with imatinib has been associated to increased event-free survival (EFS) and progression-free survival (PFS). Aim of our study was to characterize clinico-biological factors associated with the probability to reach MR4.5 (BCR-ABL/ABL ≤0.0032% IS) as a stable response (confirmed on two or more consecutive samples). In a series of 208 patients treated with imatinib first-line, after a median follow-up of 7 years, the incidence of a stable MR4.5 was 34.6%, obtained after a median time of 5.4 years. We revealed a difference in male/female ratio in the cohort of patients who achieved this response (1/1) compared to the rest of population (1.2/0.8, p=0.02) and for median age (56.4 *vs* 58.6, p=0.03). Moreover, we found a difference in Sokal risk stratification with 50% low risk, 38% intermediate and 8% high risk categories in patients who achieve MR4.5 compared to 46%, 40% and 13%, respectively, in the cohort who did not reach this response (p=0.01). At 3 months, in the cohort who reached stable MR4.5, only 1% of patients had not achieved an EMR (compared to 6% in the cohort that did not achieve MR4.5, p=0.001). The rate of MR3 at 3 months was 15% in the cohort of patients who did achieve the molecular response *vs* 4% in the cohort who did not (p=0.02). At 6 months, an MR3 was obtained by 43% of patients in the cohort of patients achieving MR4.5 compared to only 19% in the cohort who did not reach this response (p=0.01). At 12 months, this difference was again significant (69% *vs* 26%, p=0.001). None of the patients who achieved MR4.5 developed resistance with EFS of 100%, whereas EFS in the cohort who did not achieve MR4.5 was 81% (p=0.01). None of the patients who reached stable MR4.5 progressed to blast phase, whereas 6 patients (4.4%) progressed to blast phase in the cohort of patients who did not reach MR4.5 (p=0.001). Estimated 7 year overall survival (OS) was 96% in the cohort of patients with stable MR4.5 and 89% in the cohort of patients who did not reach such a response, with a favourable trend for the first cohort but without statistical significance (p=0.087). In this analysis, we found that female sex, lower age, Sokal risk and rapid reduction of molecular burden at earlier time points were the main predictive factors for achievement of stable deep molecular response. Those factors should be considered for a possible treatment-free remission decision-making.

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APPLICATION OF CARDIOVASCULAR RISK ASSESSMENT IN CHRONIC PHASE CML PATIENTS TREATED WITH NILOTINIB ALLOWS IDENTIFICATION OF SUBJECTS AT HIGH RISK OF EVENTS

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In the last few years several communications of arterial thrombotic events have been described during nilotinib therapy, but the exact pathogenetic mechanisms are still unknown. Thus, it has become of great importance to evaluate the benefit/risk ratio at baseline, considering the overall cardiovascular risk profile of patients, taking into account pre-existing comorbidities and factors predisposing to atherosclerotic events. We retrospectively applied several cardiovascular risk assessments (the SCORE chart, the Framingham risk score, the QRisk2) in order to identify patients at risk of atherosclerotic events during nilotinib treatment. We included in this study 82 CML-CP patients treated frontline with nilotinib (42 patients, with the dose of 600 mg BID) or after failure to other tyrosine kinase inhibitors (40 patients, of whom 33 after imatinib and 7 after dasatinib, with the dose of 400 mg BID). In this cohort there were 48 males and 34 females with a median age of 51 years (range 22-84). The cumulative incidence of cardiovascular events (CV) at 48 months was 8.5% (95% CI: 4.55-14.07). We retrospectively classified the whole population according to the SCORE chart and found that 28 patients (34%) were in the low risk category and none of them developed CV events, 30 patients (36%) were in the moderate risk with 10% of CV events and 24 patients (29%) were in the high risk with 29% of events (p=0.002). Atherosclerotic-free survival (AFS) was 100%, 89% and 69% in the low, moderate and high-risk population, respectively (p=0.001). The same population was then stratified according to the Framingham score and the QRisk score. According to the modified Framingham score, 51 patients were classified as having low risk and none of them experienced CV events; 17 patients were classified as intermediate risk and 17.6% experienced an event, whereas 14 patients were high risk and 36% of them experienced an event (p=0.001). According to the QRisk2 stratification, 33 patients had a cardiovascular risk less than 10% and none of them had an event; 33 were classified as having a risk between 10 and 20% and 8% of them had CV events, whereas 16 were stratified as having a risk above 20% and 40% of them had a CV event (p=0.0001). In summary, in our retrospective analysis all cardiovascular risk assessments seem to well identify patients that should

be considered at high risk of arterial thrombotic events, but in particular we found that QRisk evaluation, which includes also diabetes and obesity as predisposing risk factors, could be particularly useful in patients treated with nilotinib.

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LONG-TERM VALUE OF FLUCTUATION OF MOLECULAR RESPONSES IN CHRONIC MYELOID LEUKEMIA PATIENTS TREATED WITH IMATINIB

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Definite evidences relating real significance of fluctuation of molecular response in the long-term outcome during imatinib treatment in chronic phase chronic myeloid leukemia (CP-CML) patients have not been so far reported in details. Aim of our study was to attempt to correlate, over a median follow-up period of 7 years, how instability of molecular response can affect overall survival and failure-free survival. We retrospectively analysed a series of 208 patients treated with imatinib first-line: there were 144 males and 94 females, median age was 57.7 years. We defined stable response, a response that persisted over 3 consecutive RQ-PCR monitoring tests. From a statistical point of view, we considered overall survival the time elapsed from diagnosis to death for any cause, and failure-free survival the timespan that follows therapy without signs of recurrence (loss of hematologic and/or cytogenetic response, acquisition of clonal cytogenetic abnormalities or mutations). Overall incidence of major molecular response or MR3 was 64.4%: during the course of treatment, 17 patients (11.6%) had a fluctuation above and then below the cut-off of 0.1%. Of these 17 patients, 13 (76%) lost the response and 3 subsequently developed secondary resistance (17.6%) with an estimated FFS of 82.4% and an OS of 94.6% at 7 years. Of the remaining 117 patients that never had a fluctuation and maintained a stable response over time, only 3 (2.5%) subsequently lost the response and developed secondary resistance with an estimated FFS of 93.2% (p=0.02) and OS of 94% (p=ns). Cumulative incidence of MR4 was 51% and of MR4.5 was 34.6%, obtained after a median time of 3.8 and 5.4 years, respectively. Overall, 7 patients (6.5%) showed a fluctuation of MR4 response and 4 subsequently lost the response. None of these patients showed secondary resistance or acquired mutations of ABL kinase domain and main reason for loss of response was low adherence to long-term treatment. Eighteen patients (25%) showed an unstable MR4.5, but none of them developed secondary resistance or progressed to blast phase. For deep molecular responses, fluctuation of molecular burden was associated to FFS of 100% and OS of 98.2% as compared to patients with stable response that showed a FFS of 99.4% and OS of 97.8%, without any statistical significance. Our results showed that unstable MR3 was indeed associated to an increased probability to develop resistance, whereas long-term fluctuation of deeper molecular response (MR4-4.5) did not influence the long-term outcome of CML patients treated with imatinib.

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DASATINIB FIRST-LINE: UPDATE DI UNA ESPERIENZA "REAL-LIFE" ITALIANA

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Dasatinib was approved for the treatment of chronic phase (CP) chronic myeloid leukemia (CML) patients in first line therapy, based on the demonstration of efficacy and safety observed in patients enrolled in sponsored clinical trials. Recently, also data from cooperative groups were reported which confirmed the efficacy of the drug as observed in previous studies. We here report safety and efficacy of dasatinib used as frontline treatment outside clinical protocols in a multicentric Italian trial. One hundred and nine patients (median age 54 years) were treated from January 2012 to December 2013. Median WBC count at diagnosis was 74 x 109/l (range 15.3-401). Prognostic scores stratification was as follows: (i) according to Sokal risk, 29% were low, 45% were intermediate and 26% were high risk; (ii) according to Hasford score, 40% of patients were low risk, 41% were intermediate and 19% were high (risk); (iii) according to Eutos score, 84% of patients were classified as low and 16% as high risk. Hence we observed an increased incidence of high-risk patients when compared to sponsored trials: this unbalance may probably reflect the trend to allocate preferentially non low risk patients to second generation TKIs frontline. Median time from diagnosis to start of dasatinib therapy was 18 days. Ten patients received unscheduled dose (6 patients had 50 mg and 4 patients had 80 mg QD) whereas 99 patients received 100 mg QD. At 3 months (108 patients evaluable), 92% of the patients achieved a ratio less than 10% and the rate of CCyR was 68%. At 6 months (106 patients evaluable), rate of CCyR was 91% and the incidence of MR3 was 43% with 8% of patients reaching a MR4.5. Ninety-three patients were evaluable at 12 months: the rate of MR3 was 62% with MR4.5 being achieved by 19% of patients. At a median follow-up of 12 months, 27 patients (24.7%) were receiving the drug at reduced dose. Two patients (1.8%) experienced a lymphoid blast crisis whereas the incidence of resistance was 8%. Two patients died for CML-unrelated reason; estimated OS, EFS and PFS were 97%, 80% and 93%, respectively. As regards safety, the major side effects recorded were thrombocytopenia, neutropenia and pleural effusions, which occurred in 22%, 10% and 8% of patients, respectively. Present results confirm the efficacy and safety of dasatinib as firstline treatment even in patients treated outside clinical trials.

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CASE REPORT OF A PATIENT WITH HIGH SOKAL RISK Ph+ CML WITH ADDITIONAL CHROMOSOME ABERRATIONS [+8, del(7q)] IN Ph- CELLS AND LATE RESPONSE TO NILOTINIB

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The occurrence of additional cytogenetic abnormalities (ACAs) in Philadelphia chromosome negative (Ph-) during tyrosine kinase inhibitors (TKIs) therapy for Chronic Myeloid Leukemia (CML) is a well-known event. Some abnormalities, including monosomy 7 and del(7q), have prognostic implications. We describe here the appearance of ACA clones, including a del(7q) in a patient with CP CML, who reached a late complete remission after frontline therapy with Nilotinib. A 46 year-old woman was admitted because of a painful splenomegaly on February 2012. According to clinic and hematologic conditions she was diagnosed a CP CML with a Sokal score of 1,75 (high risk). After an initial cytore-

duction with Hydroxycarbamide, Nilotinib 300mg bid was started with poor tolerance due to hepatic and hematologic toxicity. For this reason, the total dose of nilotinib, in the first 3 months never exceeded 75% of the programmed dose. Table 1 shows the cytogenetic and molecular data, according to ELN CML recommendations. A complete cytogenetic response (CCR) was achieved at month +18 and a major molecular response (MMR) at month +22, both in the peripheral blood and in the bone marrow. Point mutations were never detected all over the course of the disease. A trisomy 8 was observed in 20% of the Ph- metaphases at +12, in 60% at +14, and in 10% at +18. A del(7q) was observed in 5-10% of the Ph- metaphases between +14 and +25 months and no more thereafter. At +18, UDS analysis showed a 35 INS. This sequence variation consists of the retention of 35 nucleotides from intron 8 at the exon 8 to exon 9 border. It is an inactive kinase and should not play any role in TKI resistance (1). The patient, with a cumulative follow up of 37 months (19 months after the CCR and 15 after the MMR) is now in MMR (MR3) under nilotinib treatment. Ph- clones carrying trisomy 8 and del(7q) appeared and disappeared during the course of the disease, apparently with no adverse effect. As previously described, these abnormalities may be only the expression of genetic instability (confirmed by the detection of a 35 INS). Moreover, a long lasting CCR and MMR may be achieved also very late under nilotinib treatment. A strict molecular follow up is pursued.

Reference

1. The BCR-ABL 35INS insertion/truncation mutant is kinase-inactive and does not contribute to tyrosine kinase inhibitor resistance in chronic myeloid leukemia. Blood. 2011 Nov 10;118(19):5250-4.

Table 1.

Time	Cytogenetics	Molecular Biology (IS)	Point mutation research
+3 months	46,XX,t(9;22)(q34;q11) [19] / 46,XX [1]	BM = 10.268 PB = 9.026	SS = Negative
+6 months	46,XX,t(9;22)(q34;q11) [15] / 46,XX [5]	BM = 14.107 PB = 9.639	SS = Negative
+12 months	46,XX,t(9;22)(q34;q11) [4] / 46,XX,+8 [4] / 46,XX[12]	BM = 0.171 PB = 0.263	SS = Negative
+14 month further examination saw the cytogenetic outline	47,XX,+8 [12] / 46,XX,del(7)(q22 ;q34) [2] / 46,XX,t(9;22)(q34;q11) [1] / 46,XX [5]	BM = 0.469 PB = 0.915	SS = Negative
+18 months	47,XX,+8 [2] / 46,XX,del(7)(q22 ;q34) [1] / 46,XX [17]	BM = 0.171 PB = 0.263	SS = Negative UDS = 35INS
+22 months	Not done	BM = not done PB = 0.091	SS = Negative
+25 months	46,XX,del(7)(q22 ;q34) [1] / 46,XX [19]	BM = 0.040 PB = 0.047	SS = Negative
+28 months	Not done	BM = not done PB = 0.053	SS = Negative
+31 months	Not done	BM = not done PB = 0.012	SS = Negative
+ 37 months	46, XX	BM = 0.006 PB = 0.019	Not done

Abbreviations: BM= Bone Marrow; PB Peripheral Blood; SS= Sanger Sequence ; UDS=Ultra Deep Sequence (NGS); IS=International scale

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LONG-TERM OUTCOME TO FIRST-LINE IMATINIB ACCORDING TO 2013 EUROPEAN LEUKEMIANET RESPONSE CRITERIA: A GIMEMA CML WP ANALYSIS

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Despite other TKIs have been approved for frontline CML treatment, imatinib (IM) is an important therapeutic option. Response criteria at given time points have been proposed by the ELN panel, irrespectively of the first-line TKI used, to decide when the treatment should be continued, optimal response (OR), or changed, failure (F); warning (W) is an intermediate zone. To investigate the significance of 2013 ELN response criteria in CML treated frontline with IM, 559 patients enrolled within 3 prospective studies (CML021-022-023) were analyzed (ITT population of each study). ELN criteria at 3 months: not fully evaluable due to missing cytogenetic analysis in 452/559 patients; we focused on the early molecular response (EMR, BCR-ABL <10% at 3 months, corresponding to OR). ELN criteria at 6 and 12 months: F, BCR-ABL >10% and/or Ph+ >35% at 6 months, BCR-ABL >1% and/or Ph+ >0 at 12 months; OR, BCR-ABL <1% and/or Ph+ 0 at 6 months, BCR-ABL <0.1% at 12 months; W: intermediate conditions. Progression: according to 2013 ELN criteria. Molecular response: according to 2015 EUTOS recommendations. Leukemia-unrelated death (LRD): known cause of death, no progression, CCyR or MMR <6 months prior to death; all other deaths were classified as leukemia-related. Median follow-up, 76 (66-99) months. The EMR rate was 82%. The progression-free survival (PFS) and the probability of LRD according to the presence-absence of EMR were 91%-87% (p=0.035) and 11%-5% (p=0.019), respectively. Combining Sokal score and EMR, the patients were divided into 4 groups, LR-IR resp, LR-IR not resp, HR resp, HR not resp: the probability of LRD was 3%-9%-10%-20% (p<0.001). The patients remaining on IM according to the response at 6 months (OR-W-F) were 77%-52%-26%, respectively. The PFS and the probability of LRD according to ELN response at 6 months (OR-W-F) were 93%-92%-77% (p<0.001) and 2%-5%-28% (p<0.001), respectively. The patients remaining on IM according to the response at 12 months (OR-W-F) were 80%-72%-35%, respectively. The PFS and the probability of LRD according to ELN response criteria at 12 months (OR-W-F) were 95%-96%-85% (p<0.001) and 1%-1%-16% (p<0.001), respectively. Patients without OR at 3 months (particularly high risk patients) had poorer outcome compared to patients with OR. Patients classified as W at 6 and 12 months have similar outcome to OR patients, both significantly better than F. The subsequent treatment may explain, at least in part, the absence of differences.

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COMPARISON OF DROPLET DIGITAL PCR AND STANDARD PCR IN CHRONIC MYELOID LEUKEMIA PATIENTS IN MR4

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Background: Treatment-free remission is a major goal in Chronic Myeloid Leukemia and it is object of intense investigation. The definition of undetectable disease has recently changed, also due to the increasing sensitivity of the methods of analysis. High sensitivity techniques like droplet digital (dd)PCR may help to discriminate patients who still present a significant amount of disease despite undetectable at standard real time (RT)PCR from patients who have achieved a more profound depletion of leukemic cells and remain in molecular response after discontinuation. *Aims:* To compare ddPCR with standard RT-PCR for low levels of disease. *Methods:* Total RNA was extracted from 19 pa-

tients at least in MR4. Both standard PCR and ddPCR analysis were carried out in duplicate using 300 ng of cDNA for each replicates. Standard PCR was performed according to European Against Cancer Program. In ddPCR the reaction mixture was partitioned into around 20,000 water-in-oil micro-droplets subjected to end-point PCR; after amplification the droplets were analyzed in a QX100™ droplet reader (Bio-Rad), and the absolute copy number of targets were determined using the Quantasoft software. The Wilcoxon test was used for the comparison of the total ABL medians; the Agreement Coefficient of the 2 methods was calculated with Cohen's K test. *Results:* There was no difference in the median total ABL copies [determined according the recent EUTOS guidelines (Cross, Leukemia 2015)] between the 2 methods with 67560 copies (34440-126000) by ddPCR and 64720 (28140-136400) by standard PCR (p 0.28). Four patients resulted BCR/ABL positive by both methods, 5 patients by ddPCR only and 1 patient by RT-PCR only. The Agreement Coefficient between the 2 methods was only 35%. Where both methods scored positive, the calculated BCR-ABL/ABL ratios were comparable with a median of 0.0044% (0.0021-0.0112) by ddPCR and 0.00417% (0.0037-0.0157) by RT-PCR. Using the ddPCR 7 analyses had a sensitivity of MR4.5, whereas with the routine PCR protocol the same degree of sensitivity was reached in 4 tests. *Conclusions:* Despite the small number of cases, our results confirm that the ddPCR may be potentially more sensitive than RT-PCR for low levels of disease. This may be due to the higher efficiency of the endpoint PCR carried out on droplets containing single targets that undergo absolute quantization.

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NANOG: ITS ROLE IN THE TKI RESISTANCE OBSERVED IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA

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Introduction: Treatment of patients with Chronic Myeloid Leukemia in chronic phase (CML-CP) with tyrosine kinase inhibitors (TKIs) showed a substantially improving of patient life expectancy. However, it is becoming evident that persistent leukemic stem cells, which are insensitive to TKIs in their quiescent state, can lead to CML recurring. Nanog is a pluripotency gene associated to a vital role in neoplasia, correlating with cell proliferation, clonogenic growth, tumorigenicity, and therapeutic resistance. Our group carried out microarray experiments on Ph+ KCL22 cell line with a sensible (Kcl22-S) or resistant (Kcl22-R) phenotype to Imatinib (Ima). The gene expression of Nanog was significantly increased in the Kcl22-R. Thus, we sought to investigate the role of Nanog in the TKI resistance observed in patients with CML-CP. *Methods:* Real Time RT-PCR (RT-qPCR) for the expression of Nanog was conducted on Ph+ K562 cell line treated with increasing doses of Ima. Western blotting (WB) analysis was conducted for the protein expression of Nanog on K562 cells treated with 5uM Ima. RNA was purified from mononuclear cells of 27 CML patients at diagnosis and after 3 months of TKI treatment. Patients were monitored by RT-qPCR for the expression of the fusion BCR-ABL mRNA. RT-qPCR for the expression of Nanog, was conducted. RT-qPCR results were normalized by the expression of Gus mRNA (Normalized mRNA copy Number: NCN). *Results:* We observed a significant increase of Nanog mRNA expression in K562 cells treated with 0.5 uM of Imatinib. Moreover, we were also able to observe a significant increase of Nanog protein expression in K562 cells treated with 1-5uM Imatinib by WB. In peripheral blood samples of CML patients at diagnosis, we observed a significant higher mRNA expression of Nanog in No-Optimal Responder compared to Optimal Responder patients (NANOg mRNA: 0.3±0.25 NCN by GUS mRNA *vs* 0.6±0.7 NCN by GUS mRNA) *Conclusions and Summary:* These data suggest that the expression analysis of Nanog at CML patient baseline, may assist in the early prediction of molecular response in patients treated with TKI. Further studies are ongoing to functionally evaluate whether Nanog is regulated by endogenous or exogenous signals in leukemic cells and evaluate the role of Nanog stemness power in induction of transformation of hematopoietic stem cell.

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CYTOGENETIC AND MOLECULAR RESPONSES IN ITALIAN PATIENTS WITH CHRONIC-PHASE CHRONIC MYELOID LEUKEMIA IN A PROSPECTIVE OBSERVATIONAL STUDY

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Introduction: SIMPLICITY (NCT01244750) is a global observational study of CP-CML patients (pts) outside clinical trials, receiving first-line (1L) imatinib (IM), dasatinib (DAS) or nilotinib (NIL). This analysis presents clinical response data from Italian SIMPLICITY pts. *Methods:* Clinical response following start of 1L TKI was assessed by cytogenetic response (CyR; karyotype or FISH) and molecular response (MR; PCR on the international scale). *Results:* 1207 pts (35% European) were enrolled prospectively through 22Sept2014, receiving IM (n=415), DAS (n=416) or NIL (n=376). Of these, 230 were enrolled at Italian centres and received IM (n=106), DAS (n=57) or NIL (n=67). Italian pts were of a similar age to the total cohort (median age [interquartile range (IQR)] 58 [46–70] *vs* 57 [46–68] years), had fewer comorbidities (mean±standard deviation, 1.6±1.6 *vs* 3.1±2.7) and were followed mainly in academic centres (98% *vs* 51%). Median (IQR) follow-up was 26 (16–34) mos for Italian pts and 26 (15–35) mos for all pts. Of pts followed for 6 and 12 mos from start of 1L TKI, complete CyR (CCyR) was documented in 28% and 45% of Italian pts. Of those tested (6 mos: n=77/210; 12 mos: n=95/185), 77% and 88% achieved CCyR by 6 and 12 mos. By 12 mos, CCyR was documented in 49%, 38% and 43% of IM, DAS and NIL pts, respectively. Of those tested for CyR (IM: 61/106, DAS: 14/37, NIL: 20/42), more DAS-pts achieved CCyR than IM or NIL (IM: 85%, DAS: 100%, NIL: 90%). Of pts followed, 21% and 47% achieved major MR (MMR) and 2% and 16% achieved MR^4.5 by 6 and 12 mos. Of those tested for MR by 6 and 12 mos (6 mos: n=130/210; 12 mos: n=152/185), 33% and 57% achieved MMR and 4% and 20% achieved MR^4.5. By 12 mos, 13%, 24% and 17% of IM, DAS and NIL pts followed achieved MR^4.5. Of the 83%, 84% and 79% of IM, DAS and NIL pts tested for MR by 12 mos, 16%, 29% and 21% achieved MR^4.5. The proportion of pts with optimal, warning and failure responses, according to 2013 ELN guidelines, varied by time and 1L TKI. Of those tested by 12 mos, more IM pts experienced failure compared with DAS and NIL. More Italian pts achieved optimal response by 12 mos than the total cohort (Table 1). *Conclusions:* In all TKI groups, optimal response by 12 mos was achieved by a similar proportion of Italian pts and was higher compared with the total cohort. Compared with pts receiving second-generation TKIs, a higher proportion of IM pts experienced failure by 12 mos and fewer IM pts achieved CCyR and MR^4.5.

Table 1. Proportion of SIMPLICITY Patients Achieving 'Optimal', 'Warning' and 'Failure' by 12 Mos.

	Imatinib		Dasatinib		Nilotinib	
	Patients Followed Through Period	Patients Tested During Period	Patients Followed Through Period	Patients Tested During Period	Patients Followed Through Period	Patients Followed Through Period
Italian cohort, N	106	92	37	31	42	33
Optimal, n (%)	48 (45.3%)	48 (52.2%)	20 (54.1%)	20 (64.5%)	18 (42.9%)	18 (54.5%)
Warning, n (%)	24 (22.6%)	24 (26.1%)	7 (18.9%)	7 (22.6%)	11 (26.2%)	11 (33.3%)
Failure, n (%)	20 (18.9%)	20 (21.7%)	4 (10.8%)	4 (12.9%)	4 (9.5%)	4 (12.1%)
Total SIMPLICITY cohort	404	276	286	197	278	203
Optimal, n (%)	127 (31.4%)	127 (46.0%)	108 (37.8%)	108 (54.8%)	113 (40.6%)	113 (55.7%)
Warning, n (%)	57 (14.1%)	57 (20.7%)	47 (16.4%)	47 (23.9%)	50 (18.0%)	50 (24.6%)
Failure, n (%)	92 (22.8%)	92 (33.3%)	42 (14.7%)	42 (21.3%)	40 (14.4%)	40 (19.7%)

P190

BCR-ABL DECREASES EXPRESSION OF THE PROAPOPTOTIC FACTOR ZNF224 VIA THE PI3K-Akt SIGNALLING PATHWAY

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The Kruppel-like protein ZNF224 is a co-factor of the Wilms' tumor 1 protein, WT1. We previously showed that ZNF224 regulates WT1-dependent transcription of apoptotic genes in chronic myelogenous leukemia (CML) K562 cells; furthermore, the induction of ZNF224 expression by cytosine arabinoside enhances apoptotic cell death (1). Here we demonstrate that the expression level of ZNF224 is significantly lower in BCR-ABL positive cell lines, compared to BCR-ABL negative cells (Figure 1 A). Moreover, inhibition of BCR-ABL tyrosine kinase activity in K562 cells by imatinib triggers the up-regulation of ZNF224 expression (Figure 1 B). The phosphatidylinositol-3 kinase (PI3K)-Akt pathway is one of the three major signaling pathways constitutively activated by the BCR-ABL tyrosine kinase activity (2). Inhibition of PI3K or Akt in K562 cells with imatinib, PI3K inhibitor (LY294002) or dual PI3K/Akt /mTOR inhibitor (BEZ235) induces an increased expression of ZNF224, while mTOR inhibition with rapamycin and everolimus does not affect ZNF224 expression, thus providing evidence that BCR-ABL-induced downregulation of ZNF224 expression is dependent on the PI3K-Akt signalling pathway (Figure 1 C).

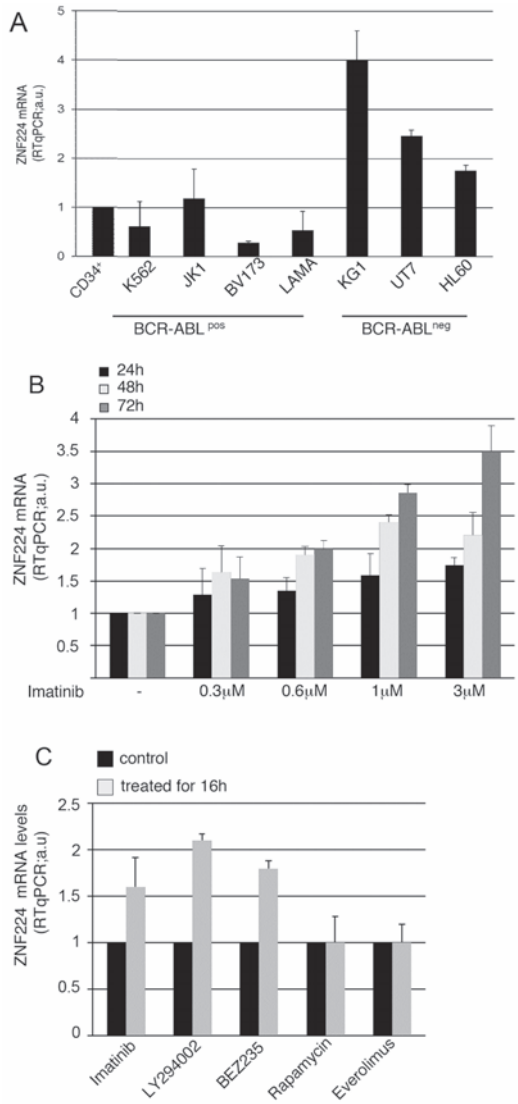

Figure 1.

The identification of ZNF224 as a downstream target of BCR-ABL kinase activity may lead to important new insights in the molecular mechanisms underlying CML. Furthermore, our data strongly suggest that the induction of ZNF224 expression by tyrosine kinase inhibitors could pave the way for the development of new therapeutic approaches in imatinib-resistant CML. 1) Montano G, Cesaro E, Fattore L, Vidovic K, Palladino C, Crescitelli R, Izzo P, Turco MC, Costanzo P. Role of WT1-ZNF224 interaction in the expression of apoptosis-regulating genes. Hum. Mol. Genet. 2013; 22: 1771-1782 2) Ren R. Mechanisms of BCR-ABL in the pathogenesis of chronic myelogenous leukaemia. Nat. Rev. Cancer 2005; 5: 172-183.

P191

VERY ELDERLY CP-CML PATIENTS TREATED WITH IMATINIB FRONTLINE: HAVE CONCOMITANT THERAPIES AN IMPACT ON OUTCOME AND TOXICITY?

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With the introduction of tyrosine-kinase inhibitors (TKIs) the expected survival of CML patients is approaching that of the general healthy population, so that a large number of CML patients are elderly or very elderly. However, the latter are frequently not eligible for clinical trials. Imatinib is effective even in this setting despite of concomitant therapies that may more frequently require dose reductions, as well as pharmacologic adjustments, to avoid drug interactions. We wanted to assess if and which concomitant drugs have an impact on both outcome and hematologic and extra-hematologic toxicity in CP-CML very elderly patients (age >75 years). Two hundred and two very elderly CP-CML patients treated with imatinib frontline were retrospectively evaluated using data collected from 31 Italian Institutions. Median age at imatinib start was 78.7 years (range 75-93); 109 (54.0%) were male. According to the Sokal Scoring System, 60 patients (29.7%) were high risk. Sixty-four (31.7%) were treated with reduced dose imatinib (<400 mg/day), and the remaining patients with imatinib >400 mg/day. Complete cytogenetic response (CCyR) was obtained in 33 (16.3%) patients within 12 months and in 85 (42.1%) after 12 months. Concomitant drugs were 1-2 in 76 (37.6%) patients, 3-4 in 56 (27.7%), and >5 in 41 (20.3%); 29 (14.4%) did not assume any concomitant medications. Antihypertensive drugs and PPIs were the most frequent therapies associated with imatinib in our population. Thus, we focused on the effects of these two classes of drugs. We did not find any significant correlation between antihypertensive drugs and

CCyR rate (p=0.50), nor with grade 3-4 hematologic and extra-hematologic toxicities (p=0.28 and 0.34, respectively). Similarly, PPIs did not correlate neither with outcome (p=0.33), nor with hematologic or extra-hematologic toxicities (p=0.33 and 0.59, respectively). However, in this preliminary analyses, considering antihypertensive classes, CCyR was obtained later in patients assuming β-blockers (p=0.017), while angiotensin II receptor blockers showed a trend toward a significant association (p=0.056). Our preliminary results confirm the well-known safety and efficacy of imatinib also in very elderly patients who frequently take other medications. Further analyses will be done to investigate single classes of concomitant drugs as predictors of outcome or toxicities to help clinician selection of the most appropriate combination therapies.

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PROTEOMIC SERUM PROFILE IN CHRONIC MYELOID LEUKEMIA: PRELIMINARY RESULTS

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Recent advances in the proteomic field have allowed us to better understand the biology of several cancer types and/or discover new candidate biomarkers. Very few data are available about the serum protein expression of patients (pts) with CML. The purpose of this study was to evaluate a possible correlation between depth of Molecular Response (MR) and proteomic profile in sera samples obtained from the peripheral blood and bone marrow of CML pts. Samples were obtained from 23 CML pts observed at the Hematology Unit of National Cancer Research Centre, Istituto Tumori "G. Paolo II" in Bari. Overall 23 sera from peripheral blood and 12 sera from bone marrow were collected. All patients at diagnosis displayed the classic t(9;22) Ph chromosome according to standard cytogenetics. The BCR/ABL transcript at RT-PCR was b3a2 in 15 pts and b2a2 in 8 pts. Pts were grouped in two cohorts: the first (including 2 pts at diagnosis) comprised those with lower molecular response to MR3 (group A: 11 pts) and the second greater than or equal to MR3 (group B: 12 pts).The association of proteomic profile with molecular response was investigated using the SELDI ToF Mass Spectrometry platform. 14 differentially expressed peaks were highlighted when comparing peripheral sera from group A and group B, but none was statistically significant. When comparing serum samples from the bone marrow of groups A(7) and B(5), 4 peaks were reported as differentially expressed in a statistically significant way (p<0.05). Focusing the differential expression analysis in peripheral sera only on MR1 pts(including 2 pts at diagnosis) *versus* MR4 pts, 1 peak at m/z 11092 was identified as significantly and differentially (p <0.05) under expressed in MR1 pts (Figure 1).

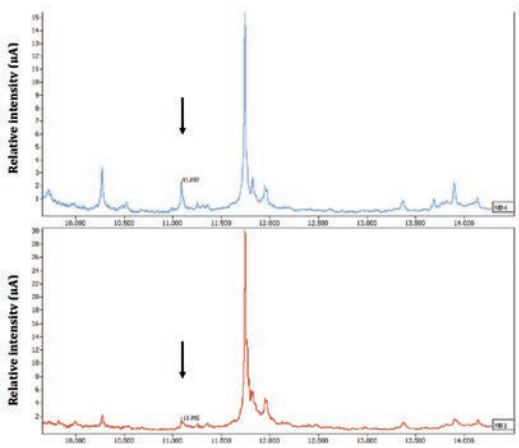

Figura 1. Representative peripheral blood serum semple's spectra of MR4 amd MR1 patients. The arrow indicates the peak at m/z 11092 under-expressed in MR1 patients.

Similarly, comparing bone marrow sera only from MR1 and MR4 pts respectively, 32 peaks were differentially expressed. Once again the peak at m/z 11092 resulted under expressed in MR1 pts, and interestingly the pts at diagnosis had the lowest value. These preliminary data suggest that an under expression of m/z 11092 in serum obtained from peripheral blood and bone marrow appears to be associated with diagnosis or worst response; at the moment identification of these peaks is "in progress" as is the planning of further investigations on a larger number of patients in order to confirm or refute our results.

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LONG-TERM OUTCOME OF ALTERNATING NILOTINIB 400 MG TWICE DAILY AND IMATINIB 400 MG ONCE DAILY AS FRONTLINE TREATMENT OF CHRONIC MYELOID LEUKEMIA: A PHASE 2 STUDY OF THE GIMEMA CML WORKING PARTY

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Background: Imatinib (IM) is the standard treatment for Ph+ Chronic Myeloid Leukemia (CML) in early Chronic Phase (ECP). Nilotinib (NIL) has demonstrated superior efficacy to IM (ENESTnd trial). The treatment with more than one TKI, may improve the response rates and may decrease the frequency of drug-resistance. The combination of different TKIs is potentially toxic, while the alternating administration of IM and NIL may be better tolerated. *Aims:* To evaluate the response rates and the long-term outcome of ECP Ph+ CML pts treated with the alternating administration of NIL and IM. *Methods:* Phase 2 study (ClinicalTrials.gov. NCT00769327). Schedule: NIL 400 mg BID for the first 3 months; IM 400 mg QD for the next 3 months; then, NIL and IM rotating every 3 months, for at least 24 months. The primary end-point was the Complete Cytogenetic Response (CCyR) rate at 12 months. The pts remained on study unless both drugs were discontinued. Failures defined according to 2013 ELN recommendations. *Results:* 123 pts were enrolled; median age 56 years (range 18-84); 22% high Sokal score; median follow-up 60 months. The CCyR rate at 12 months (primary efficacy variable) was 75%. The cumulative MMR rates by 12, and 60 months were 82% and 84%, respectively. The cumulative MR4.0 rates by 12, and 60 months were 43%, and 62%, respectively. At 60 months, 69% of pts were on study, being the majority on monotherapy with NIL (35%) or IM (23%), and only 11% still on alternating schedule. Twenty-three failures were observed, including 6 (4.8%) progressions to accelerated/blast phase that occurred after a median time of 8 months (4-25 months); 4 pts had an ABL mutation (2 T315I, 1 Y253H, 1 F359V); all pts subsequently died. Athero-thrombotic adverse events (AAE) were observed in 6 (4.8%) pts: 3 acute myocardial infarctions, 1 unstable angina, 1 peripheral arterial occlusive disease and 1 aortic atherosclerosis; 5/6 pts discontinued permanently NIL. Overall, 12 pts died. The 5-year overall survival, progression-free survival, and failure-free survival were 90%, 90%, and 78%, respectively. *Summary:* The molecular responses observed in this study are in the high range of the previously published studies with TKI first-line, while the long-term outcome measures are comparable. In conclusion, first-line therapy with a rotation policy was at least as good as any other treatment policy, thus providing an alternative to the choice between IM alone and second-generation TKIs alone.

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COMPARISON OF DASATINIB AND NILOTINIB AS SECOND-LINE THERAPY AFTER INATINIB FAILURE IN CHRONIC PHASE CHRONIC MYELOID LEUKEMIA PATIENTS

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Background: Second-generation tyrosine kinase inhibitors (2G-TKIs) dasatinib (DAS) and nilotinib (NIL) are effective in patients with chronic myeloid leukemia (CML) in chronic phase failing imatinib (IM) treatment. However, it has not yet been established if there is a significant difference in efficacy and safety of the two 2G-TKIs, and data on direct comparison are lacking. *Aims and Methods:* We have retrospectively analysed 73 CML patients who were resistant or intolerant to IM and who were treated with DAS or NIL while still in first chronic phase. We compared the characteristics of the two cohorts at the time of CML diagnosis and at the time of IM failure, including the cause of switch to 2G-TKI, duration of IM therapy, IM dose escalation and the Hammersmith's score to predict the probability of response to 2G-TKIs. Time to treatment failure (TTF) was measured from the start of 2G-TKI to any of the followings: progression to accelerated or blastic phase (AP/BP), death for any cause at any time, primary or secondary resistance leading to treatment discontinuation. Progression free survival (PFS) was measured from the start of 2G-TKI to AP/BP or death. Overall survival (OS) was measured from the start of 2G-TKI to death. *Results:* Considering CML characteristic at diagnosis, the DAS and NIL cohorts were comparable, except for a trend towards an older age (58.6 *vs* 50.9, p=0.06) and a higher incidence of high Sokal (28% *vs* 4%, p=0.02) in the DAS group, while no difference was found according to the EUTOS score (high risk: 11% *vs* 4%). Median duration of IM was similar (DAS 16 months, NIL 13 months), but 15/46 patients (33%) had IM dose escalation before DAS compared to only 1/27 (4%) before NIL (p=0.01). Reasons for switch to 2G-TKI (primary or secondary resistance, intolerance) were comparable in the 2 groups. With a median follow-up of 38 months (range 2–96), median TTF was similar for DAS (median 58 months, 5-years 49%) and NIL (median 88 months, 5-years 77%) (p=0.42) (Figure 1). Also probability of survival and progression were almost identical, with a 5-year PFS of 82% for DAS and 92% for NIL (p=0.43) and a 5-year OS of 90& and 93% (p=0.53), respectively. *Conclusions:* With the limits of a retrospective analysis and a limited number of patients, our data suggest similar efficacy of DAS and NIL after IM failure in CP-CML, with excellent long-term survival.

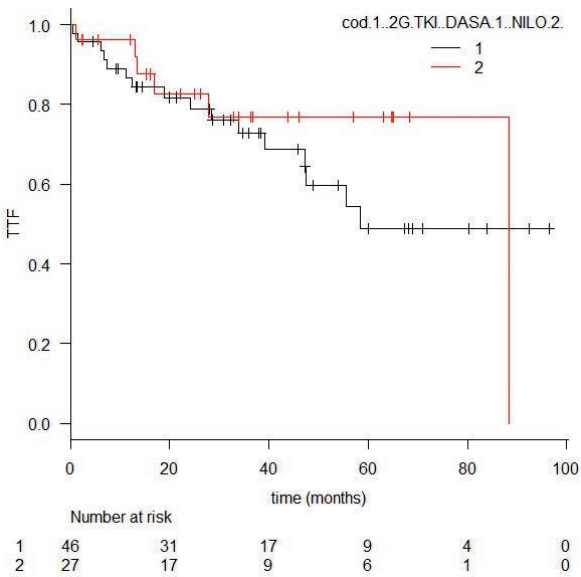

Figure 1.

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PONATINIB FOR RELAPSE AFTER ALLOGENEIC TRANSPLANTATION IN BCR-ABL+ LEUKEMIAS: PRELIMINARY DATA ON SAFETY AND EFFICACY

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Background: Hematological relapse of BCR-ABL+ leukemias after allogeneic stem cell transplant (allo-SCT) for advanced phase disease is associated with a poor prognosis, in particular when BCR-ABL mutations may confer resistance to 1st and 2nd generation TKI. Ponatinib has recently shown to be highly effective for the treatment of advanced BCR-ABL+ leukemias, however data on its use after allo-SCT are very scarce. Here we present a preliminary experience on few cases with advanced BCR-ABL+ leukemias who relapsed after allo-SCT. *Patients and Methods:* From June 2013 to July 2014, 5 pts with advanced BCR-ABL+ CML (n=4) and ALL (n=1) were treated with Ponatinib. Median interval from diagnosis to ponatinib treatment was 15yrs (range 1-19 yrs). All but one CML were transplanted in advanced phase (>CP1) and, before allo-SCT, all pts had received prior intensive chemo including SCT (auto=1, allo=2). Before the use of Ponatinib, all pts had shown to be resistant at least to one 2nd generation TKI and had BCR-ABL mutations (T315I=1, F317 isoforms=2, V299L=1, G250E=1). Starting dose was 45mg/day only in hematological relapses (ALL=1 and CML=1). The remaining in molecular relapse received 15 to 30mg/day. DLI infusion was given in 2 CML pts as consolidation while on Ponatinib. The ALL was treated with anti-CD22 Inotuzumab, achieving complete hematological remission before starting Ponatinib. *Results:* Ponatinib treatment was well tolerated regardless of the dosage. No thromboembolic events were recorded. In all 5 pts BCR-ABL transcripts became undetectable after 3 months of treatment. Of the 3 pts with molecular response, the T315I had disease progression 9 months after beginning Ponatinib and died 8 months later. The 2 with F317L, G250E are alive and well, still on Ponatinib with deep molecular response (>MR4) at 10 and 15 months. The 2 pts with hematological relapse achieved >MR4 after 3 months of treatment. The F317I received 3 DLI while on Ponatinib and maintain response >MR4 at 16 months of treatment. The V299L with ALL progressed at 9 months, and is alive with active disease at 14 months still on Ponatinib. *Conclusions:* Our data on the use of Ponatinib after allo-SCT are too small to draw any conclusion. However, it showed to be well tolerated and effective in heavily pre-treated pts with advanced BCR-ABL+ leukemias. It would be desirable to collect a larger database of BCR-ABL+ leukemias relapsed after allo-SCT to refine a better management of ponatinib in this setting.

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ROLE OF THE TRANSCRIPTION FACTOR ZNF224 IN CELL SURVIVAL AND CHEMORESISTANCE OF CHRONIC LYMPHOCYTIC LEUKEMIA

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Chronic Lymphocytic Leukemia (CLL) is an indolent non-Hodgkin's lymphoma and the most common type of leukemias in adults, characterized by the accumulation of lymphocytes with impaired apoptosis. ZNF224 is a KRAB-zinc finger transcription factor, which controls the expression of its target genes through the recruitment of enzyme activities modifying chromatin. Moreover, ZNF224 acts as a transcriptional co-regulator of the zinc finger protein Wilms'tumor 1 (WT1), playing a critical role in the positive regulation of apoptotic events in Chronic Myelogenous Leukemia. On the other hand, ZNF224 is able to interact with the cancer testis antigen DEPDC1. The ZNF224/DEPDC1 complex plays a critical role in bladder carcinogenesis by repressing the transcription of A20 gene, an inhibitor of the NF-kB pathway. In this study, we analyzed the expression levels of ZNF224 and Cyclin D3 (CCND3), a protein with oncogenic potential and a key cell cycle regulatory component, in peripheral blood sample obtained from 60 CLL patients. We observed that samples obtained from CLL patient exhibited significantly higher ZNF224 and CCND3 expression levels than lymphocytes from healthy donors ($p<0,001$). Furthermore, a positive correlation was observed between ZNF224 and CCND3 expression and between ZNF224 and absolute lymphocyte number (ALC) in CLL patients, while, there was no association between ZNF224 and the lymphocyte doubling time (LTD), thus leading us to speculate a survival role for ZNF224 in malignant B-cells. Moreover, we demonstrated a strong down-regulation of ZNF224 combined with the induction of apoptosis in leukemia cell lines after treatment *in vitro* with fludarabine, a drug extensively used in B-cell malignancies; finally, we revealed a different modulation of ZNF224 expression in chemoresistant CLL cell lines with respect to sensitive cells. These data strongly suggest that high levels of ZNF224 expression are related to B cell malignancies, through the transcriptional regulation of specific genes involved in cell cycle control, as CCND3, and/or survival. The *in vitro* experiments lead us to speculate that ZNF224 down-modulation enhances the response to fludarabine treatment and that ZNF224 has a role in the suppression of apoptosis and survival of CLL cells. Further studied are needed to elucidate the role of ZNF224 in the pathogenesis and/or progression of CLL, thus paving the way to the development of new therapeutic approach in leukemia.

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AUTOIMMUNE HAEMOLYTIC ANAEMIA DURING BENDAMUSTINE PLUS RITUXIMAB TREATMENT IN CLL PATIENTS

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Immune dysregulation with autoimmune phenomena, especially autoimmune haemolytic anaemia (AIHA), is a common complication over the lifetime of CLL patients. Drug-induced haemolytic anaemia mostly related to the use of fludarabine has been often reported. To date, this complication was rarely registered in association with bendamustine. We reported the experience of 5 Italian haematological centres focusing on AIHA during treatment with bendamustine plus rituximab (BR) in patients affected by CLL. Two hundred-thirty-five CLL patients were treated with BR in our centres. Of these, 97 patients were treated for progressive CLL as first-line therapy and 138 patients as second-line therapy. No patient experienced autoimmune phenomena when bendamustine was used with rituximab as part of front-line therapy. On the opposite in CLL patients treated with BR as second-line therapy, 6 episodes of AIHA (4.35%) were reported, as shown in Table 1. Three of six patients who experienced AIHA had positive direct antiglobulin test (DAT), AIHA or immunological auto-antibodies at the onset of CLL. Three cases used fludarabine based regimens as front-line therapy. Four of six patients developed DAT positive AIHA; low median lymphocytes count and the use of rituximab could justify DAT negative AIHA in two patients. The onset of AIHA was observed during the BR treatment in 5 patients and at the end of planned therapy in one patient. The course of AIHA was mild, it responded promptly to steroid or rituximab therapy and it was solved in a few days. Unexpectedly for the incidence of CLL in the general population, 4 out of 6 CLL patients who presented AIHA during BR treatment were female. Patients who experienced previous autoimmune phenomena or underwent fludarabine as front-line therapy, probably for its similitude in the drug structure to bendamustine, showed an increase risk of AIHA. AIHA is probably due to depletion of CD4 cells, which can lead to failure to control auto-reactive T-cells that are capable of creating autoimmunity. BR is a safe and effective regimen in CLL patients, careful observation should be taken in patients in which BR was used as second or further line therapy. Previous autoimmune phenomena, prior use of fludarabine, CD4 depletion and female sex in CLL patients treated with BR in second line should increase the physician's attention so as to discover the onset of bendamustine related AIHA.

Table 1. Characteristics and biological profile of AIHA/CLL patients.

Patient number	1	2	3	4	5	6
Sex/Age	F/58	F/62	M/68	F/64	F/63	M/68
Binet/Rai stage	B/II	B/II	B/II	B/II	C/III	A/I
Zap-70/CD38	neg/pos	pos/pos	neg/pos	neg/neg	n.a/n.a.	neg/neg
IgVH	unmutated	unmutated	unmutated	unmutated	mutated	unmutated
FISH	del13/del11	del13	del13	del17	del11/+12	neg
First-line treatment	BR	CtxR	FCR	FCR	FCR	Chl
Autoimmunity at first-line treatment	DAT+ thyroiditis	AIHA thyroiditis	DAT+ thyroiditis	DAT-	DAT-	DAT-
Response to 1st line	PR	PR	CR	SD	CR	PR
Type of treatment prior AIHA	BR	BR	BR	BR	BR	BR
Time of insurgence of AIHA respect bendamustine	30 days after the end of therapy	after 4th cycle	after 2nd cycle	after 3rd cycle	after 4th cycle	after 1st cycle
Type and specificity of DAT	4+/-	2+/ IgG	4+/-	neg	neg	2+/IgG
Lymphocyte count at AIHA (mmc)	550	2510	1000	680	2500	41720
Minimum Hb level during AIHA gr/dL	7.7	7.5	5.4	9.4	8.5	4.7
Type of therapy for AIHA	steroids	steroids	R-CVP	rituximab	steroids	steroids

IgVH: Immunoglobulin Heavy chain Variable genes; FISH: Fluorescent In Situ Hybridization; BR: Bendamustine-Rituximab; CtxR: Cyclophosphamide-Rituximab; FCR: Fludarabine-Cyclophosphamide-Rituxiamb. Chl: Chlorambucil; DAT: Direct Antiglobulin Test; AIHA: Autoimmune Haemolytic Anaemia; CR: Complete Remission; PR: Partial Remission; SD: Stable Disease; R-CVP: Rituximab-Cyclophosphamide-Vincristine-Prednisone.

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OUTCOME AND TOXICITY OF THE BENDAMUSTINE AND RITUXIMAB REGIMEN IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA TREATED IN HEMATOLOGY CENTERS OF THE LATIUM REGION

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Previously reported studies (Fisher *et al.*, JCO 2011 and 2012) have demonstrated that bendamustine and rituximab (BR) is an effective treatment for treatment naïve (TN) and relapsed/refractory (R/R) patients with CLL, particularly for elderly patients who experience a high rate of infections after fludarabine, cyclophosphamide and rituximab (FCR). Because of its activity and relatively low toxicity, BR is now a widely used treatment approach for the management of CLL. The aim of this retrospective study was to evaluate the outcome and toxicity of CLL patients treated with BR in the clinical practice. Data of 100 patients treated with BR in 12 hematology centers of the Latium region were retrospectively analyzed. CLL diagnosis and treatment requirement were confirmed according to the 2008 iwCLL criteria. The median age was 71 years (range 49-85), the median CIRS score 3 (range, 0-10), a creatinine clearance <70ml/min was present in 43 cases and a Binet stage B or C in 41. Thirty-one patients were TN and 69 R/R with a median number of 1 prior treatment (range,1-6) including FCR in 42% of cases. The IGVH mutational status was unmutated in 34/60 (68%) cases, TP53 mutations were detected in 4/38 (10.5%) analyzed cases. Bendamustine was given on d1-d2 at the dose of 90 mg/sqm in TN patients and 70 mg/sqm in R/R patients. The median number of administered courses of BR in TN and R/R patients was 6. A response to BR was achieved by 90% of TN patients and by 60% of R/R patients. Since only 20% of patients had a bone marrow biopsy at response, the rate of complete responses was not evaluable. The treatment-free survival at 36 months was 82% for the TN patients and 55% for the R/R patients. At present, no deaths have been recorded in the group of 31 TN patients, while the overall survival at 36 months of the R/R patients is 68%. Grade 3-4 granulocytopenia was recorded in 25% of TN patients and in 55% of R/R. AHIA occurred in 1 relapsed patient. During the BR regimen, 13% of TN patients experienced a FUO and 17% of R/R a pneumonia. During the 24 months following treatment discontinuation, a severe infection occurred in 13% of TN patients and in 29% of R/R. Taken together, our findings, as for response rate and toxicity, are similar to those reported in previously published trials, confirming the efficacy of BR in unselected TN and R/R CLL patients.

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EFFICACY AND SAFETY OF CHLORAMBUCIL PLUS RITUXIMAB AS FRONT-LINE THERAPY IN ELDERLY AND/OR UNFIT PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA. A GIMEMA RETROSPECTIVE STUDY

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Elderly chronic lymphocytic leukemia (CLL) patients (pts) or pts with comorbidities are usually treated with Chlorambucil (Chl), though low response rates are observed with Chl alone. The combination of Chl with Rituximab (R) allows higher response rate and is also very well tolerated in this subset of pts. Overall response (OR) rates between 66 and 84% have been reported for this combination, with complete responses (CR) ranging between 8 and 26%. These differences could be explained by the varying doses of Chl used or the different biological features of the treated pts. The GIMEMA conducted a retrospective study collecting data from Italian hematological centers that used Chl-R for the front-line treatment of elderly (>65 years) and/or unfit (CIRS >6) CLL pts. We included 103 pts from 15 Italian centers (Table 1). Pts were treated with Chl (1 mg/kg divided in a fixed dose of 10 mg a day or 8 mg/sm for 7 consecutive days per 28-day cycle for 8 cycles) plus R (375 mg/sm for the first course and 500 mg/sm for the subsequent cycles up to the 6th cycle). FISH analysis showed karyotype abnormalities in 79% of cases: 38% had del(13q14), 21% trisomy 12, 12% del(11q) and 7% a complex karyotype. None had 17p deletion. Zap-70 and CD38 were positive in 42% and 40% of cases, respectively. Seventy-seven pts were studied for the IgVH mutation status: 39 had an unmutated profile, while 38 had mutated IGHV genes. An OR was obtained in 86% of cases: 31% of pts achieved a CR and 55% a partial response (PR). Response to treatment was evaluated by CT in 60% of pts and by ultrasound in 31%. The median follow-up was 53.7 months (range 1.9-79.3). Median progression-free survival (PFS) and time to retreatment (TTR) were reached at 43.7 and 72.3 months, respectively. Median overall survival was not reached; 86% and 81% of pts were alive at 48 and 60 months. The most frequent serious adverse effect was grade 3-4 neutropenia (14%). Grade 3-4 extra-hematological side effects were uncommon (2%). The median administered dose of Chl was 600 mg and dose reduction of Chl because of toxicity was necessary in only a few pts (19%). Our retrospective data confirm that Chl-R is a safe and effective therapy for elderly and/or unfit CLL pts. The increased CR rates, PFS and TTR in comparison to other Chl-R published experiences could be due to the higher doses of Chl, the absence of del17 or other biological features of the treated pts. Studies on the impact of the latter are currently ongoing.

Table 1. Clinical features of the patients.

Median age at diagnosis	72 years (range 54-85)
Male:Female	64/39
CIRS score >6	36 pts (35%)
ECOG score 0	72 pts
ECOG score 1	28 pts
ECOG score 2	3 pts
Median PB lymphocytes	65.000/mmc (range 3.000-180.000)
Median BM lymphocytes	81% (range 20-95%)
Binet stage A	30 pts (29%)
Binet stage B	54 pts (52%)
Binet stage C	19 pts (19%)
Bulky disease	11 pts (11%)

PB: peripheral blood; BM: bone marrow.

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SERUM FREELITE AND HEVYLITE ANALYSES IN EARLY CHRONIC LYMPHOCYTIC LEUKEMIA

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We analyzed a cohort accounting for 60 CLL Binet stage A patients in whom FLC measurements were performed at the diagnosis using a commercially available immunoassay (The Binding Site Group Ltd, Birmingham, UK). Using the cut-off proposed by Pratt *et al.* for the polyclonal serum FLC (*i.e.*, 50 mg/L) we found that patients whose levels were below this threshold had a shorter time to first treatment (TTFT) (HR, 2.08; 95% CI, 1.00-5.53; P=0.03). Patients with lower and higher polyclonal serum FLC levels (*i.e.*, lower and higher than 50 mg/L) were alike with respect to age (P=0.24), gender (P=0.57), ß2-microglobulin (P=0.98), LDH (P=0.67), mutational status of IGHV (P=0.54), ZAP-70 (P=0.64) and CD38-expression (P=0.98). In contrast, patients with lower FLC serum levels had more frequently Rai stage 0 (P=0.006) and lower thymidine-kinase activity (P=0.02). The Hevylite assay which enabled us to accurately measure each isotype-specific heavy and light chain (HLC) (that is, IgGk, IgG, IgAk, IgA, IgMk and IgM) was available in 45 out of 60 patients and revealed that 37 (82.2%) had at least one Ig measurement below the normal value. In detail, HCL abnormalities in terms of low Ig serum concentrations, ranged from 69% (IgM) to 4.2% (IgG). Patients with FLC lower and higher than 50 mg/L had a similar likelihood to experience HCL decrease: IgMk (P=0.75), IgM (P=0.54), IgGK (P=1.00),IgG (P=1.00), IgAk (P=0.16),IgA (P=0.08). Increases of Ig serum concentrations were unfrequent (*i.e.*, IgG, 4.7%; IgA, 4.7%; IgG, 2.3%). IgG /IgG ratios were always in the normal range, while one patient (2.3%) had abnormal IgA /IgA ratio and 3 (7.1%) patients had abnormal IgM /IgM ratio. In

conclusion, this external validation analysis confirms the prognostic value of FCL measurement in CLL. The Hevylite assay demonstrates that the degree of immunoparesis was higher for IgM. Clinical implications of these results should be explored in larger CLL series.

P201

MODULATION OF SURFACE IGM AND IGD BY AUTOLOGOUS ACTIVATED T-CELLS AND ACTIVATION OF BCR SIGNALLING IN CHRONIC LYMPHOCYTIC LEUKEMIA FOLLOWING BCR CROSS-LINKING BY ANTI-(A) AND ANTI-(A) ANTIBODIES

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Introduction: BCR activation stimulates proliferation and survival pathways in CLL cells and is linked to poor outcome. Here, we investigated whether co-culture of CLL B-cells with AAT cells influences sIgM and sIgD cell density and whether this phenomenon translates into functional differences following BCR cross-linking by aIgM and aIgD. *Methods:* The intensity of sIgM, sIgD, Ki67 and phoshorylated-BLNK/BLNK expression as well as AnnexinV (BD. Biosciences) were determined in PMBC samples from 18 untreated CLL patients by flow-cytometry (FACSCantoII) and indicated as% of CD19+CD5+. AAT cells were obtained using the Dynabeads Human T-Activator CD3/CD28 for T-Cell Expansion and Activation (Life Technologies). B-CLL and AAT-co-cultures were exposed to goat anti-human Ab and anti-μAb [a Ab (25ug/mL) and aμAb (10 ug/mL), Southern Biotech] for 48h or 5-20min for BLNK phosphorylation (p-BLNK) experiments. All values indicated as mean±SEM. *Results:* Up-regulation of both sIgD (26.5±6.4% *versus* 36.4±4.8, p=.02) and sIgM (12.1±5.2 *versus* 21.6±5.9, p=.04) expression was detected in CLL B-cells after co-culturing with AAT-cells. *Ex vivo* CLL cells (N=5) undergo higher%apoptosis after cross-linking with aμAb (61.9±12.4, p=.043) than with a Ab (66.7±10.1, p=.08) compared to controls (54.4±12.9); while this tendency is abrogated in AAT co-culture. Cell proliferation measured in the same experiments using%Ki67 positivity, confirmed that co-culture with AAT cells induced significant proliferation compared to baseline (1.4±0.380 *vs* 27.6±6.3, p=.028). In this context, cross-linking with either aμAb or a Ab did not induce additional proliferative effects. Baseline expression of % CD19+CD5+ pBLNK+ (6.8±2.3), a downstream marker of BCR activation, was significantly lower than pBLNK levels measure following co-culture with AAT (33.2±9.9). Treating activated B-cells for 5-10-20 min with aμAb or a Ab did not result in further significant increase in pBLNK levels. *Conclusions:* Microenvironmental signals constituted by activated T-cells have a role in upregulating IgM and IgD expression, producing increases in cell proliferation and BCR downstream signaling (pBLNK). However, in this context, cross-linking upregulated IgM or IgD does not produce any additional effects on downstream BCR activity in this small cohort. It remains to be established whether in the context of AAT, inhibition of this pathway using therapeutic agents targeting the BCR, will provide useful information on the mechanisms that potentially lead to interruption of disease progression.

P202

EFFECT OF LENALIDOMIDE ON THE ACTIN SEQUESTERING PROTEIN, THYMOSIN 4 IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Lenalidomide (L) improves immune dysfunction in CLL by repairing F-actin polymerization and signaling at the immunological synapse. Previously, we identified T 4, a G-actin-sequestering protein as differentially regulated in CLL pts. We investigated the effects of L in CLL samples with defective levels of T 4. *Methods:* L (Celgene) was used at 0.5μM. Actin polymerization was inspected in isolated B and T cells from CLL pts. Migration assays in presence/absence of L were performed with SDF1 as chemoattractant. CD19+CD5+ CLLs were stained for T 4 and G-actin for flow cytometry (FC) and immunofluorescence assays. Apoptosis was evaluated using the AnnexinV assay. Autologous activated T (AAT) cells from CLL pts were obtained using the Dynabeads Human T-activator CD3/CD28/IL2 Kit. Cell proliferation was monitored by TruCount assay. *Results:* FC assessment of T 4 levels in CLL samples (n=64) showed heterogenous T 4 RFI values (median T 4-RFI=16.5, range1.3-126.6), allowing stratification of cases into high/low T 4 groups. Baseline G-actin (%G-actin) expression in CD19+CD5+, showed G-actin levels inversely correlated with T 4 expression (T 4-high>G-actin *vs* T 4-low). In CLL samples cell migration was strongly induced by SDF1, however cells with T 4-low responded to SDF1 2.5x better than the T 4-high group. After L pre-treatment, migration increased in the T 4-high group, while the T 4-low group showed a significant reduction in cell migration towards SDF1. Cell proliferation increased in AAT-ctrl *vs* AAT+L treated cells having low Tb4 levels. *Ex vivo* CLL cells in cell culture showed reduced viability when treated with L for 48h, but T 4 low cells appeared to be more resistant to apoptosis. Treatment with L caused an increase in%G-actin levels, thereby skewing the G/F actin ratio observed in baseline cells. FC analysis also confirmed the increase in G-actin levels in B-cells. *Conclusions:* Defective T 4 expression in B-CLL is associated with different cellular responses in cell migration, proliferation and cell viability. Treatment with L has opposite effects in function of endogenous T 4 levels likely based on defects on endogenous actin-regulation activity suggesting a subset of CLL pts are unable to effectively respond to L in terms of recovery of F-actin levels. T 4 low patients may be intrinsically susceptible a more aggressive phenotype and to a worse prognosis, given they are unable to recover the F-actin levels necessary for formation of viable immunosynapses with T-cells.

P203

NEXT GENERATION SEQUENCING ION TORRENT AMPLISEQ-TM TECHNOLOGY FOR THE IDENTIFICATION OF TP53 MUTATIONS –SCREENING OF 40 CASES OF CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: We describe the application of a TP53 NGS panel based on Ion AmpliSeqTM technology designed to analyze the full coding region of TP53 in a single workflow. *Methods:* Purified B-cells, from 40 therapy-free CLL samples were isolated using immunobead separation (MiltenyiMacsPro). DNA was quantified using the QuBit dsDNA assay kit. Library preparation and sequencing were performed on the Ion Torrent™PGM platform (LifeTechnologies) according to the manufacturer's protocols. Ten pooled samples were loaded on 4 different 316chips and sequenced (flow rate, 500x). Ion Torrent data processing, filtering and base calling was performed using the Ion Torrent server v4.0.2. We established a threshold of 50 and 100 reads, respectively for homozygous and heterozygous variants. On the basis of Variant Caller Parameters we accepted variants with a frequency >5% and high quality, rejected variants had frequency <5% and low quality due to potential PCR errors. *Results:* ION PGM sequencing run data showed a high coverage in 90% of the sequenced amplicons with average coverage uniformity of 89%, an average mean depth of 13,130 (range 7964-17821), and 97% of mapped reads on target. We have identified 88 mutations in the TP53 regions covered by two primer pools. Three

mutations were identified in the remaining allele in 3 pts presenting a 17p deletion as detected by FISH analysis. Briefly, 31/40 pts sequenced presented alterations across intro-exon regions. These consisted of a total of 88 heterozygous mutations, of these 27 in intronic regions; 42/56 mutations were indicated as deleterious missense alterations likely producing non functional protein according to in silico analysis; 5/56 mutations in 3'UTR region that could affect gene TP53 regulation by target miRNA; and 9 previously validated SNPs, three of which where present in all pts. All genomic sequences were compared to the IARC TP53 database.*Conclusions:* The ION PGM TP53 panel offers an easy to use platform for the evaluation of small clones of TP53 mutations. A total of 69/88 mutations fell were detected as small TP53 mutated subclones having a variant allele frequency <10%, that would likely be considered to be wild-type by Sanger sequencing, demonstrating the highly sensitive of the technique. The presence of subclonal mutations could anticipate the development of a chemorefractory phenotype among CLL pts requiring treatment. We are currently evaluating TP53 clonal alterations identified by NGS screening in a CLL cohort of bendamustine-treated pts.

P204

HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS IN A PATIENT WITH RELAPSED CHRONIC LYMPHOCYTIC LEUKEMIA TREATED WITH IBRUTINIB

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Introduction Hemophagocytic Lymphohistiocytosis (HLH) is a rare disorder characterized by an ineffective T-cell and NK response resulting in an exuberant cytokine production. Ibrutinib is an oral irreversible inhibitor of Bruton Tyrosine Kinase (Btk) significantly improving objective responses in patients with relapsed/refractory Chronic Lymphocytic Leukemia (CLL). We report the diagnostic work up and the clinical course of a case of HLH occurring in a patient with relapsed CLL during treatment with ibrutinib. Case report: the patient was a 77-years-old male diagnosed with CLL at the age of 70. At time diagnosis, the disease presented with unfavorable prognostic markers (CD38pos, ZAP70pos and unmutated IGVH). Two years later, the patient developed progressive lymphoadenopathy and a fludarabine-based regimen was administered. No objective response was observed and multiple lines of treatment attained only partial short lasting responses. In the presence of refractory disease with del17p Ibrutinib at a daily dose of 420 mg was started. On day 7 of treatment, febrile neutropenia occurred that did not benefit from antibiotics therapy. Due to persistent fever and deterioration of the clinical picture on day 16, Ibrutinib was hold and the patient hospitalized. Blood counts progressively dropped and disseminated intravascular coagulation complicated the clinical course. Additionaly, splenomegaly, hypertriglyceridemia and unexplainable severe hyperferritinemia (23,023 ng/mL) were encountered. The occurrence of 5 out of 8 diagnostic criteria allowed for a diagnosis of HLH to be made and treatment with dexamethasone, in combination with cyclosporin A and intravenous immunoglobulin, was started. We performed viral genome load and cytokine dosage tests on sera samples, showing high serum EBV genome title. In consideration of the activity of anticytokine treatment in the setting of HLH, the patient was treated by anti-interleukin(IL)-6 receptor Tocilizumab. Despite intensive supportive treatment, irreversible multiorgan failure developed with life-threatening bleeding and death. *Conclusions:* HLH is a rare occurrence in CLL and the relationship with ibrutinib treatment is uncertain in this patient. However it is worth noting that off-target effects of ibrutinib may include downregulation of CD8/NK activity which may be involved in initiating HLH.

P205

INHIBITION OF USP7 INDUCES GROWTH ARREST AND APOPTOSIS VIA PTEN IN CLL CELLS

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Background: PTEN was shown to play a role in Chronic Lymphocytic Leukemia(CLL). PTEN could be either phosphorylated in the tail by Casein Kinase II and PTEN downregulated in some CLL patients. In cancer, moreover PTEN functions are also regulated by its proper cellular compartmentalization. In particular, mono-ubiquitination promotes PTEN nuclear localization, while de-ubiquitination by USP7 promotes PTEN nuclear exclusion with dramatic consequences on its function. *Aims:* Assessment of the of PTEN cellular compartmentalization in CLL and development of strategies to reactivate PTEN in CLL. *Methods:* CD19 CLL cells were collected to investigate PTEN and USP7 cellular compartmentalization, expression by immunofluorescence and western immunoblot. PTEN and USP7 activity were modulated in CLL cell line. Primary CLL cells were treated with the USP7 inhibitor P5091. *Results:* We observed that a portion of CLL patients is characterized by PTEN nuclear exclusion. PTEN cellular compartmentalization is regulated by mono-ubiquitination. To assess whether mono-ubiquitination is responsible of PTEN cellular compartmentalization in CLL cells, we tested PTEN ubiquitination status. Notably, PTEN is mostly de-ubiquitinated in samples characterized by PTEN nuclear exclusion, when compared with PTEN diffused CLL samples. USP7 was shown to promote PTEN de-ubiquitination and therefore PTEN nuclear exclusion. Our data suggested a dual regulatory mechanisms that activate USP7 towards PTEN. It could be either over-expressed or aberrantly activated by CKII. Forcing PTEN expression into the nucleus of a CLL cell line, with a PTEN-NLS expressing vector, was associated with strong apoptosis induction and growth arrest. Interestingly, CLL treatment with USP7 inhibitor P5091 promotes PTEN re-localization into the nucleus, apoptosis induction, growth arrest and restore the levels of mono-ubiquitinated PTEN. Similarly, treatment with TBB, the inhibitor of CKII, promotes both PTEN re-localization and apoptosis induction. *Conclusions:* These results demonstrate that USP7 plays a dramatically role in CLL pathogenesis. Targeting USP7 promotes PTEN re-activation with a consequent cancer selective apoptosis induction.

P206

PROGNOSTIC SIGNIFICANCE ON CLINICAL OUTCOME AND ROLE ON TUMOR SURVEILLANCE OF αβ-DNTS IN HAEMATOLOGICAL DISEASE AND SOLID TUM OR: PRELIMINARY EVALUATIONS

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Lymphomas are a heterogeneous group of malignancies rising in the incidence. A new era is opening in the Lymphoma therapy thanks to the increasing knowledge of tumor immune biology that is leading to the development of an array of immunotherapy approaches. An unconventional subset of CD4-CD8- double-negative (CD56-) T cells (DNTs) has recently been shown to be involved in immune regulation and tolerance as well as in host defense and inflammation, acting as regulatory T cells and/or cytotoxic T cells contributing specifically to anti-tumor immunity. However no data are available on their prognostic significance, their modulation during the therapy response and their ability to kill tumor cells in cooperation with other immune cells. The aim of this study was to evaluate the DNTs frequency during therapy response in order to assess their role on the clinical outcome,progression and tumor surveillance. PB and BM samples of 140 Ly pts, with NHL and cHL were collected at diagnosis and during the follow-up, 20 PB samples from healthy donors (HD) as control and 30 fresh LN tissues from pts clinically suggestive of Ly. To evaluate the interaction of DNTs with tumor burden either 22 samples from RCC pts and 40 samples from MM pts were collected. 15 cases of LNs received a finally diagnosis of reactive follicular hyperplasia (RFH) so they were considered as controls. We observed a significant decrease (p=0.006) of circulating αβ-DNTs in untreated Ly pts as compared with healthy controls and their number seemed to be modulated during the follow-up.Their frequency in 1-month post-cht or disease relapsed pts was significantly decreased (p=0.006) as compared with the diagnosis as well as when compared indolent with aggressive histotypes (p=0.0001).In HL pts the frequency of -DNT was significantly increased as compared with other histotypes

(p=0.005).Furthermore,the αβ-DNTs in LNs of Ly pts were significantly reduced as compared to RFH-LNs (p=0.006) and are related to the aggressiveness of the disease. When evaluated either in MM and RRC pts the αβ-DNTs were significantly decreased (p=0.001) as compared with healthy controls and more interestingly when compared with Ly pts (p=0.001) given the greater immunological impairment of RCC/MM tumor burden. Our preliminary data, showed an inverse correlation between the frequency of αβ-DNTs and the tumor condition as well as that they could play an important role in both the development and the progression of lymphomas (Figures 1-6).

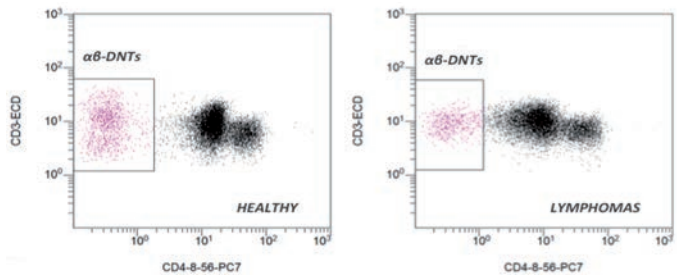

Figure 1. Circulating αβ-DNTs. Typical pattern of DNT frequencies on gated CD3+T cells. Rappresentative staining of two different subjects shown.

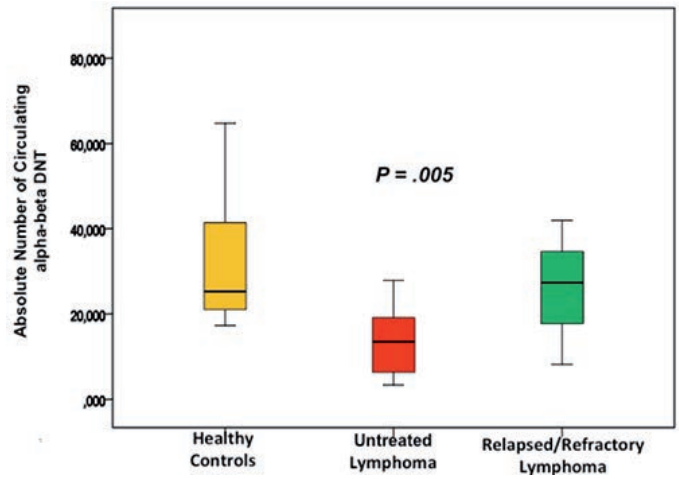

Figure 2. Absolute number of circulating αβ-DNTs: DNTs are reduced in Lymphoma patients compared to healthy controls (P=0.005) and they correlate with disease relapse/progression.

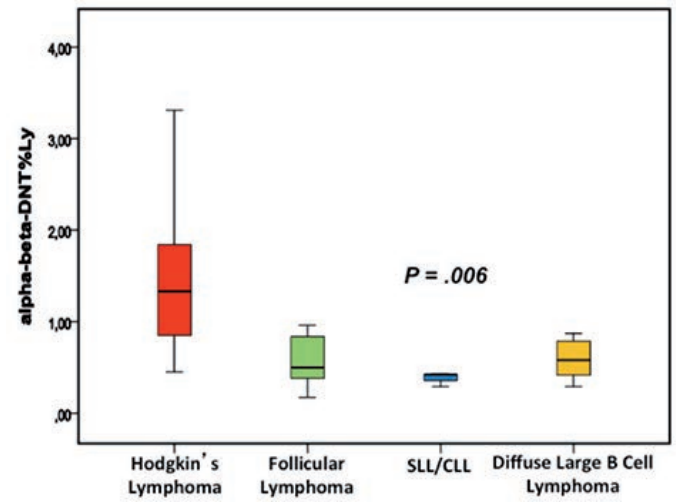

Figure 3. Percent of circulating DNTs as percent of Ly: Hodgkin's Lymphoma showed a higher number of circulating αβ-DNTs as compared with other histotypes (P=0.006).

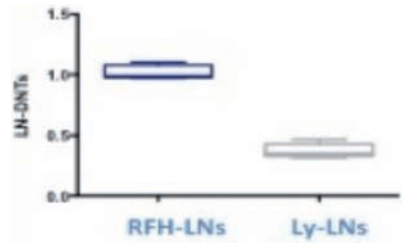

Figure 4. αβ-DNTs in RFH-LNs and malignant LNs. Significantly reduction of DNT in malignant LNs compared to RFH-LNs.(p=0.006).

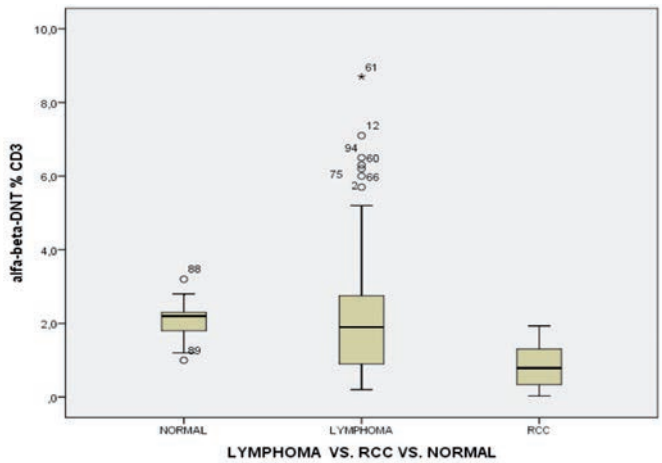

Figure 5. RCC showed a lower number of circulating αβ-DNTs as compared with controls or Lymphoma patients (P=0.001).

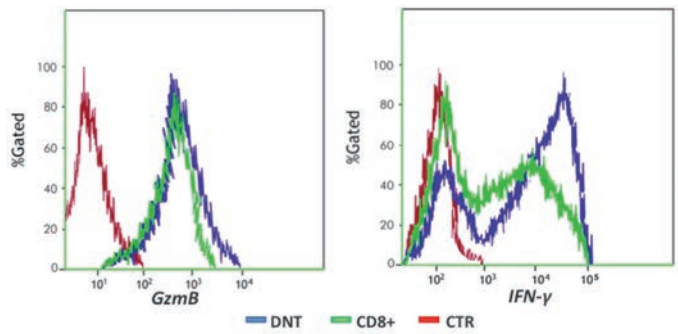

Figure 6. Expression of GzmB and IFN-γ: Typical patter of the Granzyme B and IFN-γ expression (as MFI median fluorescence intensity) in *ex vivo* expanded DNTs from Lymphoma patients as compared with autologous CD8+T cells.

Acute Myeloid Leukemia 2

P207

ARA-C SC DURING TREATMENT WITH AZACITIDINE IN ACUTE MYELOID LEUKEMIA SECONDARY TO MYELODYSPLASTIC SYNDROME: A CASE REPORT

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Azacitidine is a hypomethylating agent approved for the treatment of myelodysplastic syndromes (MDS) with Intermediate-2 or high IPPS risk and acute myeloid leukemia with myelodysplasia related changes (AMLWMRC) with blasts from 20 to 30%. We report a 77 years old patient with myelodysplastic syndrome (RAEB-2) IPSS high risk, treated with azacitidine (75 mg/mq day 1-7 every 28 days). At the start of treatment the transfusional dependence was 2 units/month. After 7 cycles, the MDS evolved to AMLWMRC with circulating blasts 35%. The neutrophil granulocytes count was 200/µl, anemia was severe, platlets count was in the normal range.The transfusional dependence increased to 8-9 units/month. Thus we started ARA-C 30 mg sc for 7 consecutive days. After 28 days, the circulating blasts decreased to 2% but the hematological profile didn't improve. So we started again azacitidine with the same schedule. After 1 cycle the neutrophil granulocytes count increased to 1500/µl and the transfusional dependence decreased to 2-3 units/month. The hematological profile is stable after further 2 cycles. The association of ARA-C sc with azacitidine can have a synergistic action to reduce the peripheral blasts count and improve hematological profile.

P208

METAPLASIA OF IMMATURE CELLS IN BONE MARROW, PANCYTOPENIA AND ELEVATED NSE: A SMALL CELL LUNG CANCER CASE REPORT

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In suspect of hematological malignancies, a 62 years-old woman came to our center and underwent a bone marrow (BM) biopsy. Her clinical presentation included smoke addiction, pancytopenia, fever, hypercalcemia, left hilar adenopathy, an history of chronic obstructive pulmonary disease and breast cancer. A total body CT scan revealed multiple mediastinal and abdominal lymph nodes, brain lesions and a lingula lung mass. Serum neuronal specific enolase (NSE) was 112000 ng/ml and chromograninеA (CgA) was 1055 ng/ml. Due to a worsening clinical status and severe thrombocytopenia, we could not perform a lung biopsy. BM sections revealed a massive infiltration of CD45-/CD34-/CD117+/CD56++/pancytokeratin+ cells; Ki-67 proliferation rate was about 80% and p63 was negative. BM smears showed an immature population with atypical features and large cytoplasm sometimes in syncytium. Flow cytometry on BM revealed a neoplastic population (6,88% of global cells) with an elevated side scatter, negative for CD45 and positive for CD117, CD15 and CD56 antigens. According to oncologist consultant, this was a case of small cell lung cancer (SCLC) at an advanced status with BM carcinomatosis; in this setting, aggressive clinical course and paraneoplastic syndromes at diagnosis are common. The diagnostic challenge relied on morphologic analysis, showing a diffuse infiltration of immature cells with scarce residual hemopoiesis, and on the expression of myeloid antigens (*i.e.* CD15, CD117), suggesting a diagnosis of acute myeloid leukemia (AML). AML can be CD45-negative and this finding does not rule out its diagnosis. CD56 is frequently seen as well. On the other hand, CT scan results and neoplastic markers posed diagnosis of BM carcinomatosis. NSE is frequently elevated in SCLC at diagnosis and linked to advanced disease; CgA levels are also correlated with metastatic disease included BM carcinomatosis; CD56 is found in a variety of cell types and it is quite common in SCLC and correlated with NSE. SCLC is a distinct histological subgroup: it occurs almost exclusively in smokers and is characterized by a high growth fraction and early development of widespread metastases. The hematological morphology and immunophenotype assays potentially guiding to a misdiagnosis of AML need to be carefully interpreted in correlation with other laboratory findings.

P209

UPREGULATION OF MIR-29A AND GENOMIC DNA HYPERMETHYLATION IN NORMAL KARYOTYPE AML SHOWING DNMT3A MUTATION

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DNMT3A, a member of DNA methyltransferases, is mutated in approximately 22% of *de novo* normal karyotype acute myeloid leukemia (NK-AML) patients leading to adverse overall survival. The highly recurrent mutation in DNMT3A is a "gain of function-like" at codon R882. To indagate about miRNA signature in NK-AML R882-DNMT3A mutated we studied by qRT-PCR the expression of 384 known human miRNA in 9 selected *de novo* AML DNMT3A mutated. We compared miRNA expression data with our previous results obtained in 31 AML DNMT3A wild type (WT) and we focused on a strong up-regulation of miR155, miR29a, miR196b and miR25. We consolidated this data in additional 24 new DNMT3A mutated AML and we confirmed the upregulation of miR29a (fold 289,201; p-value 0,000); miR29a has been demonstrated to directly target 3'UTR of DNMT3A resulting in a global hypomethylation but also directly suppress two major DNA demethylases TET1 and TDG. To understand the pathogenesis of the subgroup of AML DNMT3A mutated and the existing correlation between miR29a and its targets, we evaluated the expression levels of miR29a targets DNMT3A, TET1 and TDG in 43 AML DNMT3A mutated patients and in 43 control group AML DNMT3A WT by qRT-PCR. Results obtained revealed a no significant difference in expression of DNMT3A and of TDG; however we found a significant downregulation of the demethylases TET1 (0,661 fold; p-value 0,039). These data suggest that miR29a acts as a crucial regulator of DNA methylation and probably in presence of DNMT3A activating mutations and TET1 downregulation may cause a perturbation of methylation pattern. We analyzed the methylation status of the genomic DNA of bone marrow cells from 6 AML patients (including 3 DNMT3A-mutated and 3 DNMT3A-WT cases) and from 5 healty donors as control by Methylation Sensitive Arbitrarily Primed-PCR that provides a qualitative estimate of genome-wide DNA methylation. Results showed a global hypermethylation of genome in DNMT3A mutated patients compared to DNMT3A WT group and healthy bone marrow. The performed study increasingly suggests that the DNMT3A gain-of-function mutation, the significant upregulation of miR29a and significant downregulation of demethylase TET1 target gene would contribute to the maintenance of the hypermethylation status of the genome in patients with DNMT3A mutation. This issue may have important implications for treatment and response to hypomethylating drugs in patients affected by alterations in DNMT3A.

P210

RISK STRATIFICATION OF ACUTE MYELOID LEUKEMIA: A SICILIAN NETWORK FOR INTEGRATIVE ANALYSIS OF MULTIPLE MOLECULAR MARKERS AND KARYOTYPE

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Acute myeloid leukaemia (AML) is a cytogenetically heterogeneous disorder with acquired recurrent chromosomal alterations in about 55% of patients. The remaining normal katyotype AML (NK-AML) are characterized by molecular abnormalities; gene mutations of FLT3, WT1, IDH1, DNMT3A and high expression levels of the BAALC and

ERG show adverse prognostic value, whereas mutations in CEBPA and NPM1 genes show favourable prognosis. We performed a study involving all the Haematology Sicilian Centers evaluating the incidence of the genetic aberration to provide a prognostic stratification of patients. Cytogenetic studies were performed according to standard protocols. Molecular characterization included fusion gene (PML/RAR, BCR/ABL, AML1/ETO, CBF /MYH11, MLL rearrangements); gene mutations (FLT3, NPM1, WT1, IDH1, IDH2, CEBP and DNMT3A); gene expression (WT1, BAALC, ERG). AML1/ETO and CBF /MYH11 (CBF-AML) were also characterized for KIT gene mutation. We enrolled 733 AML cases, 45 carrying PML/RAR fusion gene; the remaining 688 were so distributed: 151 NPM1 mutated, 127 FLT3 mutated (114 with FLT3 ITD and 13 with D835), 9 WT1 mutated, 23 IDH1-R132C mutated, 16 IDH2-R172 mutated, 25 CBF traslocation, 3 of them were KIT-D816V mutated, 1 BCR/ABL traslocation, 4 MLL rearrangements, 27 CEBP mutated, 32 DNMT3A-R882H mutated. Cytogenetic studies available on 643 cases identified: 238 NK, 79 adverse karyotype (complex karyotype, inv(3)/t(3;3), t(6;9), –5, –7, del(5q), del(7q), 11q23 rearrangements), 256 intermediate (+8, +11, +21, del(20) and other abnormalities not otherwise categorized) and 70 favourable karyotype (t(8;21), inv(16), t(15;17)). Gene expression analysis of WT1 showed a median of 5423 copies/10E4 ABL copies (range from 2 to 26x10E6). 100 NK-AML were divided into quartiles by gene expression levels of BAALC and ERG: low (I and II) and high expression (III and IV). We performed BAALC and ERG expression levels on *de novo* 192 no-marker patients: 121 scored as high risk (III and IV), 57 scored as low risk (I and II). Integrated analysis of molecular and cytogenetic markers allow us to perform a prognostic stratification in high and low risk in more than 80% of patients, only 10-20% of patients remained in the less informative "intermediate" category, this may be very helpful to indicate specific therapy in the great majority of patients. *Supported by:* Ass. alla Salute (PSN 2011, 2012) and Ass. Attività Produttive (Prog. RIMEDRI) Regione Sicilia.

P211

CUMULATIVE ANALYSIS OF POST INDUCTION WT1 VALUES AND WT1 ONSET/POST INDUCTION RATIO AS AN USEFUL TOOL FOR A RISK STRATIFICATION OF ACUTE MYELOID LEUKEMIA PATIENTS

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Background: WT1 is an useful marker for minimal residual disease detection in AML. Its role in post induction risk stratification is not clear. We define a risk stratification based on post induction values of WT1 (WT1-PI) and WT1 onset-post induction ratio (WT1-R). PATIENTS AND *Methods:* Between 2008 and 2014, 110 bone marrow samples (BM) of patients at AML onset (59 male, 51 female, median age 65, range 21-89) and 17 BM of healthy subjects (11 male, 6 female, median age 58, range 23-89) were screened for WT1 using RQ-PCR. 63/110 patients received induction therapy and WT1-PI values were measured in 54 cases, (it was not possible in 5 patients because of early death and in 4 cases because of technical reasons). Of the 54 evaluable patients after induction therapy, 43 were in complete remission (CR), 6 were in partial remission (PR), 5 had progressive disease (PD). *Results:* WT1 median value at onset in AML patients was 1479,5 (0,3-34497); it was 17.6 (1.7-78.8) in healthy subjects. ROC analysis indicated 80 as optimal "threshold" of WT1 value (area of 0.9439, sensitivity of 0.89 and specificity of 1) to discriminate normal from AML bone marrow. We stratified the 54 patients, evaluable after induction, according to this value. 14/54 had a WT1-PI >80: 7 were in CR, 3 in PR and 4 with PD. 10 of these 14 patients (71%) died 5 months after onset (range 1- 19 months). 41/54 had WT1-PI value <80 (36 were in CR, 4 in PR, 1 with PD) and 36% (15/41) died within 11 months (2-38). We calculated WT1-R in all the 54 patients. According to ROC analysis, 38,55 is the cut off level indicative of probability of survival (sensitivity 0.60 and specificity 0.83). We than performed survival analysis based on WT1-PI, WT1-R and both. Results are shown in the picture. Using both the criteria, we think it is possible to distinguish three groups: unfavourable (patients with WT1-PI >80 and WT1-R <38.55), intermediate (patients with WT1-PI <80 and WT1-R <38.55) and favourable (patients with WT1-PI <80 and WT1-R >38.55). *Conclusions:* WT1-PI and WT1-R evaluation seem to be an optimal tool to stratify AML patients in favourable, intermediate or unfavourable group for survival. These, together with other evaluations could guide physicians in planning therapy (Figure 1).

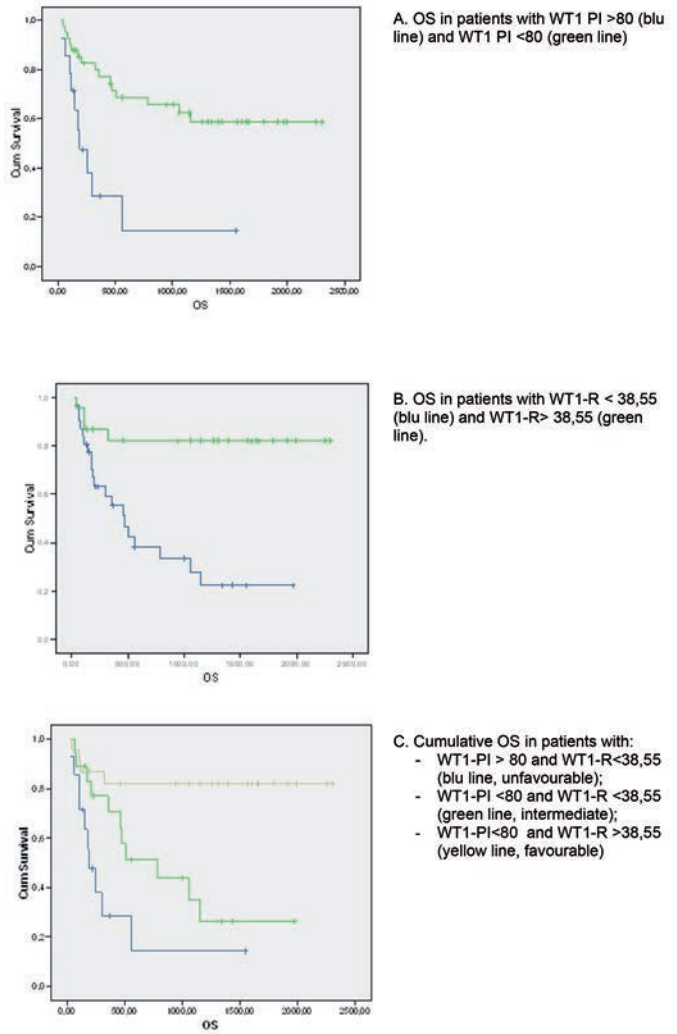

Figure 1.

P212

PRIMARY INDUCTION FAILURE IN ACUTE MYELOID LEUKEMIA PATIENTS: DOES BASELINE CHARACTERISTICS AND THERAPY REGIMENS PREDICT RESPONSE? A SINGLE CENTRE EXPERIENCE

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Acute myeloid leukemia (AML) patients resistant to first line treatment represent a subgroup with poor prognosis. Ravandi *et al.* reported that higher age, unfavourable cytogenetics and high white blood cells (WBC) count at diagnosis were significantly associated with primary induction failure (PIF). Aim of the present study was to evaluate clinical and molecular features at baseline predicting PIF in AML patients. Clinical and molecular data of 385 consecutive AML pts from 2000 to 2013 were collected in our institution. Distribution of patients' characteristics were

summarized using percentiles, for continuous variables, and percentages and frequencies for categorical variables. In order to test difference in distribution of patients' characteristics according to PIF Wilcoxon rank sum test for continuous variables and 2 test for categorical variables were performed. A logistic regression analysis identified risk factors associated of failure to achieve complete remission after induction treatment. Patients were induced with standard dose anthracycline based regimen (64%), by Fludarabine based regimen (26%) or high dose Cytarabine (Ara-C) (10%). 104 (27%) pts resulted refractory. Statistical analysis compared PIF pts with the responding control subgroup. Regarding cytogenetics 9% of controls and 2,3% of PIF pts were low risk, 23,8% of controls and 39,1% of PIF patients were high risk, the remaining pts were intermediate risk. At univariate analysis age, unfavourable cytogenetic risk and Fludarabine based regimen were predictive factors PIF (OR 1,02, 2.05 and 1,77 respectively, 1,01-1,04, 1,22-3,45 and 1,09-2,87 95% CI). In particular, high dose Ara-C regimen, NPM mutation status and favourable cytogenetic risk were associated with complete remission (OR 0,33, 0,4 and 0,23, respectively, 0,1-1,07, 0,17-0,99 and 0,05-1,02 95% CI). No statistically significant association with WBC, platelets count, presence of FLT3 mutation or PIF status was observed. In conclusion, in the present study statistically significant predictive factors of PIF in adult AML pts are higher age and unfavourable cytogenetics at baseline. FLT3 status is confirmed not to be a molecular marker of treatment failure while NPM mutation seems to have a positive predictive value. Intrestingly, in the present analysis pts treated by conditioning regimen based on high dose ARA-C showed a better outcome than pts who underwent Fludarabine based induction chemotherapy.

P213

IDENTIFICATION OF CD15 AS A MARKER FOR NEURONAL DERIVED TUMORS MIMICKING ACUTE MYEOLOID LEUKEMIA

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Background: The Lewis x antigen, or CD15, is a cell surface glycan consisting of a trisaccharide. Initially identified by monoclonal antibodies in the early 1980's, it was a useful marker for human myeloid differentiation identifying granulocyte-series cells, monocytes and myeloid progenitor cells. Otherwise known as "the stage-specific embryonic antigen-1 "(SSEA-1 antigen), CD15 has also been recognized as a positive marker for rodent multipotent neural stem cells. Pruszak *et al.* (2009) defined a CD surface antigen code CD15+/CD29HI/CD24LO for the neuronal lineage differentiation of stem cells. CD15 is present on neuronal stem cells *in vivo*, residing in stem cell niches of adult brain and is lost during differentiation into mesenchymal (CD15-/CD29HI/CD24LO) and neurons (CD15-/CD29LO/CD24HI). *Aims:* The aim of this study is to underline that CD15 can be used to identify neuronal derived tumors. *Methods:* A 32-year-old man with thrombocytopenia and a 64-year-old woman with pancytopenia and fever, were admitted in our center respectively in January and October 2015. BM smears were stained with May-Grunwald-Giemsa and cell morphology was analyzed by conventional light microscopy.Flow cytometry analysis was performed on bone marrow. The antibodies panel included: CD45, CD34, CD117, CD33, CD13, CD15, CD56 and HLA-DR. Data acquisition and analysis were performed using a FACSCANTO II flow cytometer and FACSDIVA software. *Results:* In both cases bone marrow evaluation revealed metaplasia of cells morphologically resembling blasts. By flow cytometry, these cells lacked CD45 and expressed CD117, CD15 and brilliant CD56. The presence of myeloid antigen CD117 together with CD15 suggested acute myeloid leukemia; however CD45 negativity was not suggestive of haematological malignancies. After CT scan and neoplastic markers' results, a diagnosis of neuroblastoma for the first case and small cells lung cancer BM carcinomatosis for the second one were made, respectively. Neuroblastoma derived from the neural crest and SCLC arises from neuroendocrine progenitors. These cases highlight the need for integration of data from morphology, flow cytometry and clinical data in order to avoid misdiagnoses. In this context, CD15 did not express belonging to myeloid lineage but rather neuronal derivation. In summary our results show that CD15 in combination with other antigens can be used in the diagnosis and minimal residual disease assessment in neuronal derived cancers.

P214

INVASIVE ASPERGILLOSIS DURING CONSOLIDATION CHEMOTHERAPY FOR ACUTE MYELOID LEUKEMIA: REPORT OF TWO SIMULTANEOUS CASES INVOLVING CENTRAL NERVOUS SYSTEM

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Invasive fungal diseases (IFD), in particular invasive aspergillosis (IA), contribute substantially to morbidity and mortality among AML patients. Primary antifungal prophylaxis (PAP) is a commonly used strategy. Most studies have focused on clinical efficacy and cost-effectiveness of PAP during AML induction CHT and posaconazole has been recommended as the drug of choice. There is little evidence, however, in the appropriate use of PAP in AML consolidation CHT where the risk for IFD is lower. Here we report 2 IA cases in AML patients receiving consolidation CHT after achieving complete remission(CR). They shared the same room in the Haematology Unit. Patient 1 (65 years old) received consolidation CHT with fludarabine, high dose cytarabine and mitoxantrone, while patient 2 (60 years old) was given intermediate dose cytarabin and daunorubicin. They received no PAP. At day +20 patient 1 presented fever and pleuritic chest pain. Chest CT scan showed multiple consolidative lesions suggestive of pulmonary IA with positive serum and bronchoalveolar lavage galactomannan antigen; antifungal therapy with voriconazole was started. In the subsequent week she presented dysarthria and right hemiparesis. A brain MRI scan showed wide rounded ring-enhancing lesions suggestive for septic emboli. Amphotericin B therapy was added to voriconazole but despite antifungal therapy her clinical condition worsened. Death occurred 30 days later. Patient 2 presented headache, confusion and right hemiparesis at the day +18. Brain CT scan revealed lesions consistent with acute infarcts. Due to worsening conditions a brain MRI was performed, showing a focus-bleeding infarct. Serum galactomannan antigen and chest CT scan were negative. She was treated with voriconazole and amphotericin B therapy. Excisional biopsy of the cerebral lesion was done and histopathological analysis of the tissue revealed hyphae (Aspergillum spp). Another chest CT scan was performed showing a suggestive fungal abscess. After partial lung resection the patient is now alive in CR. Despite several guidelines and multi-institutional study, the optimal duration of PAP in patients with AML is still a matter of debate. Although the small numbers of cases, our study suggests that a consistent and effective antifungal prophylaxis must be considered also for patients receiving consolidation CHT for AML.

P215

TARGETING THE LEUKEMIA CELL METABOLISM FOR THERAPEUTIC INTERVENTION

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Cancer cells are characterized by perturbations of their metabolic processes. Recent observations demonstrated that the fatty acid oxidation (FAO) pathway may represent an important alternative carbon source in different tumors, appearing therefore particularly promising for therapeutic purposes. The carnitine palmitoyl transferase 1a (CPT1a) catalyzes the rate limiting step for the entry of fatty acids into the mitochondria. It has been previously demonstrated that CPT1a interacts with members of the Bcl-2 family, and its inhibition can cause an accumulation of the toxic metabolite palmitate, resulting in mitochondrial damage and cell death. In this study we investigated the *in vitro* anti-leukemic activity of the novel CPT1a-inhibitor ST1326 (1-50 M) on acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL) and chronic lymphoblastic leukemia (CLL) cell lines and primary cells. We first demonstrated that all leukemia cell lines constitutively expressed CPT1a and that this enzyme activity was significantly inhibited by ST1326. Real-time metabolic analysis in cell lines and primary samples documented

that ST1326 was effective in inducing dose-dependent FAO inhibition. We next analyzed the cytotoxic activity of ST1326 in leukemia cell models of AML, ALL, CLL, demonstrating that the CPT1a inhibitor induced dose- and time-dependent cell growth arrest and apoptosis. To determine the effects of ST1326 on intracellular palmitate accumulation, we quantified the BODIPY-C16 uptake demonstrating a significant block of palmitate metabolism in cells exposed to ST1326. Data obtained on primary hematopoietic malignant cells confirmed the FAO inhibition and the cytotoxic activity of ST1326, particularly on AML cells: apoptotic cells increased from 20.5%±12.2 (control) to 36.6%±20.0 (p=0.23), 33.2%±18.6 (p=0.0015), 37.1%±18.4 (p=0.0006) and 67.5%±16.6 (p<0.0001) following 72 hours of exposure to 5, 10, 20 and 50 M ST1326, respectively. Conversely, no cytotoxic effects were detected on normal BM CD34+ cells. Lastly, combination experiments with ST1326 and AraC in cell lines and in primary AML samples demonstrated the favourable interaction of ST1326 with standard chemotherapy. In conclusion, we show that FAO is a crucial metabolic pathway for leukemic cells, controlling in addition to energy production, the leukemia cell growth and fate. These data suggest that leukemia treatment may be carried out by using FAO inhibitors as novel therapeutic approach.

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SAFETY AND FEASIBILITY OF CONSOLIDATION CHEMOTHERAPY WITH HIGH DOSES OF CYTARABINE IN OLDER PATIENTS WITH ACUTE MYELOID LEUKAEMIA

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Introduction: The safety and feasibility of consolidation chemotherapy with high doses of cytarabine (HD-ARAC) in older patients with acute myeloid leukaemia (AML) is not clearly defined. Aim of this study is to verify the safety and feasibility of consolidation with HD-ARAC in a population of AML patients aged more than 60 years in first complete remission (CR) after intensive chemotherapy (IC). *Patients and Methods:* Retrospectively, we reviewed 82 consecutive patients with a median age of 68 years (range 61-75 years) in first CR after induction with IC ("3+7": idarubicin and cytarabine; ICE: idarubicin, etoposide and cytarabine; FLAG: fludarabine and HD-ARAC), treated with HD-ARAC as consolidation therapy at Division of Hematology, Niguarda Hospital, Milan. At diagnosis, karyotype and mutation for NPM1 and FLT3 were available in 82 (100%), 52 (63%) and 64 (78%) patients, respectively. According to the NCCN risk based classification (2015 edition), 16 patients were included in the low risk, 45 in the intermediate and the latter 21 patients in the high risk group. According to the genetic risk, fitness status and availability of a suitable donor, patients in first CR were submitted to one course of cytarabine 1 gr/mq q12h x 3 consecutive days+idarubicin 8 mg/mq for 2 days followed by 1 or 2 courses of cytarabine 1 gr/mq q12h x 3 days. Patients submitted to allo-SCT received only one cycle of HD-ARAC+idarubicin. A database including specific data was created, reviewed for consistency and completeness and submitted for statistical analysis with the aim to study the feasibility and the mortality of our protocol. *Results:* One-hundred-sixty-seven courses of HD-ARAC were administered to 82 patients. The overall mortality rate was 1,2% (only one patient died for acute bowel perforation during the first cycle of consolidation). Sixteen patients (19.5%) interrupted the consolidation programme. Causes for drop-out were: severe organ toxicity (4 patients, 5%; namely we recorded 3 cases of pneumonitis and one abdominal abscess); early relapse (7 patients, 8.5%); other medical reasons (5 patients, 6%). *Conclusions:* This single centre experience in elderly patients with AML shows that a programme of consolidation with HD-ARAC is feasible in 80% of cases and carries a low treatment-related mortality (1%).

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NPM1 MUTATED ACUTE MYELOID LEUKEMIA WITH MONOBLASTIC DIFFERENTIATION, HEMOPHAGOCYTOSIS BY LEUKEMIC BLASTS AND ABNORMAL KARYOTYPE

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Acute monoblastic leukemia comprises <5% of cases of acute myeloid leukemia (AML). Hemophagocytosis by leukemic blasts is a rare phenomenon known to be associated with certain chromosomal changes, including t(8;16), der(8), inv(8), and t(16;21). A 41-year old man presented to the Hematology ward in February 2015 for fever, generalized bone pain and bleeding manifestations. Blood works showed a mild leukocytosis (WBC $12x10^9$/L) and a moderate thrombocytopenia (PLT $48x10^9$/L); blood coagulation parameters documented overt disseminated intravascular coagulation. Peripheral blood (PB) smear showed an altered differential count with the presence of 24% monoblasts. Bone marrow (BM) aspirate and touch imprint showed a wide population (>80% of BM cellularity) of large-sized blasts, with intense basophilic cytoplasm and frequent figures of hemophagocytosis (Figure 1). Cytochemical investigation were consistent with monocytic lineage (α-naphthyl-butyrate esterase intense positivity, Sudan Black B negativity). Several mitotic figures were also present. Molecular analysis documented the presence of NPM1 mutation, along with FLT3 D835. FLT3-ITD was absent. Surprisingly, G banding analysis showed an abnormal karyotype: 47,XY,+8[14]/ 48,idem,+8[2]/ 48,XY,+i(5)(p10),+8[2]/ 46,XY[2]. Immunohistochemical investigation on BM trephine biopsy confirmed a cytoplasmic pattern of NPM1 positivity (clone 376), consistent with molecular findings. We report the case of an adult with AML with monoblastic differentiation showing hemophagocytosis by leukemic blasts with a peculiar molecular and cytogenetic profile. To our knowledge, no previous cases of NPM1 mutated AML with this specific morphologic features have been reported in literature.

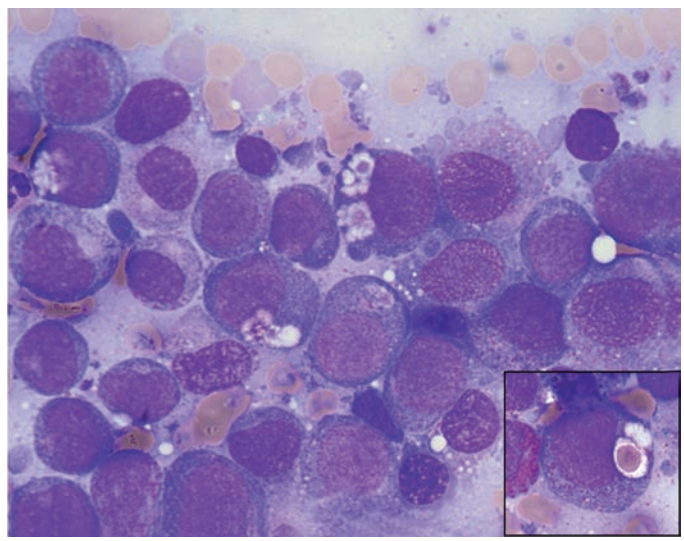

Figure 1.

P218

AZACYTIDINE THERAPY IN ELDERLY PREVIOUSLY UNTREATED AML PATIENTS: COMPARISON WITH CONVENTIONAL CHEMOTHERAPY IN A MATCHED-PAIRED ANALYSIS

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The hypomethylating agent azacytidine (AZA) is increasingly used in acute myeloid leukemia patients (AML) with low marrow blast count or in elderly patients who are not eligible for intensive chemotherapy. We

retrospectively analyzed the outcome of 42 elderly AML patients who received AZA as front line treatment, compared to a control series of patients treated with conventional chemotherapy, selected through a matched-paired analysis. Relevant comorbidities (diabetes mellitus, heart disease, hypertension, liver or lung diseases) were present in 70% of patients receiving AZA. AZA was delivered at the dosage of 75 mg/sqm s.c. with a 5+2 days schedule. AZA therapy was continued until unacceptable toxicity or disease progression, regardless of response achieved. Conventional treatment consisted in a three-days fludarabine-containing regimen with age-adjusted doses (FLAI-3). Major infection during therapy were observed in 20/42 (47.6%) patients in AZA group and 15/42 (35.7%) in FLAI-3 group (p=0.356). Four and two patients died during induction in AZA and FLAI-3 group, respectively. Complete remission (CR) rates were significantly lower in AZA-treated patients (7/38, 18%), compared to FLAI-3 (21/40, 53%) (p=0.002). Of note, none of the nine patients treated with AZA who presented with leukocytosis achieved CR, compared with 6/15 (40%) patients in the FLAI-3 cohort. However, this did not translate in a significant lower OS for patients with leukocytosis treated with AZA (p=0.120). After a median follow-up of 25 months, 2-years overall survival (OS) was 36.2% in AZA group (median 14 months), significantly higher than FLAI-3 group (11.2%, median 9 months, p=0.048, Figure1). OS duration was significantly influenced only by achievement of CR in FLAI-3 cohort (p=0.000), whereas in AZA cohort was influenced only by younger age at diagnosis (p=0.048). Achievement of CR had only a borderline effect on OS duration in AZA cohort (p=0.050). AZA was generally well tolerated, with acceptable toxicity profile, even in our cohort of very frail untreated AML patients. Even if CR rates were significantly lower when compared to a matched-paired cohort of conventionally-treated patients, OS rate were not inferior. Our data suggest that Azacytidine is a safe first-line therapy for frail AML patients, with comparable effectiveness with conventional intensive chemotherapy.

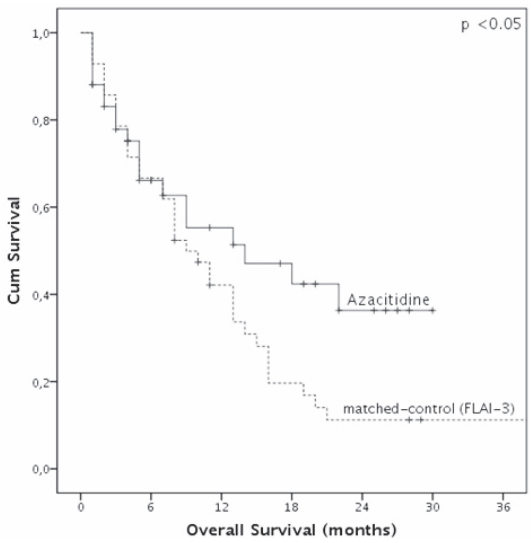

Figure 1.

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HIGH CURE RATE OF YOUNGER AML PATIENTS WITH FLUDARABINE, CYTARABINE AND IDARUBICINE INDUCTION AND RISK ORIENTED CONSOLIDATION

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Conventional induction therapy of acute myeloid leukemia (AML) is still largely based on the combination of cytarabine (ARA-C) and daunorubicin. In the last two decades alternative drug combinations have been tested in order to improve complete remission (CR) rate and quality of remission. Fludarabine has been shown to enhance ara-CTP accumulation in leukemic blasts and to inhibit DNA repair mechanisms, thus providing a rationale for combination with DNA damaging agents. One hundred-five consecutive non M3 AML patients (age 17-72 years) treated in our center between 2004 and 2013 were retrospectively analyzed. Induction regimen included fludarabine 30 mg/sqm and ARA-C 2g/sqm on days 1 to 5 plus idarubicin (IDA) 10 mg/sqm on days 1-3-5, with or without gemtuzumab ozogamicin (3mg/sqm) on day 6. Patients achieving complete remission received a second course including ARA-C 2g/sqm on days 1 to 5 and IDA at an increased dose of 12 mg/sqm on days 1-3-5. Patients were stratified in prognostic risk groups according to a comprehensive score based on karyotype, *de novo* or secondary disease, NPM and FLT3 status. High-risk and selected intermediate-risk patients underwent allogeneic bone marrow transplantation (BMT) in first CR if a donor was available. The other patients were scheduled to receive 3 courses of consolidation therapy with high doses ARA-C. Five (4.8%) and nine patients (8.6%) died during the first 30 and 60 days, respectively. After 1st induction cycle 83 patients achieved CR (79%) and 17 did not respond (16%). After cycle 2, CR rate was 84% (88/105). FLAI-5 was generally well tolerated, with negligible non-hematological toxicity. Most patients were able to receive subsequent therapy at full dose and in a timely manner; 29/88 CR patients underwent BMT in first CR whereas 41 out of the 59 not scheduled for an early transplant (70%) received the full programmed therapy. In the whole cohort, 3 years DFS and OS were 42.8% and 48.3% (median 34 and 35 months, respectively). Patients not undergoing early allo-BMT and receiving the full planned dose had a very good outcome, with a 3-year DFS and OS of 71.2% and 100%, respectively (medians not reached). Our results are comparable to those recently published and show that delivering only one fludarabine containing regimen may significantly reduce hematological toxicity therefore allowing patients to proceed along the risk tailored consolidation program in a good performance status (Figure 1).

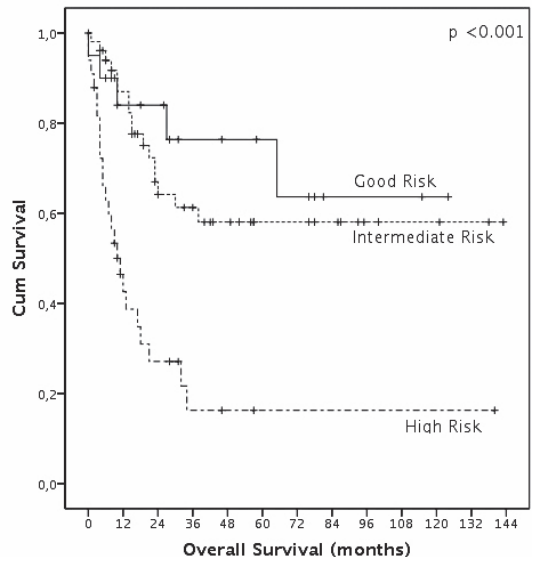

Figure 1.

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

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The negative prognostic impact of FLT3-ITD mutations in acute myeloid leukemia (AML) patients is well known. On the contrary, NPM1 gene mutation has been shown to confer a good prognosis. The co-existence of both mutations is common with most groups reporting a predominant negative prognostic impact of FLT3. One-hundred twenty four

consecutive FLT3-ITD and/or NPM1 positive non M3 AML patients (age 17-78 years) were retrospectively included in this study. Induction regimen consisted either in FLAI-5 schedule (fludarabine 30 mg/sqm and ARA-C 2g/sqm on days 1 to 5 plus idarubicin 10 mg/sqm on days 1-3-5, accounting for 90 patients) or standard 3+7 induction. (34 patients). In both centers, patients with FLT3-ITD mutation underwent allogeneic bone marrow transplantation (BMT) in first complete remission if feasible. Demographical, cytogenetic and molecular characteristics were comparable in the series of patients treated in the two centers and in patients receiving the two induction regimens. Two patients (5.9%) died during first induction in the 3+7 arm,whereas two patients (2.2%) died in the FLAI arm, mostly due to infections. One further patient in the FLAI arm died during second cycle. Overall 60-days mortality was therefore 4%. After two induction cycles, 105 patients achieved CR (88%) and 14 did not respond (16%). CR rate after cycle 1 was significantly higher in FLAI-5 cohort (86% *vs* 69%, p<0.05). Patients treated with FLAI-5 had significant higher probability of achieving MRD clearance, both on NPM and MFC-based evaluation (p<0.001 and p<0.05, respectively). After a median follow up of 51 months, 2-year DFS was 53.4% (median 101 months). DFS duration was significantly influenced by induction type (p <0.001, Figure 1), risk group, status for FLT3 and NPM1, NPM-MRD and MFC-MRD status (p <0.03, p<0.05, p<0.05, p<0.001, p<0.01, respectively). Survival did not significantly differ between patients achieving MFC-MRD negativity with FLAI 5 or "3+7". Among patients with FLT3-ITD and concomitant NPM mutation, the difference between FLAI and 3+7 cohorts in term of DFS was higher (p <0.0001, Figure 1). No significant DFS difference was observed in FLT3-ITD/NPM wild type patients. Overall Survival analysis led to similar results. Our study seems to indicate that fludarabine containing induction improves quality of response in patients carrying NPM1 mutation compared to 3+7 regimen, thus possibly overcoming the negative prognostic impact of FLT3-ITD.

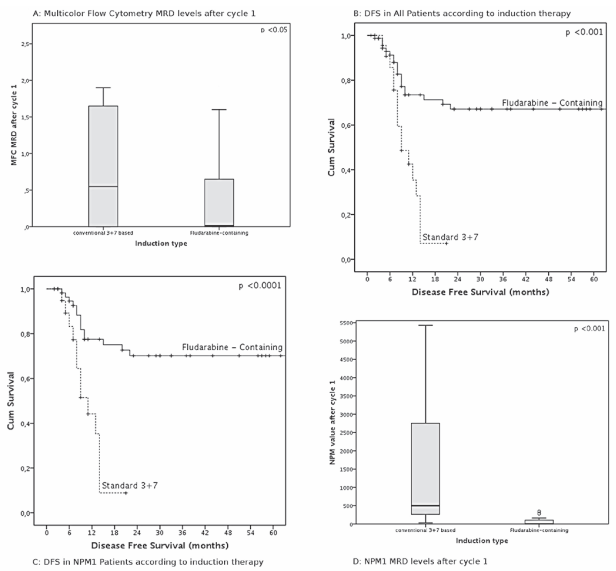

Figure 1. MRD and DFS according to induction regimen.

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MRD ANALYSIS IN 126 ACUTE MYELOID LEUKEMIA PATIENTS: POST-INDUCTION WT1 STATUS SIGNIFICANTLY PREDICTS EARLY RELAPSE

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Post induction and consolidation MRD might represent a new prognostic factor predicting AML outcome besides patients and disease characteristics. We have evaluated post induction and consolidation bone marrow mimimal residual disease (MRD) in 126 AML patients (median age: 61.5 years, range: 17-89) with 20 months median follow-up (range 2-79.9). We analysed abnormal leukemia immunophenotype (ALIP) by multiparameter flow cytometry (MPFC) and WT1 by RT-PCR as described by Buccisano *et al.* and Cilloni *et al.* Cytogenetic, NPM and FLT3 status were performed in 111, 97 and 119 patients respectively, defining the molecular cytogenetic risk in 96 patients. WT1 was +ve in 91/114 patients (70%) at diagnosis (median 1,231.5; range:2 -268,784), in 11/71 (15.5%) post induction (median 1216; range:260-134,633) and in 8/66 (12,1%) post consolidation (median 627.8; range:258-45,338). MPFC MRD was +ve in 33/66 (50%) patients after induction and in 18/48 (37.5%) after consolidation. We analysed overall, 3 and 12 month Cumulative Incidence of Relapse (CIR) adjusted by MRD status, patients and disease characteristics. 82/99 patients achieved CR, 40 relapsed in a median of 8 months (1-52 months) with 52.5% 3 yr CIR: 13 relapsed at 3 months, 29 at 12 months. Patients receiving chemotherapy (38), Autologous (14) and Allogeneic Transplant (39) as post consolidation treatment had 51,2%, 45,7% and 60% 3 yr CIR respectively. NPM+FLT3- patients had 55% 3 yr CIR, compared to 35,5% in NPM-FLT3-, 50% in NPM+FLT3+ and 47% in NPM-FLT3+. Patients with WT1 positive post induction and consolidation had a 3 month CIR respectively of 60% and 44% and a 12 month CIR of 90% and 72.5% respectively. Patients with MPFC positive post induction and consolidation had a 3 month CIR of 26.3% and 34.5% respectively and a 12 month RFS of 48.6% and 44.5% respectively. Multivariate analysis identified post induction WT1 positive status as the main predictor of 3 and 12 month CIR. Patients with WT1 positive after induction had a 30.4 RR of 3 month CIR (p<0.0001) and a 15.8 RR of 12 month CIR(p<0.0001) in comparison to WT1 negative patients (Figure 1). Hyperleukocytosis (WBC count >50,000/mcl at diagnosis) and age>60 yrs also significantly predicted 12 month CIR with a RR of 7.22 (p=0.002) and 9.1 (p=0.001) respectively. In conclusion WT1 post induction status confirmed its prognostic significance in our series targeting a subset of early relapsing patients with an extremely poor outcome.

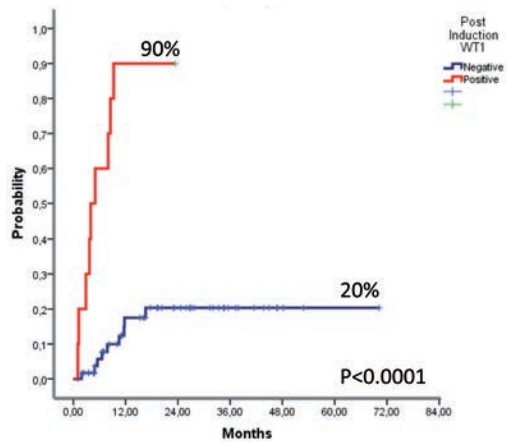

Figure 1. 12 months CIR* by WT1 post-induction status.

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PERSISTENCE OF MULTIPLE CLONAL CYTOGENETIC ABNORMALITIES OVER A 22-YEAR PERIOD IN A PATIENT WITH AML IN SECOND HEMATOLOGIC REMISSION WITHOUT RELAPSE OF LEUKEMIA

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We describe a person with acute myeloid leukaemia who achieved a complete haematological remission but who had persisting clonal cytogenetic abnormalities for 22 years and in whom leukaemia never recurred.

AML, FAB M2, cytogenetic unknown was diagnosed in a 50 years old patient in November, 1988. Complete remission (CR) was achieved after cytarabine, daunorubicin and etoposide. Autologous marrow transplant after TBI/Cyclophosphamide was performed as consolidation. In June, 1992 relapse occurred with a complex karyotype and a second CR was achieved with HD-cytarabine and daunorubicin. However, cytogenetic abnormalities persisted and he received interleukin-2 from September, 1992 until January, 2009. In spite of normal peripheral blood counts, cytogenetic abnormalities always persisted in yearly marrow controls up to December, 2014, in a variable percentage, some of which persisted longer, others for only small periods. In particular del(20)(q11.2) from 1995, with additional recurrent translocations, persisted and increased over time (Figure 1).

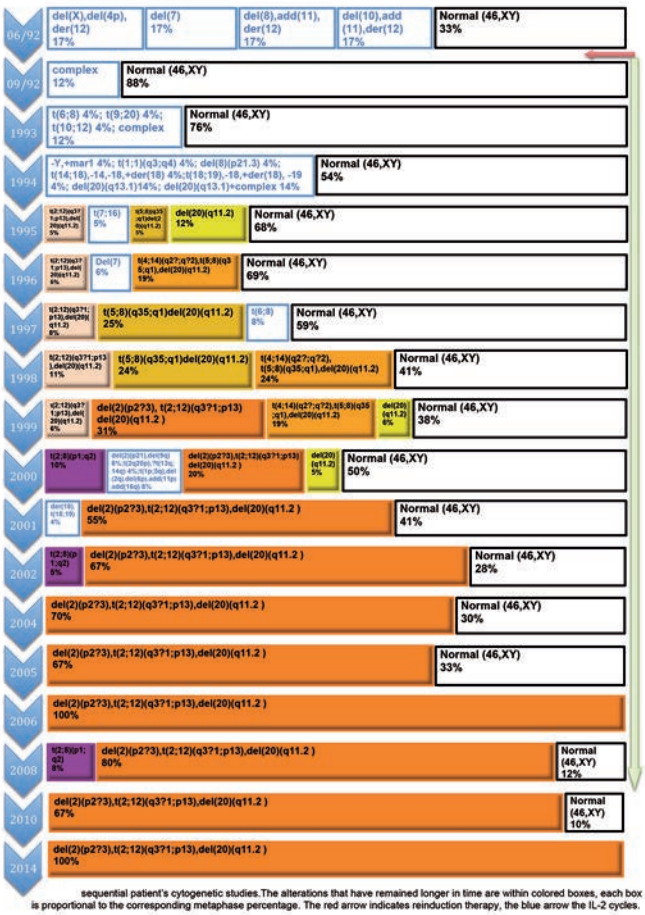

Figure 1.

Bone marrow histology was always normocellular with slight dysplastic maturation of erythroid and megakaryocyte lineages, less than 5% blasts and some infiltration of mature B- and T-lymphocytes. CD34-positive cells were typically 1% and WT-1 levels were normal. He died in February, 2015 of metastatic undifferentiated neoplasm of unknown epithelial origin, diagnosed on a biopsy of a liver metastasis showing cytokeratin positivity (no evidence of blood or bone marrow involvement). While further molecular studies are in progress to analyse the involvement in the cytogenetic abnormalities of genes of potential interest (*e.g.* ETV6 on 12p13) and to detect further kryptic abnormalities by array-CGH, these case demonstrates that cytogenetic abnormalities, even when they are clonal and even when they are abnormalities typically-associated with leukaemia do not automatically eventuate in leukemia. Potential explanations include their occurrence in a cell lacking the biological capacity to cause leukaemia such as no or limited self-renewal potential, their compatibility with differentiation and death of cells from the transformed clone, the impairment of self-renewal such that clonal expansion does not occur and leukaemia does not develop at a clinically-detectable level. *Conclusions:* caution in interpretation of clonal cytogenetic abnormalities in persons with AML with normal blood and bone marrow parameters is needed.

P223

BAALC GENE EXPRESSION LEVEL DURING INDUCTION PREDICTS RELAPSE IN CBF-LAM

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Acute myeloid leukemia (AML) is a heterogeneous disease with outcome dependent upon several factors. Core-binding factor acute myeloid leukemia (CBF-AML) –including AML with t(8;21) and with inv(16)– accounts for about 15% of adult AML and is associated with a relatively favorable prognosis. Nonetheless, relapse incidence may reach 40% in these patients. In this context, identification of prognostic markers is considered of great interest. A lot of work has been directed toward identification of the modulated expression of genes important for proliferation and differentiation of hematopoietic cells, such as the human brain and acute leukemia, cytoplasmic (BAALC) gene. Higher BAALC mRNA levels have been observed in blasts in AML and high expression has been shown to be an adverse risk factor in CN-AML. *Aims:* In the present study we investigated whether assessment of BAALC transcript levels in bone marrow during standard induction therapy correlates with CR achievement, outcome and relapse. We compared BAALC expression and AML1ETO e CBFB/MYH11 gene levels at diagnosis and during follow up. *Methods:* We serially monitored BAALC gene levels by RQ-PCR at diagnosis and during follow-up on BM samples from 4 CBF-AML patients (n=5-19 detections), measuring at the same time points CBF-gene levels. *Results:* BAALC expression levels at diagnosis and in serial follow up samples of four CBF-AML patients has been compared to CBF gene levels. 4 patients were negative to c-kit mutation, to FLT3-TKD and TID mutation and to additional chromosomal abnormalities, then showed haematological and complete morphological remission. In all patients the morphological relapse was always subsequent to an increase of CBF-gene level monitored by RT-PCR. All patients showed high BAALC levels at diagnosis and a marked reduction at the first complete remission; BAALC level remained below the median value during the follow up, with a rapid increase at the relapse, preceding significantly both molecular CBF-gene analysis alteration and morphological relapse (about a month for each patient, Figure 1, patient #1).

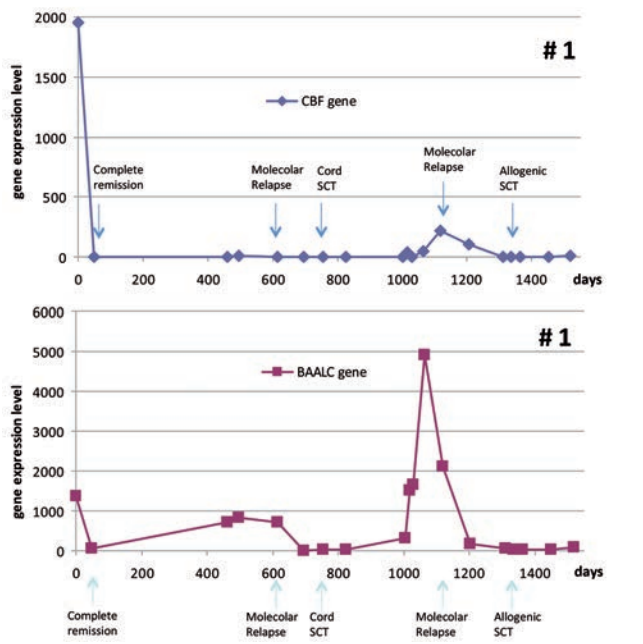

Figure 1.

Conclusions: Despite the low number of cases analyzed our data suggest the utility of BAALC expression as a marker for prediction of clinical outcomes and meaningfull for MRD in CBF-AML. Patients with low reduction of BAALC have high probability of being refractory or early relapsing, and could be immediately switched to intensification of therapy. A larger prospective study should confirm our results.

Non-Hodgkin Lymphoma 2

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A COMBINATION OF LENALIDOMIDE AND RITUXIMAB AS SALVAGE THERAPY IN ELDERLY PATIENTS AFFECTED BY DIFFUSE LARGE B CELLS LYMPHOMA RELAPSED AND REFRACTORY

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Background: The incidence of non Hodgkin's lymphoma (NHL) increases with age, over one third of NHL cases involves elderly patients >70 years of age. As a matter of fact, many elderly patients (pts) are not enrolled in controlled studies because they do not meet the inclusion criteria. This is the reason why the data from trials on elderly cases are not representative for the whole elderly population. Consequently, many of these patients do not benefit of new therapeutic progresses and the treatment is not yet adequate. Moreover in case of relapse, salvage therapy in the elderly is virtually absent, and the prognosis is extremely poor. Lenalidomide is an immunomodulatory drug with anti-angiogenetic and anti-neoplastic action on cancer cells and also has other anti-tumor activity by acting on the neoplastic microenvironment. Both monotherapy and combination of Lenalidomide with rituximab have shown efficacy in terms of overall response rate (ORR, Overall Response Rate) in the setting of salvage therapy in relapsed/refractory DLBCL, with an acceptable rate of hematologic and extra-hematological toxicities. *Patients and Methods:* In the period 2013-2014 we consecutively treated 12 elderly patients affected by advanced DLBCL relapsed/refractory. Median age at the start of treatment was 79 years (yrs) (62-86), 5 out 12 was over 80 yrs and 4 out 12 over 75 yrs. The median number of previous treatment was 3 (2-4), 3 pts were transplanted and 4 patients was refractory to previous line of therapy. The treatment scheme included at first cycle: Rituximab 375 mg/m^2 i.v. days 1, 8, 15, 22, Desametasone 5 mg p.o. days 1, 8, 15, 22 and Lenalidomide 15 mg/die p.o. from day 2 to 22. From the second to the sixth cycle we administered Rituximab 375 mg/m^2 i.v. days 1, 14, Lenalidomide 20 mg/die p.o. from day 2 to 22. *Results:* The overall response rate has been 75% (CR,PR,SD), 3 patients out 12 (25%) achieved a CR and 3 PR (25%). The median PFS was 6 months and with a median follow up of 1 year the overall survival is 25%.(Figure 1) All deaths are due to lymphoma progression and the 3 patients in CR are still alive in CCR. *Conclusions:* In this elderly and heavily pretreated group of patients our scheme containing Lenalidomide-Rituximab has shown an high rate of response with a good rate of CR and a promising trend in overall survival. The treatment appears feasible and safe also in this particular group of frail patients and even if we need more data to confirm we think it will be possible to extend this therapy in frail elderly patients in a most precocious line of treatment.

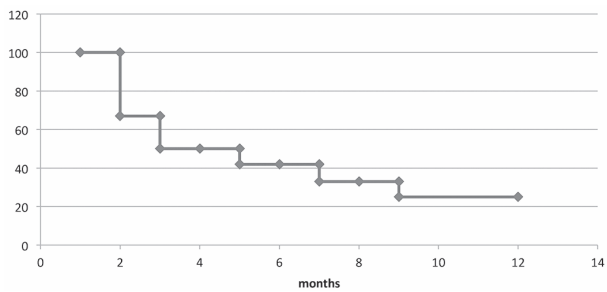

Figure 1. Overall Survival.

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LONG TERM OUTCOME OF PATIENTS WITH GASTRIC MARGINAL ZONE LYMPHOMA RECEIVING FLUDARABINE, MITOXANTRONE AND RITUXIMAB AS FIRST-LINE TREATMENT

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Background: Gastric mucosa-associated lymphoid tissue (MALT) B-cell lymphoma is characterized by a well established association with Helicobacter pylori (HP) infection. There is no consensus on the standard management for patients with HP negative or persistent disease after HP eradication. Current approaches include radiation therapy, chemotherapy and immunotherapy with rituximab, alone or in combination. Evidence about the superiority of a specific regimen toward others is still lacking. *Aims:* We have investigated long-term efficacy and safety of fludarabine and mitoxantrone in association with rituximab (R-FM) as first-line treatment for gastric MALT lymphoma with HP negative or persistent disease after HP eradication. *Methods:* A cohort of 13 patients (median age 65 yrs) diagnosed with gastric MALT lymphoma was retrospectively analyzed. Five patients had stage I disease, 7 stage II and 1 stage IV, HP was positive in 9/13 patients. Induction treatment consisted of fludarabine (25mg/m^2 i.v. on days 2 to 4), mitoxantrone (10mg/m^2 i.v. on day 2) and rituximab (375 mg/m^2 i.v. on day 1), for up to 6 cycles every 28 days. Final response assessment including esophagogastroduodenoscopy with random biopsies and CT scan was done according to the 2007 Revised Response Criteria. *Results:* All patients (13/13, 100%) achieved a complete remission (CR), a median of 4 cycles (range 3-6) of R-FM were given. Treatment-related toxicities were mainly hematologic, with grade 3-4 neutropenia in 11/13 patients (84.6%), grade 2 thrombocytopenia and anemia in 1 and 2 patients. All but one cases required secondary neutropenia prophylaxis with filgrastim, 1 patient had grade 3 febrile neutropenia and was hospitalized while on treatment because of non-neutropenic fever with detection of Candida Albicans by stool culture. Two patients developed prolonged pancytopenia, grade II nausea was documented in 4/13 patients. After a median follow-up of 60 months (range 24-110) 1/13 had disease relapse after 8 months; estimated 9-year progression-free survival and overall survival were 92.4% (Figure 1) and 100%, respectively. Summary/*Conclusions:* R-FM regimen has a high long-term efficacy as front-line treatment for gastric MALT lymphoma. The high incidence of grade 3-4 hematological toxicity makes this treatment less safe compared to other regimens such as rituximab in combination with chlorambucil or bendamustine. It could remain a suitable option for advanced-stage disease, while exluding stage I.

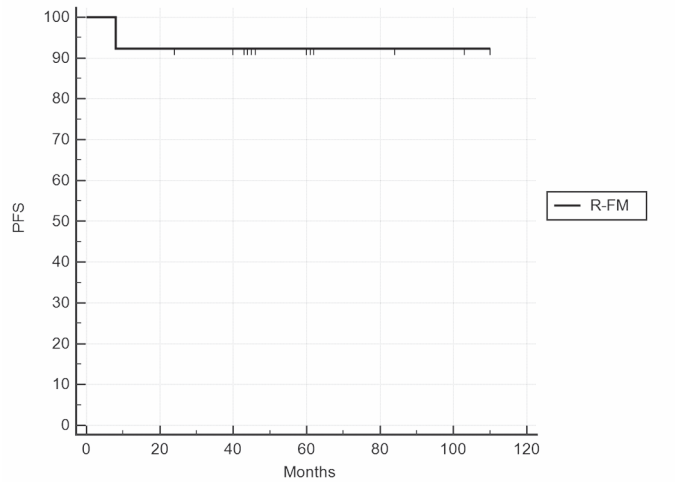

Figure 1.

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PRIMARY CARDIAC LYPHOMA: CLINICAL MANAGEMENT OF A RARE ENTITY

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Primary cardiac lymphoma (PCL) is a rare entity, defined as a lymphoma involving the heart, with the main bulk of the disease localized inside pericardium. The prognosis is poor, since diagnosis is often de-

layed, and median survival is less than 12 months. We observed 3 PCL over 458 aggressive lymphomas newly diagnosed from 2002 to 2014 (0.6%). Histology was DLBCL in all PCL pts. Two pts were male, one pt was 44 while two pts were older than 65. None of the pts had a previous history of cardiac disease and HIV was negative in all cases. Diagnosis was performed by right heart mass biopsy in 2/3 pts and by mediastinal lymphnode biopsy in the remaining pt. Cardiac biopsy was performed after sternotomy and after transjugular catheter in one case each. Clinical presentation included dyspnea and precordial pain in 2 pts, palpitations and cough in 1 pt, and 2 pts had B symptoms. Staging at baseline and at the end of treatment consisted in CT, PET, Cardiac Magnetic Resonance Imaging (MRI) and trans-thoracic echocardiography (TTE) in all pts. Two pts had mediastinal lymphnodes in addiction to heart localization, which involved right chambers in all cases. All pts received pre-treatment with steroid (prednisone 1 mg/kg/day from day-7) and vincristine (1 mg ev fixed dose day-7), followed by 6 cycles of COMP (Myocet® not pegilated Lyposomal Doxorubicin) and Rituximab ev. No acute cardiac event occurred during treatment. At final restaging all pts obtained Complete Remission (CR) PET negative and no evidence of disease at cardiac MRI. With a median follow-up of 16 months, two pts are alive and in continue CR, while the youngest pt experienced an early extra-cardiac relapse, underwent salvage therapy with R-DHAP and is now receiving Autologous Transplantation in second CR. Asymptomatic late cardiac toxicity developed in 2 pts during post-remission surveillance: one pt started low dose β-blocker due to biventricular hypokinesia, while a 70 years old pt started ACE-inhibitors due to arrhythmia and troponin increased blood levels with findings at TTE of cardiac remodeling. Our data suggest that PCL outcome has improved in the modern era, thanks to better diagnostic tools, which allow earlier diagnosis, and more effective treatment, including Rituximab and not pegilated Lyposomal Doxorubicin. Steroids and vincristine pre-treatment allowed to deliver full dose chemotherapy without acute cardiac morbidity, and should be considered in the management of this rare entity.

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EXTRANODAL MARGINAL ZONE NON HODGKIN'S LYMPHOMA OF THE LUNG: OUR EXPERIENCE WITH RITUXIMAB AND CHLORAMBUCIL-CONTAINING REGIMENS

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Purpose: Bronchial-associated lymphoid tissue (BALT) lymphoma is a rare subtype of low grade marginal zone B-cell lymphoma representing 10% of all MALT lymphomas. The aim of this study was to review data of patients with BALT lymphoma treated in our Institution. *Patients and Methods:* An observational retrospective study was performed on clinical data from all patients with histologically confirmed diagnosis of BALT lymphoma. We focus our attention on all the patients treated with Rituximab and Chlorambucil according to this scheme: rituximab 375 mg/sqm weekly for 4 doses, then monthly for 4 infusions in combination with chlorambucil at the dosage of 0,1 mg/Kg/die for 45 days, then on days 1 to 15 monthly for 4 months. *Results:* From January 2000 to February 2013, 16 patients were diagnosed and treated in our Institution, 14 of them uniformly treated with the scheme RC. The median age at diagnosis was 72 years (range 56-85). The diagnosis of BALT in 1 patients was associated with Sjogren Syndrome, in 1 patients with a positive HBV.Bone marrow biopsy was negative in 10 patients, positive in 1 and not performed in 3 patients. Stage was IA in 9 patients (64%), IB in 1, IIA in 1 patients and IIB in 1, IVA and IVB in 2 patients respectively. At the end of treatment 11 patients (79%) achieved a complete remission and 3 (21%) a partial remission with an overall response rate of 100%. With a median observation period of 52 months (range 1-133) the overall survival was 79%. The 3 patients who obtained a partial remission died, 1 for disease progression (18 months from the initiation of therapy), and 2 for causes not related to lymphoma (respectively 22 months and 1 months from the date of diagnosis). No relevant toxicities were registered, in particular no pulmonary infection or other type of infections were reported. *Conclusions:*After a long follow-up our results support the combination of Rituximab and Chlorambucil as first-line therapy of BALT lymphomas as low toxic, feasible and effective treatment.

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RITUXIMAB WITH OR WITHOUT CHLORAMBUCIL FOR THE TREATMENT OF EXTRANODAL MARGINAL ZONE B-CELL LYMPHOMA: RESULTS OF A RETROSPECTIVE MONOCENTRIC STUDY

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Purpose: Apart from localized gastric disease, there is no consensus on standard initial treatment of mucosa-associated lymphoid tissue lymphoma. This study was launched to evaluate the use of Rituximab (R) alone or plus Chlorambucil (RC) in patients not previously given systemic anticancer therapy. *Patients and Methods:* All patients with histologically confirmed diagnosis of MALT lymphoma were selected from our data base. Only patients treated with R or RC were analyzed and evaluated. The scheme RC was the follow: rituximab 375 mg/sqm weekly for 4 doses, then monthly for 4 infusions alone or in combination with chlorambucil at the dosage of 0,1 mg/Kg/die for 45 days, then on days 1 to 15 monthly for 4 months. *Results:* From January 2000 to February 2013, 136 patients had a diagnosis of MALT lymphoma and treated in our Institution, 76 of them uniformly treated with R or RC. These cohort of patients were considered for the study. The median age at diagnosis was 68 years (range 32-85). In 20 patients the disease was localized in conjunctiva; 17 in the stomach; 14 in the lung; 13 in salivary glands; 3 intestine; 2 respectively in lacrimal gland, liver, skin; 1 in breast, cheek and tongue. Stage was IA in 63 patients (83%), IB in 1, IIA in 5 patients and IIB in 1, IIIA in 1 patient, IVA in 4 patients and IVB in 1. Bone marrow biopsy was negative in 59 patients, positive in 5 and not performed in 12 patients. The diagnosis of MALT in 4 patients was associated with Sjogren Syndrome, in 4 patients with a positive HCV and in 1 with sclerodermia. According to treatment, 61 patients were treated with Rituximab plus Chlorambucil; 15 were treated with Rituximab alone. At the end of treatment 68 patients (89%) obtained a complete remission and 7 (11%) a partial remission with an overall response rate of 100%. With a median observation period of 65 months (range 1-158) the overall survival was 88%. 9 patients died, 6 for disease progression (3 after a relapse) and 3 for causes not related to lymphoma. 12 patients (16%) relapsed, 8 of these patients were retreated with Rituximab and obtained a new complete remission, 1 is still on watch and wait regimen. Both treatments were well tolerated without unexpected toxicities. With a long observation period no second neoplasms were registered. *Conclusions:* After a long follow-up the combination of Rituximab and Chlorambucil or Rituximab alone proved to be low toxic, feasible and effective therapy for MALT lymphomas.

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PSYCHOLOGICAL DISTRESS AND ORAL CHEMOTHERAPY: A PILOT STUDY

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Oral chemotherapies (OC) have recently been introduced as support or replacement of the classic intravenous therapies, especially in elderly patients with lymphoma, in which relapses are frequent and intravenous chemotherapies give more difficulties. Even if OC are well-tolerated and easy to administer, given the commitment of the management of the therapy to the patients could lead to an increase in the psychological distress. In order to better prepare the patients to self-management the OC, at the Oral Chemotherapy Clinic of the "AOU Città della Salute e della Scienza" Hospital of Turin an hospital pharmacist assist the hematologist and the psycho-oncologist and provide detailed information regarding the treatment and the possible side effects. This longitudinal study aimed to assess anxiety, depression and the general well-being in patients with lymphoma, at the beginning (T0) and after three cycles (T1) of treatment with OC. It also investigated the levels of worry of the patients about the treatment management and the levels of satisfaction related to the presence of the pharmacist. Twenty-five patients were recruited and as-

sessed at T0 and T1 with the Functional Assessment of Cancer Therapy Scale-General (FACT-G) for quality of life and the Hospital Anxiety and Depression Scale (HADS) for psychological distress. In addition, at T1, seven Visual Analog Scales (VAS) were used to measure the level of worry about the treatment management and to judge the quality of the clinic. Patients had a mean (SD) age of 80.6 (5.4) years. 68% (17/25) of them had a low grade whereas 32% (8/25) had a high grade lymphoma. The FACT-G showed a mean score of 67.0 (12.0) at T0 and of 63.5 (15.6) at T1, suggesting that, even if the patients' quality of life is partially compromised by the cancer, the OC do not further worsen it. The HADS revealed a low presence of psychological distress, both at T0 (11.8 (6.1)) and at T1 (11.8 (8.2)). These scores are, in fact, below the cut-off of 15, generally used to identify the presence of psychological distress in cancer patients. The VAS scores showed a high satisfaction regarding the quality of the services and, in particular, regarding the presence of the pharmacist and the detailed information provided. In conclusion, although preliminary, our data suggested that OC seems not to negatively impact patients' quality of life and that a multidisciplinary approach contribute to prevent an increased in the psychological distress.

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THE TREATMENT OF PRIMARY MEDIASTINAL B CELL LYMPHOMA IN THE ERA OF DA-EPOCH-R

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Background: Primary mediastinal large B-cell lymphoma (PMBCL) is a B-cell lymphoma variant that arises from thymic cell. It occurs most frequently in young females and it is characterized by mediastinal bulky mass. At the moment the standard treatment is considered immunochemotherapy in association with mediastinal radiotherapy. *Purpose:* To compare standard treatment with regimens as R-CHOP14 or R-MACOP-B plus radiation therapy and DA-EPOCH-R protocol recently defined as the most effective treatment in large series of aggressive lymphoma. Moreover we want to see, in our monocentric casistic, if R-CHOP14 plus radiotherapy is equivalent to R-MACOP-B plus radiotherapy. *Patients and Methods:* We have retrospectively analysed all patients with PMBCL diagnosis between May 1999 and April 2014 treated in our Hematology Unit. Twenty previously untreated patients with PMBCL were selected. They were treated with a combination of immunochemotherapy regimen (R-MACOP-B or R-CHOP14) with mediastinal radiation therapy. *Results:* The patients had a median age of 35 (range, 21 to 53); 70% were women. Ten were treated with R-MACOP-B (arm A) and radiotherapy was performed in all but one patient due to mycosis pulmonary infection, ten patients were treated with R-CHOP14 (arm B) and radiotherapy. In arm A 9/10 patients obtained a complete remission and in arm B 10/10 patients obtained a complete remission after induction therapy. With a median follow-up of 39 months (range, 1 to 134) one patient die and the overall survival rate was 95%. Nineteen patients manteined the complete remission and we did not observe any relapse. No secondary neoplasms are reported in our cohort of patients. *Conclusions:* This retrospective study showed the equivalence of R-CHOP14/R-MACOP-B regimens *versus* DA-EPOCH-R in first line PMBCL treatment. Each regimen has, obviously, possible side effects, both in the short and long term in particular those associated to radiation therapy. To date, however, we have to consider new radiation techniques less harmful to the patient. Moreover we must consider the crucial role of PET in determining whether the RT is needed or not and we are waiting for results of prospective randomized study. The internal evaluation of the two schemes confirms that R-CHOP14 is absolutely comparable with R-MACOP-B in term of response to therapy, progression free survival and overall survival.

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ARE THE FOLLICULAR LYMPHOMAS CURABLE DISEASES? RETROSPECTIVE STUDY ON 146 PATIENTS WITH AT LEAST 10 YEARS OF OBSERVATION

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Purpose: Follicular lymphomas are usually defined as incurable disease. This study was launched to evaluate how many pts don't relapse or don't experience a new chemotherapy treatment. We aim to identify which clinical characteristics or therapeutical approaches are associated with this cohort of favourable patients. *Patients and Methods:* All pts with diagnosis of FL grade I-II or IIIa were selected starting from January 2000 till December 2004 in such a way to have at least 10 years of observation for alive pts. We considered pts obtaining at least a PR and we divided them in 2 cohorts, cohort 1 with pts relapsed or progressed and cohort 2 with pts never relapsed or progressed. *Results:* From January 2000 to December 2004, 146 patients were diagnosed and treated in our Institution. 13 pts were excluded from the analysis, 8 because of lost to the follow-up and 5 did not obtained at least a PR. Finally 133 pts were selected for the study. The median age at diagnosis was 61 years. Stage I-II in 47 patients, III-IV in 86. Bone marrow biopsy was positive in 87 pts, FLIPI 0-1 in 35, FLIPI 2-3 in 83 and FLIPI 4 in 15 patients. 96 patients were treated with antracycline containing regimens, 24 with fludarabine containing regimens and 13 were observed or treated with RT. Rituximab was used in 92 patients, as sequential treatment in 70 or chemotherapy combined in 22; 41 pts did not use rituximab. We analysed cohort 1 (85 pts) and cohort 2 (48 pts) and the statistically significant differences between the them were: elderly pts (p 0.05), symptomatic pts (p 0.05), FLIPI and FLIPI2 high score (p 0.005), lack of CR (p 0.0000) all observed in cohort 1. The OS with a median period of observation of 115 months was 71%, considering the two groups the OS in cohort 1 was 62% with a median of 142 months and it was 94% in cohort 2 with median not reached. In univariate analysis normal value of 2 microglobulin (p 0.05) and the use of rituximab (p 0.01) were associated with a better OS; in multivariate analysis treatment with rituximab manteined a statistically significance. *Conclusions:* This retrospective monocentric study confirms that about 1/3 of FL pts could be considered cured particularly if rituximab was used. At present all pts with FL are treated with combined immuno-chemotherapy, moreover after induction therapy patients are started on manteinance. We can therefore hope for the future in an improvement of survival results.

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EFFICACY AND SAFETY OF THE GERMAN SHORT INTENSIVE RITUXIMAB-CHEMOTHERAPY PROGRAM IN BURKITT LYMPHOMA AND LEUKEMIA, AND HIGHLY AGGRESSIVE DLBCL: A SINGLE CENTER EXPERIENCE

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Introduction: The short German intensive rituximab-chemotherapy regimen (B-NHL 2002) is highly effective in the treatment of Burkitt lymphoma and leukemia (BL-L). Diffuse large B-cell lymphoma (DLBCL) is a clinically and molecularly heterogeneous disease with disparate outcomes. A subgroup of DLBCL is often characterized by more aggressive clinical course, and one-third of the patients present with disease that is either refractory to initial standard therapy, such as R-CHOP regimen, or relapses. *Methods:* Adults and consecutive patients with newly diagnosed Burkitt lymphoma and leukemia,and Highly Aggressive DLBCL, were treated with the GMALL B-ALL/NHL2002 protocol. We considered DLBCL Highly Aggressive when characterized by high proliferative index Ki67 >90% ((B-cell lymphoma unclassified with features intermediate between diffuse large B-cell lymphoma and BL; ABC/non GC DLBCL; Double Hit Lymphoma; Richter Syndrome). Patients >55 years old received a reduced regimen. Rituximab was given before each cycle and twice as maintenance. We evaluated response and outcomes in all patients. *Results:* From February 2011 to March 2015 25 patients, 15 with BL-L and 10 with DLBCL (10) were treated with the GMALL protocol as first line therapy. Three patients were not included in the statistical analysis because their treatment was not yet completed (details on baseline characteristics of patients are reported in the Table 1). After a median follow up of 9 months (5- 46), Complete Remission (CR) was achieved in 81% of the patients (18 patients); 3 patients had refractory or relapsed and shifted to further treatments. 18 Patients who achieved CR are still alive, and 17 patients are in CCR; only one patient with BL relapsed after 12 months as a Follicular Lymphoma. Major grade III/IV toxicity was hematological: 100% suffered from neutropenia in at least 1 of the performed courses, thrombocytopenia (86%), and anemia (70%). Treat-

ment was complicated by neutropenic fever requiring hospitalization in 41%, and by grade III-IV mucositis in 54%. One patient developed acute renal failure, but regular course of treatment was not discontinued. One patient had treatment-related mortality. *Conclusions:* Our data indicate that a short intensive rituximab-chemotherapy regimen (B-NHL 2002) is highly effective and well tolerated in Burkitt lymphoma and leukemia, such as in particularly poor prognosis category of patients like highly aggressive NHLs.More studies with this combination are warranted.

Table 1. Baseline characteristics of patients.

Characteristics	BL-Leukemia	DLBCL	Total
N (%)	13(59%)	9 (40.9%)	22
Age, years median (range)	47 (22 – 72)	67 (51 – 71)	55 (22 – 72)
Sex, male/female	7 / 6	6 / 3	13 / 9
Complete remission N.(%)	11 (85)	7 (78)	18 (81)
Refractory/relapsed N.(%)	1 (7.6)	2(22)	3 (23)
Complete Continuous remission N.(%)	10	7	17 (94)
< 55 years old	10	1	11 (50)
>55 years old	3	8	11 (50)
Neutropenic fever	5	4	9 (41)
Grade III-IV mucositis	7	5	12(54)
Terapy-related mortality N.	1	0	1

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PERIPHERAL BLOOD LYMPHOCYTE TO MONOCYTE RATIO AT DIAGNOSIS PREDICT SURVIVAL IN ADULT PATIENTS WITH BURKITT LYMPHOMA

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The peripheral blood lymphocyte to monocyte ratio (LMR) at diagnosis is a powerful prognostic factor in patients with diffuse large B-cell lymphoma (Rambaldi *et al.*, 2013) but its value is unknown in other aggressive B-cell lymphomas. We evaluated the prognostic implications of LMR in a retrospective cohort of adult patients with Burkitt lymphoma. Clinical characteristics and outcome of 43 patients treated between 2002 and 2013 at our Center were gathered from electronic charts. Patients were characterized by a median age of 49 years (range 23-78) with 35% of the cases >55 years, M/F ratio was 2.07. Nineteen patients had a diagnosed as Burkitt-like lymphoma. There was a prevalence of advanced Ann-Arbor stage (III-IV in 70%), elevated LDH (65%), good performance status (ECOG 0-1 in 81%) and IPI >2 (53%). Nine patients were HIV-positive. All patients were treated according to GMALL protocol with the use of Rituximab (Intermesoli *et al.*, 2013). Overall survival (OS) at 2-years of the whole cohort was 88% (IC 95%, 78% to 99%). By univariate analysis age ≤55 years (P=0.0108) was associated with a better prognosis, while IPI>2 (P=0.304), HIV status (P=0.7204) and Burkitt-like diagnosis (P=0.1672) did not show any prognostic value. LMR was analyzed as a clinical biomarker by ROC analysis and a cut-off of 2.6 confirmed to be associated to the best predictive value. LMR >2.6 proved to be a strong predictor for survival with a 2-years OS of 100% (IC 95% 100% to 100%) as compared to 75% (IC 95%, 58% to 97%) observed in patients with LMR ≤2.6, (P=0.0388, Figure 1). In conclusion, LMR is a potent predictor of survival in BL treated with rituximab-containing chemotherapy.

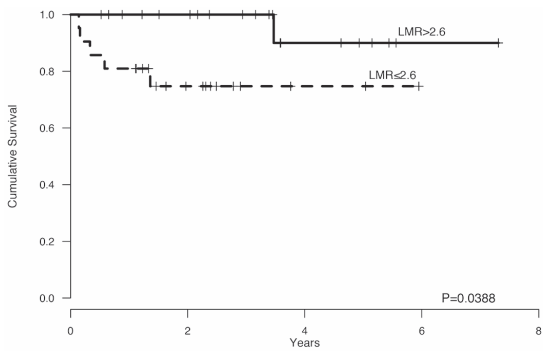

Figure 1. Overall survival (N=43).

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LOCALIZED FOLLICULAR LYMPHOMA TREATMENT WITH RITUXIMAB AND RADIOTEHRAPY

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*Background:*Early-stage follicular lymphoma (FL) has been treated with involved-field radiotherapy (IFRT) alone. 10-year OS rates ranging from 62%–79% are seen and relapse in these patients is generally outside the radiation field with a local PFS of 80% at 5 years. Moreover, in this setting, no data on the role of rituximab in association with IFRT are available. *Purpose:* We retrospectively analyzed data from patients (pts) with newly diagnosed FL referring to Hematology Divisions of Florence and Siena University Hospitals from 2007 to 2014 treated with rituximab and IFRT. *Results:*We evaluated in this study 41 consecutive pts with median age of 63 years (range 23-88), 19/41 (46%) were male, all with diagnosis of Follicular Lymphoma according to WHO. 35/41 pts (80,5%) had a stage I, six (19,5%) had a stage II, none had B symptoms and one pts had bulky disease (2%). According to the The FL International Prognostic Index 2(FLIPI2) 18/41 (44%) pts presented low risk, 22 pts (54%) intermediate risk and one (2%) high risk. Doses ranged from 20 Gy to 44 Gy, with a median dose of 24 Gy PCR-BCL2 in the bone marrow were positive in 3/41 pts. The complete response rate was 100%, 3/41 pts (7%) relapsed (one pts presented positive PCR-BCL2 and 2 had stage IIA with FLIPI2 intermediate). After a median follow-up of 33 months (range 7-88 months) progression free survival was 74% (CI 95% 67-81), after a median observation of 33 months (range 1-88 months) all patients (100%) were alive. *Conclusions:*In our experience the combination with rituximab - IFRT represents a valuable treatment in terms of PFS and toxicity for early stage FL. This therapy seems to allow a better long-term control of the disease without significant additional toxicity, but longer follow - up and prospective studies are needed to verify these results.

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ADVANTAGE OF UPFRONT AUTOLOGOUS STEM CELL TRANSPLANTATION IN DIFFUSE LARGE B CELL LYMPHOMA PATIENTS WITH HIGH-INTERMEDIATE/HIGH RISK AGE-ADJUSTED IPI: A SINGLE CENTER EXPERIENCE

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The prognosis of young DLBCL patients (pts) with HI/H aaIPI score is still unsatisfactory and the efficacy of consolidation ASCT after immune-chemotherapy (CT) remains controversial. We report a single center experience over a ten-year period (2002 -2012) during which pts were treated with intensified CT +/- ASCT according to ongoing studies and/or to current institutional guidelines. Among 74 consecutive HI/H aaIPI risk DLBCL pts, 31 received six courses of bi-weekly rituximab-cyclophosphamide-epirubicin/adriblastine-vincristine-prednisone (R-MegaCEOP14 /R-MegaCHOP or R-CHOP14) and 43 four courses of the same regimens followed by two courses of rituximab-mitoxantrone-cytarabine-dexamethasone (R-MAD) and carmustine-etoposide-cytarabine-melphalan (BEAM) with ASCT. The clinical characteristics of the whole population were: median age 50 (range 29-65); stage III/IV 70 pts; aaIPI HI/H 47/27 pts; they did not significantly differ between the two treatment groups. With a median follow up of 54 months, 5-year PFS was 86% *vs* 61% (p 0.04) and OS 88% *vs* 65% (p 0.09) in ASCT *vs* no-ASCT group respectively (Figure 1). Treatment-related deaths occurred in 4 pts, only during the CT phase (R-CHOP/MegaCHOP: 3 pts; R-MAD 1 pt), in 3 cases assigned to ASCT and in 1 case assigned to CT programs. Relapse occurred in one pt in the ASCT group as a down-grading lymphoma and in 7 pts in the no-ASCT group, with 5-years DFS of 97% *vs* 70% respectively (p 0.0065). Differential treatment effect according to risk level was detected: in HI-risk pts, DFS, PFS and OS in ASCT *vs* no-ASCT group were 100% *vs* 78% (p 0.02), 96% *vs* 65% (p 0.006) and 96 *vs* 73% (p 0.05), respectively, while in H-risk pts they were 90 *vs* 64% (p 0.2), 64 *vs* 59% (p 0.9) and 71 *vs* 51% (p 0.7), respectively. In conclusion, upfront ASCT was safe and significantly reduced the risk of progression among DLBCL pts with HI/H aaIPI who had a response to induction therapy with a trend of improvement in overall survival. The benefit was

more evident in HI than in H-risk, probably due to the lower number of H-risk pts, since the frequency of progression was comparable between the two groups. Lack of response to induction therapy and toxic deaths during dose-dense treatment still remain a challenge. FISH analysis could help in recognizing poor responders that should be addressed to experimental programs while careful monitoring and appropriate supportive therapy should reduce the early toxic fatalities.

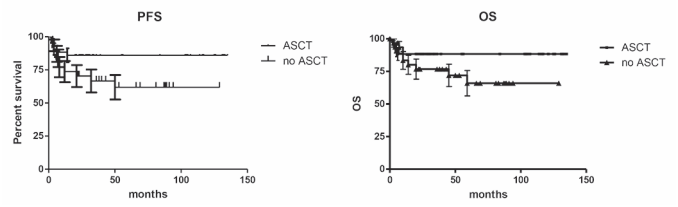

Figure 1. PFS and OS in HI/H aaIPI risk DLBCL patients who received or not upfront consolidative ASCT.

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RITUXIMAB, MITOXANTRONE, ETOPOSIDE AND CYCLOPHOSPHAMIDE REGIMEN AS FIRST-LINE TREATMENT IN ELDERLY FRAIL PATIENTS WITH AGGRESSIVE NON-HODGKIN'S LYMPHOMA

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Introduction: The R-CHOP chemotherapy is the standard first-line treatment regimen in patients with aggressive non-Hodgkin's lymphoma (NHL). However, elderly and frail patients are not suitable for standard treatments due to the presence of comorbidities, and high risk of hematologic and extrahematologic toxicity. Therefore, alternative regimens should be tested in these patients. *Methods:* Between September 2010 and December 2014, 25 elderly frail patients with a diagnosis of diffuse large B-cell lymphoma not eligible for R-CHOP therapy were treated using a combination immunochemotherapy regimen R-MEC. The regimen included the following agents: mitoxantrone given intravenously (IV) at a dose of 10 mg/m^2 on Day 1, etoposide 60 mg/m^2 IV on Days 1-2, cyclophosphamide 300 mg/m^2 IV on Days 2-3, Rituximab 375 mg/m^2 D0, and prednisone 40 mg/m^2 administered orally on Days 1-3, primary prophylaxis with G-CSF. Cycles were repeated every 21 days. *Results:* The patients' ages at diagnosis ranged from 74 to 85 years, with a mean age of 81 years. The comprehensive geriatric assessment classified 76% of patients as unfit or frail. 40% of patients had an IPI score ≥3. The average number of cycles completed was 3,5 (range 1-6 cycles). Fifteen patients (65%) achieved complete remission, and 1 patient (4%) obtained partial remission, for an overall response rate of 69%. Two patients (8%) were resistant to therapy. Two patients were not evaluable for response: 1 patient interrupted treatment due to acute perforated diverticulitis and 1 patient was switched to other treatment program after two cycles because of toxicity. The overall survival rate, with a median follow-up of 17 months (range 1-52), was 64% among the evaluable patients. The progression free survival rate was 52%, with a median follow-up of 8 months (range 1-46). However, ten patients (40%) were alive without disease at 24–50 months from the start of treatment. Hematologic and non-hematologic toxicity were acceptable, 3 deaths (12%) due to complications related to therapy were observed. *Conclusions:* The management of frail elderly patients with aggressive lymphomas requires special consideration because of the increased risk of toxicity and death from treatment and disease. The combination chemotherapy regimen R-MEC designed specifically for elderly patients with aggressive NHL offers high response rates, durable remissions, and acceptable toxicity.

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FIRST LINE TREATMENT WITH BENDAMUSTINE, BEFORE NATIONAL HEALTH SYSTEM APPROVAL, IN SELECTED NON HODGKIN'S LYMPHOMA PATIENTS: PROSPECTIVE EXPERIENCE IN TUSCANY REGION

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Bendamustine was approved by FDA on October 2008 for the treatment of indolent rituximab-refractory B-cell lymphoma. Bendamustine as front-line off label therapy was available in Tuscany from 2011 even before the Italian National Health System approval according to regional law. The aim of this study was to evaluate all consecutive patients treated in first-line with bendamustine in 7 Tuscany centers. From June 2011 to August 2013, a total of 72 patients were prospectively enrolled in the study. The histologies included small lymphocytic lymphoma (34%), follicular lymphoma (25%), diffuse large B-cell (15%), mantle-cell (14%), lymphoplasmacytoid (7%) and MALT (5%) lymphomas. The overall median age was 69 years (range 45-89), 81 years in DLBCL and 78 years in MCL. The geriatric score assessment classified 25% of patients as unfit or frail. In 56 patients bendamustine was used in combination with rituximab, in 12 patients with mitoxantrone or cytarabine, 4 patients were treated with a single-agent bendamustine. Thirty-nine patients received bendamustine on Day 1, 2 at a dose of 90 mg/m^2, 28 patients received 70 mg/m^2 and 5 patients 120 mg/m^2. The median number of cycles administered was 4 (range 2 - 6). In the 70 patients evaluable for response an ORR 89% was achieved. 50% had a complete remission, 39% partial remission, 9% progressive disease and 3% stable disease. According to histotype 95% of indolent NHL obtained a response; in aggressive lymphomas only 36% DLBCL and 55% MCL reached a complete but not durable remission. Toxicity was mainly hematological with grade 3-4 neutropenia (25%). The most frequent non-hematologic events included infection (4%) and skin rash grade 1 (7%). No toxic deaths were observed. After a median follow-up of 18 months the overall survival was 83%; 10 patients died due to progressive disease. Progression free survival, after a median observation period of 12 months, was 60%; sixty-two patients are alive, 31 in continuous complete remission, 3 relapsed and 28 with disease under control. In conclusion, in this 'real life' negatively selected population, the use of bendamustine confirmed a promising clinical activity with acceptable toxicity, particularly in indolent lymphomas. Bendamustine is effective and well tolerated and should therefore been considered in very elderly patients with aggressive lymphomas who do not qualify for standard chemotherapy.

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A CLINIC-PATHOLOGICAL RETROSPECTIVE STUDY OF 43 CONSECUTIVE PATIENTS WITH PRIMARY BONE LYMPHOMA

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Background: Primary lymphoma of bone (PLB) is a rare clinic-pathological entity which constitute 2% of adult lymphomas and 3-5% of extranodal non Hodgkin lymphomas (nHL). The disease usually involves a single bone but can be multifocal. Improvements in radiological tools, particularly the combined use of computed tomography (CT), magnetic resonance (MRI) and positron emission tomography (PET), have increased the quality of staging and of assessment of treatment response. Although there are not standardized therapies, the best results have been achieved by anthracycline-based chemotherapy followed by consolidative radiotherapy. Addition of monoclonal antibodies anti CD20 (Rituximab) since 2001 has remarkably improved the overall survival (OS). *Aims and Methods:* In our Lymphoma data base we have found 43 consecutive patients with PLB attending our Department in the period 1987-2013; we have analyzed histological, demographical and clinical features and their correlation with response parameters. Overall survival (OS) and progression free survival (PFS) were calculated with the method of Kaplan and Meier (K-M). Outcome predictions were estimated by the log-rank test. *Results:* From 1987 to 2013 in our Institution 2410 patients with NHL have been diagnosed and treated. Forty-three of them had PLB, accounting for 2%. Median age at diagnosis was 50 years (range 18-81) with prevalence of males (28 males and 15 females). The majority of cases (95%) were diffuse large B cell Lymphomas (DLBCL). According to the Ann Arbor stage system, stage was limited (I-II) in the majority of patients (72%) and

advanced (III-IV) in the 28%. Most cases showed a good performance status (PS) and a low international prognostic index (IPI). Thirty five patients were treated by CHOP or CHOP like regimen, six by a third generation regimen and two by sequential high doses (HDS) therapy followed in one case by autologous stem cell transplantation. Half of patients had Rituximab and 32 have been undergone radiation therapy. Five and ten years K-M OS were 78% (±6.5) and 71% (±8,6), respectively. Five and ten years K-M PFS were 73% (±7.2) and 65% (±8,7), respectively. Low IPI score has been identified as favorable prognostic factor (P<0.0001) while isolated disease showed a not statistically significant trend (P=0.14) toward better OS. *Conclusions:* PLB are mainly DLBCL and have a good outcome when treated with R-CHOP and radiotherapy. Low IPI score is associated to good prognosis (Figure 1).

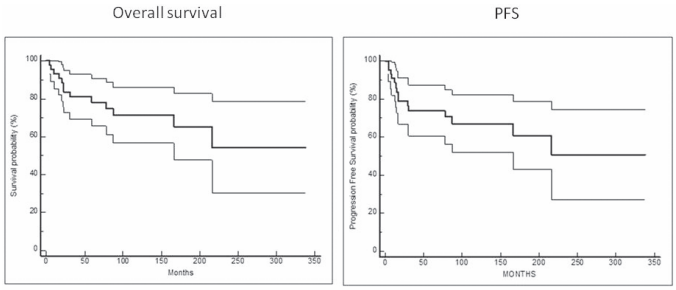

Figure 1.

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MANAGEMENT OF PRIMARY HEPATIC NON-HODGKIN'S LYMPHOMA

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Primary Hepatic (PHL) is a rare form of NHL, characterized by the exclusive involvement of the liver at the moment of the diagnosis, whereas other localizations, such as lymph nodes, spleen, bone marrow and peripheral blood, or other tissues are free of disease at least for 6 months after diagnosis. Even if its occurrence is rare, PHL should enter in the differential diagnosis of every space-occupying liver lesion, in particular if they are present in concomitance with normal levels of AFP and/or CEA and in absence of liver cirrhosis. Only small series of PHL have been investigated in literature, nevertheless a non-fortuitous association with Hepatitis C Virus (HCV) infection has been reported among these series. The prognosis is believed to be dismal, with early recurrence and short survival. Patients diagnosed with PHL at our institution between 1990 and 2014, among a population of 600 NHL, were retrospectively analyzed. 11 PHL were defined, 10 patients had a B-cell lymphoma, DLBCL in 6. The prevalence of HCV infection was 72%. Combination CHT was the mainstay of treatment for PHL. 10 patients were treated with CHT (10/11: 90%). In 7 patients, the multi-drug regimen we used consisted of the CHOP or a CHOP-like scheme; in 2 of them (diagnosed after 2002) rituximab was added, at standard schedule. In the indolent types a fludarabine-based scheme was used. One patient with a single-focal lesion underwent to surgical treatment, with a CR. 11/11 patients achieved complete remission of the disease after the frontline therapy (six CHT courses/surgical treatment) (CR: 100%). Only one patient, 47 years old, Diffuse Large B-Cell Lymphoma, relapsed 144 months after CR: she underwent to retreatment with Rituximab+CHOP with the result of SD, so she underwent to third line treatment with R-Bendamustine, but, due to cardiotoxicity, she had to stop treatment and in the same month died for heart failure. At time, 10/11 (90%) of patients are alive with a median overall survival (OS) was 123 months (r. 40-228) and median disease- free survival (DFS) was 120 months (range 36-223), no statistically significant differences were found between PHL and other types of NHL in terms of OS and DFS (Figure 1). HCV infection did not appear to influence the results of therapy. Our study confirms the rarity of PHL, shows a high prevalence of HCV infection, and demonstrates that the outcome of patients with PHL may be favorable.

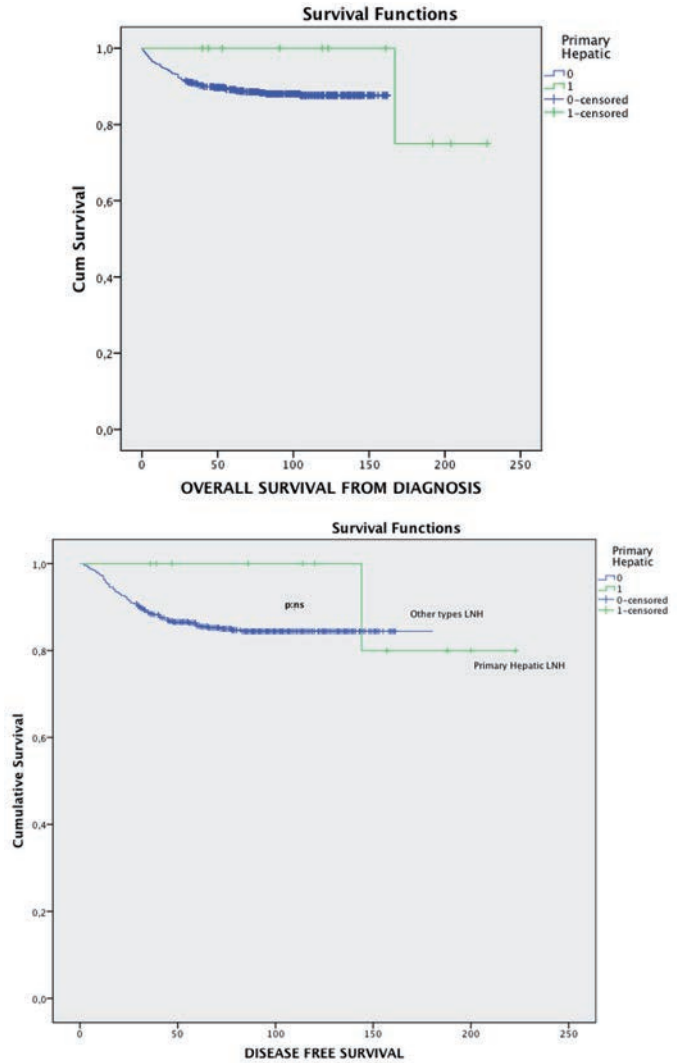

Figure 1. OS and DFS

Non-Hodgkin Lymphoma 3

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LENALIDOMIDE PLUS RITUXIMAB IN ELDERLY PATIENTS WITH RELAPSED OR REFRACTORY FOLLICULAR LYMPHOMA

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Background: Follicular lymphoma (FL) is the second most common subtype of lymphoma and despite recent chemotherapeutic advances up to half of all patients relapse. Rituximab plays an important role in the treatment of FL. In spite of high response rates achieved with this monoclonal antibody, however, many patients tend to relapse and become refractory to rituximab over time. Lenalidomide, an immunomodulatory agent, had direct tumoricidal and antiangiogenetic actions on tumor cells and was able to modulate tumor-cell microenvironment, with the restoration of impaired T-cell activity and the formation of immuno-synapsis. Based on these actions, lenalidomide represented an active drug on FL. Aim. We report the results obtained in three patients, aged >70 years, affected by FL and treated with lenalidomide and rituximab. *Patients and Methods:* From January 2013 to January 2014, we treated 3 elderly patients (male, 71, 72 and 75 years) with relapsed/refractory FL who had been heavily pretreated (more than 5 lines of treatment, including ASCT). Oral lenalidomide (15 mg/d for 21 days of each 28-day cycle) was initiated for four cycles and 375 mg/m(2) intravenous rituximab was administered on day 1 and day 21 of each 28-day cycle for four cycles. After this induction phase, two patients achieved a complete response (CR) and one partial response (PR). Lenalidomide maintenance therapy was administered at the same schedule for another 6 months. At the end of treatment all three patients achieved complete remission. *Results:* To date (+14 months after the end of therapy) all three patients are alive and still in CR. Adverse events were manageable and the most common included neutropenia and thrombocytopenia. *Conclusions:* Our experience, although on a very small number of patients, seems to confirm the preliminary data of the literature showing good efficacy of lenalidomide plus rituximab in relapsed follicular lymphoma. In fact, oral lenalidomide in combination with rituximab seem to be active and well tolerated in elderly patients with relapsed/refractory FL with a high percentage of patients achieving a continuous CR after lenalidomide maintenance.

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A CASE OF PRIMARY RECTAL NON-HODGKIN'S LYMPHOMA TREATED ONLY WITH CHEMOTHERAPY IN A PATIENT WITH RHEUMATOID ARTHRITIS

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Abstract: Primary rectal non-Hodgkin's lymphoma (NHL) is a rare disease. We report a case of rectal NHL, B cell large cell type, Stage 1E, in a patient with aggressive rheumatoid arthritis (AR), treated with chemotherapy alone. *Case Report:* A 63 years old woman in treatment for AR was undergone to colonoscopy for rectum bleeding, tenesmus and diarrhea: highlights and ulcerated lesion of the anorectal canal was showed; istologically diagnosis of NHL – B cell large cell type, anaplastic CD30+ was performed. Computed tomography total body revealed thickening of the walls of the rectum with low infiltration of adipose tissue and mesorectal. PET/TC total body showed a pulmonary nodule of the left lung (SUV 5.2) and the high level of FDG (Suv 4.0) at the rectum. The surgery resection of the little node of lung showed a chronic inflammation due to AR. Bone marrow biopsy was negative. A final diagnosis of primary rectal NHL, B cell large cell type, 1E was made. The patient started chemotherapy with the R - CHOP regimen and after four cycles PET/TC will performed to evaluate the response. *Discussion:* NHL presenting as a primary rectal localized lesion is the rarest disorder accounting for 0.1–0.6% of all colonic malignancies, and 0.05% of all primary rectal tumor. The limited number of cases in the literature makes it difficult to formulate a cohesive treatment strategy; surgery has been proposed as the treatment of choice. Only one case report of successful treatment of rectal NHL with chemo-radiotherapy, without operative intervention, has been reported in the literature. A CT scan total body, and bone marrow examination, is usually necessary for staging. HP infection, immunosuppression, inflammatory bowel disease and human immunodeficiency virus infection have been mooted as risk factors for primary GI NHL. Diffuse large B cell type is the most common histologic type in rectal NHL. Surgery with or without chemotherapy and radiotherapy has been opined as the treatment of choice for colorectal NHL. This case reinforces the feasibility of management of localized rectal NHL with non-surgical modalities. *Conclusions:* The rectum is a fairly uncommon site for lymphoma compared to other gastrointestinal sites. Here we discussed about a patient treated with chemotherapy alone. Probably the immunosuppression for AR and its treatments therapy have importance as risk factor for primary rectal NHL of our patient.

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COMBINED CHEMOIMMUNOTHERAPY IS AN EFFECTIVE TREATMENT FOR HIV-NEGATIVE, HHV-8 POSITIVE PATIENTS WITH MULTICENTRIC CASTLEMAN'S DISEASE

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Castleman disease is a rare and heterogeneous lymphoproliferative disorder: the unicentric type includes hyaline vascular and plasma cell variants, while the multicentric disease (MCD) is predominantly of the plasma cell or mixed variant. The aetiology is poorly understood; the potential roles of HHV-8 are suggested in several studies. MCD is also associated with HIV infection. No standard treatment has been established for MCD patients: steroids, chemotherapy, rituximab, thalidomide, bortezomib, antiviral therapy, and IL-6 inhibitors have been used. We have reported a complete hematologic remission and virologic response to chemoimmunotherapy R-CVP in a HIV negative, HHV-8 positive patient; in the following years we have treated MCD patients with R-CVP in order to assess the effectiveness of this treatment. Six HIV negative, HHV-8 positive patients (5 men, 1 woman, median age 77 years, range 69-80 years) with MCD were diagnosed in our Hematology Unit over a eight- year period. The patients' characteristics are described in Table 1.

Table 1.

Patient	Sex	Age (years)	Histologic diagnosis	Manifestation	Laboratory	Associated diseases
1	M	78	Mixed cell type	Constitutional symptoms, edema and pleural effusion, hepatosplenomegaly	Anemia, monoclonal gammopathy	
2	F	79	Hyaline-vascular type	Constitutional symptoms, lymphoadenomegaly	Anemia	Kaposi's sarcoma HCV infection
3	M	76	Plasma cell variant	Constitutional symptoms, lymphoadenomegaly, hepatosplenomegaly	Anemia, thrombocytopenia	Thrombotic thrombocytopenic purpura
4	M	75	Mixed cell type	Constitutional symptoms, lymphoadenomegaly, hepatosplenomegaly	Anemia, monoclonal gammopathy	Diabetes mellitus
5	M	69	Mixed cell type	Constitutional symptoms, lymphoadenomegaly	Monoclonal gammopathy	Kaposi's sarcoma
6	M	80	Mixed cell type	Constitutional symptoms, lymphoadenomegaly, hepatosplenomegaly	Anemia, thrombocytopenia	

Five patients were initially treated by chemoimmunotherapy R-CVP combination every 3 week for 6-8 cycles. Patient n° 3 was initially diagnosed with a associated thrombotic thrombocytopenic purpura (TTP) and treated with steroids, plasma exchange and rituximab. Four patients out of five completed the planned treatment. In patient n°1 to clear residual disease a maintenance treatment with rituximab every 2 months for 4 cycles was made. In the 4 patients CT scan and PET scan documented a complete remission (CR); the HHV-8 clearance was also obtained. Patient n° 1 died after a 63 months follow up with a ischemic cardiopathy. Patients n°4, n°5, n°6 are alive with a follow up respectively of 4, 3 and 64 months. Patient n° 2 initially refused treatment; 1 year later chemoim-

munotherapy was started because of the disease worsening with transfusion-dependent anemia. After the first cycle this treatment was stopped for hematologic toxicity. With his informed consent the patient was treated with bortezomib and valganciclovir, but she died one month later with pseudomonas aeruginosa sepsis. Patient n° 3 obtained a partial remission with 9 plasma exchange and rituximab, but refused further therapies and died one month later. In HIV negative HHV-8 positive MCD patients the illness can be fatal if not or lately treated. Chemoimmunotherapy is an effective treatment for these patients and is able to attain a clinical response with associated undectectable HHV-8 viral load.

P243

RITUXIMAB PLUS ABVD IN NEWLY DIAGNOSED NODULAR LYMPHOCYTE-PREDOMINANT HODGKIN LYMPHOMA

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Background: Nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL) is characterized by malignant lymphocytes positive for the B-cell antigen CD20 and a favorable prognosis but delayed relapses and transformation in aggressive B-cell lymphomas, especially diffuse large B-cell lymphoma, are frequently observed. No universal agreement regarding primary therapy exists to date. ABVD regimen has demonstrated similar results compared to classical Hodgkin Lymphoma (cHL); the anti-CD20 monoclonal antibody rituximab as single agent has already shown high efficacy with mild toxicity both in relapsed NLPHL patients and as frontline regimen. Moreover, in cHL patients, rituximab showed high efficacy and feasibility in association with ABVD (R-ABVD) in 2 phase II trials. *Aims:* In this study we have investigated efficacy and safety of R-ABVD to newly diagnosed NLPHL patients. *Methods:* A cohort of 6 consecutive patients (median age 41 yrs) diagnosed with NLPHL was retrospectively analyzed. One patient had stage I disease, 3 stage II and 2 stage III, none had "B" symptoms. Induction treatment plan consisted, according to staging, of 2-6 courses of rituximab 375mg/m^2 i.v. on day 1 and ABVD on day 2 and 16 of a 28-day cycle and, if indicated, involved field radiation therapy (RT). Final response assessment was done according to the 2007 Revised Response Criteria. *Results:* Overall response rate (ORR) was 100% with 5 complete remissions (CRs) and 1 partial response (PR), the patient in PR received consolidation RT achieving CR. All patients completed the treatment, no grade 3-4 hematological toxicity and no infectious complications were observed. Extra-hematological toxicity was mild and mainly consisting of grade ≤2 nausea/vomiting. After a median follow-up of 36 months (range 12-72) all patients were alive, 1/6 had disease relapse after 36 months and was successfully retreated with rituximab and bendamustine. Estimated 6-year progression-free survival and overall survival were 75% and 100%, respectively. *Summary and Conclusions:* There is no consensus about NLPHL first-line treatment, rituximab monotherapy is active but probably not sufficient to achieve long term remission and some reports showed clinical efficacy of R-CHOP regimen at least for advanced-stage disease. According to this background and our small experience, we suggest R-ABVD regimen is effective and well tolerated and could be suitable and less toxic than R-CHOP as first-line NLPHL therapy.

P244

AN UNUSUAL CASE OF COMPOSITE LYMPHOMA WITH CUTANEOUS LOCALIZATION IN VERY OLD PATIENT TREATED WITH RITUXIMAB AND BENDAMUSTINE

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Introduction: Composite lymphoma (CL) is a rare disease which is defined as the coexistence of two morphologically and phenotypically distinct types of lymphoid neoplasms occurring in a single anatomic organ or tissue. The incidence of CL is low, varying from 1% to 4.7%. The simultaneous occurrence of Hodgkin disease and non-Hodgkin lymphoma in a single lymph node is extremely rare. We present an unusual variant of composite lymphoma composed of nodular sclerosing Hodgkin lymphoma and diffuse large B-cell localized also at the skin. *Case Report:* We report an unusual case of composite lymphoma arising in a nineteen years old woman, localized at the skin of the thorax and fronto-temporal region. The neoplasm was composed of LNH large cell lymphoma 70% (CD79A+/BCL6-/CD10-/OCT2+/BOB1+/BOB1+/PU1+/KI67+) and nodular sclerosing Hodgkin disease 30% (large vaguely nodular areas with characteristic lacunar-type Reed-Sternberg (RS) cells typical of nodular sclerosing HD were identified, CD30+, CD15+/-, CD79a-, PAX5+, CD3-, Ki67/MIB1 +++). Physical examination revealed a lymph nodes in left axillary site and deep ulceration of skin at the thorax and an evident tumefation at the cranium. PET/TC showed high concentration of FDG in this sites. The patient began treatment with Rituximab and bendamustine and actually after three cycles is in partial response and the revaluation with TC/PET total body will be made after fourth cycles. *Discussion:* CLis a separate types of non-Hodgkin's lymphoma or its rare association with Hodgkin's lymphoma within a single organ or tissue. The incidence of CL varied between 1 - 4.7%. The combination of 2 different types of NHL composed of small cleaved cell lymphoma and diffuse large cell lymphoma is the most common variant, accounting for up to 58% of cases but the presence of composed of HD and one of the components of NHL in the same tissue, are very rare. Only six cases showing combination of classical Hodgkin lymphoma and DLBCL within the same site simultaneously were described. The prognosis and the therapeutic decision depended by the unfavourable and major component of CL. *Conclusions:* CL is the occurrence of two or more distinct lymphoma types in a single anatomic site. Extranodal composite lymphoma of the is rare and are not reported cases in the literature treated with Rituximab and bendamustine. The good response after the first cycle predicts a good response at the end of chemotherapy.

P245

REAL CLINICAL PATWAYS OF NON HODGKIN-LYMPHOMAS

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A clinical pathway defines the optimal care process, sequencing and timing of interventions In 2014 the health service of Regione Lombardia attempted to define an optimal care process in performing diagnostic procedures in Non-Hodgkin Lymphomas (NHL) as follows: "Performing lymph node biopsy, bone marrow biopsy, computed tomography (CT) or magnetic resonance imaging (MRI) or positron emission tomography (PET) during the 3 months preceding the first hospital admission for NHL in 70% of cases". We analyzed 29 NHL real clinical pathways in our institution from 11/1/2013 to 12/31/2014. In this period 19 patients (65.5%) were admitted to the hospital as inpatient (12 in surgical and 7 in medical units) and 10 (45.5%) were followed as outpatient Of the12 patients admitted in surgical units all underwent surgery during hospitalization. There were 2 axillary and 1 inguinal lymphadenectomies, 2 cervical and 1 submandibular lymph node resections, 1 submandibular gland resection, 2 laparoscopic intraabdominal lymphadenectomies, 1 laparotomic ileal resection, 1 nefrectomy and 1 resection of residual ovarian mass In these surgical patients 8 bone marrow biopsies were subsequently performed in an outpatient setting after NHL diagnosis. Moreover 13 CT scans were performed in 11 patients (2 patients underwent 2 CT scans for staging completion). Of these 8 (61.5%) during the 3 months before admission and 5 (38.5%) within 3 months after discharge. Finally 6 PETs were performed, 2 and 4 respectively within 3 month before and 3 months after hospitalization 7 patients were admitted in a medical unit. In these patients 7 bone marrow biopsy and other 11 histological investigations were performed during, before or after hospitalization (1 pt had 4 biopsies) Bone marrow biopsies were performed as follows: 1 (14.3%) in the 3 months prior to admission, 1 (14.3%) during hospitalization, 3 (71.4%) within 3 months after discharge The other 11 histological analysis were performed 1 (9.1%) in the 3 months prior to, 5 (45.5%) during and 5 (45.5%) within 3 months after first hospitalization. These 7 patients underwent also 11 CT scans (2 pts had 3 TC scans): 4 (36.4%) in the 3 months prior to admission, 6 (54.5%) during first hospitalization, 1 (9.1%) within 3 months after discharge. 2 PETs were performed both within the 3 months after dis-

charge. We examined the real clinical pathways of 29 NHL patients. This analysis can help to better define the optimal clinical pathways for NHL patients

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TYPE B LACTIC ACIDOSIS IN A PATIENT WITH VARIANT PLEIOMORPHIC MANTLE CELL LYNPHOMA, IV STAGE, LOW MIPI SCORE IN PROGRESSION DISEASE

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Introduction: Type B lactic acidosis is a rare complication of cancer, most frequently reported in hematologic malignancies and in adult patients. The pathogenesis and treatment are discussed. It is associated with a poor prognosis. *Methods:* A 68-years-old man with variant pleiomorphic MCL, IV, low MIPI, positive for amplification Bcl 6 gene, traslocation (11;14), immunoglobulin heavy-chain gene rearrangement. Peripheral smear showed atipical lymphocytes, the results of immunostaining of bone marrow biopsy were compatible with MCL. CAT scan showed spleno-haepatomegaly, multiple adenopathy. Colonscopy was positive for lymphoma. He was syntomatic and pancytopenic, high LDH levels. Chemotherapy with cyclophosphamide, prednisone, doxorubicin, vincristine and rituximab was started; after three cycles the disease was stable and LDH was normal. After the fourth cycle the patient was admitted to hospital with dispnea, tachycardia, lower extremity edema, altered mental status. His laboratory studies showed elevated lactate levels (15 mmol/L), LDH was increased, pancythopenia was present. CAT scan and bone marrow biopsy revealed a progression disease. He was started therapy with bicarbonate, fluid, chemotherapy (bendamustine, rituximab); after two cycles the lactate levels improved (2.8 mmol/L) but after some days the patient required hospitalitazion for severe lactic acidosis, lactate was 20 mmol/L; he died lymphoma progression. *Conclusions:* The causes of lactic acidosis can be divided into those associated with impaired tissue oxygenation (type A) and those in which systemic impairment in oxygenation does not exist or is not readily apparent (type B). We found a severe type B lactic acidosis in haematological malignancies and rarely in solid tumors during diagnosis or in progression disease. The proposed mechanisms by which it occurs include an aberrant IGF pathway, abnormal liver clearance of lactic acid from impaired gluconeogenesis with or without malignant infiltration, microembolization of malignant cells in the vasculature of the tumor, and thiamine deficiency. There are no randomized trials comparing treatment options, which thiamine supplementation, renal replacement therapy with bicarbonate and chemotherapy. Further studies are needed to evaluate the role of thiamine in the therapy of type B lactic acidosis secondary to malignancy, especially regarding its possible paradoxical increase in tumor growth that results from increased nucleic acid synthesis.

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BENDAMUSTINE AS FRONT LINE OR SALVAGE TREATMENT IN AGGRESSIVE NON HODGKIN LYMPHOMAS: RESPONSE RATE AND TREATMENT SAFETY

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Bendamustine is very effective in indolent non-Hodgkin's lymphomas, multiple myeloma and some solid tumors but hasn't yet been well studied for efficacy in aggressive lymphomas. Prospective trials specifically investigating the efficacy of bendamustine in patients with aggressive lymphomas were sparse. We investigated if patients with aggressive lymphoma could be appropriate candidates for therapy with bendamustine, either as first or salvage therapy. We observed 16 lymphomas: 11 Diffuse Large B cells Lymphomas (DLBCL): 6 first line and 5 salvage therapy; 3 Peripheral T Cells Lymphomas (PTCL): 2 salvage therapy; 2 Richter's Syndromes (RS) in first line. 8 Men and 8 Females. Median age: 74 years (range: 51-87). The population was at high risk: 100% stage III-IV disease, 75% high International Prognostic Index (IPI)) and 63% with poor Performance Status (PS)=2. The median number of prior therapies was 3. 56% treated in first line and 44% in salvage therapy. We examined the use of bendamustine as single agent (PTCL) or in combination with Rituximab (DLBCL, RS). Patients received Bendamustine (B) 90 mg/m^2 (days 1,2)±Rituximab (R) 375mg/m^2 (day 1), every 21 days for up to 6 cycles. The study evaluated objective response rate (ORR) and treatment safety. ORR in first line was 62% (complete response (CR), 25%; partial response (PR), 37%; stable disease (SD), 25%; progressive disease (PD), 13%). ORR in salvage therapy was 37% (CR, 25%; PR, 13%; SD, 37%; PD, 25%). Grade 3 or 4 hematological toxicities included neutropenia (31%), thrombocytopenia (25%), anemia (31%). There were 2 cases of cytomegalovirus (CMV) reactivation. We didn't observed severe extrahematological toxicities neither toxic deaths. At a median follow-up of 7 months (range: 4-29), 32% of patients (n=5) are alive, 2 DLBCL are in continuous CR, after BR as first line therapy (respectively with a follow-up of 7 and 27 months). 67% of patients (n=11) died (9 for progressive disease and 2 for other causes). Despite the limit of the small number of patients and short follow up, our experience suggests modest activity in relapsed/refractory DLBCL and inefficacy in PTCL and RS. Better results seem feasible for DLBCL, in first line therapy. Although the population was at high risk and with poor PS, bendamustine showed an acceptable toxicity profile, in first line and in salvage subset.

P248

MORPHOLOGIC VARIANTS OF MANTLE CELL LYMPHOMA: A RETROSPECTIVE SINGLE CENTER EXPERIENCE

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Mantle cell lymphoma (MCL) is a B-cell neoplasm characterized by cyclin D1 (CCND1) rearrangement. A spectrum of morphologic variants is listed in the 2008 WHO Classification of tumours of hematopoietic and lymphoid tissues. Classical MCL is generally composed of monomorphic small to medium-sized lymphoid cells with irregular nuclear contours. Besides classical MCL, the other morphologic entities are: small cell, marginal zone-like, blastoid and pleomorphic variants. These two latter variants are characterized by an unfavourable clinical course. Moreover, these aggressive morphologic variants may represent a diagnostic pitfall for hematopathologists. We retrospectively analyzed the newly diagnosed MCL cases, consecutively observed at the Hematology Unit of the Azienda Ospedaliero-Universitaria Policlinico of Modena over a period of 5 years (2010-2014). Peripheral blood (PB) smears, bone marrow (BM) aspirates and/or BM touch imprints and trephine biopsy were observed at our Hematopathology Laboratory, whereas lymph node biopsy specimens were analyzed in collaboration with the Pathology Section laboratory. A total of 21 patients with MCL were observed. Median patient age was 66 years (range 52-84 years); males and females were 13 (62%) and 8 (38%), respectively. Cyto-histological diagnosis was obtained by lymph node tissue examination in 12 (57%) cases, whereas in the remaining cases morphologic, flow-cytometry, immunocyto- or immunohistochemical examinations on PB and/or BM samples, were sufficient for the diagnosis. FISH analyses documented Cyclin D1 rearrangement in all the cases. Morphologic variants frequency was as follows: classical 13 (62%), marginal zone- like 2 (9%), blastoid 5 (24%) and pleomorphic 1 (5%) (Figure 1). A leukemic phase was documented on PB smear morphologic analysis, in 15 patients (71%), subsequently confirmed by flow cytometry assays. Nineteen patients (90%) were in stage IV at disease onset, while one patient was allocated to early stage (IIA). In a very elderly unfit patient, who underwent palliative care only, staging was not completed. In our small monocenter series the pleomorphic variant of MCL was rarely observed. Of note, the blastoid variant was quite

frequent, morphologically mimicking acute leukemia at disease onset. Therefore, when cells characterized by blast morphology are observed, waiting for the flow-cytometry results, MCL should always be included in the differential diagnosis by hematopathologists.

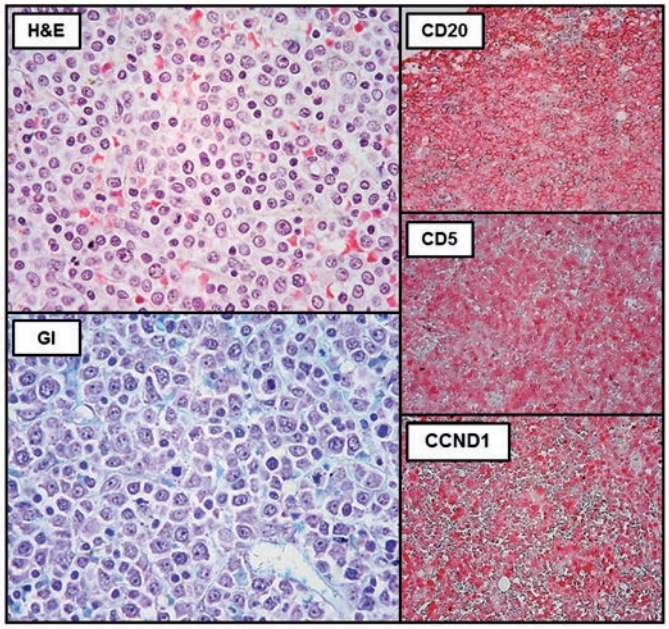

Figure 1.

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RITUXIMAB INDUCES HYPOGAMMAGLOBULINEMIA IN PATIENTS WITH NON HODGKIN LYMPHOMA

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Background: Rituximab (R) is a monoclonal antibody that binds the CD20 antigen on all peripheral B cells. Its favorable toxicity profile and effectiveness have led to its wide use in induction and maintenance regimens for Non Hodgkin Lymphoma (NHL). AIM. This retrospective single center study aimed to evaluate the hypogammaglobulinemia (hypoIg) associated with R use. *Patients and Methods:* We performed serial quantitative serum immunoglobulin (SIg) concentration at the baseline, after chemotherapy, during and after R maintenance therapy. IgG, IgA and IgM deficit were respectively defined by level below 700 mg/dL, 70 mg/dL and 40 mg/dL. Symptomatic patients were defined as having 2 or more non-neutropenic infections in a 6-month period after or during R. *Results:* 123 patients with indolent NHL and SIgG studies were analyzed, 47,1% were relapsed or refractory. The median age of patients was 60 years (range: 28-80). The histologies included follicular lymphoma (FL) (n=77), small lymphocytic lymphoma (SLL) (n=14), marginal zone lymphoma (ML) (n=20), mantle cell lymphoma (MCL) (n=12). Patients received a median of 13 doses of R (range: 6-27). The median follow-up of surviving patients was 4,4 years. Before treatment with R, 11/123 (8,9%) had low SIgG levels (5 FL, 1 MCL, 4 SLL, 1ML) and 3/11 (27,2%) required, during R maintenance treatment, IVIG administration. After R-chemotherapy, IgG deficiency appeared in 29/123 (28,4%), 2/29 needed IVIG. After or during R maintenance 25/123 (20,3%) showed IgG deficiency after a median of 9 R cumulative doses; the deficit occurred in the 80% (20/25) within the fourth R maintenance dose and in no one after the sixth R administration. In this category, 10/25 (40%) were symptomatic and 4/25 (16%) required IVIG. All 10 patients who needed IVIG showed at least two Ig isotypes deficiency. *Conclusions:* We observed that R administration was associated with a high risk of hypoIg. In addition, we found that the number of R doses correlated to the development of symptomatic hypoIg. Finally we observed that the risk of hypoIg increased in patients who received maintenance R. The decision to introduce therapy with IVIG in non-neutropenic patients was related to repeated episodes of infection. HypoIg often is underestimated also for the presence of confounding symptoms. Our study suggests that the baseline and periodic Ig monitoring should be considered in these patients subset.

P250

18F-FDG-PET/CT IN THE EVALUATION OF PATIENTS WITH FOLLICULAR LYMPHOMA

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Follicular lymphoma (FL) is characterized by slowly progressive adenopathy with variable FDG avidity. Recent studies support the use of functional imaging by 18F-FDG-PET/CT at diagnosis to identify patients with indolent or more aggressive disease according to tumor metabolic behavior. *Aims:* Evaluation of lymph node involvement in patients with FL by PET/CT, and correlation of the imaging parameters derived by this methodology with standardized clinical, biochemical and haematological variables used in the staging of these diseases. *Methods:* We evaluated retrospectively 24 patients (9 males, 15 females; mean age±SD, 57±8.7 years) with FL who had undergone whole-body PET/CT and contrast-enhanced CT alone or as part of PET/CT examination at the time of diagnosis. Patients were staged according to standard criteria including the evaluation of parameters belonging to the Follicular Lymphoma International Prognostic Index (FLIPI) 1 such as age, stage, lactate dehydrogenase (LDH), haemoglobin and number of pathological nodal sites or FLIPI 2 such as the diameter of the largest lymph node involved. *Results:* In the 24 patients studied, the number of pathological lymph node basins at PET/CT ranged between 1 and 33 and the SUVmax of the lymph node with the highest metabolic rate varied from 2.9 to 14.6 while at CT the number of pathological lymph node basins ranged between 1 and 28 and the maximum diameter of the largest involved lymph node varied from 17 to 75 mm. The number of pathological lymph nodes at PET/CT was significantly correlated with stage and FLIPI1 score (Spearman rank correlation coefficient r=0.76, P=0.0002 and r=0.56, P=0.0068, respectively). Likewise, the number of pathological lymph nodes at CT was significantly correlated with stage and FLIPI1 score (Spearman rank correlation coefficient r=0.65, P=0.0016 and r=0.64, P=0.0022, respectively). Moreover, a statistically significant direct correlation was found between the maximum diameter of the largest pathological lymph node at CT and the SUVmax of the lymph node with the highest tracer uptake (r=0.55 P=0.0075). The largest lymph node size showed a significant correlation with stage (Spearman rank correlation coefficient r=0.48, P=0.0252) while the highest SUVmax value was directly correlated with LDH levels (r=0.42 P=0.0464). *Conclusions:* In patients with FL PET/CT can be useful in evaluating lymph node involvement and therefore can be of aid in the prognostic assessment of these patients.

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EBV-POSITIVE MUCOCUTANEOUS ULCER: TWO CLINICAL CASES WITH UNFAVORABLE OUTCOME

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EBV MCU is a rare form of EBV-related lymphoproliferative (LP) disorder, generally associated with a known immunocompromised state or with senile or iatrogenic immunosuppression. Cases reported in the literature have often an indolent and self-limiting course, therefore the suggested approach is usually conservative. We describe two cases in which the course was more aggressive than expected and required a chemotherapeutic approach. A 70-year-old woman initially presented with an unique ulcerated, necrotic lesion at the right tonsil and tongue base. The biopsy showed a diffuse, polymorphic lymphoid infiltrate with numerous EBV+ CD20+ CD30+ cells. Her medical history wasn't

notable for infection predisposition, excepted for relapsing bacterial conjunctivitis and a VZV reactivation episode. Laboratory tests weren't significant and peripheral blood (PB) EBV-DNA was negative (Table 1). Prednisone treatment (25 mg/die) was prescribed with benefit for about three months, but, during the tapering phase, the lesion progressed and the patient needed an emergency tracheotomy due to massive bleeding. At this time, PB EBV-DNA was relevant. Four cycles of R-CHOP followed by radiotherapy were given, obtaining a CR which is persisting at three month from the end of the treatment. The second case is relative to a 68-year-old woman taking hydroxyurea since 1986 for polycythemia vera, then evolved in myelofibosis, and splenectomised in 2012. Her infectious anamnesis included relapsing HSV, VZV reactivation and upper airway and urinary tract infection susceptibility. Polyclonal hypergammaglobulinemia was detected post-splenectomy. On January 2014, she developed ulcerated lesions at the shoulder, oral mucosa and scalp. The biopsy showed an EBV-positive LP process. Unexpectedly, PB lymphocyte phenotyping documented a severe B cell-deficit. She received a course of rituximab (4 weekly doses at 375 mg/mq) associated with low-dose cyclophosphamide for two months, obtaining a CR. About 40 days after the stop of treatment, the scalp lesions reappeared. A new biopsy confirmed the diagnosis; molecular tests documented the clonal nature of the lesions. EBV–DNA was never found in the PB. Now she will start chemotherapy with R-COMP scheme. In conclusion we show that EBV MCU need to be strictly monitored because their course may not be self-healing and may even lead to life-threatening complications. Efforts should be made to identify prognostic markers and a correct therapeutic strategy.

Table 1. Patient's immunological and virological characteristics at baseline.

PAZIENT	AGE	WBC/mmc (Ly %)	Ly CD4+/mmc	Ly CD19+/mmc	Ig G (mg/dl)	EBV-DNA (copie/ml)
1	70	7200 (29)	396	53	799	<100
2	68	10700 (10)	450	3	1770	<100

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MAINTENANCE THERAPY WITH RITUXIMAB IN PATIENT WITH INDOLENT NON HODGKIN LYMPHOMA CD20+: IS IT A RISK FACTOR FOR HBV REACTIVATION?

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Introduction: Reactivation of hepatitis B virus(HBV)is an important cause of morbidity and mortality in patients with NHL undergoing chemotherapy. This risk increased with the introduction of anti CD20 antibody(Rituximab)based chemotherapy regimens. We evaluated how many HBV reactivation occurred among patients Hepatitis B core antigen positive(HBcAb+)and Hepatitis B surface antigen negative(HBsAg) who received maintenance therapy with Rituximab. *Patients and Methods:* Here we report our experience about 128 patients with indolent non Hodgkin Lymphoma CD20+ who received maintenance therapy with Rituximab (schedule:375 mg/mq every 2 months for 2 years)from January 2007 to February 2015. Patients received different chemotherapy regimens during induction: 38,2%(49/128) with RCHOP, 28%(36/128) with R-FN, 18% (23/128) RBendamustine, 6,2% (8/128) with RFludarabine, 3,1% (4/128) with RLeukeran, 3,1% (4/128) with Rituximab monotherapy and 1,5% (2/128) with RCVP. Only 1 patient received induction with RFC and another 1 with RCyclophosphamide. We performed blood tests for HBV (HBsAg, HBsAb, HBeAg, HBeAb, HBcAb) in all patients before starting maintenance therapy and liver function tests before each administration of Rituximab. None of these patients received prophylactic therapy with antiviral drugs during induction and maintenance therapy. *Results:* 29% of the patients(38/129)were HBcAb positive. 59,3% of the patient (76/128) completed or interrupted therapy with Rituximab and 27% of them (21/76) were HBcAb positive; two of these patients occurred HBV reactivation; 40,6% of the patients (52/128) are still in maintenance therapy and 32% of them (17/52) are HBcAb positive with risk of HBV reactivation too. *Discussion and Conclusions:* In patients HBcAB+/HBsAg- treated with Rituximab is indicated prophylaxis with lamivudine. In our single centre experience HBcAB+/HBsAg- patients didn't received therapy with antiviral drugs during maintenance therapy with Rituximab; two of our patient occurred HBV reactivation and started therapy with lamivudine. None needed hospitalization or other specific therapy. In terms of cost-benefit, we reported an advantage in the monitoring approach that was used in our patients in respect to universal prophylaxis with a total savings of about € 3.400,00 for each patient. More study are necessary to estabilish the clinical utility of prophylactic therapy with lamivudine during the maintenance therapy with Rituximab.

P253

USE OF NON-PEGYLATED LIPOSOME-ENCAPSULATED DOXORUBICIN IN NON HODGKIN LYMPHOMA PATIENTS WITH CARDIOVASCULAR COMORBIDITIES

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Anthracyclines-based regimens remain the gold standard for the treatment of Lymphomas, although the associated cardiac toxicity may limit their use, especially in frail and elderly patients. From October 2008 to January 2015 we treated 44 newly diagnosed patients with poor-risk lymphoma and cardiovascular comorbidity using the R-COMP regimen (rituximab, cyclophosphamide, non-pegylated liposome-encapsulated doxorubicin, vincristine and prednisone). Median age was 74 years (range:46-83 yrs; 45% ≥75 years). As for histology, 22/44 (50%) were Diffuse Large B-cell Lymphoma; 8/44 (18%) Mantle-cell Lymphoma; 5/44 (11%) Follicular Lymphoma; 3/44 (7%) Peripheral B-cell Lymphoma; 4/44 (9%) Nodal Marginal Zone Lymphoma; 2/44 (4%) other B-cell indolent Lymphoma. IPI score was Intermediate-High/High in 61,3% of the patients, 75% had an advanced Ann-Arbor stage and 43% presented at diagnosis with extranodal involvement. The median age adjusted Charlons comorbidity index was 6 (range: 3 to 9). Cardiovascular risk factors were considered: hypertension (30/44pts: 68%), 14/44 pts (31,6%) had a history or recent acute myocardial infarction and 4/44 (10%) suffered of Atrial fibrillation. Treatment was well tolerated and toxicities were limited grade III/IV cytopenia. Complete remission was achieved in 30/44 (68%) and partial response in 6/44 (14%). Progressive disease was present in 8/44 (18%). After a median follow up of 18 months, the median OS (Overall Survival) was not reached and 4/36(11%) responders relapsed. Cardiac toxicity was observed in one patients who died for pulmonary edema, while two patients developed arrhythmias. This study confirm the efficacy and tolerability of R-COMP regimen in elderly patients with cardiovascular comorbidities.

P254

ABDOMINAL CT SCAN IN DIAGNOSIS OF HEPATIC FOLLICULAR LYMPHOMA IN A PATIENT WITH CHRONIC HEPATITIS C

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Introduction: Follicular lymphoma (FL) is one form of lymphoma non Hodgkin. The initial symptoms of FL include painless swelling in one or more lymph nodes. Some people with FL develop large tumors in the abdomen. Initial presentation of hepatic FL can be given by abdominal pain and light increase in serum aminotransferase activity. We report a 62-year-old man with abdominal pain and history of chronic hepatitis C. Abdominal CT scan detected hypodense lesions in the V and VI hepatic segment and lomboaortic nodes >1 cm. *Methods:* A 62-year-old

man with history of chronic hepatitis C was admitted to our hospital for abdominal pain, light increase in serum aminotransferase activity and presence of hypoechoic lesions in the liver. CT scan confirmed hypodense lesions in the V and VI hepatic segment and showed lomboaortic nodes >1 cm (Figure 1). Bone marrow aspirate showed increased number of B lymphocytes 65%, with dysplastic features. Immunophenotype was CD22+, CD5-, CD23-, CD10+. Histology of liver lesions and abdominal nodes prompted the diagnosis of FL. *Results:* The patient was treated with R-CHOP (rituximab, cyclophosphamide,doxorubicin, vincristine and prednisone) obtaining a remission of symptoms. *Conclusions:* Abdominal CT scan may be helpful for a rapid diagnosis of hepatic FL in patients with chronic hepatitis. In our opinion, a multidisciplinary collaboration between haematologist, radiologist and cytologist is essential in order to obtain the diagnosis and rapidly to start treatment.

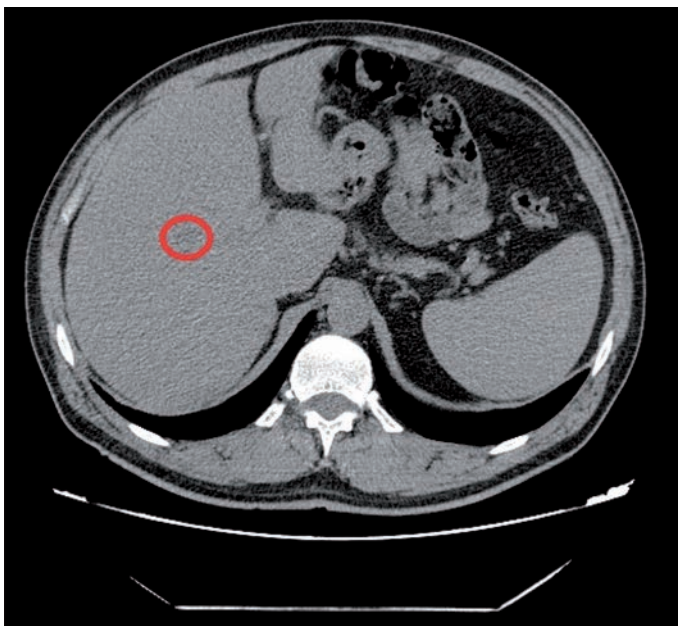

Figure 1. Hepatic Follicular lymphoma visualyzed as hypodense lesions.

P255

PERSONALIZED TREATMENT IN ELDERLY PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA: A SINGLE CENTRE EXPERIENCE

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The combination of rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone (R-CHOP) is considered the standard of therapy for diffuse large B-cell lymphoma (DLBCL). However, every-day clinical practice is different in elderly patients in which therapy is modulated on frailty and comorbidities. We analyzed treatments and survival in patients aged from 60 years old with DLBCL who were diagnosed between 2010 and 2014. Data were collected prospectively and analyzed retrospectively. Among 180 patients with DLBCL, 111 (62%) were aged >60 years. Median age was 74 years old (range 60-89 years) and median follow up from diagnosis was 35 months (range 1,7-63,1 months). Patients were divided in 3 group according to age; their characteristics and disease features are summarized in Table 1. The use of lyposomial anthracycline was considered in patient with previous cardiac comorbidities. Patient received R-CHOP had 35/41 (85%) complete remission (CR) and patient treated with R-COMP (R-CHOP with lyposomial anthracycline) had 30/42 (72%) CR. On the other hand, patient treated with R-CVP (R-CHOP without anthracycline) had a CR in 9/17 (52%) cases. All patients has received Granulocyte-Colony Stimulating Factor as neutropenia prophylaxis. No cardiac toxicities were observed. Only 4/51 patients in the late elderly group did not end the planned treatment for disease progression (n=2) or septic shock (n=2). Deaths were 4/40 in early elderly group (3 related to lymphoma), 15/51 in late elderly group (12 for disease progression) and 11/20 in the very elderly (10 for DLBCL). 5-years overall survival (OS) and progression free survival were 72% and 70%. If patients were divided in the three class of age (60-70 years old, 70-80 years old and >80 years old), 5-years OS was respectively 88%; 65%, 56%. Treating DLBCL in elderly patients is feasable and can improve quality of life among patient even in very elderly age. Anthracycline is confirmed to be an important drug in this setting with better response rate. Lyposomial anthracycline should be considered in patients with cardiac comorbidities with good response rate and no further cardiac complications.

Table 1.

	Median age	Sex	Stage	B symptoms	IPI	Bulky disease	Extranodal disease	ECOG at diagnosis	Type of treatment (CR)
Early elderly (n=40)	64 years old (range 60-69)	17 F 23 M	I-II =14 III-IV= 26	Yes=8	0=1 1= 7 2= 9 3= 14 4= 9	Yes= 8	Yes= 17	0= 6 1= 13 2= 7 3= 8 NA= 6	RCHOP = 29 (25) RCOMP= 6 (6) RCVP= 2 (1) Other= 3 (2) Palliation = 0
Late elderly (n=51)	75 years old (range 70-79)	25 F 26 M	I-II= 23 III-IV= 28	Yes= 10	1= 9 2= 15 3= 16 4= 11	Yes= 14	Yes= 29	0= 2 1= 22 2= 15 3= 7 NA= 5	RCHOP = 10 (8) RCOMP= 32 (22) RCVP= 7 (2) Other= 1 (0) Palliation = 1 (0)
Very elderly (n=20)	82 years old (range 80-89)	13 F 7 M	I-II= 10 III-IV= 10	Yes= 2	1= 1 2= 10 3= 7 4= 2	Yes= 8	Yes= 8	0= 2 1= 2 2= 6 3= 4 4= 1 NA= 5	RCHOP = 2 (2) RCOMP= 4 (2) RCVP= 7 (6) Other= 3 (0) Palliation = 4 (0)

Myeloma and Monoclonal Gammopathies 2

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ROLE OF NUCLEAR HEME OXYGENASE 1 IN BORTEZOMIB INDUCED CELL DEATH AND GENOMIC INSTABILITY OF MULTIPLE MYELOMA CELLS

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Despite recent advances in proteasome inhibitor and immunomodulatory drug-based therapies, MM remains largely incurable, primarily owing to acquired resistance. HO-1 is a cytoprotective microsomal enzyme that catalyzes the degradation of heme. We have recently shown that the protective effect of HO-1 on drug-induced cytotoxicity in leukemic cells does not involve its enzymatic byproducts, but rather its nuclear translocation following proteolytic cleavage. It has been recently described that Bortezomib (BO) is able to increase HO-1 expression. We investigated about the role of BO-induced HO-1 in MM cell lines (U266, SKM-M1). As expected, we observed that BO induced apoptosis after 24h ($p<0.001$). Flow cytometric analysis revealed increased levels of ROS after 1h ($p<0.0001$) of treatment with a peak after 3h ($p<0.001$). BO was able to induce a significant increase in HO-1 mRNA levels after 3h ($p<0.0001$) of treatment with a maximum peak after 6h ($p<0.0001$). Since HO-1 is one of the major endoplasmic reticulum (ER) associated heme protein, we analyzed the ability of BO to induce ER stress. BO was able to induce the expression of ER stress proteins (Bip, IRE1, Ero1, PERK and CHOP) in MM cells after 6h ($p<0.001$) with a peak after 24h ($p<0.0001$). By confocal microscope we observed that HO-1 localized both in the cytoplasm and in the nucleus of MM cells. Interestingly, blockage of nuclear translocation by E64, a selective inhibitor of the protein cleavage, induced MM cells to become more sensitive to BO. In addition, BO-resistant U266 showed increased HO-1 expression. Since nuclear HO-1 it has been reported to be a regulator of DNA repair activities, we also explored its role in genomic instability of MM cells. Using the cytochinesis-block micronucleus (CBMN) assay, we observed that pre-treatment of U266 with E64 for 24h led to a significant reduction of the percentage of micronuclei ($p<0.01$) and nucleolasmic bridges ($p<0.05$) observed in binucleated cells. Next, we evaluated U266 ability to activate G2/M checkpoint after UV damage using CBPI (cytochinesis block proliferation index) assay. The percentage of monucleated cells (G2/M checkpoint activated) was higher in cells pre-treated with E64 than control ($p<0.05$). Our data suggest that BO-induction of HO1 is probably due to the ability of BO to induce ER stress. HO-1 nuclear translocation may be involved in MM BO resistance. In addition, nuclear HO-1 may be involved in genomic instability of MM cells.

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LIGHT/TNFSF14 INVOLVEMENT IN MULTIPLE MYELOMA-OSTEOLYTIC BONE DISEASE

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Background: The TNF superfamily member LIGHT, known to play a major role in T-cell homeostasis, has been reported in erosive bone disease associated with rheumatoid arthritis. Herein, we investigated whether LIGHT is implicated in the mechanisms leading to Multiple Myeloma (MM)-osteolytic bone disease. *Methods:* PB and BM aspirates were obtained from 60 patients (38M/22F, median age: 66 years), newly diagnosed as having symptomatic MM with or without bone disease, smoldering MM (sMM) or MGUS. The control group included PB and BM aspirates from 15 patients with non-neoplastic disease without any skeletal involvement, and PB from 25 healthy-donors matching for age and sex with the MM group. The study was approved by Ethical Committee of Bari University Hospital. LIGHT expression was assessed by flow cytometry, western blotting and real-time PCR. Osteoclasts (OCs) were obtained from unfractionated PBMC cultures treated or not with an anti-LIGHT neutralizing monoclonal antibody (mAb). In cultures from BM mononuclear cells (BMNCs), the formation of CFU-F and CFU-OB was evaluated in the presence or absence of anti-LIGHT neutralizing mAb. Further, in CFU-F and CFU-OB cultures, the expression of OB differentiation markers was analyzed by real-time PCR. *Results:* We found overexpression of LIGHT on CD14+ monocytes, CD8+ T-cells and neutrophils of PB and BM from MM-bone disease patients, in whom LIGHT induced osteoclastogenesis and inhibited osteoblastogenesis, as we demonstrated by culture treatment with an anti-LIGHT antibody. Moreover, in cultures from healthy-donors, we found that LIGHT induced osteoclastogenesis in RANKL-dependent and –independent manners. In particular, in the presence of a sub-optimal RANKL concentration, LIGHT and RANKL showed synergic effects on osteoclast formation, associated to early and sustained activation of Akt, NF B and JNK pathways. Otherwise in cultures of BM samples from patients without bone disease, LIGHT treatment inhibited the formation of CFU-F and CFU-OB, and the expression of osteoblastic markers such as collagen-I, osteocalcin and bone sialoprotein-II, supporting a LIGHT indirect inhibition of osteoblastogenesis, possibly and to some extent, through sclerostin expression by monocytes. *Conclusions:* LIGHT overexpression seems to be implicated in the increased osteoclastogenesis and decreased osteoblastogenesis characterizing MM-osteolytic bone disease.

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MUTATIONAL SPECTRUM OF BRAF, NRAS AND KRAS IN MULTIPLE MYELOMA

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Multiple myeloma (MM) is a clinically and genetically heterogeneous plasma cell (PC) malignancy. Whole-exome sequencing has identified therapeutically targetable mutations such as those in the mitogen-activated protein kinase (MAPK) pathway, which are the most prevalent MM mutations. To analyze BRAF, NRAS and KRAS mutations in untreated MM, we investigated by next generation sequencing (NGS) highly purified plasma cells (PC) of a retrospective cohort of 132 cases at onset, 14 of whom were also tested at relapse. Moreover, we examined 11 patients with secondary PC leukemia (sPCL). NGS of the mutational hotspots of the genes (BRAF exons 11 and 15, NRAS exons 2 and 3, and KRAS exons 2-4) was performed by Roche 454 pyrosequencing on the Genome Sequencer Junior instrument. Mutations were validated in an independent PCR product by conventional Sanger sequencing or NGS. Median depth of coverage was 233x (range: 100-962). Overall, the MAPK pathway was affected in 60.1% of the patients (63.6% of those with sPCL, and 59.8% of those with MM); in particular, 10.5% of patients were found mutated in BRAF (10.6% of MMs and 9.1% of sPCLs), 27.3% in NRAS (26.5% of MMs and 36.4% of sPCLs), and 31.5% in KRAS (32.6% of MMs and 18.2% of sPCLs). Confirming recent data indicating multiple mutations within the same pathway, we identified 13 samples with simultaneous mutations in two genes: three cases in BRAF and NRAS, five in BRAF and KRAS, and five with NRAS and KRAS. Co-existing mutations tended to occur at different variant allele frequencies (VAFs), thus supporting the occurrence of tumor subclones. Sequential analyses revealed different mutation patterns, *i.e.*: the presence of clonal variants at both timepoints; the acquisition/clonal expansion of variants in the later sample; and even the disappearance of variants at relatively high VAF values, but always occurring concurrently with the emergence/clonal expansion of an additional mutation in another gene of the pathway. The majority of BRAF variants, tested also on cDNA, were comparably expressed at genomic and transcript levels. Our data confirm and extend previous published evidence that MAPK pathway activation is recurrent in myeloma, and the finding that it is mediated by

BRAF mutations in a significant fraction of patients has potentially immediate clinical implications.

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DIAGNOSIS OF MALIGNANT MELANOMA METASTASIZING TO THE BONE MARROW SUGGESTED BY FLOW CYTOMETRY: A CASE REPORT

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Multicolor flow cytometry is widely used in the diagnosis of hematological malignancies but recently there have been an increasing number of reports describing the detection of circulating tumor cells, based on the lack of expression of the pan leukocyte antigen CD45. On the other hand, some non-hematological malignancies, such as melanoma, show surface expression of CD56. Here, we report a case of bone marrow metastasis, with non cohesive tumor cells promptly identified during flow cytometry analysis. A 42-year-old woman was referred to our hospital due to weakness and bone pain. PB tests showed normocytic anemia, mild leukocytosis and thrombocytopenia. Additional laboratory tests showed an elevated calcium level and kidney failure, but serum protein electrophoresis was normal. CT scan showed an osteolytic vertebral compression of D10. Clinical examination revealed a subcutaneous lesion in the right scapular region but dermoscopic imaging was negative. BM biopsy was performed for suspected multiple myeloma. BM aspiration showed a hypercellular marrow with diffuse involvement of large atypical cells and suppressed hematopoiesis; FCM showed 15% of nucleated cells with strong CD56 expression but lacking CD38, CD45 and CD138. Cells were stained with a large panel of MoAbs but were found positive only for NG2 and myeloperoxidase, ruling out hematological malignancies and suggesting metastasis of a non-hematological malignancy such as melanoma. BM biopsy showed diffuse infiltration of rounded neoplastic cells with granular cytoplasm. Immunohistochemical examination demonstrated tumor cells positive for tryptase, CD25, S100 protein and vimentin and negative for Melan-A, HMB45, CD138, CD30, CD45, CD117, CD2, keratin, muscle actin and desmin. PET/CT scan revealed a uniform increased FDG uptake throughout the bone marrow space; a more circumscribed area of high FDG uptake was revealed in the cutaneous and subcutaneous layers of the right scapular region. A biopsy was taken from the subcutaneous lesion and, finally, metastatic melanoma was diagnosed, with a strong expression of S-100 and weak expression of Melan-A and HMB-45 proteins. The patient died approximately six weeks after the diagnosis during palliative treatment. Our case underlines the clinical relevance of FCM also in non-hematological malignancies, as a means of promptly ruling out a hematological origin of neoplastic cells and providing useful information to better address clinical investigations.

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BONE MARROW PLASMA CELLS ASSESSMENT DOES NOT IMPACT ON PERIPHERAL BLOOD STEM CELLS MOBILIZATION IN MULTIPLE MYELOMA PATIENTS IN THE NOVEL AGENTS ERA

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Background: Immunomodulatory drugs and proteasome inhibitors have been incorporated in front line multiple myeloma (MM) treatment. These drugs did not negatively affected the efficacy of stem cell harvest. Few data are available on the prognostic role of residual bone marrow plasma cells (BMPC) assessment on peripheral blood stem cell (PBSC) harvest after induction therapy in pts undergoing autologous stem cell transplant (ASCT). *Aims:* The aim of this retrospective single-centre study was to determine the impact of BMPC before PBSC mobilization on the efficacy of CD34+ cells collection and number of leukapheresis. *Methods:* 138 newly diagnosed MM pts underwent ASCT in our center from January 2006 to April 2015; induction therapy included dexamethasone/ thalidomide (TD) and/or bortezomib (VTD/VD). Bone marrow biopsy was performed before PBSC mobilization; BMPC were evaluated after CD138+, kappa and lambda immunostaining. All pts were mobilized with EDX 3-4 g/m^2 plus G-CSF 5-10 g/kg daily from day 2 until the end of the collection procedure. Leukapheresis was scheduled to start on day 10 and be performed until at least 2 106 CD34+ cells/kg were collected. CD34+ cell counts were assayed using a single platform method. Statistical analysis was made by Mann-Whitney and Kruskal-Wallis test. *Results:* Median age was 59 (range, 37-68); 52 pts were females. Induction therapy was VTD in 85 pts (62%), TD in 40 (29%), VD in 13 (9%). In 70 pts (51%) the percentage of BMPC was <5%; in 49 (35%) between 5% to 20% and in 19 pts (14%) higher than 20%. Mobilization was performed in all pts: 16 with EDX 3 g/m^2 (22%), 122 (88%) with EDX 4g/m^2. In 104 pts (75%) the mean number of CD34+ cells collected was 11.8±8.67x106/kg within 1 leukapheresis; 25 (19%) pts needed a second procedure to collect a mean CD34+ cells number of 7.33±3.8x106/kg, whereas 9 pts (6%) required 3 or 4 procedures and collected 4.9±2.1 106/Kg (p=0.0001). The BMPC percentage was not significantly correlated with the CD34+ cell count or numbers of leukapheresis. The CD34+ cells count was significantly higher in pts younger than 55 yrs (n=49; 12,6±6.6 106/Kg) than in the older pts (n=89; 9,48±8.5 106/Kg) (p=0.0003). The mean CD34+ cells count was significantly higher in pts treated with VTD *vs* TD (11.80 106/Kg, *vs* 8.21 106/Kg). *Discussion and Conclusions:* In our study the residual BMPC percentage did not adversely impact on CD34+ collection. Younger age and VTD induction were associated with higher CD34+ cell count.

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TARGETING THE NOTCH PATHWAY IN MULTIPLE MYELOMA THROUGH SMALL MOLECULES UNCOUPLING NOTCH-JAG INTERACTION

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Multiple myeloma (MM) is still an incurable plasma cell malignancy, mainly due to the localization of MM cells in the bone marrow (BM), where they establish complex interactions with the local milieu, resulting in severe osteolysis, drug resistance (DR) and consequent relapse. Notch signaling is involved in MM cell migration, proliferation, survival and DR. In MM Notch is dysregulated due to the aberrant expression of Notch1-2 receptors and Jag1-2 ligands. The relevance of this dysregulation in MM is evident since through the interaction of Notch receptors and ligands, MM cells shape the nearby niche, promoting key features of MM progression such as osteolysis and DR induced by BM stromal cells (BMSCs). The evidence of our group and literature data showed that all these effects can be interfered by knocking down Jag1-2 in MM cells. This provides a strong rationale for disrupting Notch-Jagged interaction in MM cells and the nearby BMSCs in order to set up a novel therapeutic strategy for relapsing myeloma and advanced bone disease. We have identified in silico 100 small candidate molecules able to disrupt the Notch-Jag complex by screening a large combinatorial database of commercial compounds. The effect of two candidate compounds on Notch transcriptional activity was preliminary analyzed. Notch signal activation was measured in HEK293T, using a Notch responsive Luciferase reporter assay. The compounds efficacy in inhibiting Notch activity was comparable to common anti-Notch agents, *i.e.* -secretase inhibitors (GSIs). Moreover, getting advantage of co-culture systems and Notch responsive luciferase assay, we verified that the compounds in analysis were effective in inhibiting the ability of MM-derived Jag ligands to activate, by heterotypic interaction, Notch signaling in BMSCs. Finally, using different cell lines, we verified that the treatment with compounds induced a dose-dependent cell growth inhibition comparable to GSIs. In conclusion, the proposed approach, aiming at disrupting the Notch-Jag complex using small molecules, promises to be effective to overcome the effect of Notch activation in MM cells as well as in the surrounding stromal cells. We aim to expand the number of candidate compounds in order to successfully identify a lead clinical drug candidate.

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HIGH-THROUGHPUT SEQUENCING FOR THE IDENTIFICATION OF DIS3 MUTATIONS IN MULTIPLE MYELOMA

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DIS3 is a catalytic subunit of the human exosome complex, containing exonucleolytic (RNB) and endonucleolytic (PIN) domains and recently found mutated in about 10% of patients with multiple myeloma (MM). To analyze DIS3 mutation in untreated MM, we investigated by next generation sequencing (NGS) highly purified plasma cells (PC) of a retrospective cohort of 130 cases at onset, 14 of whom were also tested at relapse. Moreover, we examined 10 patients with secondary PC leukemia (sPCL). Deep sequencing of the PIN and RNB domains was performed by Roche 454 pyrosequencing on the Genome Sequencer Junior instrument. Mutations were validated in an independent PCR product by conventional Sanger sequencing or NGS. Median depth of coverage was 264x (range: 102-870). The analysis revealed the presence of 30 different tumor-specific non-synonymous variants, identified in 27 patients: of these, 23 mutations were missense, five introduced a frameshift, and two an in-frame deletion. Seven of these variants have been already reported by others, also specifically in MM patients, while 23 were novel. Mutations were predominantly localized in the RNB domain; the most recurrently affected residues were R780 and D488, mutated in five and four cases, respectively. Globally, 18.5% of MM and 30% of sPCL patients were found mutated. DIS3 mutations were preferentially carried by IGH-translocated patients, and were often detected at low variant allele frequency (VAF): in particular, a VAF of around 100% was exclusively observed in a fraction of patients with 13q deletion. In the rest of the cases, *i.e.* the remaining del(13q14) samples and all the patients disomic for chr 13, the VAFs were respectively suggestive of a mutation present in hemizygosis in a tumor subclone (0.8%<VAF<57%), or in heterozygosis either in all MM cells (if around 50%) or in a tumor subclone (0.5%<VAF<40%). Sequential analysis highlighted a few instances of increase of DIS3 mutation burden during disease progression. By means of NGS of DIS3 cDNA in mutated cases, we found that the majority of variants were comparably detectable also at transcript level. Furthermore, gene expression profiling analysis identified in DIS3-mutated patients a transcriptional phenotype apparently compatible with impaired RNA exosome function. In conclusion, these data demonstrate the relevance of DIS3 mutations and suggest it as a potential tumor suppressor in PC dyscrasias.

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OSTEONECROSIS OF THE JAWS IN MULTIPLE MYELOMA PATIENTS TREATED WITH ZOLEDRONIC ACID: A SINGLE-CENTER EXPERIENCE

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It has been demonstrated that bisphosphonate-based supportive therapy reduces skeletal events (onset or progression of osteolytic lesions) both in patients with multiple myeloma and in cancer patients with bone metastasis. Bisphosphonates are generally well tolerated and associated with minimal adverse effects: fever, renal function impairment, myalgias and hypocalcemia. In recent years, several cases of jaw bone necrosis associated with long-term use of bisphosphonates have been reported. The estimated incidence varies from 1.8% to 12.8% (Hematology 2006). The pathogenesis of this complication is unknown; however, several predisposing factors have been identified: poor oral hygiene, periodontal disease, dentoalveolar surgery, corticosteroid therapy, immune-compromised state predisposing to increased risk of infection. We performed a retrospective study on osteonecrosis of the jaws in 34 multiple myeloma patients with a history of chronic zoledronic acid therapy. Between January 2008 and December 2014 we observed four patients with osteonecrosis of the jaws (14.7%). Diagnosis was radiological and clinical. CT scan confirmed the presence of an osteolityc area with signs of periosteal reaction. Microbiology showed actinomycetes and mixed bacteria. The characteristic of the patients were the following: median age=75 years (43-84), M/F=3/2, IgG/IgA=3/2 kappa/lambda=3/2. Steroids use=5 patients. Thalidomide use=3 patients. Autologous stem cell transplantation=1 patient. Previous dental extraction=1 patients. Median time of exposure to zoledronic acid=5 months (3-13). Aminobisphosponates exert several antitumor effect, including induction of tumor cell apoptosis, inhibition of tumor cell adhesion to the extracellular matrix, and inhibition of tumor invasion. Zoledronic acid also have antiangiogenesis properties and can activate γ-δ T cells. It has resulted in a statistically significant reduction in skeletal complication. We have initiated the following guidelines in an effort to ameliorate the incidence of jaw bone necrosis. Patients have a screening dental examination and an appropriate radiographic study before the administration of zoledronic acid. They are encouraged to practice good dental hygiene and see a dentist promptly if oral or dental symptoms appear. In addition, zoledronic acid are held for a period of 3 months prior to invasive dental procedures to allow for the osteoclastic recovery. Following the dental procedure we re-introduce bisphosphonates only after the healing process is complete.

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MULTIPLE MYELOMA-ASSOCIATED DRUG RESISTANCE: TARGETING THE NOTCH PATHWAY

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Multiple myeloma (MM) represents 11% of hematological malignancies and is caused by the accumulation of malignant plasma cells in the bone marrow (BM). Although treatments with new drugs, such as immunomodulators and proteasome inhibitors, are increasing patients' survival, MM is still incurable due to the development of endogenous or BM mediated drug resistance. Therefore it is important to find new therapeutic targets. The dysregulated expression of two Notch ligands, Jagged1 and 2, causes the hyperactivation of the Notch signaling both in MM cells and in bone marrow stromal cell (BMSC). The aim of this study was to investigate the role of Notch signaling in endogenous and BMSC-induced drug resistance in MM. At this purpose, we silenced two Notch ligands, Jagged1 and 2, in the MM cell lines OPM-2 and U266. Jagged1/2 silencing causes a reduction in the expression of anti-apoptotic genes, *i.e.* SDF-1α, Bcl-XL, Bcl-2, Survivin and ABCC1. In accordance, MM cells with reduced levels of Jagged1 and 2 showed an increased sensitivity to different drugs commonly used in MM therapy such as Bortezomib, Mitoxantrone and Melphalan. In addition, Jagged1/2 knockdown affects the pathological interaction between MM and BMSCs resulting in the activation of Notch signaling in both cell types. Indeed, when co-cultured with human BMSCs, MM cells displayed an higher level of drug resistance due to: 1) an increased expression of anti-apoptotic genes in MM cells, *i.e.* Bcl-XL, Bcl-2, Survivin and ABCC1; 2) the BMSC-mediated release of soluble factors, *i.e.* SDF-1α and VEGF, relevant for MM cell growth and survival. Jagged1 and 2 silencing in MM cells could reverse all these effects. These *in vitro* results were confirmed in co-culture experiments performed with primary human CD138+ multiple myeloma cells and BMSCs isolated from patient's bone marrow aspirates. The evidence that Jagged-1/2 silencing affects endogenous and BMSC-induced drug resistance in MM cells supports the use of a Jagged-targeted approach in MM therapy alone or in a combination with standard of care drugs.

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OUTCOME OF NEWLY DIAGNOSED SYMPTOMATIC MULTIPLE MYELOMA IN VERY ELDERLY PATIENTS (AGED 80 YEARS OR MORE)

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MM is a plasma cell neoplasm typical of the elderly, with a median age at diagnosis of 65 years. The increase in median age in western countries has led to an increase in the incidence of this disease. The aim of our study is a retrospective analysis of the outcome of very elderly (80 years or more) patients (pts) with newly diagnosed symtomatic MM to determine the characteristics of this subset of pts. We collected data from 47 very elderly (80 years or more) pts diagnosed and treated from January 2008 to December 2014. Median follow-up was 31 months after diagnosis. Median age at diagnosis was 83 years and PS was <2 in 37 cases (79%). One or two concomitant diseases requiring specific treatments were present in 29 pts (62%), and 3 or more concomitant diseases were present in 8 pts (17%). According to the ISS, 17 pts were classified as III stage, 10 as II stage and 20 pts as I stage. Bone lytic lesions were present at diagnosis in 34 pts (72%), representing the most CRAB feature. First line therapy was bortezomib (once-weekly administration)/dexamethasone in 10 pts (21%), melphalan/prednisone +/- thalidomide in 9 pts (19%) and bortezomib/melphalan/prednisone in 28 pts (60%). According to IMWG response criteria, 19 pts (40%) achieved CR, 12 (26%) achieved PR, 5 (11%) achieved VGPR, and 8 (17%) achieved stable disease; 3 pts (6%) experienced progressive disease. Hematologic toxicity was infrequent but usually weak/moderate and 24 pts received erythropoiesis-stimulating agents. Extrahematologic toxicity was observed in 17 pts (36%), and neuropathy was the most common adverse event for treatment. 25 pts (53%) had at least one disease progression since diagnosis and were therefore switched to second-line therapy. The median time to first disease progression was 21 months since start of first-line therapy. Second line therapy was bortezomib (once-weekly administration)/dexamethasone in 5 pts, and lenalidomide/dexamethasone in 20 pts. 5 pts received bendamustine/dexamethasone as third line of treatment in disease progression. 31 pts (66%) are still alive for a median OS of 22 months. 14 pts died due to disease progression and 2 died due to causes not related to MM. A study in a larger series of pts is warranted but our experience showed that no upper age limit should be applied for the administration of new drugs with MM; these treatments could be offered to very elderly pts, including those with severe concomitant diseases.

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FUNCTIONAL EXPRESSION OF CD47 AND ITS LIGANDS IN TUMOR PLASMACELLS OF MULTIPLE MYELOMA

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CD47 is an integrin-associated transmembrane protein that functions as a ligand for the signal regulatory protein- (SIRP /CD172), expressed on macrophage and dendritic cells, and for thrombospondins (TSP)-1-2, representing matricellular proteins synthesized and secreted by a variety of cells in the hemopoietic microenvironment. While CD47 is present at surface of different cell types, its engagement by SIRP inhibits the phagocytosis of CD47-expressing target cells by SIRP -expressing macrophages. Overexpression of CD47 may then represent a mechanism for cancer cells to evade phagocytosis. We explored the putative role of CD47-mediated cellular interactions in the pathophysiology of human multiple myeloma (MM). To this end, we evaluated expression of CD47 and of its ligands in a panel of human MM cell lines and purified CD138+ BM tumor plasmacells (PCs) from 20 patients with newly diagnosed MM and plasma cell leukemia (PCL). The relative expression of CD47 and ligands was initially evaluated by QRT-PCR using the THP1/HL60 cells as internal comparators. Most MM cell lines (LP-1, U266, RPMI 8226, AGM1, AGM4) expressed CD47 transcripts at 2- to 4-folds higher levels than THP1 and very low to undetectable levels of SIRP,g and TSP1. In contrast, KMS12-BM and KMS21-BM cells showed low CD47 expression coupled to increased levels of SIRP and TSP1 transcripts. Interestingly, all MM cel lines overexpressed TSP2. Results were confirmed by surface proteins detection through flow cytometry. While CD138+ tumor PCs showed a consistent overexpression (up to 7-fold higher) of CD47 transcripts and protein, as compared to CD138- cells (P=0.0020), the expression of CD47 ligands was by far more heterogenous, with samples displaying SIRP,g and TSP1 at significantly higher levels than CD138- cells (40% of cases) or at low/undetectable levels. TSP2 levels were invariably upregulated in CD138+ tumor cells. Intriguingly, the 2 PCL cases analyzed were characterized by the highest and lower expression levels of CD47 and SIRP,g and TSP1/2, respectively. Notably, exposure of MM cell lines to anticancer agents used in MM resulted in CD47 upregulation on tumor cells. Blocking CD47-SIRP interactions with CD47-Fc prevented THP1-mediated phagocytosis of primary tumor PCs and MM cell lines, before and after exposure to alkylators. These results suggest that inhibitory targeting of CD47 on malignant PCs may represent a valuable strategy to boost anti-tumor immune response in MM.

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BORTEZOMIB WITH HIGH DOSE MELPHALAN CONDITIONING FOR AUTOLOGOUS STEM CELL TRANSPLANTATION IS SAFE AND IMPROVES THE RESPONSE RATE IN PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA

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High dose therapy followed by autologous stem cell transplantation (ASCT) is the standard of care as first-line treatment in eligible patients with newly diagnosed Multiple Myeloma (MM). High-dose Melphalan (HDM) is the standard conditioning regimen before ASCT. Recent evidences suggest that the depth of response after the induction therapy influences the progression free survival (PFS) and, in most studies, overall survival (OS). To improve the depth of response after ASCT in transplant–eligible MM patients the introduction of Bortezomib (BOR) to high-dose Melphalan (HDM) in conditioning regimen has shown to be effective without increased hematological toxicity. In this study we retrospectively analyzed, in a single center, 22 patients treated with BOR+HDM as conditioning regimen as compared to a historical cohort of 22 patients treated with HDM alone in order to evaluate the safety and the response rate. Any significant difference was not observed between the two cohorts of MM patients analyzed regarding median age (58.7 *vs* 60.5 years), sex and dose of melphalan. Distribution of ISS stage was similar in the two groups, as well as that of high-risk cytogenetic or ultra high-risk features. Moreover the response rates after the induction therapy with novel agents was not statistically different in the two groups of patients analyzed. Any significant difference on hematopoietic recovery rates was not observed in BOR+HDM as compared to HDM alone with a median time to neutrophil recovery of 12 days (range 5-18) and to platelet recovery of 12 days (range 6-22) in both groups. BOR+HDM conditioning was well tolerated, with no increase of neuropathy occurrence. We then analyzed the response rate after ASCT, showing that the overall response rate was significantly higher in BOR-HDM group as compared to HDM (P=0.029) with a significant higher number of complete response (CR) (57% *vs* 16%; P=0.019). The number of stringent CR was also significantly higher in BOR-HDM as compared to HDM alone (4 *vs* 0). Moreover an improvement of the response rate after ABMT was seen more frequently in the BOR-HDM group as compared to the HDM group (P=0.033). In conclusion, this retrospective analysis suggests that BOR-HDM is safe as conditioning regimen with a higher response rate after ABMT as compared to the standard HDM regimen giving the rational design for randomized studies needed to assess whether this conditioning regimen is superior to HDM alone.

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NOTCH PATHWAY AND INTELEUKIN-6 COOPERATE AND SUPPORT MULTIPLE MYELOMA CELL GROWTH

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Multiple myeloma (MM) is a malignant plasma cells (PCs) disorder characterized by proliferation of neoplastic cells in the bone marrow (BM). MM accounts alone for 11% of all hematological malignancies and still remains incurable despite the development of new drugs. MM cells are strictly dependent on BM microenvironment, since it supports tumor growth and progression through adhesion molecules and soluble mediators, such as interleukin-6 (IL6). The Notch family of receptors consists of 4 isoforms that, once activated by membrane-bound ligands (Jag1-2 and DLL1-3-4), work as transcription factors. Recent evidences from our group suggest that the Notch pathway participates in critical events during MM disease progression by positively regulating cell proliferation, drug resistance and BM infiltration through the overexpression of both receptors (Notch1-2) and ligands (Jag1-2). Here, we specifically investigated the cooperation between the Notch pathway and IL6 signaling in the promotion of MM cells growth. MM cells depend on IL6 secretion by BM stromal cells (BMSCs) and may later acquire the ability to produce IL6 by themselves. By using a panel of MM cell lines and an inhibitory approach through chemical inhibitors (*i.e.* DAPT) or RNA interference, we demonstrate that Notch pathway favors MM cell growth by directly controlling IL6 autocrine production, and that, upon Notch withdrawal, IL6-independent cell lines became dependent on IL6 stimulation for proliferation. On the opposite, IL6-dependent cell lines show a lower level of IL6 dependency when Notch signaling is activated. Besides an autonomous expression of IL6 by MM cells, several reports indicate that BMSCs are the most important source of IL6 in the BM. By knocking down Notch1 expression, we verified that BMSC-mediated IL6 production is positively regulated by Notch signaling. Moreover coculture systems of MM cells and BMSCs allowed us to verify that MM cell-derived Jag ligands activate Notch signaling in BMSCs, thereby inducing IL6 secretion and promoting the proliferation of MM cells. Accordingly, Jag silencing, obtained through RNA interference approach in MM cells, reduced BMSCs mediated production of IL6 and their ability to sustain MM cell growth. The present results suggest that Notch pathway activation in MM cells and the surrounding BMSCs is key to MM cell growth thus may support the rationale for a Notch-directed approach in MM therapy, suggesting Jagged ligands as promising molecular targets.

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IMAGING CT SCAN IN DIAGNOSIS OF PANCREATIC WALDENSTROM MACROGLOBULINEMIA

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Introduction: Waldenström macroglobulinemia (WM) is a rare lymphoid neoplasm which secrete a monoclonal immunoglobulin (Ig)M characterized by a wide range of clinical presentation related to the direct tumor infiltration. Pancreatic WM represent an atypical presentation of the disease. Abdominal CT scan can show the presence of a mass in pancreatic region visualyzed as an hypodense area. We describe a case of a patient with pancreatic WM at diagnosis. *Methods:* A 61-year old man with a history of chronic hepatitis HCV related was diagnosed with IgM Waldenström macroglobulinemia (WM). Initial presentation was given by abdominal pain, lymphadenoathy and fatigue. Abdominal CT scan showed the presence of a 3,1 2,3 cm mass in the pancreatic tail as hypodense area (Figure 1). CT scan confirmed also the presence of hypodense lesions in the VI, VIII hepatic segment, lomboaortic nodes >1 cm, splenomegaly and bulky mediastinal lymph nodes. Biochemical parameters showed Hb 7,4 g/dL, platelet count 30 x109/L, IgM 370 mg/dL, LDH 1500 U/mL. Monoclonal component was 3,5 g/dl. B lymphocytes represented 50% of bone marrow, immunophenotype was CD19+,CD20+, CD 22+, CD138+, CD5-, CD10-. Histology of abdominal nodes and contrast-enhancement-US guided biopsy of the pancreatic mass prompted the diagnosis of WM. *Results:* The patient started treatment with Rituximab in combination with glucocorticoids and cyclophosphamide obtaining a partial response. *Conclusions:* Pancreatic WM represent an atypical presentation of disease. CT scan visualyze pancreatic WM as an hypodense area. In our opinion, a multidisciplinary collaboration between haematologist, radiologist and cytologist is essential in order to obtain the diagnosis and rapidly to start treatment. New treatment agents including Rituximab in combination with glucocorticoids and cyclophosphamide demonstrate promising response rates especially in patients with pancreatic WM.

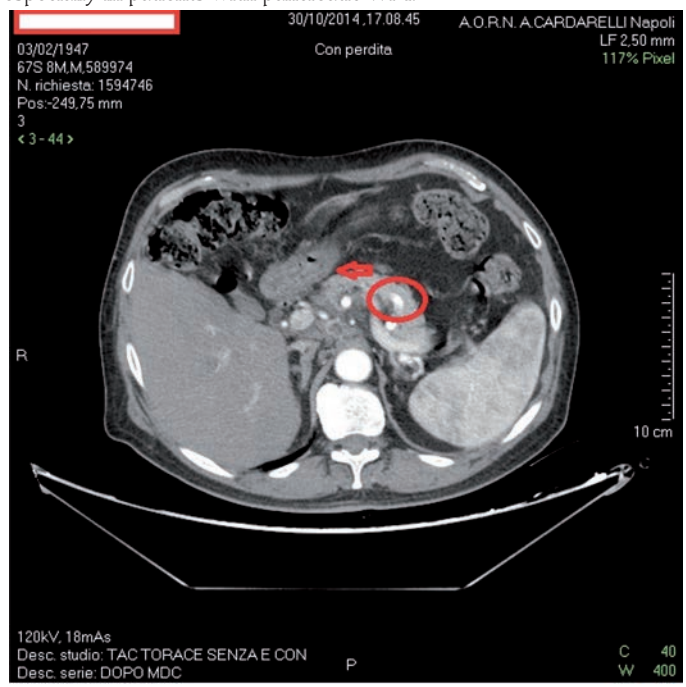

Figure 1. Pancreatic Waldenström macroglobulinemia (WM) visualyzed as an hypodense area.

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BING-NEEL SYNDROME WITH ANEMIA, THROMBOCYTOPENIA AND HYPERVISCOSITY SYNDROME: A CASE REPORT

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Introduction: Bing-Neel syndrome is rarely observed in patients with Waldenström macroglobulinemia (WM) and is characterized clinically by confusion, disorientation, dizziness, hypotension. MRI scan in Bing-Neel syndrome shows the presence of T2 hyperintense cerebral lesions. We describe a case of a patient with Bing-Neel syndrome, anemia, thrombocytopenia and hyperviscosity syndrome. *Methods:* A 73-year old man with a history of chronic hepatitis HCV related was diagnosed with IgM Waldenström macroglobulinemia (WM). Monoclonal component was 3,2 g/dl, B lymphocytes represented 68% of bone marrow, immunophenotype was CD19+,CD20+, CD 22+, CD138+, CD5-, CD10-,CD23-. Histology of bone biopsy confirmed immunophenotype and B lymphocytes infiltration. Biochemical parameters showed IgM 3570 mg/dL, LDH 2000 U/mL, Hb 7,3 g/dL, platelet count 18 x109/L. Initial presentation was given by confusion, disorientation, hypotension, oronasal mucosal bledding, dizziness. Brain MRI showed the presence of T2 hyperintense areas in hippocampal region, cerebral frontal convexity and parietal convexity. CT scan confirmed the presence of hypodense lesions in hippocampal region, cerebral frontal convexity and parietal convexity. Were present also splenomegaly, hypodense lesions in the VIII hepatic segment, lomboaortic nodes >1 cm, presence of bulky adenopathy, pleural effusion. *Results:* The patient started treatment with plasmapheresis plus Cyclophosphamide in combination with glucocorticoids and rituximab, resolving anemia, thrombocytopenia and symptomatic hyperviscosity with neurological symptoms. *Conclusions:* Bing-Neel syndrome is rarely observed in patients with Waldenström macroglobulinemia (WM). It is caused by infiltration of the central nervous system with monoclonal lymphoplasmacytic cells as infiltrates or as tumors and is primarily characterized by neurological symptoms. MRI scan shows the presence of T2 hyperdense areas in hippocampal region, cerebral frontal convexity and parietal convexity and may be helpful for a rapid diagnosis. In our opinion, a multidisciplinary collaboration be-

tween haematologist, radiologist and cytologist is essential in order to obtain the diagnosis and rapidly to start treatment.

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BENDAMUSTINE-BORTEZOMIB-DESAMETASONE IN THE MANAGEMENT OF RELAPSED AND REFRACTORY MULTIPLE MYELOMA: A REGIONAL REAL-LIFE EXPERIENCE

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Bendamustine is a bifunctional alkylating agent, with low toxicity, proved to be effective in relapsed, refractory and in new diagnosed Multiple Myeloma (MM). It has been evaluated efficacy and tolerance of Bendamustine, in combination with bortezomib-dexametasone (BVD) in patients with relapsed and refractory MM (rrMM), whose prognosis is particularly severe. A regional retrospective real-life analysis of patients with rrMM who had been treated with BVD as salvage therapy has been performed. 35 patients (19 M/16 F), with rrMM, median age at diagnosis 57 years (r. 36-82), median age at start of treatment 62 years (r.37-83) treated with several lines of treatments (median 6, r. 2-11), every refractory to all the drugs previously received (also Bortezomib), received BVD (Bendamustine 90 mg/sqm days 1,2; Bortezomib 1.3 mg/sqm days 1,4,8,11, Desametasone 20 mg days 1,2,4,5,8,9,11,12, Pegfilgrastim day +4) every 28 days, until progression. ISS was equally distributed, and cytogenetic was evaluable in 9 patients, and in particular one del13q and one t(11;14). All the patients had previously been treated with schedule containing bortezomib and IMIDs, 90% of them with melphalan, 77% with cyclophosphamide, 34% with antracyclines and 30% had also received radiotherapy. 58% of them had undergone at least to a single auSCT. All patients were relapsed and refractory to last therapies received before BVD. Bendamustine was well tolerated, with grade 3 transfusion-dependent anemia in 29% of patients, and 41% grade 3 neutropenia (no ospedalization was required, no septic shocks were observed). No severe extrahematologic toxicity was observed, only grade 1 gastrointestinal side effect (nausea), treated by common antiemetic drugs. According to IMWG, after a median follow-up of 9 months (r.2-36), ORR was 54% (1 CR, 1 VGPR, 9 PR, 8 MR) with 7 PD and 9 patients in SD, which can be considered as an impressive result in this subset of rrMM patients. In particular, for 4 patients, BVD was, after having achieved a PR, a bridge to second auSCT, and for one patient a bridge to auSCT. Median OS from diagnosis was 61.4 months (range 6-151), median OS from start of Bendamustine was 7.2 months (range 2-36). BVD has shown significant efficacy (ORR 54%) in a particular severe setting of patients, relapsed and refractory to all avaiable therapeutic resources, and in particular cases it could be considered as a bridge to a second autologous or allogenic BMT.

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PEGYLATED LIPOSOMAL DOXORUBICINE, CYCLOPHOSPHAMIDE AND DEXAMETASONE (CED): A NEW OPPORTUNITY IN RELAPSED AND REFRACTORY MULTIPLE MYELOMA

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Patients affected by multiple myeloma almost invariably become chemo-resistant: hence, one of the main topic in clinical research is the quest for therapeutic alternatives to overcome relapsed and refractory disease. Since 2009, in our Institution, patients affected by Multiple Myeloma, relapsed and refractory to most of the therapeutic options avaiable, have been treated with a chemotherapy based on a combination of pegylated liposomial doxorubicin (35 mg/sqm, day 1), cyclophosphamide (800 mg/sqm day 1) and dexamethasone (20 mg days 1-2-3-4), Pegfilgrastim day +4, cycles every 28 days (CED regimen), until progression of disease, with very interesting results, considering this subset of patients. 31 patients (16 women, 12 men), with a median age of 63.4 years (range: 43-84) affected by advanced, relapsed and progressive multiple myeloma, whose madian number of previous treatment was 5.7 lines (range 2-11) were treated with CED schedule (median number of courses: 4.3, range: 2-17). Only patients completing at least two courses of CED were considered for analysis. The toxicity profile of CED was satisfactory: grade 3 transfusion-dependent anemia in 37% of patients, and 46% grade 3 neutropenia (no ospedalization was required, no septic shocks were observed). No severe extrahematologic toxicity was observed, only grade 1 gastrointestinal side effect (nausea) in almost every patients, treated by common antiemetic drugs, and two important extrahematological side effects only in two cases: an episode of acute renal failure in one patient and a bradycardia in another, both them not necessiting of hospitalization. According to IMWG response criteria, after a median follow-up of 6 months (r.2-17), Overall response ratio (ORR) was 51% (2 CR, 2 VGPR, 8 PR, 4 MR) with 10 progressions of disease and 5 patients in stable disease, which can be considered as an impressive result in this subset of rrMM patients. Median OS from start of CED was 5.9 months (range 2-17). CED (pegylated liposomial doxorubicin, cyclophosphamide and dexamethasone) has shown significant efficacy (ORR 51%) in a particular severe setting of patients, relapsed and refractory to all avaiable therapeutic resources, and it can be considered also as a good option in the necessity of palliative treatment in rrMM.

Myelodysplastic Syndromes 2

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A POPULATION-BASED STUDY OF MYELODYSPLASTIC SYNDROMES PATIENTS IN THE LAZIO REGION (ITALY), MISCODING IN MEDICAL CLAIMS AND 11-YEAR MORTALITY FOLLOW-UP: THE GRUPPO ROMANO-LAZIALE MIELODISPLASIE EXPERIENCE OF RETROSPECTIVE MULTICENTRIC REGISTRY

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Background: The MDS are not well recognized and may be under-reported. *Objectives:* To evaluate the MDS miscoding in medical claims and to conduct a mortality follow-up for a cohort of retrospective analysis of MDS patients enrolled in GROM registry. *Methods:*Using an anonymous unique patient code (AUPC),we linked the MDS cohort with 2 regional health information system: the Hospital Information System (HIS) and the Regional Mortality Information System (ReNCaM). HIS includes information on pts demographic characteristics and discharge diagnoses according to the ICD-9-CM coding. ReNCaM includes information on demographic characteristics, as well as date, place, and cause of death (codified by ICD-9).We evaluated the proportion of pts to whom the MDS had been properly registered in the hospitalizations succeeding to the date of diagnosed MDS by the Hematology Center until the end of 2012. Furthermore, we followed-up the MDS cohort since date of diagnoses until 31/12/12, or the date of death if it occurred before. We observed the time to death in the Kaplan-Meier curve and the principal causes of death. *Results:* MDS has been diagnosed for 684 pts in 12 Hematology Centers of Lazio Region during the period 2002-2010. The mean age of these pts was 69.7 and 45.5% were female. According to WHO classification were diagnosed: SA 5%, 5q 4.2%,MDS-U, 4.3%, RA 37.3%, RAEB I 14.6%, RAEB II 12.2%,and RCMD 22.4%.

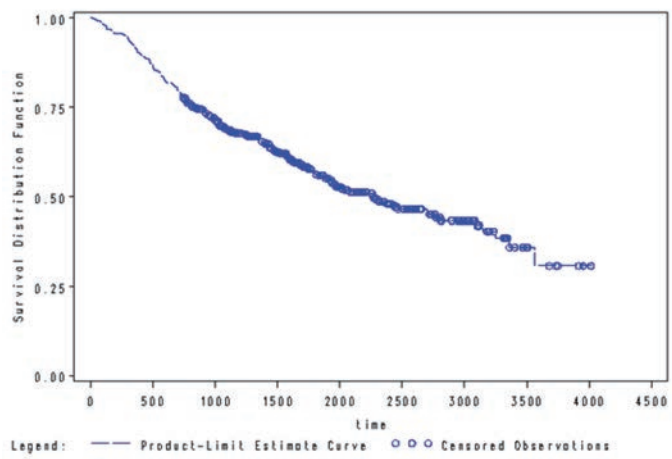

Figure 1. Kaplan-Meier curve for the 556 Myelodysplastic Syndrome patients: 11-year mortality follow-up.

The analysis was limited to 556 patients with an AUPC. For the first objective, we identified 442 patients who had at least 1 hospitalization during the period 2002-2012. Of these, 316 pts (71.5%) had at least 1 hospitalization with the ICD-9-CM code 238.7 in any principal or secondary diagnosis. Among the remaining 126 pts, 66 had a hospitalization with hematological principal diagnoses. For the second objective, minimum observed follow-up(FU) time was of 25 days and the maximum was of 10 years and 10 months. During the FU, we observed 253 deaths (45.5% pts). Among the most frequent causes of death, the first was the code 238.7 for 21.7% patients and the others were:chronic or acute leukemia and aplastic anemia. *Conclusions:* This study shows for the first time in Lazio that MDS are under-documented in the HIS archive because are not exactly codified. Moreover, our data suggest a miscoding of MDS also for reporting the most frequent causes of death (Figure 1).
The study was supported by a grant from Regione Lazio: 'sindromi mielodisplastiche dell'adulto nell'area di Roma e del Lazio: epidemiologia caratteristiche diagnostiche e clinico-terapeutiche, analisi dei costi mediante un registro onco-ematologico regionale' (Grant 2011-Progetti di farmacovigilanza – Area tematica 5).

P274

LONG-LASTING HEMATOLOGIC RESPONSE TO AZACITIDINE IN MYELODYSPLASTIC SYNDROMES: RETROSPECTIVE STUDY FROM A SINGLE INSTITUTION

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Introduction: Although Azacitidine (AZA) has proven effective in myelodysplastic syndromes (MDS), the duration of haematologic response is usually limited (median: 13.6 months) The French Group (Itzykson 2011) identified some clinical and haematologic parameters, and these 4 criteria were integrated in a 3-risk-group prognostic score. These data prompted us to retrospectively analyse our MDS pts treated with AZA who showed a favourable long-lasting response to AZA (i.e: duration of response ≥20 months). *Methods:* The type of response was defined according to IWG criteria (Cheson 2006): Complete Remission (CR), Partial Remission (PR), Marrow CR (mCR), Hematologic Improvement (HI). Moreover, we quantified the degree of phosphoinositide-phospholipase C (PI-PLC) β1 methylation and gene expression before and during AZA administration. *Results:* From September 2004 in our Institution, 70 pts were treated with AZA. 11/70 pts (15.7%) (4 males, median age: 68, range 60-84 yrs) showed a response duration ≥20 months. At AZA onset, WHO diagnosis was: RCMD-RS: 1 pt; RAEB-1: 4 pts; RAEB-2: 5 pts; MDS with Fibrosis: 1 pt. MDS was therapy-related in 3 pts. ECOG p.s. was <2 in all cases. IPSS risk was: Low: 1 pt; INT-1: 3 pts; INT-2: 6 pts; High: 1 pt. WPSS risk was: Low: 1 pt; INT: 2 pts; High: 5 pts; Very High: 3 pts; IPSS-R risk was: Low: 2 pts; INT: 3 pts; High: 4 pts; Very High: 2 pts. IPSS cytogenetic risk was: Good: 5 pts; INT: 4 pts; Poor: 2 pts. Transfusion need was high (≥4 RBC units/8 weeks) in 4 pts. No patient showed circulating blasts. Following Itzykson's AZA prognostic scoring system, the risk was: Low: 3 pts; INT: 8 pts. The pts received a median of 24 (8-59) cycles of AZA (3 lower-risk pts discontinued therapy after 8th cycle). The median duration of treatment was 35 (8-68) months. The best response achieved was: CR in 6 pts; marrow CR+HI: 1 pt; HI: 4 pts. An abnormal karyotype persisted in 3 pts. The median duration of response was 40 (21-75) months. In 8 pts the disease evolved into AML (4 pts), or into a more advanced MDS (2 pts), or into a chronic myeloproliferative neoplasm (2 pts). Median OS from the start of AZA was 50 (25-125) months. All the pts showed an increase in PI-PLCβ1 expression, that was maintained along with the hematologic response. *Conclusions:* Our data show that a limited but significant fraction of MDS pts show a long-lasting hematologic and molecular response to AZA.

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MYELODYSPLASTIC SYNDROMES: AN INTEGRATED WORKUP FOR A CORRECT DIAGNOSIS

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Background and Aims: The diagnosis of MDS is determined by bone marrow's histology and is integrated by conventional cytogenetic (CC) analyses that, although, are unable to show chromosomal abnormalities in about 30% of cases. Array Comparative Genomic Hybridization (aCGH) detects copy number aberrations in half of MDS diploid by CC. Recently, TET2, ASXL1, EZH2, CBL, IDH1/IDH2, DNMT3A, and UTX mutations have been described in MDS. Therefore, we evaluated the prognostic and predictive implications of FISH, aCGH and mutation assays when added to CC. *Patients and Methods:* Fifty patients, 15 females and 35 males, with a median age of 72-year (range 39-88) were enrolled in a single institution (Hematology Unit of University of Pisa, Italy), between March 2013 and October 2014. Patients' prognosis was determined according to IPSS in low (42%), intermediate-1 (33%), intermediate-2 (17%) and high risk (8%). Samples were evaluated by CC including, conventional karyotyping and FISH for chromosome 5, 7, PDGFRA, and PDGFRB. Moreover, aCGH was performed using SurePrint G3 Human CGH Microarray, 8x60K (Agilent), and mutations of TET2, TP53, ASXL1, and EZH2 were determined using qBiomarker somatic mutation PCR array (Qiagen). *Results:* Table 1 summarizes CC results. Using FISH, additional copy number losses of chromosome 3 and 5 were observed in 1 and 2 cases, respectively. According to aCGH, 20 patients (40%) showed copy number aberrations. Seventeen patients had a mutation: 12 in TP53, 4 in ASXL1 and 1 in TET2 sequence. *Conclusions:* 1) The study showed the reduction of CC failures (only 16%); 2) Because of FISH results, a patient received azacitidine and another one lenalidomide; 3) Three MDS patients with copy number aberrations detected exclusively by aCGH progressed in acute leukemia being lethal: one patient had copy number loss of chromosome 3, one had copy number losses of chromosomes 8 and Y and the last one had copy number loss of ETV6 locus. 4). One patient with TET2 mutation did not respond to the azacitidine. Three out of 4 patients with ASXL1 mutation had a good response to epoietin. TP53 mutations were observed in 12 cases of which 2 developed acute leukemia, 2 were resistant, and 2 sensitive to epoetin. In conclusion, our results support the relevance of an integrated workup for MDS and provide a preliminary estimation of copy number aberration and mutation frequencies. The prognostic role of aCGH and somatic mutations needs to be determined in properly sized series.

NUMBER OF PATIENTS	CHROMOSOMAL ABNORMALITIES	CYTOGENETIC RISK
1	45, -X, -Y [20]	Good
2	46, XY, del(5)(q15)[4]/46, XY[16]	
	46, XX, del(5)(q15)[20]	
4	47, XY, +8[4]/46, XY[16]	Intermediate
	47, XY, +8 [20]	
	47, XX, +8 [16]/46, XX [4]	
	47, XY, +8 [20]	
1	47, XY, +6 [4]/46, XY [16]	
1	46, XY, del(13)(q12-q14)[18]/46, XY [2]	
1	47, XY, +14[6]/46, XY[2]	
1	45, XY, -7[20]	Poor
4	47, XY, +14, del(20)(q11.2)[4]/48, XY, +14, +21, del(2)(p21), del(20)(q11.2)	
	46, XX, -5, -12, -18, -19, +/-4, -5m[16], 46, XX[4]	
	85, XX, -Y, +8m, ..., inc.[6]/92, XXYY[2]/46,XY[12]	
	92-98, XXYY,...., inc.[8]/44, XY, -4, -17, -21, del(5)(q15), del(7)(q21), +2, -3[12]	

Table 1.

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ANALYSIS OF THE ACTUAL COST OF SUPPORTIVE CARE AND HIPOMETHYLATING AGENT IN A SET OF PATIENTS WITH MYELODYSPLASTIC SYNDROMES

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The Myelodysplastic Syndromes (MDS) are clonal diseases of the bone marrow that occur primarily in elderly population. A conventional Best Supportive Care (BSC) treatment with blood cells transfusion have been associated with High costs and decreased Quality of Life (QoL) compared with transfusion independence. There are not any data on the cost-effectiveness of azacitidine treatment in the Italian reality, because the "cost" variable is different for every country. In order to performing a cost-effectiveness analysis in the Italian reality it is necessary to know the "cost" variable of the therapy in our National Health System. Therefore we analyzed the raw cost of the population of MDS patients treated with azacitidine and Best Supportive Care (BSC) in our center. In our study, we analyzed 30 patients (23 males, 7 females, mean age at diagnosis 75,1 years, range 40-91) affected by MDS treated with azacitidine and BSC, enrolled from January 2008 to December 2013. We registered age, sex, number of therapy cycles with azacitidine, number of transfusions (RBC or platelets), number of days of hospitalization including hospitalization day(DH) and the Diagnosis Related Group (DRG) for every hospital admission. We then calculated the total cost for patient assessing the standard cost of a transfusion and the DRG. Patients were hospitalized an average of 52.8 days/year and 21.2 days in DH/year. The DRG rates for admissions amounting to 1,320,978.37€, with an average effort 44,032,61€/patient, turns in a real cost for patient/year of 19,668.64€. The mean annual total cost (DRG+Pharmaceutical spending and Transfusion) per person/year was assessed to 30.971,37€.The median survival was 20 months with a global advantage for patients treated with azacitidine in DH. Our analysis showed that the use of azacitidine in MDS patients is cost-effective option compared with conventional care regimen because provides greater clinical benefit. The mean cost of 30.971,37 € is comparable to other countries, but is lacking the Quality of Life (QoL) valutation, because this was a retrospective study. It is necessary to assess the QoL in this population of patients for further analysis and finally, it is necessary a more uniform legislation in Italy on the health care costs.

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TARGETED SEQUENCING ANALYSIS OF COMMONLY MUTATED GENES IN CHRONIC MYELOMONOCYTIC LEUKEMIA USING NGS: IMPACT AND CLINICAL IMPLICATIONS

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Chronic myelomonocytic leukemia (CMML) is an overlap myelodysplastic/ myeloproliferative neoplasm characterized by a variable clinical course and well-documented prognostic factors as WBC count, splenomegaly, cytopenias, and cytogenetics. Recently, somatic mutations have been identified in CMML, and preliminary evidence suggests that selected mutated genes may influence prognosis. The aim of this study is to evaluate the frequency and confirm prognostic value of mutations of different genes in CMML. Fifty-three CMML patients diagnosed according to WHO classification were included. The mutational profile of ASXL1, DNMT3A, EZH2, RUNX1, SF3B1, SRSF2, U2FA1, TET2 and TP53 was characterized using a TruSeq Custom Amplicon panel (Illumina). Around 250 ng of gDNA extracted from mononuclear cells obtained at diagnosis were used to prepare sequencing libraries following the Illumina standard protocol. Samples were run on an Illumina MiSeq and data analysis involved the mapping of all the reads against the human reference genome hg19 and annotation of all the variants were performed by ANNOVAR. Eighty-eight percent of patients showed at least one mutation (median number per patient: 2, range 0-4). The mutational frequencies in this cohort of CMML cases were: SRSF2 (49%), ASXL1 (41%), TET2 (21%), RUNX1 (11%), DNMT3A (10%), SF3B1 (6%), U2AF1 (6%), TP53 (6%) and EZH2 (2%). The group of patients with ASXL1 mutated (mut) presented higher platelet count (109/L) (median: 105) than ASXL1 wild type (wt) patients (median: 58) (p=0.008). In the group of patients with U2AF1 mut, the hemoglobin levels (g/L) were lower than in patients wt (median U2AF1 mut: 8.5 *versus* median U2AF1 wt: 10.3, p=0.035). Univariate analysis identified higher risk IPSS (for patients with WBC <12 109/L) (p=0.02) and CMML2 subtype (p=0.001) associated with shorter survival. We also evaluated the prognostic impact of mutations. In patients with age<65

years, survival was inferior in the presence of DNMT3A mut (p=0.008) but in patients with age>65 years, worse survival (p=0.01) was associated with TP53 mut. When we considered lower risk IPSS patients none of the mutated genes influenced survival. In this study we confirm the extremely high frequency of somatic mutations in CMML patients. We could not confirm the prognostic significance of ASXL1 and SRSF2 recently demonstrated in CMML patients, but we observed that survival of young and old patients seemed to be influenced by different somatic mutations.

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ROLE OF INFLAMMATION IN *DE NOVO* MYELODISPLASTIC SYNDROMES

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Myelodisplastic syndromes (MDS) usually affect elderly patients, and they often had other comorbidities. The role of inflammation in MDS is still under-explored. We evaluated impact of 5 inflammation index ferritin,α1glycoprotein(1g),α1antripsin(1a),C reactive protein(CRP) and haptoglobin(apto) on overall survival (OS) and and correlation with WHO 2008 classification, IPSS risk, IPPSr, WPSS, presentation CBC. Sample were obtained from 76 consecutive MDS patients *de novo* in the Hematology Unit of Florence. Patients were classified according to WHO classification as follow: 15 RA, 4 RARS, 30 RCMD, 11 RAEB1, 5 RAEB2, 7 AML <30% of blasts and 4 CMML, and prognostic score was evaluated according to IPSS as follow: 79,2% lower risk, 20,8% higher risk, according to IPPSr: 70% lower risk, 15 intermediate and 15% higher risk; according to WPSSr: 56,9% lower risk, 2,4% intermediate and 20,6 higher risk. Median age:75 years (54-93), 42% were female (32/44 F/M). Median follow up:29 months.Median value of all the indexes we analysed was within the limits of laboratory.Mean value of 1g was 0.93 ±0.34 g/L, 12 patients had 1g level above normal value (n.v.) (n.v 0.5-1.2 g/L), mean value of 1a was 1.67 ±0.43 g/L, 10 patients had 1a above n.v. (n.v. 0.9-2 g/l), mean value of apto was 0.94±0.72 g/L, 5 patients had apto level above n.v. (n.v.0.3-2 g/L), mean value of ferritin was 229±826 ng/ml, 27 patients had ferritin level above n.v. (20-300 ng/ml), 15 patients had CRP level above the normal limit (9 mg/L).There was no significant difference among different WHO subtypes, IPSS IPSS-R and WPSS-R risk categories.We compared MDS cases with normal values to those with values above normal range. We observed that MDS patients with higher ferritin had a median OS of 27,1 mos while median OS was nr at 60 mos for the group with normal value of ferritin (p=0.041). High CRP levels strongly indicated shorter OS (14 mos *vs* nr) (p=0.000), while elevated 1a was associated with 12,1 mos OS *vs* nr (p=0.015) as well as 1g (14,0 mos *vs* nr). Apto level variations were not associated with significant difference in OS. Inflammation markers are not a common hallmark of MDSpatients.When patients showed elevated CRP, 1g and 1a OS was significantly shorter.These observations indicate that inflammation plays a dominant a role in determining MDS outcome, beyond risk classification.CRP may be a easy tool to implement evaluation of MDS outcome in addition to accepted scoring systems.

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INFLUENCE OF BONE MARROW FIBROSIS ON RESPONSE OF INT-2/HIGH RISK MDS PATIENTS TO 5-AZACITIDINE.

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Introduction: Azacitidine (AZA) induces responses and prolongs overall survival compared with conventional care regimens in patients with high-risk myelodysplastic syndromes (MDS). Risk factor that may influence the response to AZA are still largely unknow. It has recently been shown that different biological parameters influence response and overall survival after azacitidine treatment. Bone marrow fibrosis is another well known negative prognostic index and we investigated whether it could influence the response to 5-azacitidine therapy. *Methods:* We analyzed 73 MDS patients (72% HR-MDS) treated with subcutaneous 5-Azacitidine 75 mg/kg/day for 7 days every 28 days. Mean number of cycles was 7.4 (range: 1-54). Mean age was 72 (33-89), 61% were male. Response to azacitidine treatment was evaluated according to IWG 2006 criteria as CR, PR, HI, SD, DP. Overall response rate according IWG criteria 2006 was 45.5%, stable disease was obtained in 25,3% of MDS patients. In 16 patients response was not evaluable at the time of analysis because they received less than 4 azacitidine cycles. Overall Survival was analyzed with Kaplan Meir test. The grade of fibrosis and its extent were evaluated semiquantitatively in archival slides stained by Gomori silver impregnation according to European consesus on grading of bone marrow fibrosis and assessment of cellularity criteria (48 patients was grade 0; 18 grade 1, 5 grade 2 and 2 grade 3). *Results:* Overall response of patients with grade 0 fibrosis was 47,9%, and for patients with grade 1, 2 and 3 was 32% (p>0.05). In details: response of patients with grade 0 was: CR+PR+mCR+HI 47%, SD 35,4% and DP 16,7%. Response of patients with grade 1 CR+PR+mCR+HI 33%, SD 38,9% and DP 27,8% and 2 was: CR+PR+mCR+HI 40%, SD 40% and DP 20%. Response of patients with grade 3 was: CR+PR+mCR+HI 0%, SD 100% and DP 0%, Differences between groups are not statistically significant. The patients with fibrosis had worse median OS than patients with no fibrosis (13,2 mos *vs* 28,6 mos). With COX regression we observed that fibrosis, with response to treatment, were the only significant variables in our study. *Conclusions:* Mild bone marrow fibrosis does not influence the response of MDS patients to 5-azacitidine therapy, but it remains an indipendent negative prognostic factor for OS and LFS.

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EFFECT OF RECOMBINANT HUMAN ERYTHROPOIETIN A ON CARDIAC REMODELING IN PATIENTS WITH "LOW RISK" MYELODYSPLASTIC SYNDROMES: RESULTS FROM A PROSPECTIVE STUDY

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Introduction: Myelodysplastic syndromes (MDS) are typical diseases of the elderly. The most frequent symptom is anemia. Both age and comorbidities have a relevant negative impact on the clinical outcome. Aim of our study is to investigate of early treatment with rHuEPO α may reverse cardiac remodeling, improve Ejection Fraction (EF) and reduce cardiovascular morbidity in "lower risk" MDS pts. *Materials and Methods:* From Jan 2013 to Jan 2015, 43 MDS pts were included in a prospective observational multi-centric study; 16 F and 27 M, of mean age 75. According to the WHO 2008 classification, morphological MDS diagnosis were as follows: 16,5% Refractory Anemia (RA), 58% Refractory Cytopenia with Multilineage Dysplasia, 7% RA with Ringed Sideroblasts, 18,5% RA with excess of blasts-1. All pts had IPSS low or int-1 risk and epo serum levels <500 mU/L. ESAs treatment was allowed when hemoglobin (Hb) was lower than 10.5 g/dL. All pts were analyzed with echocardiography before starting ESAs and every 6 months. Left ventricular hypertrophy (LVH) was defined by a LVM index >47 in F and >50 in M. We also analyzed comorbidity by CIRS and cardiovascular risk factors. All pts were treated with rHuEPO α 40000 U weekly. *Results:* At baseline all pts were transfusion-free and with mean Hb concentration of 9.66 g/dl. The rate of erythroid response to rHuEPO according to IWG criteria 2006 was about 60%.One or more comorbidity of any grade of severity was seen in 90% of pts at diagnosis. The more common comorbidity was cardiac (65%).Cardiovascular disorders were more frequent among older subjects (55% in >75y *vs* 30% in <75y), and among males.The median EF at baseline was upper than 55% and half pts had normal LVM. In 90% of pts with erythroid response EF was stable, in 10% improved and in 0% decreased while LVM was reduced in 35% of pts. The EF improvement and decrease of LVM was more evident in pts with cardiovascular comorbidities. None pts among "responders group" had cardiac comorbidity worsening or hospitalization. Anemia improvement and FE increase were associated with an improvement of quality of life (QOL). The median observation time was 12 months (range 1-24). *Conclusions:* rHuEPO α tp is effective and safe also in pts with cardiac comorbidities. In responders "lower risk" MDS pts, early treatment has been shown to improve FE and reverse cardiac re-

modeling with consequence a reduction of cardiovascular risk/ hospitalization and improvement QOL.

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TET2 MUTATIONAL STATUS CORRELATES WITH PTEN UP-REGULATION IN LOW GRADE MYELODYSPLASTIC SYNDROME (MDS) PATIENTS

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Background: TET2 is the most frequently mutated gene in MDS (12-26%). We analysed TET2 (ex3-11) mutational status in 100 newly diagnosed MDS pts enrolled in an Italian multicentre prospective study (ClinicalTrials.govIdentifierNCT01291745). TET2 mutational status was correlated with changes in gene expression (GE) in a panel of stem cell related genes. *Methods:* gDNA from bone marrow was subjected to TET2 mutational screening analysis using combined high resolution melting (HRM) analysis and Sanger sequencing (SS). HRM analysis was performed on the 7900HT-RT-PCR (Applied) using primers designed for TET2 isoform A gene locus (NM_001127208). Samples displaying aberrant melting curves were subjected to SS on the ABI3130 (Applied). All mutations/alterations were first compared with published SNP data (www.ncbi.nlm.nih.gov/project/SNP). We analysed mRNA expression in TET2 mutated (N=5) *vs* wild type samples using TaqMan Array MicroFluidic Card (AppliedBiosystems). Protein levels were determined by Western blotting (WB). *Results:* Of the 100 pts, we identified 1 previously described mutation and 13 novel mutations of the TET2 coding sequence consisting of 8 InDel mutations, 5 aminoacid substitutions (Aln301Val, His578Arg, Ser820Gly, Met1028Ile, Ser1898Tyr) and one substitution coding a STOP codon predicted to alter or abrogate TET2 protein function. Patients with SNPs also displayed aberrant melting curves, allowing the identification of 10 previously described and annotated SNPs in 100 (75%) MDS pts. Three mutations fell within the highly conserved LCX1 and 1 in LCX2, and were found in patients with a low IPSS. Our incidence of detectable TET2 mutations was 14%. Of the 96 genes of the stem cell panel analyzed in low-risk TET2 mutated pts, 5 pts with TET2 mutations presented higher levels of PTEN mRNA than TET2 wild-type cases. WB analysis showed that phosphorylated-AKT (a protein negatively regulated downstream by PTEN) levels were decreased in these cases. *Conclusions:* We have identified an alteration of the PTEN pathway in pts with TET2 mutation. Previous reports have shown that high-risk MDS pts generally have downregulated expression of PTEN when compared to healthy controls and low-risk MDS pts demonstrate low levels or absence of p-AKT staining. Here, the increased expression of PTEN observed in low-risk MDS pts with TET2 mutation could in part be supported by the decreased levels of p-Akt, its downstream target. A better definition of the PI3K/Akt pathway in MDS is warranted.

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CXCL4 AND CXCL7 EXPRESSION IN BONE MARROW MICROENVIRONMENT OF CMML AND AML POST MDS PATIENTS TREATED WITH DECITABINE: A POSSIBLE NEW TOOL FOR PREDICTION OF RESPONSE

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The hypomethylating agent Decitabine (DAC) has demonstrated efficacy in the therapy of chronic myelomonocytic leukemia(CMML) and elderly acute myeloid leukemia (AML). However, the overall response rate is quite heterogeneous and mechanisms of resistance need to be clarified. In a recent study, we analyzed a cohort of 40 CMML before treatment with DAC and we demonstrated that resistance to DAC was associated with overexpression of chemokines CXCL4 and CXCL7 genes. Using immunohystochemistry (IHC) analysis, this overexpression of the cytokines was observed also in bone marrow (BM) microenvironment (Meldi *et al.*, JCI 2015).On this basis, we evaluated by IHC CMML and AML cases eligible to treatment with DAC to verify if this method could be useful to predict response based on IHC. We evaluated 17 patients:10 AML post MDS and 7 CMML diagnosed according to WHO criteria. Bone marrow biopsy was performed at diagnosis or immediately before DAC treatment (20 mg/m^2/day iv for 5 days every 28 days). After 6 cycles patients were classified as responders (R) or nonresponders (NR), according to IWG 2006 criteria. Three- m-thick formalin-fixed, paraffin embedded bone marrow sections will be deparaffined and incubated with CXCL4 antibody (1:300) or with CXCL7 antibody (1:50); megakariocytes were marked with CD61 antibody. Immunostaining was performed with BenchMark histo-stainer (Roche-Ventana Medical Systems) using a peroxidase detection kit with 3,3-diaminobenzidine substrate, the sections were counterstained with hematoxylin.We analyzed the different expression of CXCL4 and CXCL7 in BM of AML and CMML patients, responsive (R)and non responsive (NR) to DAC therapy. In all cases analyzed, we observed that CXCL4 was primarily localized in megakaryocytes, while CXCL7 was present in monocytic cells. In CMML cases we confirmed an increase of CXCL4 and CXCL7 expression in BM from NR patients respect to R (4 NR and 3 R). This increase was more evident for CXCL4 than CXCL7. On the other hand, among AML cases, we could not demonstrate a significant difference between NR (n=6) and R (n=4) patients. This simple method could be an innovative and interesting new approach to predict response to DAC therapy in CMML patients and confirms our previous observations. Moreover it seems specific of CMML and not so of AML.

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MANAGEMENT OF MYELODYSPLASTIC SYNDROMES WITH ERYTHROPOIESIS STIMULATING AGENTS: EVALUATION OF ERYTHROPOIETIC ASPECTS AND ANALYSIS OF RESPONSE

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ESAs are the frontline treatment in low-risk anemic MDS patients and an employment of this therapy in the earlier stage of the disease can delay the need for RBC transfusion, hypothetically by slowing the disease course. It's matter of debate whether the clinical response is a result of proliferation and maturation of the dysplastic clone or stimulation of residual normal erythropoiesis by ESAs. Macrocytosis is one of the cytological hallmarks of dyserithropoiesis in MDS: an analysis of the erythropoietic response to ESAs therapy in a cohort of anemic non trasfusion-dependent MDS patients, enrolled in a retrospective register, RECAMDS, subgroup of Italian register, was performed. 137 patients, treated with standard-dose ESAs, have been retrospectively analyzed (Table 1).

Table 1.

MDS PATIENTS	137
M	66 (48%)
F	71 (52%)
WHO	
AR	44 (32%)
DEL 5q	9 (6%)
RAEB-1	5 (4%)
RAEB-2	2 (2%)
RARS	20 (14%)
RCMD	57 (41%)
R-IPSS	
VERY LOW	46 (34%)
LOW	79 (58%)
INTERMEDIATE	10 (7%)
HIGH	2 (1%)
ERYTHROPOIESIS	
BASELINE HB (mean, g/dL)	9.5 g/dL (r. 7.1-11.3)
BASELINE SERUM EPO (mean, mU/mL)	41 mU/mL (r.3-30)
OVERALL RESPONSE RATIO	
RESPONDERS	114/137 (83%)
RESPONDERS AT 3 MONTHS	105/137 (76%)
RESPONDERS AT 6 MONTHS	9/137 (7%)
RESPONDERS AT 9 MONTHS (NON RESPONDERS IN IWG 2006)	2/137 (2%)
NON RESPONDERS	23/137 (17%)

Data analysis was performed, according to IWG2006, at the baseline, after 3 and 6 months of continuous treatment, with a subanalysis of the patients according to WHO and R-IPSS risk stratification. ESAs were started at mean Hb concentration of 9.5 g/dl, mean serum EPO concentration: 41mU/L, after a mean time from diagnosis of 6 months (r.1-118). Overall response rate (ORR) was 83% (114/137), no difference among WHO and IPSS subgroups was found:76% achieved response after 3 months of treatment, while other 7% after 6 months. 2 patients with SD (non responders IWG), in which treatment was continued, achieved response after 9 months. In the macrocytic-responders group 87% exhibits again macrocytosis after 3 months, while 13% become normocytic. In the normocytic-responders group 92% exhibits again normocytosis, while 4/52 (8%) become macrocytic: in these 4 patients after 3 months there was a contemporary worsening in neutropenia and thrombocytopenia, with transfusion-dependence, regarded as first signs of progression of disease. Non responders were 23/137 (17%): in the macrocytic-non responders group 89% exhibit again macrocytosis after 3 months, while 11% become normocytic; in the normocytic group 80% exhibits again macrocytosis, while 20% become normocytic: r.1-23). These preliminary data can suggest that, in the majority of MDS patients responsive to ESAs, the increase of Hb concentration occurs mainly stimulating erythroid production in MDS clones; in the minority of patients probably it happens recruiting residual polyclonal erythropoiesis. It is interesting to note that stimulating effects of ESAs last even when the expression of dysplasia progresses.

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IN VITRO CHARACTERISTICS OF HEMATOPOIETIC PROGENITORS FROM PRIMARY MYELOFIBROSIS PATIENTS CORRELATE WITH IPSS/DIPSS RISK CATEGORY

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Hyperplasia of morphologically abnormal megakaryocytes (MKs) is a hallmark of primary myelofibrosis (PMF) but the molecular events leading to MK abnormalities are still unclear. We previously demonstrated that, in thrombopoietin (TPO)-stimulated cultures, PMF CD34+ cells showed enhanced *in vitro* expansion capacity and impaired megakaryocytic differentiation compared to CD34+ cells from healthy individuals, and that the over-expression of the proto-oncogene protein kinase C epsilon (PKC) contributes to these abnormalities (Masselli *et al.* ASH 2013 abstr.114). Here we investigated whether clinical (included in the IPSS or DIPSS risk category) or biological (JAK2 mutational status) variables might impact the *in vitro* behavior of TPO-stimulated PMF CD34+ cells. We stratified 8 PMF patients according to the IPSS or DIPSS category (low/intermediate *vs* high risk) and JAK2 mutational status and evaluated: (1) Fold increase (FI) at day 14 of culture, (2) MK differentiation (% of CD41+ and CD42b+ cells and% of proplatelet-forming MKs) and (3) PKC protein levels (by western blot). CD34+ cells from high risk PMFs displayed increased proliferative capacity as compared to low/intermediate risk (FI: 44±0.2 *vs* 26.5±4.3, p=0.012), while no difference could be observed between JAK2V617F+ and JAK2V617F- PMFs (FI: 37.2±11.8 *vs* 27.9±5, p=0.39). Additionally, high risk PMFs revealed impaired MK differentiation potential, as indicated by the lower% of CD41+ and CD42b+ cells (respectively: 26.3±9.4 *vs* 54.2.6, p=0.008 and 16.1±8 *vs* 38.2±3, p=0.011) and proplatelet-forming MKs (0.67±0.21 *vs* 1.8±0.25, p=0.035). By contrast, no statistical difference was observed according to the JAK2 mutation. Finally, we found that high risk patients-derived MKs are characterized by higher expression of PKC as compared to low/intermediate risk ones (relative PKC /GAPDH OD values: 1.75±0.52 in high risk *vs* 0.82±0.29 in low risk, p=0.023). Conversely, PKC levels were comparable among JAK2V617F+ and JAK2V617F- PMFs (1.11±0.34 *vs* 1.3±0.8, p=0.72). These data indicate that the degree of *in vitro* growth and megakaryocytic commitment of PMF CD34+ cells is correlated to the aggressiveness of the disease (indicated by IPSS/DIPSS risk category) and not to the JAK2V617F mutation. Similarly, we found that PKC levels are significantly greater in high *vs* low/intermediate risk patients, leading us to speculate that PKC can be utilized as a marker of high disease burden and a more aggressive disease.

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PRIMARY HYPEREOSINOPHILIC SINDROMES AND IMATINIB THERAPY: A RETROSPECTIVE ANALYSIS OF 38 CASES

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Background: Chronic myeloproliferative neoplasms with eosinophilia are rare and heterogeneus disorders characterized by eosinophil proliferation and tissue damage. The 2008 updated "Classification of Tumors of the Hematopoietic and Lymphoid Tissues" of the World Health Organization, divides this neoplasms into two major subgroups: myeloid and lymphoid neoplasms with eosinophilia associated with Platelet-derived Growth Factor Receptor (PDGFR)-α, PDGFRβ or Fibroblast Growth Factor Receptor1 (FGFR1) and CEL, NOS. Low-dose imatinib

is an effective therapy for myeloid neoplasms with eosinophilia and abnormalities of PDGFRα/β, and prognosis of these disease has dramatically improved. CEL, NOS remains a difficult-to treat disease with a dismal prognosis. *Methods:* We retrospectively evaluated data form 38 patients affected by primary eosinophilia followed at IRCCS Ca' Granda Maggiore Hospital Foundation, Milan and Azienda Ospedaliera Papa Giovanni XXIII, Bergamo, Italy. *Results:* Median age at diagnosis was 53 years (range 18-77). Seven cases presented PDGFR-α rearrangements, while 8 were diagnosed as CEL, NOS. Diagnosis were HES and HE(US) in 17 and 6 patients, respectively. Four CEL, NOS patients presented the KITM541L somatically acquired mutation. Organ damage was present in 27 patients: pruritus and cutaneous lesions occurred in 37% of cases, 32% and 12% presented various degree of pulmonary and cardiac alterations, respectively, diarrhoea and venous thrombosis were found in 5% of cases. Thirty patients received imatinib and overall response rate was 57%. Complete hematologic response (CHR) to low-dose imatinib was obtained in all patients presenting PDGFR-α rearrangements, in 57% of CEL, NOS and 31% of HES patients, respectively. After a median imatinib treatment of 3 years (range 1-9), 6-years progression-free survival was 75% and 54% for patients with PDGFR-α rearrangement and CEL, NOS, respectively. For CEL, NOS patients harbouring the KITM541L mutation and treated with low-dose imatinib, 6-years PFS was 67%. Median OS for patients receiving imatinib was 70 months. *Conclusions:* Our data confirm that myeloprolipherative neoplasms with eosinophilia are rare disorders presenting various clinical pictures. Low-dose imatinib is an effective therapy for cases with PDGFR-α rearrangements. Furthermore, search at diagnosis for the KITM541L in CEL, NOS patients could identify an additional subgroup of patients that may benefit from low-dose imatinib.

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CARDIOPULMONARY EXERCISE TESTING FOR EARLY DETECTION OF PULMONARY HYPERTENSION IN PRIMARY MYELOFIBROSIS

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Pulmonary Hypertension (PH) is a frequently under-recognized complication of primary myelofibrosis (PMF); cardiopulmonary exercise testing (CPET) proved to be an essential and non-invasive diagnostic method in PH. We aimed to identify CPET parameters useful to earlier PH detection in PMF. Patients (pts) with diagnosis of PMF (acc. WHO 2008), age >18 years and fit to perform CPET were included. Main exclusion criteria were pulmonary or cardiac disease, portal hypertension and clinical history of PH. At enrollment (t0) pts underwent echocardiography, CPET, global spirometry and diffusing capacity of the lung for carbon monoxide (DLCO) analysis; echocardiographic assessment was repeated after 6 months (t1). During CPET we monitored ECG and beat-to-beat arterial pressure; we also used ventilatory expired-gas and transcutaneous PCO2/PO2 analyzer. Statistics was performed using T-test unpaired, T-test paired and Pearson's correlation. Twenty-three pts were compared to age and gender-matched controls. Median age was 68 years; 10 pts carried JAK2 V617F mutation, 11 a CARL mutation (7 type-1, 4 type-2), 2 were triple-negative. DIPSS was low or intermediate-1 in 9 and 14 cases, respectively. Fourteen pts were receiving hydroxyurea and 17 antiplatelets; 18 pts had a prefibrotic-stage MF, while 5 had grade 2-3 bone marrow fibrosis. During CPET PMF pts compared to controls demonstrated raised minute ventilation carbon dioxide production relationship at anaerobic threshold (AT) (38.3+3.3 *vs* 28.5+2.9; p<0.05); high PaCO2 at rest (44.2+2.1 *vs* 39.4+1.8 mmHg; p<0.02) and high difference between artery and tele-expiratory CO2-pressure [δP(a-et)CO2] at AT (+4+1.5 *vs* -5+1.8 mmHg; p<0.02). By echocardiography PMF showed slightly elevated pulmonary arterial pressures (PAPs) (34+4.2 *vs* 23+4.5 mmHg; p<0.05), short pulmonary valve acceleration time (PVAccTime) (88+11 *vs* 122+9 msec; p<0.01), mildly increased left atrial volume (42+7 *vs* 29+5 ml/mq, p<0.02). Global spirometry and DLCO analysis were normal. At t1 PMF demonstrated a significant increase of PAPs and reduction of PVAccTime when compared with t0 (37+ 5.6 *vs* 34+4.2 mmHg; 80+14 *vs* 88+11 msec; p<0.05). A positive relationship between δP(a-et)CO2 and both δPAPs and δPVAccTime was identified (Rsquare 0.45 and 0.39; p 0.025 and 0.04). This study defines δP(a-et)CO2 as an early predictor of pulmonary circulation impairment, useful for early identification of PMF most at risk of developing clinically significant PH.

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TYPE 1 *VS* TYPE 2 CALRETICULIN MUTATIONS AND THROMBOTIC RISK IN ESSENTIAL THROMBOCYTHEMIA PATIENTS: ANALYSIS OF A MONOCENTRIC SERIES

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Background: Recent advances in BCR-ABL1-negative myeloproliferative neoplasms (MPN) have highlighted the prevalence of mutations in the calreticulin (CALR) gene. This discovery enables for the first time molecular information, in addition to JAK2 V617F, to be used in the majority of MPN patients as an affirmative variable to discriminate neoplastic from reactive myeloproliferation. CALR mutations were reported in about 25% of essential thrombocythemia (ET) and in 35% of primary myelofibrosis (PMF) patients, not associated with JAK2 or MPL gene mutations. Specifically, two variants account for 85% of the CALR mutations in both ET and PMF: L367fs*46, type 1 and K385fs*47, type 2. *Methods:* In a cohort of 213 adult patients with a WHO 2008-based diagnosis of ET, baseline clinical/molecular characteristics and outcome measures (thrombosis, hemorrhages, death, overall and event-free survival) were evaluated. The JAK2 V617F mutation was detected by allele-specific PCR and confirmed by direct Sanger sequencing. CALR and MPL mutations were assessed by direct Sanger sequencing of exon 9 and 10, respectively. *Results:* In our ET series we identified mutations of CALR gene in 41 patients (19.3%): in particular, there were 20 (48.8%) and 15 cases (36.6%) of type 1 and 2 mutation, respectively, and 6 cases (14.6%) carried other distinct variants: D373fs*54, E372fs*50, E372fs*48, K368fs*51, K368fs*50 and L385fs*48, all detected in only one patient. Their main clinical and laboratory features at diagnosis are reported in Table 1.

	CALR-mutated ET			
	Total (n=41)	Type 1 (n=20)	Type 2 (n=15)	Other (n=6)
Male/female	15/26	6/14	7/8	2/4
Age (years), median (range)	49 (21-85)	52 (21-78)	44 (29-85)	40 (28-80)
Hb (g/dl); median (range)	13.4 (10.2-16.7)	13.3 (11.4-15.5)	12.8 (10.2-16.7)	13.6 (12.3-14.4)
Hct (%), median (range)	39.5 (31.8-49.2)	39.4 (34.8-46.7)	39.5 (31.8-49.2)	39.9 (36.7-43.0)
WBC count (x10^9/l); median (range)	7.42 (4-11.6)	8.55 (4-11.6)	6.9 (5-11.43)	7.35 (6-10.2)
PLT count (x10^9/l); median (range)	842 (448-1890)	852 (482-1560)	946 (448-1890)	711 (562-1020)
LDH (IU/l); median (range)	395 (210-762)	423 (335-627)	395 (210-577)	365 (276-762)
Palpable splenomegaly, n (%)	4 (9.7)	3 (7.3)	1 (6.7)	/
Conventional thrombotic score, n (%)				
Low risk	23 (56.1)	11 (55)	7 (46.7)	5 (83.3)
High risk	18 (43.9)	9 (45)	8 (53.3)	1 (16.7)
IPSET, n (%)				
Low risk	28 (68.3)	12 (60)	10 (66.6)	6 (100)
Intermediate risk	9 (21.9)	5 (25)	4 (26.7)	/
High risk	4 (9.8)	3 (15)	1 (6.7)	/
Previous thrombosis, n (%)	4 (9.8)	3 (7.3)	1 (6.7)	/
Previous hemorrhages, n (%)	2 (4.9)	1 (2.4)	1 (6.7)	/

Table 1. Baseline clinical and laboratory features of 41 CALR-mutated ET patients according to mutational types.

We then compared the three subgroups of CALR-mutated ET patients considering thrombotic risk. After a median follow-up of 11.8 years (range 1.9-31.2), we globally described 11 thrombotic events, in particular 5 arterial and 6 venous thrombosis. Of these, 10 cases (90.9%) happened in the type 1 mutation group, only one case (9.1%) in patients with a type 2 mutation, and none in those bearing other CALR mutations. *Conclusions:* With the limitations due to the retrospective nature of the present study, our data showed a different thrombotic rate in type

1 and 2 mutation groups, being the former higher than the latter. This was associated with an older age at diagnosis, but there was only a slight different distribution in the thrombotic risk according to either conventional (considering age >60 years, history of thrombosis and extreme thrombocytosis (platelet count >1500 x10^9/l) as risk factors) or IPSET-thrombosis score.

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RISK OF PREGNANCY COMPLICATIONS AND EFFECT OF DIFFERENT TREATMENTS IN WOMEN WITH ESSENTIAL THROMBOCYTHEMIA: A RETROSPECTIVE MONOCENTER ANALYSIS OF 62 PREGNANCIES

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Background: Women with essential thrombocythemia (ET) are highly prone to obstetric complications (OC), and the rate of live births are around 60%. The role of the JAK2V617 mutation as predictor of pregnancy complications and possible therapeutic driver is controversial. *Patients and Methods:* We analysed 62 pregnancies of 38 ET pts. (median 2 pregnancies/woman, range 1-3). The median age at diagnosis was 29 yrs (range 18-41); the age at conception was >35 yrs in 33 pregnancies (53%). Nineteen pts. (50%) carried the JAK2V617F mutation; 2 had hepatic vein thrombosis and TIA before first conception, respectively. One terminated pregnancy, one blighted ovum and one miscarriage due to Turner syndrome were excluded from further analysis. Antepartum antithrombotic treatment was LMWH+aspirin (ASA) in 32 pregnancies, ASA in 14, LMWH in 6, none in 7. Interferon was given during 9 pregnancies. Different antepartum strategies were estimated by a multivariate Cox regression model over the weeks of gestation. Puerperium was defined as 6 weeks after delivery at >20 week of gestation and was treated with LMWH in 45/51 cases. *Results:* The rate of live births was 83% (49/59); 17/59 (29%) OC were considered ET-related: 8 miscarriages, 2 stillbirths, 1 abruptio placentae with neonatal death, 6 intrauterine fetal growth retardations. Seven OC occurred during LMWH+ASA (22%), 2 during ASA (14%), 3 during LMWH (50%), and 5 in the untreated pregnancies (71%). Treatment reduced the OC risk by 88% *versus* untreated pregnancies (odds ratio, OR 0.12, 95%CI 0.02-0.69). The OC rate was 43% in the pregnancies of JAK2V617F-positive pts. (13/30) and 14% in those of the JAK2 V617F-negative pts. (4/29) (OR 4.77, 95%CI 1.33-17.18); however after exclusion of the untreated pregnancies the risk associated with JAK2V617F was no more significant (OR 3.33, 95%CI 0.85-13.00). In a multivariate model including age >35 yrs, JAK2V617F, and antepartum ASA, LMWH, and interferon, only ASA was associated with the outcome (OR for complications 0.28, 95%CI 0.10-0.80, p=0.01). No thrombosis occurred antepartum or during the puerperium treated with LMWH. Cerebral vein thrombosis occurred in 1/6 untreated puerperium periods (17%). *Conclusions:* Antepartum ASA is effective in preventing ET-related OC and antepartum LMWH could be reserved only to women with additional risk factors for venous thromboembolism. In ET the rate of puerperium-related venous thrombosis is high and prompts LMWH prophylaxis.

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IS HIGH PLATELET COUNT AT DIAGNOSIS OF ESSENTIAL THROMBOCYTHEMIA A PROTECTIVE FACTOR FOR THROMBOSIS?

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The protective effect of higher platelet count at diagnosis of Essential thrombocytemia (ET) was reported in some papers (Carobbio A. 2011, Palandri 2012, Montanaro M., 2014). As at our knowledge there is no study specifically addressing this point, in this retrospective analysis we examined 1250 ET patients followed in 11 Hematological centers of our region from January 1978 to December 2010. Diagnosis of ET was performed according to the period of 1st observation, respectively with PVSG, WHO 2001 and WHO 2008 criteria. Main characteristics of patients are reported in the Table 1. Survival curves were plotted according to Kaplan-Meier and independent risk factors were identified by the Cox proportional-hazards method. At the multivariate analysis predictive factors for TFS resulted: previous thrombotic events (p=0.0004), age (p=0.0044), spleen enlargement (p=0.042) and platelet count (p=0.03). Factors influencing overall survival (OS) were WBC count >15 x 10^9/L (p=0.017), previous thrombosis (p=0.0003), age ≥60 yrs (p<0.0001); no difference was seen for spleen enlargement, CVRF and platelet count (p=NS).Receiver operating characteristic (ROC) analyses based on thrombotic event during follow-up identified a baseline platelet count of 944 x 10^9/L as best threshold for predicting thrombotic events. Thrombotic events according to this cutoff were 40/384 (10.4%) in patients with platelet count >944 x 10^9/L and 109/817 (13.34%) in patients with platelet count <944 x 10^9/L, respectively. The comparison of main characteristics in these two populations showed in patients with PLT count <944 x 10^9/L an older median age (60.4 yrs *vs* 57.1 yrs, p=0.016), a lower median WBC count (8.8 x 10^9/L *vs* 10.6 x 10^9/L, p<0.0001), a higher median Hb level (14.1 g/dL *vs* 13.6 g/dL, p<0.0001)and a higher rate of JAK-2 positivity (67.2% *vs* 41.6%, p<0.0001); no differences were observed between the two groups as to thrombotic events before diagnosis, spleen enlargement and CVRFs (p=NS). In conclusion, our retrospective analysis confirmed the protective role for thrombosis of high platelet count at diagnosis. The possible explanation is still unclear, taking also in account that PLT count resulted very often near normal values when a thrombotic event occurred. The older age and the higher rate of JAK-2 V617F positivity in the group of patients with a baseline lower platelet count could in part be responsible of this counterintuitive finding.

Table 1.

N. of cases	1250
Median age	62,9 (r. 19-96)
Gender (F/M, %)	797/452 (63,8 - 36,2)
Median follow-up (y)	7,75
WBC (x10^9/L), median	8,8 (r.1,2- 57.7)
Hb (g/dL), median	14,0 (r. 6-20,5)
Plt (x 10^9/L), median	813 (r. 457-3582)
JAK2^{V617F}, mutated/performed, (%)	498/834 (59,7)
JAK2^{V617F}, quantitative (%), median	19,6 (0,2- 99,9)
Splenomegaly, number, (%)	226/1204 (18,7)
Thrombosis before diagnosis, number (%)	223/1239 (17,9)
Previous Arterial/Venous Thr. , number (%)	A: 176 (14,1%), V: 47(3,8%)

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ATYPICAL CHRONIC MYELOID LEUKEMIA: CLINICAL PRESENTATION AND PROGNOSIS IN NOT SELECTED CLINICAL CASES

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Atypical chronic myeloid leukemia (aCML) is an infrequent myeloid neoplasm characterized by leukocytosis, marked myeloid dysplasia, absence of Philadelphia chromosome and BCR/ABL rearrangement. The 2008 WHO Classification includes aCML among myelodysplastic/myeloproliferative neoplasms (MDS/MPNs) with chronic myelomonocytic leukemia (CMML), RARS with thrombocytosis (RARS-T), juvenile myelomonocytic leukemia (J-MML) and unclassifiable MDS/MPN (MDS/MPN-U). We collected data about 38 patients (pts) with MDS/MPNs observed between 1998 and 2014: 19 were CMML, 6 RARS-T and 13 had the diagnostic criteria for aCML. The characteristics of pts were as follows: mean age 74 years (range 67-87); sex 12 male, 1 female; leukocytes 61 x 1000/mcl (19-173); immature granulocytes 25%

(10-55); neutrophils 60% (44-82); monocytes 4% (1-10); eosinophils 2% (0-12); basophils 0.5% (0-5); hemoglobin 9.6 g/dl (6.4 - 15.6); platelets 170 x 1000/mcl (18-583); we detected peripheral blasts in 6/13 pts (range 1-9%). In all pts bone marrow was hypercellular (between 90 and 100% in 10/13) and with dysgranulopoiesis; 4/13 pts had bilinear bone marrow dysplasia and 8/13 trilinear dysplasia; only in 1 pt blasts were 9%, in all the others were lower than 5%. In 11 pts cytogenetics was normal; 1 pt had trisomy 8, another a complex karyotype: 45,XY,-3,t(7;9;22)(p22;q22,q11). BCR/ABL rearrangement was negative in all cases. One pt had aCML associated with systemic mastocytosis with cKIT mutation. The rearrangement of PDGFRA/PDGFRB was tested in 2 pts with eosinophilia and found negative. 6/13 pts had splenomegaly. All pts were treated with hydroxyurea and supportive therapy. 2 pts evolved to AML after 10 and 28 months from diagnosis and subsequently died; 7 pts died for complications/comorbidities (median survival 10 months). 2 pts are still alive 16 and 30 months from diagnosis; 2 pts are lost to the follow up. Our data confirmed those reported in literature: aCML is a heterogeneous disease without specific cytogenetic marker, much more aggressive than chronic myeloid leukemia and which affects elderly pts with prevalence of male, with poor prognosis and reduced median survival.

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RECEIVER OPERATING CHARACTERISTIC ANALYSIS AS A TOOL TO IDENTIFY THRESHOLD VALUES OF CONTINOUS RISK FACTORS FOR THROMBOSIS AND SURVIVAL IN ESSENTIAL THROMBOCITEMIA: A RETROSPECTIVE ANALYSIS ON 1250 PATIENTS

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Risks factors for thrombosis and survival in Essential Thrombocitemia (ET) have been well defined. In a previous paper of our group (Montanaro *et al.*, 2014), the following risk factors at diagnosis statistically significant for a shorter thrombosis-free survival (TFS) were identified at univariate analysis: age ≥60 yrs (p=0,0054, CI 95% 1.18 – 2.6), previous thrombosis (p<0.0001, CI95% 1.58 – 4.52) and the presence of at least 1 cardiovascular risk factor (p=0,036, CI95% 1.15 – 3.13). Patients with a previous thrombosis occurred ≥24 months before ET diagnosis had a significantly shorter TFS compared to patients with a previous thrombosis occurred <24 months before ET diagnosis (p=0.0029, CI95% 1.5 – 6-1); furthermore, patients with previous thrombosis occurred <24 months did not show a significant shorter TFS when compared with patients without previous thrombosis (p=0.303, CI95% 0.64 – 3.21). At the multivariate analysis for TFS, only the occurrence of a previous thrombosis maintained its prognostic impact (p=0.0004, CI95% 1.48 – 3.79, RR 2.36), while age >60 years had only a trend of significance (p=0.058, CI95% 0.99 – 2.56, RR 1.59). The thresholds we used have been obtained empirically, based on well-recognized international studies. In this work we preliminarily identified the best threshold of any continous variable with the Receiver Operating Characteristic (ROC) analyses; TFS and OS curves were then plotted according to Kaplan-Meier method and the independent factors were derived by Cox proportional-hazards method. ROC analysis based on thrombotic events identified the best threshold for age (64.17 yrs), WBC count (9.6 x 109/L), Hb level (12.7 g/dL), PLT count (944 x 109/L) and JAK2 V617F allele burden (93%). The same evaluation was performed for OS with the identification of the following cut-offs: 56.6 yrs for age, 10.6 x 109/L for WBC count, 12.3 g/dL for Hb level and 37.4% for JAK2 V617F allele burden. At the multivariate analysis using for continuous variables the above thresholds identified by ROC analysis, significant independent parameters for TFS were PLTs count <944 x 109/L (p=0.0184), spleen enlargement (p=0.040), previous thrombosis (p=0.0089) and age ≥64.17 yrs (p=0.0002), while for OS were age ≥56.6 yrs (p=<0.0001), Hb level (p=0.01) and WBC (p=0.005). In conclusion, the use of thresholds from ROC analysis led to a more correct identification of prognostically significant continuous variables than the use of empirical threshold values, refining and improving our previous observations (Table 1).

Table 1.

Variables	Area under the ROC curve (AUC) Classification variable: Thrombosis	p	Area under the ROC curve (AUC) Classification variable: Death	p
Previous Thrombosis	0.58	0.0017	0.58	0.001
Previous Thrombosis ≥ 24 m	0.58	<0.0001	0.53	0.031
Age	0.54 (>64.17yrs)	0.06	0.71 (≥56.3yrs)	<0.0001
WBCs	0,52 (>9.6 x 10^9/L)	0.72	0.54 (>10.6 x 10^9/L)	0.098
Hb	0.51 (>12.7 g/dL)	0.72	0.57 (<12.3 g/dL)	0.01
Qualitative JAK-2	0.52	0.52	0.52	0.62
JAK-2 V617F allele burden (%)	0.58 (≥93.1%)	0.11	0.65 (≥37.4%)	0.035
Spleen enlargement	0.53	0.07	0.53	0.065
CVRF (at least 1)	0.53	0.053	0.50	0.89

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GATA1, BUT NOT FOG1, FLI1 OR CALR, IS UP-REGULATED IN ESSENTIAL THROMBOCYTHEMIA INDEPENDENTLY FROM JAK2 AND CALR MUTATIONS

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GATA1 is the founding member of the GATA transcription factor family and it is essential for cell maturation and differentiation within the erythroid and megakaryocytic lineages. We and others have demonstrated that elevated GATA1 expression is found in the bone marrow of Essential Thrombocythaemia (ET) patients, independent of JAK2V617F and CALR mutations. Friend of GATA (FOG1) and the Friend leukaemia integration 1 (FLI1) transcription factors are vital for megakaryocyte and erythroid-lineage commitment and their expression largely overlaps spatiotemporally with that of GATA1. Calreticulin (CALR) mutations reported in myeloproliferative neoplasms create translation frameshifts in exon 9 which truncate the C-terminal calcium binding domain and create a novel C-terminal peptide. Initial reports support CALR mutations as early and disease-initiating mutations that favor expansion of the megakaryocytic lineage. We wanted to study the expression of GATA1 in peripheral blood (PB) of patients with ET, together with FLI1, FOG1 and CALR, trying to identify if there is a common altered pathway and/or any correlation with JAK2 and CALR mutational status. PB specimens were collected from 36 patients diagnosed with ET, 17 JAK2 mutated (47%), 4 CALR (11%) mutated, 1 MPL mutated (3%) and 14 with no molecular abnormalities, and compared with a cohort of healthy volunteers. Samples were enriched for the mononuclear fraction by Ficoll separation. Total RNA was extracted and analysed by Real Time PCR for GATA1, FOG1, FLI1 and CALR expression relative to the housekeeping gene GAPDH using the 2- CT method. We confirmed the data obtained in bone marrow demonstrating that GATA1 is significantly up-regulated in ET patients also in PB and that GATA1 overexpression is independent from JAK2V617F and CALR mutations. However, the transcription factors FOG1 and FLI1 do not appear to be subject to the same regulatory control in ET as that of GATA1, remaining at the same level of expression as the controls. Interestingly we also found a significant downregulation in CALR mRNA comparing with controls and this is indipendent from CALR mutation as well. These results suggest that GATA1 is specifically deregulated in ET. GATA1 overexpression is isolated and indipendent from its co-factor FOG1 or FLI1. Very interestingly CALR is downregulated in ET samples indipendently from CALR mutation or JAK2 matation, however more data are required to better understand the mechanisms of this deregulation.

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A NEW PHD2 VARIANT IN A PATIENT WITH ERYTHROCYTOSIS: A FUNCTIONAL STUDY

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Background: Congenital Erythrocytosis, are rare and heterogeneous clinical entities, caused by genetic deregulation of the erythroid cell line

followed by an increased production of red blood cells. Primary Congenital Familial erythrocytosis (PCFE) are related to mutations of the erythropoietin receptor (EPOR) and are associated with reduced/normal levels of serum erythropoietin (EPOs). In contrast, Secondary Congenital Erythrocytosis may result from inherited defects of the oxygen-sensing pathway (OSP) genes inducing increased production of EPOs. At present, three genes of OSP have been described to be mutated in patients with erythrocytosis: the von Hippel-Lindau (VHL), the hypoxia-inducible factor 2 α (HIF2A/EPAS1) and the prolyl hydroxylase 2 (EGLN1/PHD2) genes. While a number of VHL mutated patients have been described, only 24 cases of PHD2 and 9 cases of HIF2A mutated genes have been published. We describe here a new mutation observed in a 22 years old man who presented with RBC 6,16x10^9/L, Hb 169 g/L and Htc 50,1% and normal WBC (8,7x10^9/L) and platelet (345x10^9/L) counts. *Materials and Methods:* Sanger sequencing method was used to find mutations in the first 3 exons of PHD2 gene. The computational evaluation of the founded mutation was performed with Poliphen software. To assess its biological effect we performed an in cellulo reporter assay in order to study the HIF transcriptional activity, while the hydroxylation capacity of PHD2 variant has been tested with an *in vitro* Hydroxylation test. We compared the functional activity of the found PHD2 mutation with severe and not-severe mutations already published. *Results:* In the propositus, we found a PHD2 mutation (G349S) and we classified it as not severe. In agreement, the functional studies did not show significant differences when compared to wild type PHD2 activity, while the severe mutation (D254H) showed an important loss of activity. *Conclusions:* As already published, functional studies of PHD2 variants do not always show a clear loss of function of the PHD2 proteins, despite a clear genetic link with the disease. Our results suggest that more sensitive tests should be developed and other PHD2 partners may be tested. Moreover, to completely understand the ethiology of the disease, others candidate genes need to be investigated and high throughput sequencing, as Next Generation Sequencing (NGS), should be performed.

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PROGNOSTIC VALUE OF HEMOBLOGIN LEVELS AND CIRCULATING BLASTS IN SECONDARY MYELOFIBROSIS

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Polycythemia Vera (PV) and Essential Trombocythemia (TE) can progress to secondary myelofibrosis (SMF), named post PV (PPV-SMF) and post ET (PET-SMF), in about 5 – 15% of patients. To evaluate the role in these SMF of a simple clinical score already validated in primary myelofibrosis and based on 2 easily assessable features (Hb <10 gr/dL and circulating blasts ≥1%), we collected in the retrospective database of our cooperative group of Latium region 84 patients with SMF. The diagnosis of evolution in SMF was done according the standard IWG-MRT criteria. The main features at myelofibrotic evolution were as follows: 48 patients (57.1%) were females, median age was 68,9 years (interquartile range 61,1-74,5), 39 patients (46.4%) were PPV-SMF and 45 (53.6%) PET-SMF, median Hb level was 9.9 g/dl (interquartile range 8.7 – 11.5), median time from PV/ET diagnosis to SMF diagnosis was 146.9 months (interquartile range 73.0 – 220.0), JAK2-V617F mutation was positive in 48 out of 63 patients with an available data (76,1%). As to the 2 features considered in the score system, 42 patients (50.0%) had Hb level <10 g/dl and 18 patients (21.4%) had peripheral blasts ≥1%: according to these 2 features, 38 patients (45.2%) did not have any and were considered as low-risk, while 46 (54.8%) had at least 1 feature (32 patients) or both (14 patients) and were considered as high-risk. Median survival of the whole cohort since myelofibrotic evolution was 83.0 months (95% CI 53.6 – 112.4): median survival of low-risk patients was 125.2 months (95% CI 69.3 – 181.2) while median survival of high-risk patients was 53.6 months (95% CI 37.2 – 69.9) (p<0.001). The risk of leukemic transformation (LT) was not statistically different between low and high-risk groups (p=0.086). In conclusion, the evaluation of Hb level and circulating blasts was an important tool to predict survival in SMF but not LT. The dramatic nature of the LT event and the dismal outcome of acute leukemia evolved from myeloproliferative neoplasm justifies continuing efforts to define models predicting LT and to validate the current scores in larger series of patients.

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USE OF HOSPITAL DISCHARGE DATABASES TO ESTIMATE EPIDEMIOLOGICAL INDICATORS OF HAEMATOLOGICAL MALIGNANCIES IN ABRUZZO

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This study was aimed to provide epidemiological data of haematological malignancies (non-Hodgkin lymphoma (NHL), Hodgkin lymphoma (HL), multiple myeloma (MM), acute and chronic leukaemia) in Abruzzo. Hospital discharge records (HDR) have been used to estimate epidemiological indicators in the 2009-2013 period and were compared with those of the 2004-2008 period. HDR with haematological malignancies in primary or secondary diagnosis (ICD-9CM codes: 200.00-208.91) and their sub-types were selected: NHL (ICD-9CM codes: 200.00-200.88; 202.00-202.98), HL (201.00-201.98), MM (203.00-203.81), leukaemias (204.00-208.91). Newly diagnosed cases were defined as first diagnosis of haematological malignancy in primary or secondary diagnosis of HDR and never recognized in the 2005-2008 period. Incident cases were defined as first diagnosis of haematological malignancy only in primary diagnosis. In the 2009-2013 period, among 8,613 prevalent patients with an haematological malignancy (1,723/year), newly diagnosed cases were 791/year (total 3,955) and were similar to those observed in the 2004-2008 period (783/year) (Table 1).

Table 1. New diagnosed cases of haematological malignancies (A) and incident cases (B) in the 2009-2013 and 2004-2008 periods in Abruzzo.

New diagnosed cases (A)	2009	2010	2011	2012	2013
Haematological malignancies (any)	802	797	803	797	756
NHL	291	323	321	308	326
Leukaemias	272	259	271	267	256
MM	160	160	162	157	119
HL	83	58	53	68	55

New diagnosed cases (A)	2004	2005	2006	2007	2008
Haematological malignancies (any)	756	873	800	728	762

Incident cases (B)	2009	2010	2011	2012	2013
Haematological malignancies (any)	478	487	479	438	422
NHL	181	204	204	180	205
Leukaemias	158	165	158	155	141
MM	85	78	88	70	50
HL	54	40	29	33	26

In the 2009-2013 period, incident cases were 2,304 (461/year). Among 3,955 new diagnosed cases, 40% were NHL, 33% leukaemias, 19% MM and 8% HL. Among 2,304 incident cases, 42% were NHL, 34% leukaemia, 16% MM, and 8% HL. The 5-year age-standardised incident rate (SIR) of haematological malignancy evaluated as new diagnosed cases was 58.3/100,000 (males: 72.9/100,000; females: 47.0/100,000).

The 5-year age-SIR of haematological malignancy evaluated as incident cases was 34.1/100,000/year (males: 42.1/100,000; females: 27.9/100,000). The 5-year age-SIR in the entire population was stable for new diagnosed cases and decreased for incident cases by 4%/year ($p<0.05$). Compared to the observed 791 new diagnosed cases/year of haematological malignancies and 461 incident cases/year, expected incident cases by applying cancer registries' pool rates to the Abruzzo population were 650/year. The 5-year age-SIR of new diagnosed cases was 26.2/100,000 for NHL, 6.7/100,000 for HL, 11.6/100,000 for MM, and 21.3/100,000 for leukaemias, whereas The 5-year age-SIR of incident cases was 15.7, 3.5, 5.7, 12.1, respectively. Our study demonstrates that HDR can provide readily available information to monitor epidemiological indicators. The incidence of haematological malignancies has been stable in the 2009-2013 period as compared to the 2004-2008.

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EFFICACY OF RUXOLITINIB IN MYELOFIBROSIS PATIENTS WITHOUT SPLENOMEGALY

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Ruxolitinib (RUX) is the only commercially available JAK2-inhibitor approved as therapy for myelofibrosis (MF). The COMFORT studies enrolled patients with palpable splenomegaly and demonstrated the clinical efficacy of RUX on splenomegaly and constitutional symptoms. However, little is known on the clinical activity of RUX in patients without splenomegaly. Between June 2011 and January 2015, 103 MF patients were followed in 3 Italian Hematology Centers (Bologna, Roma, Torino) and treated with RUX for a median time of 24 months (6-36). Thirteen (12.6%) patients did not carry baseline splenomegaly but were burdened by constitutional symptoms. Within the first month of RUX therapy, all patients reported a clinical benefit on disease-related symptoms, specifically fatigue, night sweats, itching, and bone pain. Notably, best responses were associated with higher titrated doses (Table 1).

Table 1. Main patients clinical features and outcome.

Patient no.	1	2	3	4	5	6	7	8	9	10	11	12	13
Age, y/sex	62/F	60/M	54/F	63/F	67/M	61/F	71/F	40/F	72/M	53/F	66/F	46/F	85/M
MF type	PMF	PMF	PMF	PMF	PMF	PPV-MF	PET-MF	PET-MF	PET-MF	PET-MF	PET-MF	PMF	PMF
Hepatomegaly	no	no	no	no	no	no (prior splenectomy)	no	no	no	no	no	no	no
Symptoms	weight loss fatigue	sweats fatigue	sweats fatigue bone pain	sweats fatigue itching	weight loss sweats fatigue	weight loss fatigue	sweats fatigue itching	sweats fatigue	fatigue itching	fatigue	fatigue weight loss	fatigue	sweats fatigue
ECOG	1	1	1	1	1	2	1	1	1	1	1	1	1
Risk IPSS	INTM2	INTM2	INTM2	INTM2	HIGH	HIGH	INTM2	INTM2	HIGH	INTM2	HIGH	INTM2	HIGH
WBC, K/UL	8.9	11.5	7.2	8.3	6.2	80	14.5	15	4.4	16,1	6.2	6.7	4,1
Hb, g/dL	9.4	11.4	9.8	13	8.1	11.9	14.7	14	9.2	9,8	13,7	12.1	8,7
PLT, K/UL	630	539	1084	879	141	355	289	321	109	603	92	437	373
Transfusion need	yes	no	yes	no	yes	no	no	no	yes	no	no	no	yes
PB blast %	0	2	0	1	0	8	0	1	0	0	1	0	2
Cytogenetics	46, XX	46, XY	46, XX	46, XX, del(5q)	n.a.	46, XX,der(6)t(6;15), inv(15),+8	46,XX,del(20q)	46, XX	n.a.	n.a.	46, XX	n.a.	n.a.
Type of mutation	CALR, type 1	JAK2 V617F	JAK2 V617F	JAK2 V617F	JAK2 V617F	JAK2 V617F	JAK2 V617F	JAK2 V617F	MPL W515K	JAK2 V617F	TRIPLE NEGATIVE	JAK2 V617F	MPL W515K
Mutation burden	58%	55%	3.5%	39.6%	97%	99%	46%	98%	n.a.	49%		29%	n.a.
Rux titrated dose	10 BID	10 BID	10 BID	15 BID	5 OAD	5 BID	10 BID	10 BID	20 BID	25 BID	10 BID	10 BID	5 BID
Decrease of fatigue*	-80%	-80%	-50%	-20%	-10%	-10%	-100%	-100%	-80%	-80%	-60%	-80%	-20%
Decrease of sweats*	n.a.	-100%	-100%	-50%	-100%	n.a.	-100%	-100%	n.a.	n.a.	-60%	n.a.	-30%
Decrease of itching*	n.a.	n.a.	n.a.	-50%	n.a.	n.a.	-100%	n.a.	-80%	n.a.	n.a.	n.a.	n.a.
Decrease of bone pain*	n.a.	n.a.	-75%	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Weight gain*	8,7%	1,2%	5,2%	0,1%	8,0%	14,0%	1,5%	0%	1,5%	4%	10%	0.2%	2%

All patients had weight gain; also, patients in working age could resume their normal work activity. No patients developed hepato-splenomegaly over time; in one case, baseline hepatomegaly was not palpable at a 6 month-interval. Patients with marked thrombocytosis normalized their blood counts. JAK2V617F/CALR allele burden were evaluated at treatment start and at 12-month intervals: in one case a significant reduction (-71%) of JAK2V617F mutation load was observed. Treatment was generally well tolerated. No significant (grade 3 or 4) non-hematologic adverse events were observed, although 3 patients experienced a total of 5 infectious complications requiring oral antibiotics (2 bronchitis, 1 pneumonia, 1 urinary tract infection). At RUX start, 5 patients were receiving transfusion support with red blood cells; in all these patients, transfusion need remained stable during treatment. One patient became transfusion-dependent after two months of therapy and 3 additional patients finally discontinued ruxolitinib due to persistent anemia and thrombocytopenia, that did not allow to maintain an effective titrated dose. No patient, including those with hyperleucocytosis and unfavorable cytogenetics, progressed to acute myeloid leukemia. This experience confirms a positive effect of RUX on patient symptoms not related to reduction of spleen size.

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STUDY OF MIRNAS EXPRESSION PROFILE IN JAK2V617F KNOCK-IN MOUSE MODEL

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Abnormal miRNAs expression might be involved in the pathogenesis of Myeloproliferative Neoplasms (MPNs). In order to clarify the contribution of miRNAs to the pathogenesis of JAK2V617F-positive MPNs, we analysed the miRNAs expression pattern in erythroid (TER119+) and mieloid (GR1+) cells purified from BM of JAK2V617F knock-in (KI) mouse model (2 KI and 2 WT) using TaqMan® Mouse MicroRNA Array v3.0 that includes 365 miRNAs. In the validation phase, the expression level of selected miRNAs was evaluated in the same cells type of 5 JAK2V617F KI and 5 JAK2 WT mice by TaqMan® miRna assays. Relative miRNAs expression was measured using 2^- Ct method. Potential mRNA targets were predicted in silico according to Targetscan 6.1. First, we identified a list of differentially expressed miRNAs: 66 differentially modulated in GR1+ (44 up-and and 22 down-regulated) and 64 in TER119+ (6 up-and 58 down-regulated). Among them, the most differentially expressed were selected for validation: 5 were up-regulated (miR-147-3p, miR-15b-5p, 193a-3p, miR-7a-5p, 291b-5p) and 7 down-regulated (miR-29b-3p, miR-467b-5p, miR-150-5p, miR-28a-5p, miR-592-5p, miR-484, miR-10a). In the validation phase, we confirmed up-regulation of miR-7a-5p and miR-291b-5p ad down-regulation of miR-150-5p in GR1+; in TER119+, up-regulation of miR-7a-5p and down-regulation of miR-150-5p and miR-592-5p. Moreover, in not-validated miRNAs we found significant up-regulation of miR147-3p, miR467-5p in TER119+ and of miR-193-3p, miR-28a-5p, miR-484 and miR-10a in GR1+.Only 2 differentially expressed miRNAs were shared by both erythroid and myeloid cells (miR-7a-5p and miR-150-5p, up-and down-regulated respectively), suggesting a direct relationships between these miRNAs and JAK2V617F. Target prediction analysis included genes involved in pathways having functional relevance for MPN such as mTOR Signaling Pathway (PIK3R1 and EIF4B), CXCR4 Pathway (CXCR4 and PTK2), cell cycle (*e.g.* TP53, MDM2, RB1, E2F1 and CDK2), apoptosis (*e.g.* CASP9, BCL2 and BCL2L1,), epigenetic regulation (*e.g.* EZH1 and DNMT3A) and transcriptional modulation (*e.g.* SMAD1, NOTCH1 and MYB). Our study identified a list of differentially expressed miRNAs in JAK2V617F KI mouse whose deregulation might contribute to the development/phenotype of MPNs. Further studies are in progress to (i)evaluate the role of these miRNAs in CD34+ and granulocytes of MPN patients; (ii)explore the relevance, using functional approach, of these selected miRNAs.

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EVALUATION OF THE PLASMA MIRNAS EXPRESSION PROFILE IN PRIMARY MYELOFIBROSIS PATIENTS

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Introduction: MicroRNAs (miRNAs) are small non-coding RNAs crucial in cellular processes and have been shown to be abnormally expressed in cancer. Recently, miRNAs might constitute a novel class of minimally invasive biomarkers for prognosis assessement Even if several studies demonstrate specific miRNA expression pattern in cells from Primary

Myelofibrosis (PMF) patients, the significance of circulating miRNAs in PMF remains to be defined. In this study, we aimed to extensively investigate circulating miRNAs in PMF as potential biomarkers for the disease. *Methods:* Plasma samples were collected from patients with PMF and healthy donors.Total RNA was extracted using miRNeasy Serum/Plasma Kit (Qiagen®) and it was reverse transcribed using the miRCURY LNA Universal RT miRNA PCR, (Exiqon®). The miRNA detection experiments were performed by qRT-PCR on Serum/Plasma Focus micro-RNA PCR Panel. Evaluation of miRNA expression was performed with the 2^- Ct relative quantification method. *Results:* We analyzed the expression of 175 miRNAs in RNA plasma samples collected from 25 patients with PMF and 6 healthy donors. We identified differentially expressed miRNAs but only 6 of them were statistically different (P value<0.05): miR-let7b*, miR-10b, miR-424 and miR-99a were up-regulated while miR-144* and miR-375 were down-regulated in PMF patients compared to the controls. Of interest, miR-99a and miR-144* were reported to be de-regulated in granulocytes of PMF patients(Guglielmelli *et al.* Exp Hematol.2007). Comparing miRNAs and clinical data we found that BM fibrosis grade correlated with miR-let7b*, miR-10b and miR-144*(P=0.04, 0.02 and 0.02 respectively); abnormal karyotype was associated with miR-let7b*(p=0.04), miR-424 (p=0.001) and miR-99a (p=0.003), platelet count correlated directly with miR-144* and miR-375 (p=0.03 and 0.02 respectively). Results of ongoing analysis oif correlation with survival and leukemia transformation will be presented at the meeting. *Conclusions:* We were able to show for the first time a distinct plasma miRNA expression patterns in patient with PMF compared with healthy subjects. Our preliminary results showed several clinical correlation with selected circulating miRNAs.

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INFLAMMATORY MICROENVIRONMENT AND MYELOFIBROSIS: THE INTERPLAY OF IL-1 AND TNF- HIGHLY PROMOTE THE *IN VITRO* MAINTENANCE OF CIRCULATING CD34+ STEM/PROGENITOR CELLS

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Introduction: Mutations in JAK2, Calreticulin (CALR) and MPL genes are associated with Myelofibrosis (MF), a clonal disorder of the hematopoietic stem/progenitor cell (HSPC). Strikingly, HSPCs actively sense pro-inflammatory factors and the inflammatory network has been shown to modulate HSPCs functions. According to recent studies, a chronic inflammation may drive and promote the clonal evolution of myeloproliferative neoplasms. However, the key players linking inflammation and cancer in MF are elusive. Here we investigated whether crucial factors of the inflammatory microenvironment (Interleukin-1β (IL-1β), Tumor Necrosis Factor (TNF)-α, Tissue Inhibitor of Metalloproteinases-1 (TIMP-1) and Adenosine-triphosphate (ATP) may abnormally modulate the *in vitro* phenotype/function of HSPCs from MF patients. *Methods:* Circulating CD34+ HSPCs from MF patients (24 cases: 17 JAK2V617F+, 7 CALR+) or cord blood (CB) were *in vitro* incubated with or without pro-inflammatory factors (alone or in combination) and cell survival (AnnexinV/PI staining)/proliferation (colony forming unit (CFU-C) assay and cell cycle analysis) were assessed. *Results:* We found that the survival of MF CD34+38- cells was strongly stimulated by pro-inflammatory cytokines as compared with the normal counterparts. Of note, when we tested IL-1β/TNF-α in combination, the survival of CD34+38- cells from MF patients was mainly promoted independently of mutation profile. On the contrary, apoptosis of CD34+ cells of CALR+ patients was significant increased in the presence of ATP, TNF- and TIMP-1. In addition to the effect on cells survival, IL-1β/TNF-α significantly up-regulated the proportion of MF CD34+ cells entering cell cycle S-phase. Interestingly, ATP, TIMP-1 and TNF-α (in combination) significantly reduced the clonogenic potential of MF CD34+ cells as compared to CB CD34+ cells. Conversely, IL-1β plus TNF-α significantly increased the BFU-E, but not CFU-GM, growth of MF CD34+ cells as compared with untreated and CB-derived cells. According to mutations, in the presence of IL-1β/TNF-α, the CFU-C growth was significantly decreased in CALR+ patients as compared with JAK2V617F+ patients. *Conclusions:* Together these findings emphasize that selected pro-inflammatory cytokines promote the *in vitro* maintenance of the malignant hemopoietic clone by inducing a selective growth advantage in MF. Of note, the interplay of IL-1 and TNF-α may have a potential role in disease progression/evolution.

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MYELOID SARCOMA IN ADULTS. PRELIMINARY RESULTS FROM A MULTICENTER ITALIAN REGISTRY

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Introduction: Myeloid Sarcoma (MS) is a rare extramedullary haematologic myeloid neoplasm composed of immature myeloid cells that can involve any site of the body. It can occur as isolated extramedullary form (designated as *de novo*, non-leukemic or primary, MS) or it can be associated with an acute myeloid leukemia (AML) at onset or at relapse. *Patients and Results:* we report the clinical characteristics, treatment and outcome of 27 cases of MS diagnosed in the Italian Hematological Centers in the last 10 years (2005-2015) and included in an open and active national register. The median age was 47 yrs (range 15-79) and 15 (56%) patients (pts) were male. There were 18/27 primary MS while 9/27 cases derived from a previous myeloid neoplastic disease (the median time to the development of MS, in this setting, was 38 mths, range 4-61). Histologic and immunohistochemical data are available in all cases. The most common extramedullary anatomic sites of disease were: skin (with multiple papules and/or nodules), skeletal muscles, bone, spleen, lymph nodes and central nervous system. The bone marrow was involved (at onset or during the course of MS) in 18/27 (67%) of cases. Treatment: 24/27 pts (89%) are able to receive a program of intensive chemotherapy (combined with radiotherapy in 6/24 cases) including FLAI, HADC-IDA, HyperCVAD and MEC schemes, with a Complete Remission Rate of 46% (11/24). Thirteen pts (54%) underwent Allogeneic Stem Cell Transplantation (Allo-SCT). The median overall survival (OS) of the whole population was 16 mths. The median OS of the alloSCT recipients was significantly better than the OS of non transplanted cases (20 *vs* 12 mths, P <0,05). *Conclusions:* 1)The rarity of MS does not enable prospective clinical studies and therefore, a specific italian register can be very useful for the clinical and biological studies of this disease. 2)These preliminary results confirm the very poor prognosis of MS both in *de novo* and in secondary forms. 3)The OS of MS appeare to be improved by Allo-SCT.

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SIMILAR EFFICACY OF BIOSIMILAR FILGRASTIM COMPARED WITH LENOGRASTIM FOR AUTOLOGOUS PERIPHERAL BLOOD STEM CELLS MOBILIZATION IN ADULT PATIENTS WITH MYELOMA: A SINGLE INSTITUTION EXPERIENCE

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Biosimilar Granulocyte Colony-Stimulating Factor (G-CSF) Zarzio® has been approved for prophylaxis of febrile neutropenia and for autologous peripheral blood stem cell (PBSC) mobilization. However, there is still general skepticism about safety and efficacy of Zarzio® in these setting of patients. From March 2013 to March 2015, 20 consecutive adult patients with multiple myeloma (MM) underwent autologous PBSC mobilization after administration of high-dose Cyclophosphamide (HD-CY) and biosimilar Filgrastim (Zarzio®) in our Institution. Zarzio® was administered at dosage of 10 mcg/Kg/day in order to reach a target of CD34+ cell dose of 8 x 10^6/Kg recipient body weight. This cohort of 20 patients was compared with 23 consecutive MM patients who underwent autologous PBSC mobilization after administration of HD-CY and Lenograstim (Myelostim®) at the same dosage from March 2011 to February 2013. The two groups of patients were similar as baseline clinical features, including sex (P=0.893), age and body weight at leukapheresis (P=0.396 and 0.906, respectively), bone marrow involvement at leukapheresis (P=0.755), previous chemotherapy lines (P=1) and incidence of diabetes and median value of serum glucose at leukapheresis (P=0.646 and P=0.801, respectively). As for PBSC collection data, median days of G-CSF administration, median CD34+/mcL number at leukapheresis and median number of CD34+ x 10^6/Kg collected at first leukapheresis were similar between two groups of patients (P=0.954, P=0.479 and P=0.465, respectively). Moreover, in the group of patients who received Zarzio®, we observed a similar incidence of mobilization failures (5% compared to 0% of Myelostim® group; P=0.331), and a similar rate of patients unable to reach the target of CD34+ cell planned dose (5% *vs* 9%; P=0.845). Only one patient needed of Plerixafor administration in the Zarzio® group, compared with none of patients treated with Myelostim® (5% *vs* 0%; P=0.331). No difference in terms of drug-related adverse events was observed in the two cohorts of patients, with no reported serious adverse events. Despite the limitation due to the non-randomized design of the study, our data suggest that Zarzio® could be similarly effective when compared with Myelostim® for PBSC mobilization after HD-CY in adult patients with MM. Further randomized studies on a larger number of patients are warrant to better evaluate the role of Zarzio® in this setting.

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PERIPHERAL BLOOD STEM CELL (PBSC) COLLECTION MAY BE IMPAIRED BY AN INCREASED FERRITIN SERUM LEVEL IN LYMPHOMA AND MYELOMA PATIENTS UNDERGOING AUTOLOGOUS STEM CELL TRANSPLANTATION

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Iron overload represents an adverse prognostic factor for patients with hematological malignancies undergoing allogeneic stem cell transplantation (SCT). Recent observations have pointed out that pre-transplant ferritin serum level possesses a prognostic value even after autologous stem cell transplantation (ASCT) in multiple myeloma (MM) or lymphoma patients. Aim of the present study was to evaluate whether ferritin serum level, as detected prior to the chemotherapy regimen performed in order to promote PBSC mobilization, has an influence on PBSC collection procedure The records of all the patients who underwent autologous PBSC transplantation for MM or non-Hodgkin lymphoma (NHL) at our Institution were reviewed; a pre-PBSC collection serum ferritin was available in 55 transfusion -naive patients (33M, 22F, median age=59yrs), 31 MM and 24 NHL. PBSC mobilization regimens included Cyclophosphamide 4g/sqm+G-CSF in all MM patients, while NHL were treated with high-dose cytarabine+ G-CSF (10 patients), ifosfamide- gemcitabine- vinorelbine+G-CSF (9 patients), cytarabine - oxalyplatinum - rituximab (5 patients). A ferritin serum level above normal (>300ug/dl) was observed in 21 patients (14 MM=45% and 7 NHL=29%) and was associated with a lower number of collected CD34+ cells x 108/kg (9.8 ±4.7 *vs* 17.1±12.2, p=0.003) in a higher number of apheretic procedures (p=0.04). No correlation was demonstrated with pre transplant disease status and response to therapy, while response duration was shorter (22 *vs* 25.8 months) in patients showing a higher ferritin level, even though the figures did not reach statistical significance due to the low number of patients. Our results indicate that an increased serum ferritin level correlates with a reduced PBSC yield in patients undergoing PBSC mobilization, regardless of disease type and mobilization regimen; this observation deserves further investigation in a larger series of patients in order to evaluate the relative role of iron overload, chronic inflammation or other putative mechanisms and to confirm a possible influence on remission duration

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SINGLE INSTITUTION EXPERIENCE ON TREATMENT OF ACUTE LEUKEMIA AND PROCEDURE OF BONE MARROW TRANSPLANTATION IN JEHOVAH'S WITNESSES

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The therapy for acute leukemia and TMO in Jehovah's witnesses are generally considered not feasible. We present our experience accrued in 18 years on Jehovah's witnesses. Ten pts with acute leukemia; 6 had AML (5 M3 and 1 M2) and 4 pts ALL. The age ranged from 4 to 46 years; none received packed red cells. The induction therapy was unmodified for AML-M3 and ALL, instead we shortened the FLAI schema to 4 days in pt with M2. Eight pts entered CR; all AML including M2 and 2 pts with ALL. Epo was given to all pts. The nadir of Hb was 1.5gr/100mL in a pt with ALL who died in induction; the range was from 1.5 to 8.7 (5.6 mean); plt dropped under 10x10^3 in all AML pts, ranging from 15 to 70 in ALL pts. At a follow-up of 5 to 17 years 7 pts are alive and in CR; 5 with M3 and 2 with ALL. In the same period twenty-three transplantation procedures were done in Jehovah's witnesses; nine received allo-TMO and 13 auto-TMO. The age ranged from 8 to 71 year. Allo-TMO was done in patients with MDS (1), CML before TKI (3), ALL (2), NHL (1), MM (2) and MF (1); the therapy was myeloablative in 7 pts and reduced intensity in 2. The source of CD-34 cells marrow in 3 pts, PBSC in 5 pts and both marrow+PBSC in 2 pts; one was a MUD Auto-TMO was done in HL (1), MM (6), AML (1), NHL (2), Breast cancer (1); the source of CD-34 cells was PBSC in 12 pts and Marrow in 1. The conditioning regimen was that scheduled for disease with a reduction of Melphalan at 140mg/sqm instead of 200mg/sqm. The nadir for Hb in allo-TMO was 1.7 gr/100mL in a pt with MF who accepted to be transfused up to 6 gr/100mL and she then recovered; other pts had a minimum of 7.0 gr/100mL. The nadir for auto-TMO of Hb was 8.3 gr/100mL and PLT <10x10^3/μL. The recovery of Hb was in general faster and beginning in before the day 10 from infusion of CD-34 cells. We had 1 death during auto-TMO procedure for CMV infection; other deaths, 3 in auto-TMO and 5 in allo-TMO occurred for progression of disease. In conclusion the therapy for leukemia induction and the procedure of TMO in Jehovah witnesses are feasible without transfusion in selected patients.

P304

DEFERASIROX IMPROVES HEMATOPOIESIS AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION: REPORT OF 13 PATIENTS

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We report on 13 patients, transplanted for hematological diseases (12

acute leukemia, 1 aplastic anemia) heavily transfused before transplant that, considering the iron overload, were treated after HSCT with deferasirox. Before starting deferasirox, the patients were fully engrafted and in complete remission (acute leukemia), although transfusion dependent, and with incomplete hematological reconstitution after allogeneic HSCT. Patients were selected according to the following inclusion criteria: 1) transfused pre-transplant with more than 20 RBC units; 2) incomplete haematological recovery; 3) transfusion-dependence; 4) serum ferritin >1800 ng/mL; 5) normal creatinine value. All patients received an initial dose of deferasirox 10 mg/kg/day, later adjusted according to side effects. All patients experienced an increase in haemoglobin levels, with a reduction in the frequency of RBC transfusions, followed by transfusion independence (median time: 23 days from the first dose of deferasirox). In addition, it was promptly (median time: 26 days) associated with haematological improvement, with sustained values and no further platelet support or growth factors administration. Moreover, ferritin values were progressively reduced with deferasirox treatment. The workup for other aetiologies resulted negative; no concomitant infection was documented (CMV: negative; HHV-6: negative; EBV: negative). No relevant modifications with immunosuppressive or myelosuppressive drugs were made during deferasirox treatment. Deferasirox was well tolerated. The role of iron overload post transplant is not completely understood. No reports, in our knowledge, have up to now focused on the possible effect of iron chelators after transplant on the restoration of normal hemopoiesis and, in particular, transfusion independence. Basing on our results, we think that deferasirox determined stimulatory, and/or derepressive effects on hematopoiesis after allo-HSCT. In conclusion, this clinical experience raises the possibility of a potential additive benefit on hematopoiesis after transplant following iron chelation therapy with oral deferasirox. Further long term studies, in larger cohorts of patients are needed to confirm these data and design an efficient strategy to reduce iron loading after transplant. *Acknowledgements:* supported in part by AIL Pesaro Onlus.

P305

AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR MULTIPLE MYELOMA PATIENTS AGED 65 YEARS OR OLDER: A RETROSPECTIVE SINGLE INSTITUTIONAL ANALYSIS

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Background: Multiple myeloma (MM) is a disease of the elderly, with two-thirds of the affected population aged over 65 years. The introduction of novel agents in front-line treatment has improved the outcome of young as well as elderly patients. It is also contributing to an ever increasing utilization of autologous stem cell transplant (ASCT), by making a greater number of patient ≥65 years eligible for this procedure. *Methods:* From January 1997 to January 2015, 56 Multiple myeloma patients underwent ASCT at our Institute. From 1997 to 2002, 22 patients (12 M, 10 F; median age 68 years, range 65-73) had received a conditioning regimen with oral busulphan plus i.v. melphalan; this group was considered only for the survival analysis as historical control. From 2003, 34 (20 M, 14F; median age 67, range 65-72) had received a conditioning regimen with high (200 mg/m^2, n=12) or intermediate (140 or 100 mg/m^2, n=22) dose i.v. melphalan; induction therapy included novel agents (thalidomide and/or bortezomib) for 23 patients (67%); disease status at ASCT was CR/VGPR (n=16), PR (n=16), NR (n=2); the median number of reinfused CD34+ HSC was 5.8 x 10^6/Kg (range 2.3-11.5); 12 patients received a consolidation therapy with bortezomib and/or thalidomide and 5 a maintenance with interferon-α. Stem cell mobilization was carried out with high-dose cyclophosphamide (3-5 g/m^2) and G-CSG or pegfilgrastim administration. *Results:* For the historical control group, the median OS since ASCT was 45.7 months. For the 34 patients of the study group, polymorphonuclear leukocyte engraftment was reached at a median of 12.5 days (range 11-33); treatment-related mortality (<100 days from SCT) was 2.9% (n=1,Cytomegalovirus reactivation while in progressive disease); EFS was 18.2 months; for patients aged 65-70 years (n=27), median OS since ASCT was 64 months and for patients ≥70 years (n=7) 36.5 months; for patients treated with bortezomib/thalidomide in the induction phase, median OS was 64 months while it was 36.5 months for patients who had not received novel agents. *Conclusions:* Our data confirm that ASCT is a safe procedure in selected elderly patients with MM. It allows prolonged EFS and OS, mainly in populations aged 65 to 70 years. The use of new drugs before ASCT presents a survival advantage. To better evaluate the efficacy of these agents before ASCT as well as in the maintenance phase, larger studies are desirable.

P306

CD4+ CELL COUNT ON DAY+30 PREDICT OVERALL SURVIVAL AND TRANSPLANT RELATED MORTALITY IN ACUTE LEUKAEMIA PATIENTS AFTER ALLOPBSCT

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Allogeneic peripheral stem cell transplantation (alloPBSCT) from a related or unrelated donor is a well established strategy for patients with acute leukaemia. However, this procedure is associated with increased morbidity and mortality because of graft *versus* host disease (GvHD), immune reconstitution impairment and a high risk of infections. In our study we evaluated the lymphocyte subset recovery after alloPBSCT and its impact on transplant related mortality (TRM) and overall survival (OS) in acute leukaemia patients. We evaluated the immune reconstitution of CD3+/CD4+, CD3+/CD8+ and NK cells performed at 30, 100, 180 and 360 days after alloPBSCT in 122 patients with acute leukaemia. Patients were transplanted with unmanipulated PBSC from an HLA matched related donor (MRD) (n=85) or an HLA (8/8) matched unrelated donor(MUD) (n=37). Median age was 38 years (range 18-61); diagnoses were acute myeloid leukaemia (n=96) and acute lymphoblastic leukaemia(n=26); 80% of patients underwent myeloablative conditioning (busulphan, cyclophosphamide in MRD and busulphan, cyclophosphamide and ATG in MUD) and 20% underwent reduced intensityconditioning (busulphan,fludarabine, ATG). The median counts of CD3+/CD4+ were 98, 160, 200, 262 l at 30, 100,180 and 360 days, respectively. The median counts of CD3+/CD8+ were 180, 350, 500 and 670 l at 30, 100, 180 and 360 days, respectively. The median counts of NK cells were 110, 260, 270 and 260 Lat 30, 100, 180 and 360 days, respectively. The median CD3+/CD4+ cell count on day +30 for the entire patient population was 98 l (range 20-190) and TRM at 2 years was significantly higher in patients not achieving this CD4 cell count (38% *vs* 15%, p<0.001); patients with a low CD3+/CD4+ count (<98 l) on day +30 had a higher risk of dying of infections (30% *vs* 11%, p>0.003). Median OS in patients not achieving CD4+ of 98 l at 30 days was 40 months while median OS in patients with more than 98 CD4+ at 30 days was not reached. In multivariate analysis, transplant from a related donor (p=0.05) and absence of aGvHD(grade II-IV) (p=0.002) were significantly associated with a better CD4 cell recovery. CD3+/CD8+ and NK cells recovery did not correlate with a different TRM risk or OS. Our results support the relationship between immune reconstitution of CD4+, OS and TRM. The CD4+ cell count on day +30 is able to predict OS and TRM after myeloablative alloPBSCT in acute leukaemia patients.

P307

ONCE DAILY INTRAVENOUS BUSULPHAN PLUS FLUDARABINE IS A SAFE LESS TOXIC CONDITIONING FOR AML AND MDS ALLOGENEIC TRANSPLANTATION

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Allogeneic haemopoietic stem cell transplantation is the treatment of choice for HR-intermediate risk AML and HR MDS. Standard conditionings like TBI-CY and BU-CY have been widely used, but extra-hematologic toxicity remains a concern. Recently the Busulphan–Fludarabine regimen has replaced Bu-Cy to reduce toxicity. We started to use this combination for allogeneic transplantation in patients with AML and MDS in October 2008. Primary objective was evaluation of OS, DFS, TRM and relapse. Since October 2008 to April 2015, 30 patients (23 males, 7 females) entered this study. Median age was 56 years (range 35-63). Underlying diseases were AML (25 patients: CR1=20,PR2=2, 1st

relapse=1, resistant disease=2) and MDS (5 Int 2 patients). Busulphan (Busilvex 9,6-12,8 mg/kg) was given once daily in a 3-4 hour infusion while Fludarabine (160 mg/m^2) for 4 days. CSA+short MTX were used as GVHD prophylaxis and ATG were used in case of MUD transplants. Eighteen patients received HSCs from HLA identical siblings, 1 from a MM family donor, 11 from unrelated donors. Source of stem cells was bone marrow in 15 patients, peripheral blood stem cells in 14, both in 1. All patients regularly engrafted. One patient presented rejection in aplasia 2 months after a MM-unrelated donor transplant. She received rescue with an haploidentical transplant and she is alive in remission. Six patients presented Mixed Chimerism after transplant in complete remission, but no patients received DLI. Nine patients experienced GI toxicity (1 grade III mucositis, 2 grade II mucositis, 6 grade I mucositis); 3 patients had grade II cystitis. Acute grade III GI GVHD occurred in 1 patient; 15 patients presented grade I skin GVHD, associated with grade 2 GI in one. CrGVHD occurred in 7/21 evaluable patients. Incidence of infections was low; 1 patient presented early infection, 4 late infections; CMV reactivation occurred in 22/30 patients (73%). Twenty patients are alive (67%), 19 in complete remission, with a median follow-up of 16 months (range 3-46 months). Ten patients died (33%): 9 for recurrent disease, 1 for TRM (Ac.GVHD). Between the 20 AML patients transplanted in 1st Complete remission, 15 are alive in CR (75%), 5 died for recurrence of HR leukemia. This single Centre report underlines that the association IV Busulphan–Fludarabine is a safe and effective conditioning for AML/MDS patients. Intravenous Busulphan administered once daily is convenient, effective and well tolerated.

P308

LATE LEUKEMIA RELAPSE AFTER ALLOGENEIC HEMATOPIETIC STEM CELL TRANSPLANTATION

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The present study was aimed at establishing LLR incidence and origin in 202 consecutive patients(pts) submitted to allo-HSCT at a single Institution. CC and FISH studies were carried out as already reported, whereas Quantitative and nested RT PCR assays for CML monitoring were performed according to the Italian LabNet guidelines. Chimerism and Minimal Residual Disease (MRD) evaluation was performed at day +100, +180, +365 and every six months post-transplant. A LLR occurred in 5 pts and was due to recipient's leukemic cells re-growth. At the time of allo-HSCT two pts were CML in first chronic phase, two AML in first complete remission (CR) and one a resistant AML post-MDS. All pts received a standard myeloablative conditioning, a sex-mismatched HSCT from a sibling donor and standard GvHD prophylaxis. The two CML pts relapsed as Ph' positive ALL 7 and 16 years post-transplant. One month before haematological relapse one pt with no chronic GvHD (cGvHD) was still a complete chimera and MRD negative, the other one 16 years post-transplant complained of otitis, fever and showed the almost exclusive presence of CD34+, TdT+, CD10+ Ph'+ lymphoblasts in the bone marrow (BM) and a BCR-ABL/ABL ratio=214,2 (IS). The t(3;11) (q26;q23) AML in first CR developed an extensive cGvHD on day +183 and remained a complete chimera until five years post-transplant when he complained of fever and a BM marrow examination revealed the almost exclusive presence of blasts which karyotype was: 50,XY,t(3;11)(q26;q23),+4,+8,t(12;?)(p21;?),-14,+mar1,+mar2,+mar3[13]. A trisomy 8 AML with no signs of GvHD remained a complete chimera until fifteen months post-transplant when she was still on immune-suppressive treatment (IST) and developed an initial marrow relapse, but IST withdrawal determined a new CR along with an extensive cGvHD. This state was maintained until ten years post-transplant when she presented 20% BM leukemic cells with a 47, XX,+10/48XX, +8,+10 karyotype. The last FLT3-ITD AML pt developed a cGVHD one year post-transplant and remained a complete chimera until four years post-transplant when she presented 45% of BM blasts and an improvement of cGVHD. *Conclusions:* Our data confirm that LLR is a very rare event potentially caused by a leukemic stem cell re-entry into the cell cycle after they have survived to the myelo-ablative conditioning due to their quiescent state.

P309

OUTCOME OF NEWLY DIAGNOSED MULTIPLE MYELOMA: OS, PFS1, PFS2 IN PATIENTS ELEGIBLE FOR STEM CELL TRANSPLANT. A 15-YEARS SINGLE CENTER RETROSPECTIVE STUDY

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Better understanding of biological mechanisms, availability of sophisticated diagnostic tools and increasing number of therapeutic options significantly improved global outcome in MM patients, even if MM is still considered an incurable disease. The aim of our study was to evaluate global outcome in newly diagnosed MM patients eligible for autologous stem cell transplant (ASCT) treated with different induction therapy in the last 15 years. We retrospectively evaluated 177 MM patients transplanted in our Institution from November 1999 to August 2014. Median age at start therapy was 58 years (range 36-71) and median follow up time 52 months (range 7-185). Different induction therapies were administered reflecting different time period and consequently the availability of new drugs: conventional chemotherapy-based (VAD 27 pts, 15%, TT2-like 37 pts, 22%, EDAP 1 pt), thalidomide-based (TD 15 pts, 8%), bortezomib-based (VTD 74 pts, 43%; VCD 8 pts, 5%; PAD 3 pts, 1%; VD 3 pts, 1%) and lenalidomide-based (RD: 9 pts, 5%). After induction 71 pts received a single ASCT, 106 pts double ASCT; 11 pts tandem autologous and allogeneic SCT. Treatment related mortality was 1% (3 pts: 2 of them after Allogenic ASCT, 1 for aGvHD, 1 for VOD). After 1st line therapy 7 pts died: 3 for progressive disease, 4 for other causes (2 myocardial infarction, 1 intestinal ischemia and 1 breast cancer). 90 pts (51%) experienced MM relapse (median time from ASCT 24 months) and were treated according to different chemotherapy schedules. At last follow-up 115 pts were alive (65%), 61 of them in CR, 31 in VGPR, 8 in PR, 4 in SD, 11 in PD. Median global OS was 109 months, median global PFS1 51 months and median global PFS2 (time from start therapy to second disease progression or death from any cause) 80 months. According to different induction therapy (CHT *vs* bortezomib *vs* thalidomide *vs* lenalidomide-based) median OS was 117 months *vs* not reached (more than 96) *vs* 68 *vs* not reached (more than 60); median PFS1 was 47 months *vs* 60 *vs* 35 *vs* not reached (more than 60); median PFS2 was 76 months *vs* 92 *vs* 57 *vs* not reached (more than 60), respectively. As reported in published data the introduction of novel agents in treatment of MM patients in our center improved outcome considerably. Bortezomib-based therapy was the most represented induction regimen and achieved the best results in terms of Survival (OS), control of the disease (PFS) and response to 2nd line therapy after relapse (PFS2).

P310

IMMUNE RECONSTITUTION AFTER ALLOGENEIC STEM CELL TRANSPLANTATION: A PILOT FLOW-CYTOMETRY STUDY FOR T-CELL SUB-POPULATIONS

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Immune reconstitution after allogeneic hematopoietic stem cell transplantation (allo-SCT) is a complex process. From January 2014 we enrolled 6 patients with haematological disorders who were to undergo myeloablative allo-SCT (medium age: 42 years). All patients received apheretically collected stem cells, two from sibling HLA-identical donor, two from MUD full matched, two from haploidentical familiar donor. Peripheral blood samples were collected and analyzed one day before the beginning of the conditioning regimen and at days +30, +100, +180, +360 after stem cell infusion. Circulating T-cell populations are identified by 4-colour flow cytometry on the base of the co-expression of CDs according to the most recent knowledge. We studied absolute population counts and their percentage, and described the medium trend of reconstitution of different sub-populations. At time 0, CD45RA+ T lymphocytes were predominant; lymphocytes and T-reg were well represented in all patients. After allo-SCT, at day +30 memory T-cells seem to be the first expanding population. At the same time it is possible to observe a

first increase of total circulating T cells (Fiure 1 A) after an extremely low count period. At day +100, Treg cells are measurable in most patients for the first time. Their mean number increases at day +180, and again at day +360. From day +180, mean T naïve cells percentage tends to rise. Between day +180 and day +360 almost every patient shows increased naïve T cells levels, while memory T-cells reach their minimum (at day +180). The expression of CD57, considered as a marker of senescence, is high in circulating T cells after 30 days and even higher after 100 days, this percentage keeps rising at days +180. Then, it's possible to observe a plateau until +360. Some of the cases we studied seem to confirm the hypothesis of Treg cells inhibiting NK cells. The cases in which Tregs rise is the steepest seem to be the ones where NK cells levels drop more abruptly (Figure 1 B). The heterogeneous clinical history and the low number of patients do not allow to correlate a specific subtype of lymphocytes to a particular clinical feature (GvHD, outcome, CMV reactivation). In conclusion, this pilot study confirms some of the latest data on the timing of recovery after allo-SCT. A functional study of lymphocyte population, and an indirect measurement of thymic activity, linked to clinical features, could clarify the *in vivo* role of different lymphocyte populations.

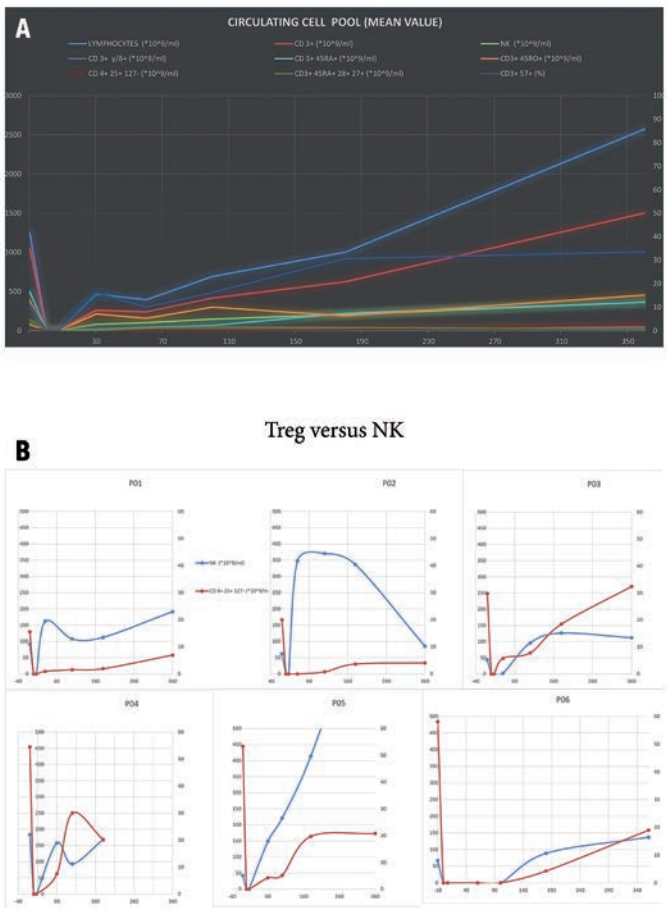

Figure 1.

P311

REVERSAL OF POOR GRAFT FUNCTION WITH IRON-CHELATING THERAPY AFTER ALLOGENEIC TRANSPLANTATION FOR SEVERE APLASTIC ANEMIA

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Poor Graft Function (PGF) after allogeneic transplantation is defined as severe cytopenia of ≥2 cell lines for ≥2 consecutive weeks beyond day +14 post-transplant, associated with transfusion requirement, hypoplastic-aplastic bone marrow (BM), complete donor chimerism and in the absence of severe GVHD and relapse. Degree of HLA matching, ABO incompatibility, cell dose, stem cell source, graft-*versus*-host disease (GVHD), viral infections, conditioning regimen, use of myelotoxic agent, such as ganciclovir, have all been associated with PGF. We report a case of a 27-year old woman who presented at our clinic because of severe aplastic anemia with high transfusion requirements of platelets (Plt) and red blood cells (RBC). As she had no HLA-matched siblings, she underwent first-line treatment with cyclosporine and horse anti-lymphocyte globulin, but had no response. She was started on deferasirox and referred for allogeneic transplantation: search on IBMDR found a 10/12 male donor with major ABO incompatibility. Conditioning included TBI-Fludarabine-Cyclophosphamide. Because of high anti-A titre, graft erythrodepletion was performed; however, due to insufficient recovery of total nucleated cells (TNC), the whole graft content was reinfused (2.8 x 108 TNC/kg) followed by plasma-exchange. Hematologic recovery was delayed (neutrophils >500 at day +16 and platelets >20.000/mm3 at day +19) but at day +14 she achieved full donor chimerism. However, at follow-up visits hematologic recovery was still uncomplete in all three lines, and transfusion requirements remained high. BM biopsy (BMB) showed aplastic BM (Figure 1 A) and increased iron stores (Figure 1 B). GVHD was absent. A diagnosis of PGF was made and procedures were started to obtain a CD34+ boost from the unrelated donor. Because of increasing ferritin, deferasirox was resumed. After two months, we noticed decrease in ferritin levels and reduction of transfusion requirements. A BMB showed improved marrow cellularity. Finally, 1 year after transplant, the patient had normal CBC count and had not been transfused in the previous 3 months; a BMB showed 70% cellularity with normal hematopoiesis (Figure 1 C) and reduced iron stores (Figure 1 D). Accumulating evidence in myelodysplastic syndromes and aplastic anemia indicates that benefits of iron-chelating therapy are not restricted to prevention of iron overload but may support recovery of hematopoiesis. This is the first report describing complete reversal of PGF with iron-chelating therapy.

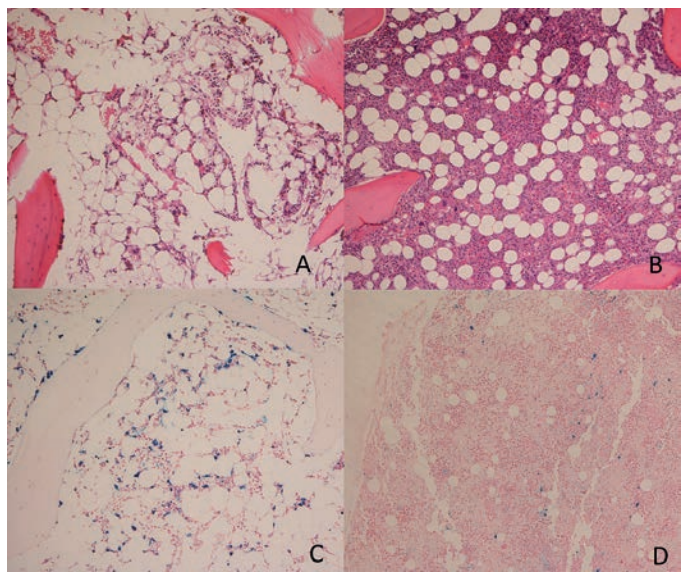

Figure 1.

P312

AUTOLOGOUS STEM CELLS TRANSPLANTATION AFTER USE OF NOVEL AGENTS AS SALVAGE TREATMENT FOR PATIENTS WITH MULTIPLE MYELOMA RELAPSED AFTER FIRST AUTOGRAFT

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Background: Therapeutic options for patients (pts) with multiple myeloma (MM) relapsed after a prior autologous stem cell transplant (ASCT) rely on a wide range of novel agents, often combined with traditional chemotherapy or a second ASCT, but without a proven and accepted standard of care. We carried out this retrospective analysis in

order to investigate the impact of novel agents followed by ASCT as salvage therapy in pts relapsed after a first autograft procedure. *Methods:* We evaluated 25 pts with Multiple Myeloma, referred and treated in our center between May 2005 and February 2015 that relapsed after a single or tandem transplant procedure. All patients received re-treatment with novel agents (proteasome inhibitor or/and immunomodulating agents). Cy and G-CSF were used for stem cells mobilization, collecting at least 5x106/kg CD34+. Conditioning regimen consisted of melphalan (200 mg/m^2 or 140 mg/m^2). Kaplan-Meier method was used to estimate OS probabilities, with differences compared by the log-rank test. *Results:* All pts received induction treatment before first high-dose chemotherapy, 20 pts with novel agents (11 thalidomide based, 9 bortezomib based) and 5 with standard chemoterapy VAD (vincristine, adriamycin and dexamethasone). 22 pts underwent a single ASCT, while 3 pts performed a tandem ASCT procedure. Three months after ASCT we observed a CR in 10 pts (40%), VGPR in 9 pts (36%) and PR in 6 pts (24%). Median time between previous high-dose treatment and first relapse was 21 mts (range 2-65). 22 pts received salvage ASCT in first relapse after a new drugs re-treatment with bortezomib (18 pts), lenalidomide (9 pts) or pomalidomide (1pt). 5 pts were in >1 relapse. Melphalan dose for salvage transplant procedure was 200 mg/m^2 in 17 pts and 140 mg/m^2 in 8 (according to clinical conditions). Median age at salvage ASCT was 60 years (range, 41 to 69), and 56% (n=14) were men. No pts died within 100 days of ASCT. Response at 3 months after salvage ASCT was CR in one pt (4%), a VGPR in 12 pts (48%) and a PR in 12 pts (48%). The median overall survival (OS) was 24 months, while median progression-free survival (PFS) was 15 months. Due to the little number of patients no significant differences were observed between novel agents re-treatment or overall response groups. *Conclusions:* Salvage ASCT, following re-treatment with novel agents, could be a safe and effective strategy for MM pts with late relapse and chemosensitive disease.

P313

PONATINIB IS A BRIDGE TO TRANSPLANT IN ALL-PH+ T315I: SUCCESSFULLY STRATEGY WITH HAPLO-CORD COMBINED STEM CELL SOURCE. CASE REPORT

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A high complete remission (CR) rate has been reported in newly diagnosed Philadelphia chromosome–positive acute lymphoblastic leukemia (Ph+ ALL) following (tyrosine kinase inhibitor) TKI combined chemotherapy. However, resistance and relapse occurred in the majority of patients. ABL-kinase domain mutations account for resistance in over 80% of cases of Ph+ ALL. In particular, T315I is quite commonly detected at the time of relapse and currently remains the most troublesome mutation. We report one case of relapse accompanied by T315I mutation during rapid disease progression. A 57-year-old female was diagnosed with Ph+ ALL in October 2014. Haematological response was achieved by induction therapy with Vincristine and Prednisone, (2 cycles) then patient received Dasatinib therapy (140 mg/daily). After 29 days of Dasatinib therapy, patient hematologically relapsed and the t315I mutation was observed. Meanwhile, Ponatinib treatment started at 45 mg/day, and Molecular Response was achieved in 6 weeks with 1% of p190 transcript amount. As the patient did not have a human leukocyte antigen (HLA) -identical sibling or a fully-matched unrelated bone marrow donor, we performed an Haplo-cord transplant with selected haploidentical CD34 cells from familiar donor and umbilical cord blood MM 4/6 with a reduced intensity conditioning regimen consisting of 30 mg/mq of Fludarabine for 5 days and Melphalan 70 mg/mq for two days, GvHD prophylaxis with ATG, tacrolimus and mycophenolate. Engrafment occurred after 10 days from the transplant, patient was discharged on day 22. Chimerism was initially almost completely haplo-donor, but in three months chimerism appeared full cord blood donor. Bcr/abl is actually undetectable (follow up 5 months from transplant). Ponatinib could be represent the bridge to transplant for patient affected by ALL-Ph+, and haplo-cord transplant can be an opportunity for the most of patient, expecially to limitate NRM.

45° Congress of the Italian Society of Hematology
Florence, Italy, October 4-7, 2015

MAIN PROGRAM

HEMORRHAGIC COMPLICATIONS OF NEW ORAL ANTICOAGULANTS

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The direct oral anticoagulants have shown promise for the prevention and treatment of thrombosis in a variety of clinical settings. Bleeding is the major complication of their use. The risk of bleeding with these agents is increased by inappropriate use; for example, the risk of dabigatran-associated bleeding increases when the drug is used in the very elderly, those with renal insufficiency or those taking medications known to increase the pharmacologic effect of any of these agents. Also, switching from vitamin K antagonists to direct anticoagulants without allowing the INR to appropriately decrease is associated with an increased bleeding risk. There is little or no good quality evidence to guide reversal of these agents. Specific antidotes have been developed (idarucizumab, andexanet α, ciraparantag), but until they will become available for clinical use, hemorrhage or overdose of any of these agents must be treated with prohemostatic interventions and generic mechanical and resuscitative interventions. Patients should be assessed for severity of bleeding. Major or life-threatening bleeding should be managed with drug withdrawal, implementation of resuscitative measures, and transfusion therapy to address identified deficiencies. Given a lack of evidence for their use in actively bleeding patients, transfusion therapy with PCC, rFVIIa or aPCC can be considered but only in patients with life-threatening bleeding. Mechanical measures must be employed promptly in patients with life-threatening bleeding, and in many patients with major bleeding. Hemodialysis effectively removes dabigatran (but not rivaroxaban or apixaban), limited availability make it unlikely that dialysis will be widely used for dabigatran-associated bleeding.

THE MOLECULAR LANDSCAPE OF THE MYELODYSPLASTIC/MYELOPROLIFERATIVE NEOPLASMS: IMPACT ON CLINICAL PRACTICE

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Introduction: Created in 2001 and retained in 2008, the World Health Organization (WHO) now recognizes a distinct category of myelodysplastic/myeloproliferative neoplasms (MDS/MPNs) for those patients diagnosed with clinical, morphologic and laboratory features that overlap with those of both MDS and MPN[1]. MDS/MPNs are characterized by combined 'cytopenias' and 'cytoses'. The bone marrow is usually hypercellular because of both ineffective (MDS-like) and effective (MPN-like) hematopoiesis. In most cases, dysplasia is seen in ≥1 hematopoietic lineages in the peripheral blood, bone marrow, or both. By definition, the percentage of blasts must be <20%. The clinical presentation may be more 'MDS-like' (*e.g.*, cytopenias with dysplasia), 'MPN-like' (*e.g.*, cytoses with organomegaly), or anywhere in between.

Four hematopathologic diagnoses exist within this category: chronic myelomonocytic leukemia (CMML), atypical chronic myeloid leukemia BCR-ABL1 negative (aCML), juvenile myelomonocytic leukemia (JMML) and myelodysplastic/myeloproliferative neoplasm unclassifiable (MDS/MPN-U). Refractory anemia with ring sideroblasts associated with marked thrombocytosis (RARS-T) is included in this category as a provisional entity. The clinical and pathologic characteristics of MDS/MPNs are challenging to recognize, and the diagnostic criteria are imperfect for the subjective interpretation of morphology. Moreover, the rarity of these disorders in combination with a symptomatology not exclusive to either MDS or MPN has resulted in the exclusion of these overlap syndromes from clinical trials. Therefore, the current therapeutic approach to the MDS/MPNs has been largely adopted from neighbouring disorders. Nowadays the spectrum of genetic mutations in MDS/MPNs is relatively well-characterized and allow to provide the evidence of clonality in most patients. Moreover, emerging data suggests that these molecular lesions have a prognostic value and are potential therapeutic targets, which might improve outcome by reducing disease-related symptoms and complications and by prolonging survival. Therefore knowledge of the molecular MDS/MPNs landscape could be helpful in the diagnosis and improve the therapeutic approach. *CMML:* It was estimated that the CMML incidence is 0.41 per 100,000 per year and account for 90% of the MDS/MPNs overlap diagnoses. The diagnosis is made based on the 2008 WHO classification criteria, with the distinction between CMML-1 and CMML-2 based on blast percentage. The bone marrow is typically hypercellular with granulocytic hyperplasia. Monocytic proliferation and dysplasia of at least one hematopoietic lineage can be observed. Flow cytometry, although not part of the diagnostic criteria, can be helpful in distinguishing CMML, CMML transforming to acute myeloid leukemia (AML), and acute myelomonocytic leukemia. CMML lacks a pathognomonic cytogenetic abnormality; karyotypic aberrations can be detected in 20-40% of patients, indicating the clonal nature of the disease and providing prognostic information. Frequent alterations include trisomy 8, monosomy 7, del(7q) and recurring abnormalities of chromosome 12p. In addition to cytogenetic abnormalities, the advent of next generation sequencing technology has allow the identification of molecular aberrations in approximately 90% of CMML patients[2-3]. These molecular aberrations can be classified into four main categories: a) mutations involving epigenetic regulator genes such as EZH2, ASXL1, TET2, DNMT3A, IDH1 and IDH2 2; b) mutations involving the spliceosome component pathway such as SF3B1, SRSF2, U2AF1 (U2AF35), ZRSR2, SF3A1, PRPF40B, U2AF2 (U2AF65) and SF1; c) mutations involving DNA-damage response genes such as TP53; c) mutations involving genes regulating cellular/receptor tyrosine kinases and transcription factors such as JAK2, KRAS, NRAS, CBL, SETBP1, FLT3 and RUNX1. Moreover, mutations involving epigenetic regulator genes can be further classified into two distinct subgroups, namely those affecting DNA methylation (TET2, DNMT3A, IDH1 and IDH2) and those regulating chromatin modification (ASXL1 and EZH2). Future prognostic scoring systems for CMML will likely use molecular analysis in combination with clinical and cytogenetic data[4-5]. For example, TET2 mutations may not offer prognostic information except for certain patients with CMML-1; moreover, there is a trend towards higher response rate in patients with TET2 mutations (when not associated with an ASXL1 mutation). The association between the TET2 and DNMT3A mutations is predictive for responsiveness to hypomethylating therapy. SETBP1 mutations are associated with poor prognosis. CBL mutations in both CMML-1 and CMML-2 are associated with poorer outcomes, and patients bearing this mutation tend to have splenomegaly. None of the mutations in SRSF2, SF3B1, and U2AF35 identified in CMML are prognostic for outcome, although the mutation of SRSF2 is associated with increased age, less pronounced anemia and a normal karyotype. Mutations in RUNX1 transcription factor are detected in 15–40% of patients with CMML; patients with CMML and RUNX1 mutations may have a higher risk of transformation to AML. Some recent prognostic models

have begun to take into account mutational information, such as the mutational status of ASXL1, which may confer an adverse prognosis when mutated. Point mutations in KRAS and NRAS genes were identified as secondary events associated with poor prognosis. *aCML:* aCML is a rare hematological malignancy with an incidence estimated at 1-2 cases per 100 patients with BCR-ABL1 positive CML. Patients with aCML often present not only with neutrophilia but also with erythrocytosis and thrombocytosis; splenomegaly can be observed in 75% of cases. The presence of peripheral blood dysgranulopoiesis is common. Blasts may be present, but are usually less than 5%. The bone marrow is hypercellular due to an increase of granulocytes and their precursors. Dysplasia in the granulocytic and, occasionally, in the erythroid and megakaryocytic lines, is commonly seen. Clonality can be established in 80% of cases by the presence of karyotypic abnormalities, most commonly +8 and 20q deletion, less commonly +13 and i17q. In patients affected by aCML survival is poor, ranging 2–3 years, with evolution to acute myeloid leukemia (AML) occuring in 20–45% of cases. Recurrent mutations in SETBP1 were identified in 24% of aCML cases. The gene encoding for colony-stimulating factor 3 receptor (CSF3R) was reported as mutated in 59% of patients with aCML and chronic neutrophilic leukemia (CNL)[6]; on the other hand, other studies showed that CSF3R mutations were essentially restricted to CNL and absent in aCML[7]. Moreover, somatic ETNK1 gene mutations were reported in 9% of aCML. RAS mutations were relatively frequent in aCML, whereas JAK2 p.V617F mutations were infrequent. CALR mutations in exon 9 were reported in 3% of aCML patients. *JMML:* JMML is an uncommon overlap MDS/MPN that occurs exclusively in young children, with a median age of diagnosis of <2 years. The spectrum of mutations reported in JMML affects genes all encoding proteins that signal through the RAS-RAF-MAPK pathway. Although largely mutually exclusive, mutations of NF1, PTPN11, KRAS, NRAS, and CBL are detected in 90% of patients with JMML[8]. Specifically, 20-25% of patients with JMML carry somatic mutations in NRAS or KRAS, about 35% carry aberrations in PTNP11 and in 11% of patients with JMML were identified NF1 gene mutations. Both somatic and germline mutations of these genes have been identified, with the latter being most associated with congenital genetic syndromes such as Noonan syndrome (PTPN11) and Neurofibromatosis type 1 (NF1). In rare children carrying germline N-RAS, K-RAS, PTPN11, and CBL mutations together with associated congenital syndrome the myeloproliferative disorder is often self-resolving and do not require the hematopoietic stem cell transplantation. In other cases characterized by RAS-mutated JMML long-term event-free survival has only been achieved following hematopoietic cell transplantation. However, the optimal therapeutic regimen, both before and during the transplantation process, has yet to be identified. *MDS/MPN-U:* Of the four 'overlap' MDS/MPNs, MDS/MPN-U is the least characterized; in fact, the MDS/MPN-U category includes patients who at initial presentation do not fulfill diagnostic criteria for a specific subtype of MDS/MPNs. Most patients present with macrocytic anemia. The bone marrow is usually hypercellular with multilineage dysplasia and show MPN-related features, including bone marrow fibrosis. MDS/MPN-U is characterized by a poor prognosis with an overall survival of 1 year from presentation. Patients affected by MDS/MPN-U show an increased incidence of isolated trisomy 8. JAK2 V617F mutation occurs in approximately 25% of MDS/MPN-U patients. SETBP1 mutations are detected in 10% of MDS/MPN-U, and correlate with higher WBC, lower hemoglobin levels, and lower platelet counts[9]. *RARS-T:* RARS-T is now considered a provisional entity with overlapping features of MDS and MPN. In fact, patients with RARS-T have features of RARS and thrombocytosis, with proliferation of large atipical megakaryocytes, similar to those observed in BCR-ABL1 negative myeloproliferative neoplasms. Molecular abnormalities in RARS-T can be divided into the following groups: a) spliceosome component pathway - SF3B1(90%), SRSF2 (6.7%), U2AF1 (5.3%), ZRSR2 (2.7%); b) signaling pathways- JAK2 V617F (57%), MPL (2.7%), CBL (4%); c) epigenetic regulators- ASXL1 (15%),TET2 (25%), EZH2 (7%), DNMT3A (15%), IDH2 (4%); d) transcription regulators- ETV6 (2.7%), RUNX1 (1.3%)[10]. Approximately 50% of patients harbored boh the JAK2 V617F and SF3B1 mutations. Conversely, most of the SF3B1 wild type patients have JAK2 V617F and ASXL1 mutations, whereas MPL and CALR gene mutations are infrequent in RARS-T. *Conclusions:* MDS/MPNs represent a class of diseases with considerable molecular complexity and heterogeneity. The presence of several gene mutations suggests the need for founding diagnostic and therapeutic approaches on the molecular profile of individual patients; hopefully, further functional genomic studies will provide insights and better understanding on the molecular pathogenesis of this disorder.

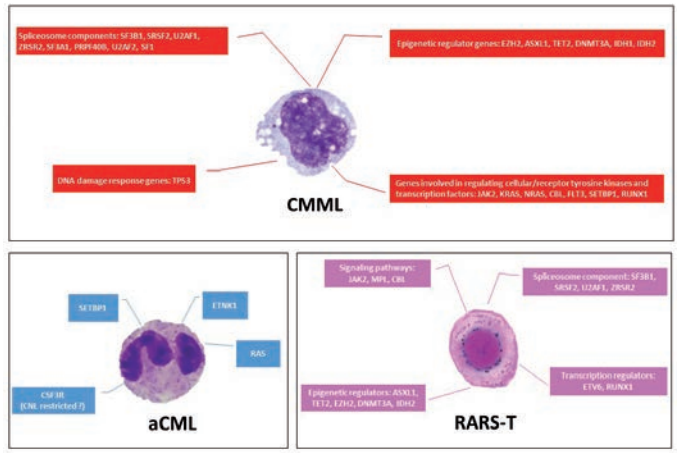

Figure 1. Frequency of gene mutations in CMML, aCML and RARS-T.

References

1. Orazi A, Bennett JM, Germin U, et al., Chronic myelomonocytic leukaemia. In: Swerdlow SH, Campo E, Harris NLJ, et al.,, editors. WHOclassification of tumours of haematopoietic and lymphoid tissues, 4th ed. Lyon: IARC; 2008. pp. 76–79.
2. Patnaik MM, Parikh SA, Hanson CA, Tefferi A. Chronic myelomonocytic leukaemia: a concise clinical and pathophysiological review. Br J Haematol. 2014;165:273-86
3. Benton CB, Nazha A, Pemmaraju N, Garcia-Manero G. Chronic myelomonocytic leukemia: Forefront of the field in 2015. Crit Rev Oncol Hematol. 2015 Mar 14. [Epub ahead of print]
4. Traina F, Visconte V, Elson P, Tabarroki A, Jankowska AM, Hasrouni E, Sugimoto Y, Szpurka H, Makishima H, O'Keefe CL, Sekeres MA, Advani AS, Kalaycio M, Copelan EA, Sauntharajah Y, Olalla Saad ST, Maciejewski JP, Tiu RV. Impact of molecular mutations on treatment response to DNMT inhibitors in myelodysplasia and related neoplasms. Leukemia. 2014; 28:78-87.
5. Itzykson R, Kosmider O, Renneville A, Gelsi-Boyer V, Meggendorfer M, Morabito M, Berthon C, Adès L, Fenaux P, Beyne-Rauzy O, Vey N, Braun T, Haferlach T, Dreyfus F, Cross NC, Preudhomme C, Bernard OA, Fontenay M, Vainchenker W, Schnittger S, Birnbaum D, Droin N, Solary E. Prognostic score including gene mutations in chronic myelomonocytic leukemia. J Clin Oncol. 2013; 31:2428-36.
6. Gotlib J1, Maxson JE, George TI, Tyner JW. The new genetics of chronic neutrophilic leukemia and atypical CML: implications for diagnosis and treatment. Blood. 2013; 122: 1707-11.
7. Wang SA, Hasserjian RP, Fox PS, Rogers HJ, Geyer JT, Chabot-Richards D, Weinzierl E, Hatem J, Jaso J, Kanagal-Shamanna R, Stingo FC, Patel KP, Mehrotra M, Bueso-Ramos C, Young KH, Dinardo CD, Verstovsek S, Tiu RV, Bagg A, Hsi ED, Arber DA, Foucar K, Luthra R, Orazi A. Atypical chronic myeloid leukemia is clinically distinct from unclassifiable myelodysplastic/myeloproliferative neoplasms. Blood. 2014; 123: 2645-51.
8. Chang TY, Dvorak CC, Loh ML. Bedside to bench in juvenile myelomonocytic leukemia: insights into leukemogenesis from a rare pediatric leukemia. Blood. 2014; 124: 2487-97.
9. Patnaik MM, Tefferi A.Refractory anemia with ring sideroblasts and RARS with thrombocytosis. Am J Hematol. 2015; 90: 549-59.
10. DiNardo CD, Daver N, Jain N, Pemmaraju N, Bueso-Ramos C, Yin CC, Pierce S, Jabbour E, Cortes JE, Kantarjian HM, Garcia-Manero G, Verstovsek S. Myelodysplastic/myeloproliferative neoplasms, unclassifiable (MDS/MPN, U): natural history and clinical outcome by treatment strategy. Leukemia. 2014; 28: 958-61.

HCV-ASSOCIATED LYMPHOMAS

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Hepatitis C virus (HCV) has a positive sense RNA genome. At least 6 distinct genotypes of HCV have been identified by nucleotide sequencing. Genotypes differ one from another in sequence homology by nearly 30%. HCV infection is a global health problem, with up to 170 million persons infected in the world (3% of global population).[1] However the prevalence of infection shows relevant geographical differences: the lowest prevalence rates are reported in Northern Europe (0.01-0.1%) while in Italy, Egypt, Japan and southern parts of United States, preva-

lence estimates exceeds 2%.[2] HCV infection is a leading cause of chronic hepatitis, cirrhosis and hepatocellular carcinoma and has been associated also to extra-hepatic manifestations, especially type II mixed cryoglobulinemia and a spectrum of B-cell NHL with or without cryoglobulinemia.[3] In 2003, a systematic review of studies evaluating prevalence of HCV infection in B-cell NHL[4] was reported. Mean HCV infection prevalence was 13%. In 10 case-control studies examined, HCV prevalence in B-cell NHL was 17% compared to 1.5% in healthy controls (Odds Ratio=10.8). Therefore, it was concluded that HCV prevalence in patients with B-cell NHL is higher than that reported in general population (15% *vs* 1.5%). Subsequently, in 2006, an updated meta-analysis of 15 case-control studies on the association between HCV infection and NHL, demonstrated a pooled relative risk of lymphoma among HCV-positive subjects of 2-2.5 depending on study design. The study, indeed, confirmed that the risk to develop NHL in the context of HCV infection is most evident in populations with high HCV prevalence and that consequently the fraction of NHL attributable to HCV infection varies greatly by country, possibly reaching 10% in highly endemic areas. The numbers of cases analyzed in these series were too small to establish a correlation between HCV and specific histotypes of lymphoma. In the Epilymph case-control study[5], the subtype most clearly associated with HCV infection was diffuse large B-cell lymphoma, followed by marginal zone lymphoma and lymphoplasmacytic lymphoma. To obtain a more robust estimate of the risk to develop specific NHL subtypes after HCV infection, the International Lymphoma Epidemiology Consortium (InterLymph), based in Europe, North America, and Australia, performed a pooled case-control study including in the analysis data of 7 previous surveys.[6] Overall, among 4,784 cases of NHL and 6,269 controls matched by sex, age and study center, HCV infection was detected in 172 NHL cases (3.6%) and in 169 (2.7%) controls. In subtype-specific analyses, HCV prevalence was associated with associated with diffuse large B-cell lymphoma, marginal zone lymphoma and lymphoplasmacytic lymphoma. The role of HCV infection in lymphomagenesis may be related to the chronic antigenic stimulation of B-cell response,[3] similarly to the well characterized induction of gastric MALT lymphoma development by *Helicobacter pylori* chronic infection.[7] In a similar way, chronic HCV infection may possibly sustain a multi-step evolution from mixed cryoglobulinemia to overt indolent NHL and, eventually, to aggressive NHL.[3,7] During this process, independence from antigenic stimulation can develop due to additional genetic aberrations. Regarding the antigenic trigger, the monoclonal component of MC is often an IgM with a rheumatoid factor activity (anti-IgG cross-reactive binding) that mirrors the expansion of a B-cell monoclonal population[8] not only in bone marrow but also in hepatic follicles.[9] Envelope protein such as E2 protein can play a role in lymphomagenesis; it interacts with the tetraspanin CD81, present also on the B-cell surface, lowering the threshold and leading to a polyclonal B-cell activation.[10] Another mechanism of HCV-related lymphomagenesis is associated with lymphotropism of HCV as demonstrated the viral replication in lymphatic tissue.[11] In addition, regarding molecular signals in HCV-related lymphomagenesis, it has been demonstrated[12] that HCV-infected cells display a mutator phenotype with increased mutation frequency of immunoglobulin heavy chain, *BCL-6*, p53, and β-catenin genes.

Table 1 - Series of patients with indolent B-cell lymphoma associated with HCV infection treated with interferon-based antiviral treatment as anti-lymphoma approach.

	Year	N. of pts	Type of NHL	N. of pts with MC	Type of anti-viral treatment	NHL response	HCV response
Mazzaro et al.[39]	1996	6	Immunocytoma	6	α -IFN-2b	3 CR	3
Bauducr[36]	1996	1	EMZL (oral cavity)	-	α -IFN	1 PR	1
Caramanchi et al.[38]	1999	1	EMZL (salivary glands)	-	α -IFN	1 CR	NA
Moccia et al.[34]	1999	3	NMZL	-	α -IFN	2 CR	NA
Patriarca et al[35]	2001	1	LPL	-	α -IFN	1 CR	1
Hermine et al.[14]	2002	9	SLVL	6	α -IFN	7 CR	7
Casato et al.[24]	2002	1	Leukemic MZL	1	α -IFN	1 CR	Decreased HCV-RNA
Pitini et al.[37]	2004	2	NMZL	-	α -IFN	2 CR	2
Tursi et al.[30]	2004	16	EMZL (stomach)	-	α -IFN-2b + RBV	16 CR	11/16
Kelaidi et al.[31]	2004	8	NMZL (n=4) Disseminated MZL (n=1) Leukemic MZL (n=1) EMZL (n=2) (1 duodenum; 1 ileus)	8	α -IFN-2b + RBV	5 CR	5 SVR, 2 PR
Vallisa et al.[32]	2005	13	NMZL (n=4) NMZL (n=2) EMZL (n=2) FL (n=1) LPL (n=4)	5	Peg-IFN + RBV	7 CR, 2 PR	7 SVR, 1 PR
Svoboda et al.[28]	2005	1	EMZL (salivary gland, liver)	-	Peg-IFN + RBV	CR	1
Saadoun et al.[27]	2005	18	SLVL	18	α -IFN (+ RBV in 10)	14 CR, 4 PR	14 CR, 4 PR
Paulli et al.[41]	2009	2	EMZL (subcutaneous tissue)	2	Peg-IFN + RBV	1 CR, 1 PR	2 CR
Mazzaro et al. [41]	2009	18	1 SLVL 1 FL 16 LPL	13	α –IFN + RBV (n=8) Peg-IFN + RBV (n=10)	9 CR, 4 PR	9 SVR
Oda et al.[42]	2010	1	B-NHL (liver)	-	Peg-IFN + RBV	CR	SVR
Saadoun et al.[43]	2010	13	8 MZL 4 LPL SLL 1	13	Peg-IFN + RBV (+ R in 7)	11 CR	
Pellicelli et al.[44]	2011	9	3 EMZL 3 SMZL 1 NMZL 2 FL	5	Peg-IFN + RBV	5 CR 2 PR	7 SVR
Mauro et al.[45]	2012	1	LPL	1	Peg-IFN + RBV	CR	SVR
Arcaini et al.[46]	2014	100	Indolent NHL		(Peg)-IFN +/- RBV	44 CR 33 PR	SVR 80 %

SMZL: splenic marginal zone lymphoma; NMZL: nodal marginal zone lymphoma; SLVL: splenic lymphoma with villous lymphocytes; EMZL: extranodal marginal zone lymphoma of MALT; FL: follicular lymphoma; LPL: lymphoplasmacytic lymphoma; SLL: small lymphocytic lymphoma; NHL: non-Hodgkin's lymphoma; NOS: not otherwise specified; pts: patients; IFN: interferon; RBV: ribavirin; R: rituximab; CR: complete response; PR: partial response; SVR: sustained virologic response

The regression of HCV-associated lymphoma with antiviral treatment (AVT) alone is the strongest argument in favor of an etiological link between lymphoma and HCV infection.[13] The first experience was conducted by Hermine *et al.*,[14] in 9 patients with splenic MZL with villous lymphocytes and HCV infection treated with IFN. After IFN treatment, 7 patients obtained both a complete hematological remission and virological response. An Italian multicenter study[15] reported results of AVT with pegylated IFN and ribavirin (RBV) in 13 indolent B-cell NHL (8 MZL) carrying HCV infection. Of the 8 assessable patients with MZL, 5 achieved complete response and two partial hematologic responses. A survey ("HCV-LNH outcome survey") was initiated in Italy under the sponsorship of the Fondazione Italiana Linfomi (FIL) in 2010 to better define the outcome and the efficacy of treatments of HCV-positive NHL.[16] A cohort study of 704 consecutive HCV-positive patients with indolent NHL; 134 patients were managed with AVT for lymphoma control. For entire cohort, 5-yr overall survival (OS) was 78% and% and 5-yr progression-free survival (PFS) was 48%. In multivariate analysis, use of AVT during the patients' life had positive impact on OS. Forty-four of the 100 patients treated with first-line AVT achieved a CR and 33 a partial response (PR). HCV-RNA clearance was achieved in 80 patients and was related to lymphoma response. At a median follow-up of 3.6 years, 5-yr PFS was 63%.[16] The favourable impact of AVT on outcome of these patients has been also reported in ANRS HC-13 Lympho-C study by Michot *et al.*,[17]: outcome analysis showed a favourable association between OS and AT in all patients (P=0.05) and in the subgroup of MZL patients only (P=0.04). It is to be underlined that there is a clear association between the lymphoma regression and the clearance of HCV across the studies, although the direct anti-lymphoma properties of IFN cannot be ruled out. Hepatitis C therapy is undergoing a revolution: after nearly 25 years of incremental improvements of interferon (IFN)-based therapies, enormous research and development efforts have produced a large number of new antiviral drugs, including direct-acting antiviral (DAA) with high curative potential.[18-20] Data on new interferon-free regimens in HCV-associated lymphoproliferative disorder are based on clinical reports and show hematological response especially in MZL.[21-23] Few data are available in literature regarding to DLBCL associated with HCV infection and generally are focused on therapeutic issues.[24-26] In a French study [24] focused on 23 HCV-positive DLBCL pts, 65% developed hepatic toxicity during chemotherapy significantly higher than matched HCV- pts. In an Italian study[25], among 132 pts with HCV-positive DLBCL only 4% had to discontinue treatment due to severe hepatotoxicity. A Japanese survey analyzed 553 pts with DLBCL (131 HCV-positive) treated with R-CHOP. HCV infection was not a significant risk factor for prognosis. Of 131 HCV+ pts, 36 (27%) had severe hepatic toxicity (grade 3-4), compared with 13 of 422 (3%) pts who were HCV-. Multivariate analysis revealed that HCV infection was a significant risk factor for severe hepatic toxicity.[26] The identification of predictive-prognostic markers in HCV-positive DLBCLs is still a matter of debate. The Fondazione Italiana Linfomi recently proposed a new "HCV Prognostic Score" that is based on 3 readily available factors (performance status, albumin level and HCV-RNA viral load), and is able to identify 3 risk-categories with different survival in HCV-associated DLBCL.[27] In a study 46 cases of HCV-positive and 64 HCV-negative DLBCL have been investigated for NOTCH, NF- B and B-cell receptor signaling mutations. NOTCH pathway molecularly deregulated in 25% of HCV-positive DLBCL by mutations affecting *NOTCH2* in nearly 20% of cases and *NOTCH1* in around 4%. A small to medium cells component, histologically resembling MZL, was detected in 1 case harbouring *NOTCH1* mutation, in 4 cases with *NOTCH2* mutation and in the single case with *SPEN* mutation. NOTCH mutational status was compared with a series of 64 HCV-negative DLBCL: *NOTCH2* resulted mutated in 1/64 (1.6%) (p=0.002) and *NOTCH1* in 0/64 (p=0.17). After a median follow up of 3.7 years for entire series, 5-year OS is 53%. In univariate analysis, a worse OS resulted associated with age >60 years, mutation of *NOTCH2*. 5-year OS for pts carrying *NOTCH1* or *NOTCH2* mutation was 29% and 60% for pts without these mutations. 5-year OS for pts carrying *NOTCH2* mutation was 22% and 60% for pts without *NOTCH2* mutation (p=0.02). In multivariate analysis, only presence of mutated *NOTCH1* or *NOTCH2* resulted associated with a worse survival.[28] In conclusion, the association between HCV infection and B-cell NHL has been demonstrated by epidemiological studies, in particular in highly endemic geographical areas. MZL and DLBCL are the histotypes most frequently associated with HCV. AVT has been proved to be effective in

the treatment of HCV-positive indolent lymphomas. It is likely to foresee that future improvements in AVT and advances in prognostication and monitoring of hepatic toxicity may directly result in increase in cure rates of HCV-associated NHL.

References

1. Wasley A, Alter MJ. Epidemiology of hepatitis C: geographic differences and temporal trends. Semin Liver Dis. 2000;20(1):1-16.
2. Shepard CW, Finelli L, Alter MJ. Global epidemiology of hepatitis C virus infection. Lancet Infect Dis. 2005;5(9):558-567.
3. Marcucci F, Mele A. Hepatitis viruses and non-Hodgkin lymphoma: epidemiology, mechanisms of tumorigenesis, and therapeutic opportunities. Blood;117(6):1792-1798.
4. Gisbert JP, Garcia-Buey L, Pajares JM, Moreno-Otero R. Prevalence of hepatitis C virus infection in B-cell non-Hodgkin's lymphoma: systematic review and meta-analysis. Gastroenterology. 2003;125(6):1723-1732.
5. Nieters A, Kallinowski B, Brennan P, et al., Hepatitis C and risk of lymphoma: results of the European multicenter case-control study EPILYMPH. Gastroenterology. 2006;131(6):1879-1886.
6. de Sanjose S, Benavente Y, Vajdic CM, et al., Hepatitis C and non-Hodgkin lymphoma among 4784 cases and 6269 controls from the International Lymphoma Epidemiology Consortium. Clin Gastroenterol Hepatol. 2008;6(4):451-458.
7. Suarez F, Lortholary O, Hermine O, Lecuit M. Infection-associated lymphomas derived from marginal zone B cells: a model of antigen-driven lymphoproliferation. Blood. 2006;107(8):3034-3044.
8. Agnello V, Zhang QX, Abel G, Knight GB. The association of hepatitis C virus infection with monoclonal rheumatoid factors bearing the WA cross-idiotype: implications for the etiopathogenesis and therapy of mixed cryoglobulinemia. Clin Exp Rheumatol. 1995;13 Suppl 13:S101-104.
9. Sansonno D, De Vita S, Iacobelli AR, Cornacchiulo V, Boiocchi M, Dammacco F. Clonal analysis of intrahepatic B cells from HCV-infected patients with and without mixed cryoglobulinemia. J Immunol. 1998;160(7):3594-3601.
10. Pileri P, Uematsu Y, Campagnoli S, et al., Binding of hepatitis C virus to CD81. Science. 1998;282(5390):938-941.
11. Zignego AL, Macchia D, Monti M, et al. Infection of peripheral mononuclear blood cells by hepatitis C virus. J Hepatol. 1992;15(3):382-386.
12. Machida K, Cheng KT, Sung VM, et al. Hepatitis C virus induces a mutator phenotype: enhanced mutations of immunoglobulin and protooncogenes. Proc Natl Acad Sci U S A. 2004;101(12):4262-4267.
13. Arcaini L, Bruno R. Hepatitis C virus infection and antiviral treatment in marginal zone lymphomas. Curr Clin Pharmacol. 2010;5(2):74-81.
14. Hermine O, Lefrere F, Bronowicki JP, et al. Regression of splenic lymphoma with villous lymphocytes after treatment of hepatitis C virus infection. N Engl J Med. 2002;347(2):89-94.
15. Vallisa D, Bernuzzi P, Arcaini L, et al. Role of anti-hepatitis C virus (HCV) treatment in HCV-related, low-grade, B-cell, non-Hodgkin's lymphoma: a multicenter Italian experience. J Clin Oncol. 2005;23(3):468-473.
16. Arcaini L, Vallisa D, Rattotti S, et al. Antiviral treatment in patients with indolent B-cell lymphomas associated with HCV infection: a study of the Fondazione Italiana Linfomi. Ann Oncol. 2014;25(7):1404-1410.
17. Michot JM, Canioni D, Driss H, et al. Antiviral therapy is associated with a better survival in patients with hepatitis C virus and B-cell non-Hodgkin lymphomas, ANRS HC-13 lympho-C study. Am J Hematol. 2015;90(3):197-203.
18. Afdhal N, Zeuzem S, Kwo P, et al. Ledipasvir and sofosbuvir for untreated HCV genotype 1 infection. N Engl J Med. 2014;370(20):1889-1898.
19. Gane EJ, Stedman CA, Hyland RH, et al. Nucleotide polymerase inhibitor sofosbuvir plus ribavirin for hepatitis C. N Engl J Med. 2013;368(1):34-44.
20. Zeuzem S, Dusheiko GM, Salupere R, et al. Sofosbuvir and ribavirin in HCV genotypes 2 and 3. N Engl J Med. 2014;370(21):1993-2001.
21. Rossotti R, Travi G, Pazzi A, Baiguera C, Morra E, Puoti M. Rapid clearance of HCV-related splenic marginal zone lymphoma under an interferon-free, NS3/NS4A inhibitor-based treatment. A case report. J Hepatol. 2015;62(1):234-237.
22. Sultanik P, Klotz C, Brault P, Pol S, Mallet V. Regression of an HCV-associated disseminated marginal zone lymphoma under IFN-free antiviral treatment. Blood. 2015;125(15):2446-2447.
23. Carrier P, Jaccard A, Jacques J, et al. HCV-associated B-cell non-Hodgkin lymphomas and new direct antiviral agents. Liver Int. 2015.
24. Besson C, Canioni D, Lepage E, et al. Characteristics and outcome of diffuse large B-cell lymphoma in hepatitis C virus-positive patients in LNH 93 and LNH 98 Groupe d'Etude des Lymphomes de l'Adulte programs. J Clin Oncol. 2006;24(6):953-960.
25. Visco C, Arcaini L, Brusamolino E, et al. Distinctive natural history in hepatitis C virus positive diffuse large B-cell lymphoma: analysis of 156 patients from northern Italy. Ann Oncol. 2006;17(9):1434-1440.
26. Ennishi D, Maeda Y, Niitsu N, et al. Hepatic toxicity and prognosis in hepatitis C virus-infected patients with diffuse large B-cell lymphoma treated with rituximab-containing chemotherapy regimens: a Japanese multicenter analysis. Blood. 2010;116(24):5119-5125.
27. Merli M, Spina M, Luminari S, et al. Prognostic models for outcome prediction in non-Hodgkin's lymphomas associated with hepatitis C virus Infection: a multicenter study of 1,123 patients on behalf of the fondazione italiana linfomi (FIL). Ann Oncol 2011;Volume 22 | Supplement 4 |
28. Arcaini L, Rossi D, Lucioni M, et al. The NOTCH pathway is recurrently mutated in diffuse large B-cell lymphoma associated with hepatitis C virus infection. Haematologica. 2015;100(2):246-252.
29. Mazzaro C, Franzin F, Tulissi P, et al. Regression of monoclonal B-cell expansion in patients affected by mixed cryoglobulinemia responsive to alpha-interferon therapy. Cancer. 1996;77(12):2604-2613.
30. Bauduer F. MALT non-Hodgkin's lymphoma associated with hepatitis C virus infection treated by interferon alpha. Am J Hematol. 1996;53(3):209.
31. Caramaschi P, Biasi D, Carletto A, et al. [MALT lymphomas of the salivary glands. Review of the literature apropos of a case in a patient with hepatitis C virus infection]. Recenti Prog Med. 1999;90(11):585-591.
32. Moccia F, Tognoni E, Boccaccio P. The relationship between splenic marginal zone B-cell lymphoma and chronic liver disease associated with hepatitis C virus infection. Ann Ital Med Int. 1999;14(4):288-293.
33. Patriarca F, Silvestri F, Fanin R, Zaja F, Sperotto A, Baccarani M. Long-lasting complete remission of hepatitis C virus (HCV) infection and HCV-associated immunocytoma with alpha-interferon treatment. Br J Haematol. 2001;112(2):370-372.
34. Casato M, Mecucci C, Agnello V, et al. Regression of lymphoproliferative disorder after treatment for hepatitis C virus infection in a patient with partial trisomy 3, Bcl-2 overexpression, and type II cryoglobulinemia. Blood. 2002;99(6):2259-2261.
35. Pitini V, Arrigo C, Righi M, Scaffidi M, Sturniolo G. Systematic screening for HCV infection should be performed in patients with splenic marginal zone lymphoma. Br J Haematol. 2004;124(2):252-253.
36. Tursi A, Brandimarte G, Torello M. Disappearance of gastric mucosa-associated lymphoid tissue in hepatitis C virus-positive patients after anti-hepatitis C virus therapy. J Clin Gastroenterol. 2004;38(4):360-363.
37. Kelaidi C, Rollot F, Park S, et al. Response to antiviral treatment in hepatitis C virus-associated marginal zone lymphomas. Leukemia. 2004;18(10):1711-1716.
38. Svoboda J, Andreadis C, Downs LH, Miller Jr WT, Tsai DE, Schuster SJ. Regression of advanced non-splenic marginal zone lymphoma after treatment of hepatitis C virus infection. Leuk Lymphoma. 2005;46(9):1365-1368.
39. Saadoun D, Suarez F, Lefrere F, et al. Splenic lymphoma with villous lymphocytes, associated with type II cryoglobulinemia and HCV infection: a new entity? Blood. 2005;105(1):74-76.
40. Paulli M, Arcaini L, Lucioni M, et al. Subcutaneous 'lipoma-like' B-cell lymphoma associated with HCV infection: a new presentation of primary extranodal marginal zone B-cell lymphoma of MALT. Ann Oncol;21(6):1189-1195.
41. Mazzaro C, De Re V, Spina M, et al. Pegylated-interferon plus ribavirin for HCV-positive indolent non-Hodgkin lymphomas. Br J Haematol. 2009;145(2):255-257.
42. Oda Y, Kou T, Watanabe M, et al. Regression of B-cell lymphoma of the liver with hepatitis C virus infection after treatment with pegylated interferon-alpha and ribavirin. Dig Dis Sci;55(6):1791-1793.
43. Saadoun D, Resche Rigon M, Sene D, et al. Rituximab plus Peg-interferon-alpha/ribavirin compared with Peg-interferon-alpha/ribavirin in hepatitis C-related mixed cryoglobulinemia. Blood. 2010;116(3):326-334; quiz 504-325.
44. Pellicelli AM, Marignani M, Zoli V, et al. Hepatitis C virus-related B cell subtypes in non Hodgkin's lymphoma. World J Hepatol. 2011;3(11):278-284.
45. Mauro E, Pedata M, Ermacora A, Mazzaro C. An additional line of therapy with pegylated interferon and ribavirin after rituximab in a patient with hepatitis C virus-related mixed cryoglobulinaemia and indolent non-Hodgkin's lymphoma previously treated with interferon. Blood Transfus;10(1):101-103.

ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN PEDIATRIC PATIENTS WITH NON-MALIGNANT DISORDERS

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Although HLA haploidentical hematopoietic stem cell transplantation (HSCT) has been largely employed in children with life-threatening non-malignant disorders, the survival of patients given this type of allograft has been reported to be inferior to that of patients transplanted from a compatible, unrelated volunteer (UV). To overcome this hurdle, we implemented a novel method of *ex vivo* T- and B-cell depletion based on the selective elimination of αβ+ T cells through labeling with a biotinylated anti-TCR αβ+ antibody, and anti-CD19 antibodies followed by incubation with an anti-biotin antibodies conjugated to paramagnetic beads. We herein report an update of 31 children with non-malignant disorders who were given this type of allograft. Twenty-two patients were males and 9 females, median age at HSCT being 3.5 years (range 0.3-13.2). Nine patients had severe combined immunedeficiency (SCID), 8 Fanconi Anemia (FA), 4 Severe Aplastic Anemia (SAA), 2 Thalassemia Major, 2 Hemophagocytic Lymphohistiocytosis (HLH) and 1 each Immunedeficiency with Polyendocrinopathy Enteropaty X-linked (IPEX), Kostmann Syndrome, Hyper IgE Syndrome, Osteopetrosis, Swachmann-Diamond Syndrome and Congenital Amegakaryocytic Thrombocytopenia (CAMT). All patients were transplanted from 1 of the 2 parents (21 from the mother and 10 from the father), the median number of $CD34^+$ and αβ+ T cells infused being $22.57x10^6$/kg and $4x10^4$/kg. The original conditioning regimen consisted of treosulphan and fludarabine (FLU) ± Thiotepa in 13 (9 SCID, 1 IPEX, 1 CAMT, 1 Kostmann Syndrome and 1 Swachmann-Diamond Syndrome), FLU and cyclophosphamide ± single dose

TBI in 12 (8 FA and 4 SAA) and busulphan, FLU and thiotepa in 6 (2 Thalassemia, 2 HLH, 1 Osteopetrosis and 1 Hyper IgE Syndrome). No patient received immunosuppression after HSCT. All patients received anti- thymocyte globulin (ATG Fresenius; 4 mg/kg/day) on days -5 through -3 before allografting and rituximab (200 mg/m^2) to prevent PTLD on day -1. All patients but 6 engrafted, the median time to reach neutrophil and platelet recovery being 13 days (range 9-23) and 9 days (range 7-40), respectively. The 6 patients (2 with SAA and 1 each with thalassemia, FA, HLH and Osteopetrosis) who had primary graft failure were successfully re-transplanted (2 from the same parent, 3 from the other relative and 1 from an UV). Grade I/II skin acute GVHD occurred in 4 patients, while no patient had chronic GVHD. Three patients died due to multi-organ failure secondary to cytomegalovirus (1 SAA) and adenovirus infection (1 CAMT and 1 SCID). No patient developed EBV-related PTLD. With a median follow-up of 17.6 months (range 4-46), 28 out of the 31 (90.3%) patients are alive and disease-free. Recovery of γδ+ T cells was prompt, but αβ+ T cells progressively ensued over time. These data confirm that infusion of B-cell and αβ+ T-cell-depleted hematopoietic progenitors from an HLA-haploidentical parent is an effective option for children with life-threatening either congenital or acquired non-malignant disorders. Acute GVHD involved only the skin and the absence of chronic GVHD is particularly noteworthy, considering the detrimental impact of this complication on quality of life. Moreover, the transplantation-related mortality of 9.7% observed in this cohort is comparable to that observed using an HLA-matched UV. Infusion of titrated numbers of donor-derived αβ T cells transduced with a suicide gene for controlling possible alloreactive reactions could further optimize this type of allograft, by accelerating recovery of adaptive immunity.

THE EXPERIENCE IN MANAGING PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA: IMPLICATIONS FOR ADULT THERAPY

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I risultati nel trattamento della Leucemia Linfoblastica Acuta (LLA) in età pediatrica sono migliorati in modo progressivo negli 10-15 anni, raggiungendo oggi oltre l'85% di guarigione almeno nei Paesi con adeguate risorse. Tale risultato è da ascriversi a diversi fattori tra cui una migliore classificazione della malattia alla diagnosi, l'ottimizzazione nell'uso di chemioterapici ed il miglioramento delle terapia di supporto. Nel delineare le possibili implicazioni per la terapia dell'adulto certamente l'introduzione della valutazione molecolare della Malattia Residua Minima (MRM) costituisce uno degli aspetti più rilevanti. Il miglioramento e la progressiva standardizzazione delle tecniche (mediante analisi dei geni dei "Recettori-T per l'Antigene"-TcR e delle Immunoglobuline-Ig) e della loro applicabilità praticamente estendibile a tutti i pazienti con LLA (sia del lineage B che T) sono gli elementi che hanno determinato il successo della MRM nella LLA . Dall'esperienza pediatrica, la valutazione della MRM come marcatore molecolare *in vivo* della risposta iniziale al trattamento è divenuto il fattore di rischio più significativo nel delineare il trattamento post-induzione anche nel trattamento della LLA dell'adulto. Il secondo aspetto di impatto della ricerca pediatrica è relativo alla definizione di nuove entità genetiche, come risultato della disponibilità di piattaforme di genomica sempre più avanzate. L'aspetto certamente più rilevante non è solo da ascriversi ad una migliore definizione di nuove entità genetiche (che ricorrono nel bambino come nell'adulto pur con diversa incidenza), ma alla possibilità che per alcune delle nuove lesioni identificate siano oggi disponibili farmaci come inibitori delle tirosin-chinasi (TKI) o del pathway di JAK/STAT (inibitori di JAK2), che potrebbero essere valutati come efficacia in aggiunta a schemi di chemioterapia, come già sperimentato con successo nel trattamento della LLA Ph+. L' approccio diagnostico e terapeutico nelle LLA del bambino e dell'adulto, si delinea dunque con significative opportunità e prospettive di ulteriore miglioramento. In questa prospettiva verranno discussi i seguenti aspetti:i.Le nuove tecnologie basate su NGS e le possibili implicazioni; ii. Come integrare l'uso della MRM negli studi clinici; iii. Come integrare la definizione di nuove lesioni genetiche nel work-up diagnostico di pazienti arruolati a studi clinici; iv. Quale possibili implicazioni dell'uso della MRM come marcatore "surrogato" di risposta all'introduzione di nuovi farmaci.

CHRONIC GVHD AFTER PERIPHERAL BLOOD STEM CELL TRANSPLANTATION FROM HLA IDENTICAL SIBLING

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Chronic graft *vs* host disease (cGVHD) is one of the major complications after allogeneic stem cell transplantation (SCT): patients who experience extensive or severe cGVHD have a significant reduction of survival and a negative impact of quality of life too. Several prospective randomized studies and meta-analyses [1-2] clearly demonstrated that peripheral blood stem cells (PBSC) significantly increase the risk of cGVHD in comparison to bone marrow. The use of PBSC in allogeneic SCT anyway increased over past two decades, representing roughly 70% of all transplants. The European survey showed also that HLA identical sibling represents less than 40% of the allogeneic transplants performed in 2013 and that the 73% of all sibling transplants are performed using PBSC as stem cell source. Irrespective of the source used, when GVHD is refractory to first line therapy (with steroids) no clear evidence of efficacy of further lines of therapy have emerged since now, and it still considered as orphan disease. Several strategies has been applied to reduce GVHD including as *ex vivo* or *in vivo* T-cell depletion. The agents mostly used for *in vivo* T- cell depletion are alemtuzumab and ATGs. Alemtuzumab is a humanised monoclonal antibody against CD52 which is expressed in a variety of cells in addition to lymphocytes. No randomized studies are available in the setting of sibling PBSC transplants, but several reports showed that the use of alemtuzumab is capable of reducing both acute and chronic GVHD but with controversial effects on relapse, infections and NRM [3]. ATGs are an heterogeneous set of molecules with different specificities, because the entire process of manufacturing and production is substantially different, and no formal comparisons can be done, at the moment. The most frequently used ATGs in the setting of prevention of GVHD are both anti-rabbits polysera, one ATG-Fresenius, ATG-F, Grafelon, Germany) obtained against a T-lymphoblastic acute leukaemia (Jurkat cell line), the other instead is produced against human thymocytes (Thymoglobulin, Sanofi, France). The benefits of these approaches depend on the setting where they are used as well as on the brand, dose and timing of such drugs. With regard to setting, the unrelated transplants, because of a greater HLA distance between the donor and the patient, have been rightly considered to be more prone to GVHD prevention than the HLA identical sibling setting, and have been firstly evaluated as ideal setting to explore the benefit of an intensification of GVHD prophylaxis. In 2001 the GITMO trial [4] randomized 109 patients receiving BM from a matched unrelated donor after a myeloablative conditioning regimen between CsA plus MTX and CSA plus MTX plus (two doses of) thymoglobulin (7.5 and 15 mg/kg as total doses). The reduction of GVHD was significant in the experimental arm but with a significant increase of infections which lead to unchanged survival. In the long term follow up anyway the late mortality, chronic GVHD and chronic lung dysfunction were reduced in patients who received thymoglobulin in comparison with patients who were in the control arm. In the Finke study [5] 202 patients with haematological malignancies (mainly acute leukemias) were randomized to receive or not ATG-F in addition to the standard GVHD prophylaxis with cyclosporine and methotrexate after a myeloablative transplant from a matched unrelated donor. More than 80% of patients received PBSC as stem cell source. The use of ATG.F at a total dose of 60 mg/kg was found to be effective in reducing acute and chronic GVHD without increasing the relapse risk and death from infection. No significant advantages in survival were found but the proportion and the probability to be free from immunosuppressive therapy at three years was 16.9% *vs* 52.9% in the control and experimental arm, respectively. In the setting of HLA identical sibling transplants, where PBSC are the prevalent source of stem cells the need to intensify GVHD prophylaxis with ATG has been a debated issue. The incidence of cGVHD after a myeloablative transplant with PBSC from an HLA identical sibling donor using after standard GVHD prophylaxis (cyclosporine and methotrexate) is 67% at three years [1]. The impact of ATG on GVHD in the setting of sibling transplants with PBSC was evaluated by several non randomized studies and one randomised study. In the majority of the non randomised analysis ATG didn't increase the relapse risk except from the case of reduced intensity conditioning regimens but only with higher doses of thymoglobulin [6]. The experience with thymoglobulin, mainly from the French groups, suggests that in the context of sibling PBSC transplants the best dose is less than/up to 5 mg/kg and some preliminary results from a new randomised study has been recently presented at 2014 ASH meeting utilising

4.5 mg in the unrelated setting. As to ATG-F the used doses range from 15 to 90 mg/kg. In the Hamburg experience [7] both acute and chronic GVHD decreased with a median dose of 30 mg/kg (range 20-90) but a trend for increased relapse for the ATG group was recorded, without a full statistical significance. Our group started with ATG in the sibling setting with low doses ATG-F in a phase II study in patients transplanted for Chronic Myeloid Leukemia [8]. The incidence of cGVHD significantly decreased in comparison to the hystorical control. No significant increases of infections and relapse or minimal residual disease (MRD) positivity was observed after transplant. The clinical findings with low doses were supported by evaluation, on those patients, of the plasma level of post transplant ATG which reached the peak value at day zero (plasma level) and at day -4 (ATG-bound to lymphocytes); ATG administration was also associated with an increased level of IL-4 and IL-10. (data unpublished). The use of ATG was then extended to all diseases and we finally retrospectively evaluated the outcome of 245 patient receiving an allotransplant from a related HLA identical donor with PBSC, with bone marrow and with PBSC plus ATG-F (doses ranged from 15 to 30 mg/kg) showing that cGVHD was significantly lowered by ATG-F without increase of relapse and infection. Acute GVHD was not statistically reduced and survival was similar [9]. The effect of ATG is not fully understood, but data suggest that there is a dose-dependent effect. The majority of the analyses showed that no detrimental effect of ATGs was seen if the conditioning regimen is myeloablative and the phase of disease is early, but in the setting of reduced conditioning regimens where the antileukemic effect relies mainly on the graft *versus* tumor effect any types of *in vivo* T cell depletion could have a greater impact. This is particularly clear in the comparison between the cIBMTR study [9] and Acute Leukemia Working Party of the EBMT [10] evaluating the impact of *in vivo* T cell depletion in the setting of RIC transplants; in the former a significant proportion of patients were in advanced phase of various diseases, and received a sensible higher dose of ATG while in the latter instead all patients where transplanted in 1st complete remission of acute leukemia with PBSC from the HLA identical related donor. The effect of alemtuzumab was similar but the impact of ATGs on relapse was significantly adverse only in the cIBMTR study. So far no definitive conclusion can be drawn on the effect of ATGs in RIC transplants from a related identical donor and the dose and timing for each brands should still be determined. Data now available suggest that low doses ATGs should be added in this setting due to their beneficial effect on cGVHD and GVHD related mortality and morbidity. New data are emerging from a prospective randomized study which has run in the last decade in Europe and Israel which compared the effect of the addition of ATG to the standard GVHD prophylaxis (with cyclosporine and methotrexate) in patients with acute leukemia in remission undergone PBSC transplant after a myeloablative conditioning regimen from an HLA identical donor. Preliminary data showed that cGVHD is significantly reduced by ATG-F 30mg/kg without increased relapse and infection risks. The results of this study will have the potentiality to change the clinical practice, a new standard which will be the reference arm of the comparison with the new appealing strategies of GVHD prophylaxis that are strongly emerging in these years.

References

1. Stem Cell Trialists' Collaborative Group. Allogeneic peripheral blood stem-cell-compared with bone marrow transplantation in the management of hematologic malignancies: an individual patient data meta-analysis of nine randomized trials. J Clin Oncol 2005; 23: 5074-5087.
2. Anasetti C, Logan BR, Lee SL, Waller EK, Weisdorf DJ, Wingard JR et al. Peripheral-blood stem cells *versus* bone marrow from unrelated donors. N Engl J Med 2012; 367: 1487-1496.
3. Kharfan-Dabaja MA, Nishihori T, Otrock ZK, Haidar N, Mohty M, Hamadani M. Monoclonal antibodies in conditioning regimens for hematopoietic cell transplantation. Biol Blood Marrow Transplant. 2013 ;19:1288-300.
4. Bacigalupo A, Lamparelli T, Barisione G, Bruzzi P, Guidi S, Alessandrino PE, et al. Thymoglobulin prevents chronic graft-*versus*-host disease, chronic lung dysfunction, and late transplant-related mortality: long-term follow-up of a randomized trial in patients undergoing unrelated donor transplantation. Biol Blood Marrow Transplant. 2006; 12:560-565.
5. Finke J, Bethge WA, Schmoor C, Ottinger HD, Stelljes M, Zander AR, et al. Standard graft-*versus*-host disease prophylaxis with or without anti-T-cell globulin in haematopoietic cell transplantation from matched unrelated donors: a randomised, open-label, multicentre phase 3 trial. Lancet Oncol. 2009; 10:855-864.
6. Devillier R, Fürst S, El-Cheikh J, Castagna L, Harbi S, Granata A, et al. Antithymocyte globulin in reduced-intensity conditioning regimen allows a high disease-free survival exempt of long-term chronic graft-*versus*-host disease. Biol Blood Marrow Transplant. 2014; 20:370-374.
7. Wolschke C, Zabelina T, Ayuk F, Alchalby H, Berger J, Klyuchnikov E et al. Effective prevention of GVHD using *in vivo* T-cell depletion with anti-lymphocyte globulin in HLA-identical or -mismatched sibling peripheral blood stem cell transplantation. Bone Marrow Transplant. 2014; 49:126-130.
8. Bonifazi F, Bandini G, Arpinati M, Tolomelli G, Stanzani M, Motta MR, et al. Intensification of GVHD prophylaxis with low-dose ATG-F before allogeneic transplantation from HLA-identical siblings in ault patients with haematological malignancies: results from a retrospective analysis. Bone Marrow Transplant 2012; 47: 1105-1111.
9. Soiffer RJ, Lerademacher J, Ho V, Kan F, Artz A, Champlin RE, et al. Impact of immune modulation with anti-T cell antibodies on the outcome od reduced intensity allogeneic hematopoietic stem cell transplantation for hematologic malignancies. Blood 2011; 117: 6963-6970.
10. Baron F, Lobopin M, Blaise D, Lopez-Corral L, Vigouroux S, Craddock C et al. Impact of *in vivo* T cell depletion on outcome of AML patients in first CR given peripheral blood stem cells and reduced-intensity conditioning allo-SCT from a HLA-identical donor: a report from the Acute leukaemia Working Party of the European Group for Blood and Marrow Transplantation. Bone Marrow Transplant 2014; 49: 389-396.

ROLE OF ALLOGRAFTING IN LYMPHOMAS

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Introduction: Lymphomas are a heterogeneous group of malignancies with varied aggressiveness and several therapeutic options. Both autologous (auto-SCT) and allogeneic transplantations (allo-HCT) play roles in the management of these diseases especially in the setting of relapse or disease refractoriness to upfront therapy. In this manuscript, we will particularly focus on the current role of allo-SCT in the era of targeted therapies. Overall, the numbers of allo-SCT in lymphomas has been rising in recent years in Europe. In Italy, between 2010 and 2014, 440 patients with HL and 747 with NHL received an allo-SCT. In HL, donors were HLA-matched sibling in 23%, unrelated in 36%, haploidentical/ family mismatched in 36% and in 4% of the patients cord blood units were used. In NHL donors were HLA-matched sibling in 37%, unrelated in 44%, haploidentical/ family mismatched in 17% and in 2% of the patients cord blood units were used. A brief overview of the existing indications for an allograft is the topic of this article. *Allografting for Hodgkin limphoma:* The past few decades have seen significant progress in HL treatment. The disease is now curable in about 80% of patients. Unfortunately, 20% to 30% of patients show either primary refractoriness to induction chemotherapy, early or late disease relapse. In this setting, salvage high-dose chemotherapy (HDC) and auto-SCT have become standard of care and durable remissions are observed in approximately 50% of them. Disease recurrence or progression after auto-SCT are associated with poor prognosis. In this setting, the antibody-drug conjugate brentuximab vedotin (BV) has shown promising results, but relapse eventually occurs in all patients. Given the young age of the vast majority of HL patients, allo-SCT maintain a role in patients who failed auto-SCT. Historically, results of myeloablative conditioning (MAC) allo-SCT in HL were poor, partly due to the inclusion of heavily pre-treated patients, or patients transplanted with active disease. In the late nineties, the introduction of reduced-intensity conditioning (RIC) regimens dramatically increased the proportion of patients eligible to allo-SCT. In 2002, Robinson *et al.* [88] reported results of RIC allo-SCT in 188 patients from the database of the European Bone Marrow Transplant (EBMT) Lymphoma Working Party (LWP). The vast majority of patients were conditioned with fludarabine-based regimens. The estimated 1-year overall survival (OS) and progression free survival (PFS) were 62% and 46%, respectively. Day 100 and 1-year transplant-related mortality (TRM) were around 13%. Older patients had higher TRM. Probability of disease progression at 1 year for patients with chemo-resistant and chemo-sensitive disease were 75% and 25%, respectively (p=0.001). Burroughs *et al*. compared outcomes of 90 relapsed/refractory HL patients who received a low-dose TBI-based non-myeloablative conditioning before allo-SCT from related (MRD), unrelated (MUD) or haploidentical (HAPLO) donors. After a median follow-up of 25 months, 2-year estimate of OS and PFS were 53% and 23%, respectively. There were no significant differences in terms of relapse incidence or survival among the various stem cell sources.In a retrospective study on 285 patients treated with RIC allo-SCT, factor associated with higher TRM were chemorefractory disease, older age, and poor performance status (PS). Sarina *et al.* [100] performed a retrospective analysis on 185 HL patients relapsed after auto-SCT. This comparative study was based on the time of HLA typing and donor availability. A donor was identified in 122 patients.

Donor type was MRD for 55%, MUD for 32%, or HAPLO for 13%. After a median follow-up of 48 months, 2-year estimates of PFS and OS were better in the donor group (39.3% vs. 14.2%, and 66% vs. 42%, respectively, p <0.001). The advantage was confirmed by multivariable analysis. The largest prospective trial on relapsed/refractory HL and RIC allo-SCT was published by Sureda *et al*. [102]. Seventy-eight patients were enrolled in a phase II study. Day 100 and 1-year TRM were 8% and 15%, respectively. Relapse was the major cause of treatment failure. One-year and 4-year estimates of OS were 71% and 43%, whereas 1-year and 4-year estimates of PFS were 48% and 24%, respectively. The data of RIC regimens are encouraging when compared to the TRM up to 60% historically reported with MAC allo-SCT. Recent data also suggest that HAPLO donors may be a valid option.. Raiola *et al*. [109] reported on 26 patients with advanced HL who underwent HAPLO SCT with the Baltimore approach [108]. Incidence of grade II-IV acute graft-vs.-host disease (GVHD) and chronic GVHD was 24% and 8%, respectively. After a median follow-up of 24 months, 21 patients were alive, 20 of them disease free. Cumulative incidence of TRM and relapse were 4% and 31%, respectively. Actuarial 3-year survival was 77% and actuarial 3-year PFS was 63%. However, data have not yet been confirmed by prospective clinical trials. Importantly, robust prognostic factors should be identified to guide patient selection for auto-SCT or allo-SCT and to develop a risk-adapted therapy algorithm. For allo-SCT candidates, main predictors of outcome are currently chemo-sensitive disease and negative FDG-PET at the time of transplant. Only patients at least in stable disease (SD) or partial remission (PR) may be considered ideal candidates for allo-SCT. In the light of its efficacy and toxic profile, BV may be prospectively evaluated as a bridge to RIC allo-SCT. The optimal timing of allo-SCT after BV response remains to be defined. In summary, HL patients who relapse after HDC and auto-SCT have poor prognosis. Durable responses are rarely achieved even with novel targeted drugs. Allo-SCT is the only cure available at the moment. Moreover, BV and other promising agents such as PD-1 inhibitors, may become an optimal bridge to allo-SCT. *Non-Hodgkin lymphomas:* The existence of a graft-vs-lymphoma effect (GVL) effect mediated by donor T cells is supported by the observation of lower relapse rates compared with auto-SCT for non-Hodgkin lymphoma (NHL) patients. The strength of the GVL effect varies widely among lymphoma histologies, with indolent histologies such as follicular lymphoma (FL) being the most sensitive to GVL effect and the high-grade lymphomas being the least sensitive. In addition, for patients with indolent lymphoma who relapse after allo-SCT, disease regression has been reported after withdrawal of immunosuppression and after donor leukocyte infusions (DLI). *Follicular lymphoma:* There is currently a large number of therapeutic options for patients with follicular lymphoma (FL). However, there is no consensus on the optimal treatment sequence. Auto-SCT has extensively been employed and there are evidences of superiority compared to conventional chemotherapy. Long-term follow-up studies showed that only a small subset of patients may have reached cure with auto-SCT. Therefore, allo-SCT remains the only known curative treatment for patients with FL Due to the long natural history of FL and concerns on TRM, allo-SCT has been investigated in younger patients later in the disease course. Improved supportive care, better donor selection, and the introduction of RIC regimens have lowered TRM and broadened patient eligibility. No conclusive data on allo-SCT vs. auto-SCT for FL are available. Studies reported lower relapse risk but higher TRM for allo-SCT patients. A comparison of 726 patients who underwent an auto-SCT and 149 who underwent a RIC allo-SCT as first transplant procedure for relapsed FL were reported by the EBMT LWP. One-year TRM was 15% for the allo-SCT cohort and 3% for the auto-SCT cohort (p<0.001). The relapse/ progression rate was significantly worse after auto-SCT (p<0.001). Five-year estimates of PFS were 57% and 48% for allo-SCT and auto-SCT, respectively. There was no significant difference in OS between the two cohorts. Similarly, a smaller study through the National Comprehensive Cancer Network (NCCN) lymphoma outcomes project evaluated 136 patients with relapsed/refractory FL who underwent auto-SCT and 48 who underwent allo-SCT after rituximab-based therapies. Patients in the auto-SCT cohort were older (54 vs. 51 years, respectively p=0.01) and they had more commonly grade 3 FL (35% vs. 8%, respectively, p=0.006). Allo-SCT recipients received more prior therapy lines (4 vs. 3, respectively, p<0.0001) and had more frequently resistant disease at transplant (19% vs. 6%, respectively, p=0.008). Three-year TRM was 3% vs. 24% (p<0.0001) for auto-SCT and allo-SCT, respectively. Cumulative incidence of relapse/progression/transformation were 32% vs. 16% (p=0.03) for auto-SCT and allo-SCT, respectively. The higher TRM translated into a worse 3-year estimate of OS for allo-SCT recipients (61% vs. 87%, p<0.0001. The OS advantage for the auto-SCT cohort was confirmed in multivariate analysis. In this study, auto-SCT was shown to be an effective therapy for patients with FL whereas TRM in allo-SCT remained an issue. A recent study by Klyuchnikov *et al.* compared long-term clinical outcomes in patients with relapsed/refractory grades 1-2 FL treated with auto-SCT vs. allo-SCT in the rituximab era. SCTs were performed between 2000 and 2012. Five-hundred-eighteen patients were evaluated. In the allo-SCT cohort patients were younger, more heavily pre-treated, and with more advanced disease. The 5-year TRM was 5% and 26% (p<0.0001) for auto-SCT and allo-SCT groups, respectively. Relapse/progression rates were 54% and 20% (p<0.0001); PFS was 41% and 58% (p<0.001), and OS was 74% and 66% (p=0.05) for auto-SCT vs. allo-SCT, respectively. Of note, 10-year cumulative incidence of second hematological malignancies after allo-SCT and auto-SCT were 0% and 7%, respectively. The authors concluded that both front line auto-SCT and RIC allo-SCT may provide long-term disease control in relapsed FL. However, continued disease recurrence risk after auto-SCT determines improved PFS and OS after allo-SCT in long-term survivors. RIC regimens allow TRM reduction and clear plateaus in survival and relapse are now observed. Several trials prospectively evaluated the role of RIC allo-SCT in FL. The Fred Hutchinson Cancer Research Center of Seattle reported results of 62 NHL patients (54 FL) conditioned with fludarabine and low dose TBI. Overall, OS in prospective trials ranged between 52% and 81% with a very wide TRM range. The number of patients enrolled was relatively small and further analyses are necessary to better define the role of RIC allo-SCT in FL and the best conditioning regimen. Chemosensitivity prior to allo-SCT emerged as the most important prognostic factor for OS. Whether a relapsed FL patient with chemo-sensitive disease should be directed toward an auto-SCT or an allo-SCT remains a clinical dilemma. The main disadvantage of auto-SCT is that subsequent relapses may not respond to salvage therapies which would greatly decrease the potential efficacy of allo-SCT. In the light of the current knowledge, allo-SCT should be considered early in the disease course, especially in younger FL patients. *Diffuse large B cell lymphoma:* Diffuse large B cell lymphomas (DLBCL) accounts for about 30% of NHL. After the introduction of rituximab, about 60% of patients achieve a cure with first-line treatment. For patients experiencing relapse and for those with refractory disease, HDC followed by auto-SCT may be curative. A proportion of patients eventually relapses after auto-SCT. In this setting allo-SCT is the only cure. The factor associated with the strongest impact on post allo-SCT outcome is the disease status at the time of transplant. Therefore, in a subgroup of patients with refractory disease, allo-SCT may not be an option because of an excessively high relapse risk. Moreover, TRM remains problematic, particularly for patients older than 65 or those with comorbidities. The first evidence of the superiority of HDC followed by auto-SCT vs. chemotherapy alone in relapsed DLBCL was reported by Philip *et al.* in 1995. Fifty-five patients were included in the auto-SCT arm and 54 patients in the standard chemotherapy arm. Five-year estimate of OS and PFS for auto-SCT patients were 53% and 46%, respectively. On the other hand, OS and EFS of the standard chemotherapy arm were significantly shorter (32%, p=0.038 and 12%, p=0.001, respectively). This study was performed only in patients with chemo-sensitive disease. Conclusive data of auto-SCT in patients with refractory disease are not available at the moment. Recently, the prospective CORAL study provided outcome of relapsed/refractory DLBCL patients in the rituximab era. The study was designed with the intention to further consolidate second remissions with auto-SCT. Only about 50% of the initial cohort actually underwent auto-SCT. The 3-year estimate of PFS by intention to treat of the entire cohort was 31%. The number of patients and published studies using allo-SCT in refractory/early relapsed DLBCL are small and do not allow to draw definitive conclusions. No prospective comparative studies are available. In a large retrospective study, Lazarus *et al.* compared the clinical outcomes of DLBCL patients who underwent a first auto-SCT (n=837) or a MAC allo-SCT from a MRD (n=79) between 1995 and 2003. The data were derived by the Center for International Blood and Marrow Transplant Research (CIBMTR) database. In the allo-SCT cohort there were more patients with intermediate-high or high-risk international prognostic index (IPI), extranodal involvement, marrow involvement, and B symptoms. Allo-SCT was associated with higher TRM but with a similar risk of disease progression compared to auto-SCT. The EBMT registry published a retrospective analysis of 101 DLBCL patients. The majority of patients received a RIC allo-SCT from MRD. Clinical outcomes at 3 years were encouraging.The TRM was 28.2%,

and the relapse rate was 30%. PFS and OS were41% and 53%, respectively. Overall, allo-SCT led to a long-term survival in a subset of patients with poor-risk features. Factor associated with better prognosis were long remission after auto-SCT and chemo-sensitive disease at the time of the allo-SCT. The role of allo-SCT in DLBCL is likely to be investigated in prospective studies on relapsed/refractory patients or frontline in patients considered to be at high-risk of relapse after auto-SCT (Relapse within 1 year from diagnosis or age-adjusted IPI 2-3). *Mantle cell lymphoma:* Allo-SCT has typically been used in younger patients with multiply relapsed mantle cell lymphoma (MCL). No large trials have been conducted so far. A retrospective study compared auto-SCT and allo-SCT in 97 patients. Patients who underwent allo-SCT were younger but more heavily pretreated. By contrast, patients in the auto-SCT cohort, were more commonly in first CR (34% vs. 18%). Overall, allo-SCT resulted in higher day 100 TRM (19% vs. 0%, p<0.01), lower response rate (21% vs. 56%, p=0.11), and similar PFS (44% vs. 39%, p=0.85) and OS (49% vs. 47%, p=0.51) at 5 years. In a study of the British Society for Blood and Marrow Transplantations the role of RIC allo-SCT in 70 R/R MCL patients was evaluated. Of note, 34% previously failed an auto-SCT. Most patients were prepared for transplant with an alemtuzumab-containing conditioning. At 5 years, TRM, PFS, and OS were 21%, 14% and 37%, respectively. Cumulative risk of relapse was 65% at 5 years, which was significantly associated by disease status and chemo-sensitivity at the time of transplant. Fifteen of the 18 patients who relapsed were rescued with DLIs or a second RIC allo-SCT. Seventy-three % of patients arrived in CR at last follow-up.. In another study on 33 R/R MCL patients prepared with fludarabine and low dose total body irradiation (TBI) reported a 2-year TRM of 24%, overall response rate of 85% and OS of 65%. A recent report by the CIBMTR on 519 chemo-sensitive patients compared clinical outcomes of RIC allo-SCT and auto-SCT. Patients received auto-SCT or allo-SCT in first PR or CR after no more than 2 chemotherapy lines (early SCT), or later in the disease course (late SCT). Allo-SCT was associated with lower relapse/progression but higher TRM. Five-year OS was similar between allo-SCT and auto-SCT in both early SCT (62% vs. 61%, p=0.95) and later SCT (31% vs. 44%, p=0.20) groups. This study validates the current practice of auto-SCT in patients with MCL in first CR. *Peripheral T cell lymphoma:* Allo-SCT may be useful in relapsed peripheral T cell lympoomas PTCL or after failure of auto-SCT. A CIMBTR study on 241 patients compared clinical outcomes between patients who underwent auto-SCT with those who underwent allo-SCT. In the auto-SCT cohort, patients were more frequently in CR at the time of transplant, they had more chemo-sensitive disease, and they had received a maximum of 2 prior therapy lines. Three-year PFS and OS of auto-SCT recipients beyond first CR were 42% and 53%, respectively. In allo-SCT recipients who received transplantations beyond CR1, 31% remained progression-free at 3 years, though more heavily pretreated and with more refractory disease. TRM was 3.5 times higher after allo-SCT (p<0.001). TRM and PFS at 3 years were not different for MAC and non-myeloblative/RIC allo-SCT. By multivariate analysis, chemo-sensitivity and 2 or fewer therapy lines before SCT were independent predictors of OS. An Italian study on R/R PTCL (no=52) resulted in TRM of 12%, PFS of 50%, and OS of 40% at 5 years. Of note, 66% of patients with disease progression (no=12) responded to DLI. By multivariate analysis, refractory disease and age older than 45 years were associated with poor prognosis. A more recent phase II study by Corradini *et al.* evaluated feasibility and efficacy of chemo-immunotherapy in young (60 years) and elderly (>60 and 75 years) patients with newly diagnosed PTCL. Younger patients received induction chemotherapy and, on the basis of donor availability, patients in response received allo-SCT or auto-SCT. Thirty-seven patients received allo-SCT (no=23) or auto-SCT (no=14). After a median follow-up of 40 months, 4-year OS, PFS and DFS rates were 49%, 44% and 65%, respectively. The study was not powered to compare auto-SCT and allo-SCT. Overall, allo-SCT may result in long-term disease control in a subset of PTCL patients. *Conclusions:* The introduction of novel targeted therapies has broaden the armamentarium to treat lymphoproliferative diseases. The management of relapsed/refractory patients remains however highly challenging. In this setting, allo-SCT may play an important role in at least a subset of patients. An important novel concept is the combination of allo-SCT and prior therapy with new drugs as a bridge to the allograft. This sequential plan may become a key factor to improve clinical outcomes and increase the number of patients who potentially reach a cure. Finally, the timing of allo-SCT may be crucial to evoke maximal graft-vs.-lymphoma effects. With this view, lymphoma study groups and transplant teams should closely work together to include allo-SCT in the therapeutic algorithm and, possibly, in the context of prospective clinical trials.

Table 1. Allografting in Hodgkin Lymphoma: suggested readings.

Author	Title	Source	Year
Gajewski JL, Phillips GL, Sobocinski KA, et al	Bone marrow transplants from HLA identical siblings in advanced Hodgkin's disease	J Clin Oncol	1996
Milpied N, Fielding AK, Pearce RM, et al.	Allogeneic bone marrow transplant is not better than autologous transplant for patients with relapsed Hodgkin's disease	J Clin Oncol	1996
Aversa F, Tabilio A, Velardi A, et al	Treatment of high-risk acute leukemia with T-cell-depleted stem cells from related donors with one fully mismatched HLA haplotype	N Engl J Med	1998
Champlin R, Khouri I, Kornblau S, et al	Biomarking bone marrow transplantation: Nonmyeloablative preparative regimens and induction of graft-vs-malignancy effect	Oncology (Williston Park)	1999
Woolfrey A, Pulsipher MA, Storb R	Nonmyeloablative hematopoietic cell transplant for treatment of immune deficiency	Curr Opin Pediatr	2001
Akpek G, Ambinder RF, Piantadosi S, et al	Long term results of blood and marrow transplantation for Hodgkin's lymphoma	J Clin Oncol	2001
Robinson SP, Goldstone AH, Mackinnon S, et al	Chemosensitivity at appearance lymphoma predicts for a poor outcome following reduced-intensity allogeneic progenitor cell transplantation: an analysis from the Lymphoma Working Party of the European Group for Blood and Bone Marrow Transplantation	Blood	2002
Sureda A, Schmitz N	Role of allogeneic stem cell transplantation in relapsed or refractory Hodgkin's disease	Ann Oncol	2002
Porter DL, Stadtmauer EA, Lazarus HM	'GVHD' Graft-versus-host disease or graft-versus-Hodgkin's disease? An old acronym with new meaning	Bone Marrow Transplant	2003
Mehta J, Singhal S, Gee AP, et al	Bone marrow transplantation from partially HLA-mismatched donors for acute leukemia: single center experience of 201 patients	Bone Marrow Transplant	2004
Spitzer TR	Haploidentical stem cell transplantation: the always present but overlooked donor	Hematology Am Soc Hematol Educ Program	2005
Peggs KS, Hunter A, Chopra R, et al	Clinical evidence of a graft-versus-Hodgkin's lymphoma effect after reduced-intensity allogeneic transplantation	Lancet	2005
Alvarez I, Sureda A, Caballero MD, et al	Nonmyeloablative stem cell transplantation in an elderly patient for refractory or relapsed Hodgkin lymphoma: results of a spanish prospective cooperative protocol	Biol Blood Marrow Transplant	2006
Corradini P, Dodero A, Farina L, et al	Allogeneic stemcell transplantation following reduced intensity conditioning can induce durable clinical and molecular remissions in relapsed lymphomas: pre-transplant disease status and histotype heavily influence outcome	Leukemia	2007
Peggs KS, Sureda A, Qian W, et al	Reduced-intensity conditioning for allogeneic haematopoietic stem cell transplantation in relapsed and refractoryHodgkin lymphoma: impact of alemtuzumab and donor lymphocyte infusionson long term outcomes	Br J Haematol	2007
Peggs KS, Sureda A, Qian W, et al	Reduced-intensity conditioning for allogeneic haematopoietic stem cell transplantation in relapsed and refractoryHodgkin lymphoma: impact of alemtuzumab and donor lymphocyte infusionson long term outcomes	Br J Haematol	2007
Lucini L, O'Donnell PV, Symons HJ, et al	HLA-haploidentical bone marrow transplantation for hematologic malignancies using nonmyeloablative conditioning and high-dose, posthansplantation cyclophosphamide	Biol Blood Marrow Transplant	2008
Burroughs LM, O'Donnell PV, Sandmaier BM, et al	Comparison of outcomes of HLA-matched related, unrelated, or HLA-haploidentical related hematopoietic cell transplantation following non- myeloablative conditioning for relapsed or refractory Hodgkin lymphoma	Biol Blood Marrow Transplant	2008
Thomson KJ, Peggs KS, Smith P, et al	Superiority of reduced-intensity allogeneictransplantation over conventional treatment for relapse of Hodgkin'slymphoma following autologous stem cell transplantation	Bone Marrow Transplant	2008
Sureda A, Robinson S, Canals C, et al	Reduced- intensity conditioning compared with conventional allogeneic stem cell transplantation in relapsed or refractory Hodgkin's lymphoma: an analysis from the Lymphoma Working Party of the European Group for Blood and Marrow Transplantation	J Clin Oncol	2008
Sahebi F, Cooper N, Rao K, et al	Reduced intensity conditioning and allogeneic stem cell transplantation in childhood malignant and nonmalignant diseases	Bone Marrow Transplant	2008
Burroughs LM, O'Donnell PV, Sandmaier BM, et al	Comparison of outcomes of HLA-matched related, unrelated, or HLA-haploidentical related hematopoietic cell transplantation following non- myeloablative conditioning for relapsed or refractory Hodgkin lymphoma	Biol Blood Marrow Transplant	2008
Anderlini P, Saliba R, Acholonu S, et al	Fludarabine-melphalan as a preparative regimen for reduced-intensity conditioning allogeneic stem cell transplantation in relapsed and refractory Hodgkin's lymphoma: the updated M.D. Anderson Cancer Center experience	Haematologica	2008
Sureda A, Robinson S, Canals C, et al	Reduced- intensity conditioning compared with conventional allogeneic stem-cell transplantation in relapsed or refractory Hodgkin's lymphoma: an analysis from the Lymphoma Working Party of the European Group for Blood and Marrow Transplantation	J Clin Oncol	2008
Devetten MP, Hari PN, Carreras J, et al	Unrelated donor reduced-intensity allogeneic hematopoietic stem cell transplantation for relapsed and refractory Hodgkin lymphoma	Biol Blood Marrow Transplant	2009
Robinson SP, Sureda A, Canals C, et al	Reduced intensity conditioning allogeneic stem cell transplantation for Hodgkin's lymphoma: identification of prognostic factors predicting outcome	Haematologica	2009
Farina B, Castagna L, Farina L, et al	Allogeneic transplantation improves the overall and progression free survival of Hodgkin lymphoma patients relapsing after autologous transplantation: a retrospective study based on the time of HLA typing and donor availability	Blood	2010
Farina B, Castagna L, Farina L, et al	Allogeneic transplantation improves the overall and progression free survival of Hodgkin lymphoma patients relapsing after autologous transplantation: a retrospective study based on the time of HLA typing and donor availability	Blood	2010
Johansson JE, Rembergerr M, Lazarevic VL, et al	Allogeneic haematopoietic stem-cell transplantation with reduced intensity conditioning foradvanced stage Hodgkin's lymphoma in Sweden: high incidence of posttransplant lymphoproliferative disorder	Bone Marrow Transplant	2011
Peggs KS, Kapahi I, Edwards N, et al	Donor lymphocyte infusions mediate relapse risk in mixed chimeras and induce durable salvage in relapsed patients after T cell-depleted allogeneic transplantation for Hodgkin's lymphoma	J Clin Oncol	2011
Sureda A, Corolo C, Arranz R, et al	Allogeneic stem-cell transplantation after reduced intensity conditioning in patients with relapsed or refractory Hodgkin's lymphoma. Results of the HDR-ALLO study – a prospective clinical trial by the Grupo Español de Linfomas/Trasplante de Médula Ósea (GEL/TAMO) and the Lymphoma Working Party of the European Group for Blood and Marrow Transplantation	Haematologica	2012
Chon R, Palmer JM, Thomas SH, et al	Brentuximab Vedotin enables successful reduced-intensity allogeneic hematopoietic cell transplantation in patients with relapsed or refractory Hodgkin lymphoma	Blood	2012
Gopal AK, Ramchandren R, O'Connor OA, et al	Safety and efficacy of Brentuximab Vedotin for Hodgkin lymphoma recurring after allogeneic stem cell transplantation	Blood	2012
Chen YB, McDonough S, Hasercjian R, et al	Expression of CD30 in patients who acute graft versus host disease	Blood	2012
Gibb A, Jones C, Bloor A, et al	Brentuximab Vedotin in refractory CD30+ lymphomas: a bridge to allogeneic transplantation in approximately one quarter of patients treated on a Named Patient Programme at a single UK center	Haematologica	2013
Theurich S, Mailcher J, Wennhold K, et al	Brentuximab Vedotin combined with donor lymphocyte infusions for early relapse of Hodgkin lymphoma after allogeneic stem-cell transplantation induces tumor-specific immunity and sustained clinical remission	J Clin Oncol	2013
Arreckx A, Pimpinelli N, Grassi A, et al	Skin CD30(+) T cells and circulating levels of soluble CD30 are increased in patients with graft versus host disease	Auto Immun Highlights	2013
Canellos GP, Rosenberg SA, Friedberg JW, et al	Treatment of Hodgkin lymphoma: a 50-year perspective	J Clin Oncol	2014
Van Tresckow B, Diehl V	An update on emerging drugs for Hodgkin lymphoma	Expert Opin Emerg Drugs	2014
Rasia A, Domenetto A, Varaldo R, et al	Unmanipulated haploidentical BMT following non-myeloablative conditioning and post-transplantation CY for advanced Hodgkin's lymphoma	Bone Marrow Transplant	2014
Chon R, Palmer JM, Tsai NC, et al	Brentuximab Vedotin is associated with improved progression-free survival after allogeneic transplantation for Hodgkin lymphoma	Biol Blood Marrow Transplant	2014
Garciaz S, Coso D, Peyrade F, et al	Brentuximab Vedotin followed by allogeneic transplantation as salvage regimen in patients with relapsed and/or refractory Hodgkin's lymphoma	Hematol Oncol	2014
Moskowitz CH, Ribrag V, Michot JM, et al	PD-1 blockade with the monoclonal antibody pembrolizumab (MK-3475) in patients with classical Hodgkin lymphoma after Brentuximab Vedotin failure: preliminary results from a phase 1b study (KEYNOTE-013)	Blood	2014
Zirlio F, Pini M, Bunca A, et al	Safety and efficacy of Brentuximab Vedotin (SGN- 35) in Hodgkin Lymphoma patients undergoing-reduced intensity allogeneic stem cell transplant following a relapse after autologous transplant	Haematologica	2014
Castagna L, Carlo-Stella C, Mazza K,et al	Current role of autologous and allogeneic stem cell transplantation for relapsed and refractory Hodgkin lymphoma	Mediterr J Hematol Infect Dis	2014
Zinzani PL, Corradini P, Gianni AM, et al	Brentuximab Vedotin in CD30 positive lymphomas. A SIE, SIES, GITMO position paper	Clin Lymphoma, Myeloma Leuk	2015
Perales MA, Ceberio I, Armand P, et al	Role of cytotoxic therapy with hematopoietic cell transplantation in the treatment of Hodgkin lymphoma: guidelines from the American Society for Blood and Marrow Transplantation	Biol Blood Marrow Transplant	2015
Ragoobir S	Nivolumab shows clinical activity in Hodgkin's lymphoma	Lancet Oncol	2015
Ansell SM	Targeting immune checkpoints in lymphoma	Curr Opin Hematol	2015
Ansell S	Novel agents in the therapy of Hodgkin lymphoma	Am Soc Clin Oncol Educ Book	2015
Ansell SM, Lesokhin AM, Borrello I, et al	PD-1 blockade with nivolumab in relapsed or refractory Hodgkin's lymphoma	N Engl J Med	2015
Crocchiola R, Castagna L, Garciaz S, et al	Tandem autologous allogeneic stem cell transplantation as a feasible and effective procedure in high risk lymphoma patients	Haematologica	2015
Michallet AS, Guillerman Y, Dreyfus B, et al	Sequential combination of gemcitabine, vinorelbine, pegylated liposomal doxorubicin and Brentuximab as a bridge regimen to transplant in relapsed or refractory Hodgkin lymphoma	Haematologica	2015
Ridge T, Boudabdallah R, Chon R, et al	Allogeneic transplant following Brentuximab Vedotin in patients with relapsed or refractory Hodgkin lymphoma and systemic anaplastic large cell lymphoma	Leuk Lymphoma	2015
Steinberg A, Romeria AS, Scigliano E, et al	Bendamustine brentuximab: Bridging to transplant	Biology of Blood and Marrow Transplantation	2015
Stoltha A, Younes A	The new therapeutical scenario of Hodgkin lymphoma	Ann Oncol	2015
Carlo-Stella C, Ricci F, Dalto S, et al	Brentuximab Vedotin in patients with Hodgkin lymphoma and a failed allogeneic stem cell transplantation: results from a named patient program at four Italian centers	Oncologist	2015
Martino M, Festuccia M, Cremmaelle M, et al	Salvage treatment for relapsed/refractory Hodgkin lymphoma: the role of allografting and Brentuximab Vedotin.	Submitted	2015

Table 2. Allografting in Non-Hodgkin Lymphomas: suggested readings.

Author	Title	Source	Year
Philip T, Guglielmi C, Hagenbeek A, et al	Autologous bone marrow transplantation as compared with salvage chemotherapy in relapses of chemotherapy-sensitive non-Hodgkin's lymphoma	N Engl J Med	1995
van Besien KW1, de Lima M, Giralt SA, et al	Management of lymphoma recurrence after allogeneic transplantation: the relevance of graft-versus-lymphoma effect	Bone Marrow Transplant	1997
Van Besien K1, Sobocinski KA, Rowlings PA, et al	Allogeneic bone marrow transplantation for low-grade lymphoma	Blood	1998
Toze CL, Shepherd JD, Connors J, et al	Allogeneic bone marrow transplantation for low-grade lymphoma and chronic lymphocytic leukemia	Bone Marrow Transplant	2000
Van Besien K, Loberiza FR Jr, Bajorunaite R, et al	Comparison of autologous and allogeneic hematopoietic stem cell transplantation for follicular lymphoma	Blood	2003
Hosing C1, Saliba RM, McLaughlin P, et al	Long-term results favor allogeneic over autologous hematopoietic stem cell transplantation in patients with refractory or recurrent indolent non-Hodgkin's lymphoma	Ann Oncol	2003
Toze CL, Barnett MJ, Connors JM,et al	Long-term disease-free survival of patients with advanced follicular lymphoma after allogeneic bone marrow transplantation	Br J Haematol	2004
Maris MB, Sandmaier BM, Storer BE, et al	Allogeneic hematopoietic cell transplantation after fludarabine and 2 Gy total body irradiation for relapsed and refractory mantle cell lymphoma	Blood	2004
Lenz G, Dreyling M, Schiegnitz E, et al	Moderate increase of secondary hematologic malignancies after myeloablative radiochemotherapy and autologous stem-cell transplantation in patients with indolent lymphoma: results of a prospective randomized trial of the German Low Grade Lymphoma Study Group	J Clin Oncol	2004
Ganti AK, Bierman PJ, Lynch JC, et al	Hematopoietic stem cell transplantation in mantle cell lymphoma	Ann Oncol	2005
Hari P, Carreras J, Zhang MJ, et al	Allogeneic transplants in follicular lymphoma: higher risk of disease progression after reduced-intensity compared to myeloablative conditioning	Biol Blood Marrow Transplant	2007
Rezvani AR, Storer B, Maris M, et al	Nonmyeloablative allogeneic hematopoietic cell transplantation in relapsed, refractory, and transformed indolent non-Hodgkin's lymphoma	J Clin Oncol	2008
Kuruvilla J, Pond G, Tsang R,et al	Favorable overall survival with fully myeloablative allogeneic stem cell transplantation for follicular lymphoma	Biol Blood Marrow Transplant	2008
Bloor AJ, Thomson K, Chowdhry N, et al	High response rate to donor lymphocyte infusion after allogeneic stem cell transplantation for indolent non-Hodgkin lymphoma	Biol Blood Marrow Transplant	2008
Armand P1, Kim HT, Ho VT,et al	Allogeneic transplantation with reduced-intensity conditioning for Hodgkin and non-Hodgkin lymphoma: importance of histology for outcome	Biol Blood Marrow Transplant	2008
Rezvani AR, Norasetthada L, Gooley T, et al	Non-myeloablative allogeneic haematopoietic cell transplantation for relapsed diffuse large B-cell lymphoma: a multicentre experience	Br J Haematol	2008
Thomson KJ, Morris EC, Bloor R, et al	Favorable long-term survival after reduced-intensity allogeneic transplantation for multiple-relapse aggressive non-Hodgkin's lymphoma	J Clin Oncol	2009
Winter JN, Inwards DJ, Spies S, et al	Yttrium-90 ibritumomab tiuxetan doses calculated to deliver up to 15 Gy to critical organs may be safely combined with high-dose BEAM and autologous hematopoietic stem cell transplantation in relapsed or refractory B-cell non-Hodgkin's lymphoma	J Clin Oncol	2009
Thomson KJ, Morris EC, Milligan D, et al	T-cell-depleted reduced-intensity transplantation followed by donor leukocyte infusions to promote graft-versus-lymphoma activity results in excellent long-term survival in patients with multiply relapsed follicular lymphoma	J Clin Oncol	2010
Gisselbrecht C, Glass B, Mounier N, et al	Salvage regimens with autologous transplantation for relapsed large B-cell lymphoma in the rituximab era	J Clin Oncol	2010
Cook G, Smith GM, Kirkland K, et al	Outcome following Reduced-Intensity Allogeneic Stem Cell Transplantation (RIC AlloSCT) for relapsed and refractory mantle cell lymphoma (MCL): a study of the British Society for Blood and Marrow Transplantation.	Biol Blood Marrow Transplant	2010
Sirvent A, Dhedin N, Michallet M, et al	Low nonrelapse mortality and prolonged long-term survival after reduced-intensity allogeneic stem cell transplantation for relapsed or refractory diffuse large B cell lymphoma: report of the Societe Francais de Greffe de Moelle et de Therapie Cellulaire	Biol Blood Marrow Transplant	2010
Shutov AR, Gooley TA, Sandmaier BM, et al	Allogeneic haematopoietic cell transplantation after nonmyeloablative conditioning in patients with T-cell and natural killer-cell lymphomas	Br J Haematol	2010
Pazdur, Wagman LD, Camphausen KA, et al	Cancer management: a multidisciplinary approach	Medical, surgical and radiation oncology	2010
Lazarus HM, Zhang MJ, Carreras J, et al	A comparison of HLA-identical sibling allogeneic versus autologous transplantation for diffuse large B cell lymphoma: a report from the CIBMTR	Biol Blood Marrow Transplant	2010
Shea T, Johnson J, Westerveit P, et al	Reduced-intensity allogeneic transplantation provides high event-free and overall survival in patients with advanced-indolent B cell malignancies: CALGB 109901	Biol Blood Marrow Transplant	2011
Kharfan-Dabaja MA, Cutler CS,	Rituximab for prevention and treatment of graft-versus-host disease	Int J Hematol	2011
Thieblemont C, Briere J, Mounier N, et al	The germinal center/activated B-cell subclassification has a prognostic impact for response to salvage therapy in relapsed/refractory diffuse larag B-cell lymphoma: a bio-CORAL study	J Clin Oncol	2011
Van Kampen RJ, Canals C, Schouten HC, et al	Allogeneic stem-cell transplantation as salvage therapy for patients with diffuse large B-cell non- hodgkin's lymphoma relapsing after autologous stem-cell transplantation: an analysis of the European Group for Blood and Marrow Transplantation: an analysis of the European Group for Blood and Marrow Transplantation Registry	J Clin Oncol	2011
Pasquini MC, Wang Z,	Current use and outcome of hematopoietic stem cell transplantation: CIBMTR summary slides		2011
Stewart DA, Duan Q, Carlson L, et al	A Prospective Phase II Study of RICE Re-Induction, Then High-Dose Fludarabine and Busulfan, Followed by Autologous or Allogeneic Blood Stem Cell Transplantation for Indolent B-Cell Lymphoma	Clin Lymphoma Myeloma Leuk	2011
Dodero A, Spina F, Narni F, et al	Allogeneic transplantation following a reduced-intensity conditioning regimen in relapsed/refractory peripheral T-cell lymphomas: long-term remissions and response to donor lymphocyte infusions support the role of a graft-versus-lymphoma effect	Leukemia	2012
Khouri IF, Saliba RM, Erwin WD,et al	Nonmyeloablative allogeneic transplantation with or without 90yttrium ibritumomab tiuxetan is potentially curative for relapsed follicular lymphoma: 12-year results	Blood	2012
Gisselbrecht C, Schmitz N, Mounier N, et al	Rituximab maintenance therapy after autologous stem-cell transplantation in patients with relapsed CD20(+) diffuse large B-cell lymphoma: final analysis of the collaborative trial in relapsed aggressive lymphoma	J Clin Oncol	2012
Laport GG	Changing role of stem cell transplantation in follicular lymphoma.	Hematology Am Soc Hematol Educ Program	2012
Jantunen E, Sureda A	The evolving role of stem cell transplants in lymphomas	Biol Blood Marrow Transplant	2012
LeMaistre JA, Bachier C, Smith B, et al	Once daily busulfan cyclophosphamide is well tolerated and effective as a preparative regimen for allogeneic hematopoietic stem cell transplant	J Oncol Pharm Pract	2012
Smith SM, Burns LJ, Van Besien K, et al	Hematopoietic cell transplantation for systemic mature T-cell non –Hodgkin lymphoma	J Clin Oncol	2013
Vose JM, Carter S, Burns LJ, et al	Phase III randomized study of rituximab/carmustin, etoposide, cytarabine, melphalan (BEAM) compared with 131-iodine tositumomab/BEAM with autologous stem cell transplantation for relapsed diffuse large B-cell lymphoma (DLBCL): Results from the Blood and Marrow Transplant Clinical Trials Network (BMT CTN) 0401 Trial	J Clin Oncol	2013
Fenske TS, Zhang MJ, Carreras J, et al	Autologous or reduced-intensity conditioning allogeneic hematopoietic cell transplantation for chemotherapy-sensitive mantle-cell lymphoma: analysis of transplantation timing and modality	J Clin Oncol	2014
Klyuchnikov E, Bacher U, Kroll T, et al	Allogeneic hematopoietic cell transplantation for diffuse large B cell lymphoma: who, when and how?	Bone Marrow Transplant	2014
Corradini P, Vitolo U, Rambaldi A, et al	Intensified chemo-immunotherapy with or without stem cell transplantation in newly diagnosed patients with peripheral T cell lymphoma	Leukemia	2014

References

See tables 1 and 2.

CURRENT APPROACH TO TREATMENT OF FEBRILE NEUTROPENIA IN HEMATOLOGIC PATIENTS

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Introduction: Febrile neutropenia (FN) represents a clinically important complication that can impact the outcome of hematologic patients receiving chemotherapy or hematopoietic stem cell transplantation (HSCT): it can be assumed that FN might occur in more than 80% of the patients with hematologic malignancies. Bacterial, fungal and viral infections are responsible for the majority of febrile episodes in hematologic patients, although it should be underscored that at least one quarter of febrile episodes may be due to non-infectious causes, namely toxic effects of chemotherapy, drug reactions, antitumor responses or engraftment syndrome. The definition of risk factors and an accurate diagnostic work-up are the steps necessary to start an effective treatment, which has not to be delayed while awaiting results (Figure 1).

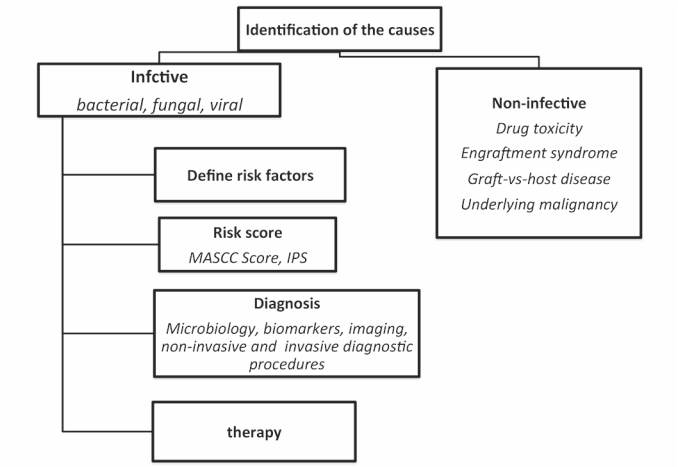

Figure 1. Approach to febrile neutropenia.

Risk factors: In order to secure a correct approach to patients with chemo-induced FN, it is of great relevance to define the risk factors for FN. The quantitative relationship between neutrophils and infections in hematologic patients is a well known concept since the mid of nineties. More recently a consistent body of evidence supports the role of mucosal barrier injury (MBI). Perturbation of the mucosal barrier secondary to chemotherapy treatments may facilitate the translocation of micro-organisms to invade blood circulation and cause bacteremia. In addition, micro-organisms, pathogen-associated molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs) released on the occurrence of tissue damage, are able to induce an inflammatory response by activating pattern recognition receptors (PRRs) eventually resulting in fever (1). According to these observations, fever may be considered as a response to MBI irrespective of the fact that an infection is involved or not. Looking more specifically at the risk of invasive fungal infections (IFI), two different clinical conditions define patients at high risk for IFI, acute myeloid leukemia (AML)/myelodysplastic syndrome (MDS) receiving induction chemotherapy and allogeneic HSCT recipient, although other hematologic malignancies have emerged as potentially at risk for IFI such as chronic lymphoproliferative disorders. Table 1 summarizes key risk factors for IFI. *Risk index scoring systems:* Several risk index scoring systems have been developed to stratify patients with febrile neutropenia eventually facilitating more targeted application of treatment strategies. The multinational association of supportive care in cancer (MASCC) index score demonstrated that seven characteristics (burden of illness, no hypotension, no COPD, solid tumor or no previous fungal infection in hematologic tumors, outpatient status, no dehydration, age <60 years) assessed at the onset of FN could identify low-risk group patients with a score ≤21 which was predictive of less than 5% risk to progress to a severe infection (2). However, this study included more than 1100 patients with FN mainly with solid tumors, thereby limiting the applicability of the analysis to hematologic patients. Several attempts have been made to improve the predictive value of the MASCC score for high-risk febrile neutropenic patients, especially hematologic patients, by adding variables (*i.e.* CRP, procalcitonin) or combining different risk-index scores (*i.e.* PACI model) (3), The infection probability score (IPS) has been developed and validated in the intensive care unit and included 6 parameters (temperature, heart rate, respiratory rate, white blood cells, CPR, organ failure). Apostolopoulou *et al*. (4) demonstrated that the IPS had the best sensitivity in predicting bloodstream infections when compared to APACHE and Karnofsky performance scores.

Table 1. Key risk factors for invasive fungal infections (IFI) in patients with acute leukemia and hematopoietic stem cell transplant (HSCT) recipients.

	Pretreatment Risk Factors		Posttreatment Risk Factors	
	Acute leukemia	HSCT	Acute leukemia	HSCT
Disease-related	-Adverse cytogenetics -Low probability of CR -Neutropenia at diagnosis	-active disease at HSCT	-	-
Treatment-related	-clofarabine	-clofarabine -rituximab	-duration of neutropenia -lymphopenia	-duration of neutropenia -lymphopenia -steroids Anti-TNF
Host-related	-age	-age -genetic factors	-	-
Donor-related	-	-MUD including CBT -haploidentical donor	-	-
Comorbidity	-poor PS -COPD	-EBMT risk score -iron overload	-mucosal barrier injury	-mucosal barrier injury -GVHD -CMV infection
Exposure to opportunistic fungi	-colonization by *Candida* and *Aspergillus* species -building construction and renovation -high exposure job	-colonization by *Candida* and *Aspergillus* species -building construction and renovation -IFI in the 6 months before HSCT	-	-

Abbreviations: CR, complete remission; MUD, matched unrelated donor; CBT, cord blood transplant; PS, performance score; COPD, chronic obstructive pulmonary disease; GVHD, graft-versus-host disease; CMV, cytomegalovirus.

Diagnostic work-up: Blood culture represents the first step of the diagnostic strategy, although it should be emphasized that it may result positive in less than 30% of hematologic patients with FN. Several serologic biomarkers are currently included in the daily clinical practice. Galactomannan (GM), a component of the fungal cell wall, can be detected in the serum, and has been endorsed by international guidelines as a noninvasive tool to implement the diagnosis of IFI. The sensitivity and specificity of the test may regarded as of 70% and 90%, respectively, although a recent study suggests that in patients receiving mold-active antifungal prophylaxis, the use of serum GM may contribute to a diagnostic benefit when adopted as a diagnostic-driven strategy rather than as a surveillance monitoring (5). It should be emphasized the both GM and 1,3-β-D glucan (BDG) may be assessed also in the CNS fluid. In hematologic patients persistently febrile after >48 hours of broad-spectrum antibacterial therapy, 10% of chest xRay are abnormal, whereas HR-CT scans at this time may reveal pathologic findings in 50% of patients. CT scan may assume a considerable importance to detect occult fungal disease. The halo sign represents an early stage of IPA and although not specific, the presence of the halo sign should be considered as highly suggestive of an invasive pulmonary aspergillosis (IPA). Two different radiological patterns have been described in hematologic patients with IPA: the angioinvasive disease, characterized by the presence of a nodule with halo sign, is associated with low leukocyte counts and is more frequent in patients with acute leukemia; by contrast, the airway-invasive or bronchoalveolar disease characterized by centrilobular micronodules and tree-in-bud infiltrates is typically associated with non-neutropenic patients, namely HSCT recipients (6). In addition, it is worthwhile recalling that hematologic patients may present radiological findings that do not necessarily qualify as the EORTC/MSG diagnostic criteria. When lung infiltrates have been documented, bronchoscopy with bronchoalveolar lavage (BAL) should be arranged, although critical hypoxemia might represent the main obstacle to the procedure. Table 2 summarizes the microbiological workup recommended for hematologic patients. BAL GM seems to improve the diagnostic performance with sensitivity and specificity ranging between 57 and 100% and 89 and 99% respectively, although recent studies recommended caution in using this assay as a tool for initiating antifungal treatment. Approximately 50% of the patients may be colonized by *P. jirovecii*: in this respect, a negative PCR in the BAL is highly suggestive of the absence of the infection, while a quantitative PCR >1450 copies/ml has a 98% PPV; similarly a semi-quantitative PCR (≥++) may be helpful to differentiate between colonization and infec-

tion as well as a negative BDG in the serum makes the diagnosis of *P. jirovecii* infection unlikely. CMV disease is a life-threatening complication particularly among HSCT recipients; CMV PRC in the blood is routinely assessed during the post-transplant course allowing the initiation of pre-emptive treatment; when performed in the BAL, CMV PCR has high NPV, but low PPV.

Table 2. Febrile neutropenia in hematologic patients: recommended diagnostic work-up.

	Diagnostic procedure
MICROBIOLOGY	Blood culture Urine and stool cultures Culture specimens from any site of suspected infection, as clinically indicated
BIOMARKERS	CRP, procalcitonin, Galattomannan index, 1,3-β-D glucan
IMAGING	Chest CT scan CT scan of paranasal sinus Abdominal CT scan
NONINVASIVE DIAGNOSTIC PROCEDURES	Broncoscopy and BAL *Recommended microbiological procedures:* - GRAM stain - Bacteriological and fungal cultures - Mycobacteria: solid and liquid medium - actinomycetes - Galattomannan index - *P. jirovecii:* PCR (quantitative) Direct immunofluorescence - *M. tubercolosis:* PCR - *Legionella:* PCR - *Mycoplasma:* PCR - *Clamydia:* PCR - VIRUS: CMV, EBV, HSV, HZV: PCR RESPIRATORY VIRUSES (PCR): adenovirus, coronavirus, influenza, parainfluenza, rhinovirus, enterovirus, RSV, metapneumovirus
INVASIVE PROCEDURES	Open lung biopsy, percutaneous needle biopsy

Abbreviations: CRP, C-reactive protein; BAL, bronchoalveolar lavage; CMV, cytomegalovirus; EBV, Epstein Barr Virus; HSV, Herpes simplex virus; HZV, Herpes zoster virus; RSV respiratory syncytial virus;

Treatment strategies: Broad spectrum antibiotic treatment represents the up-front therapy of febrile neutropenic patients. In this respect two factors have a paramount importance, namely timing and appropriateness. Legrand *et al.* (7) showed that each hour of delay in antibiotic treatment during the first 6 hours of a septic episode, may result in a 7.6% reduction of the survival probability, while an inappropriate therapy is defined as the lack of at least one antibiotic active *in vitro* against the infecting micro-organism. The choice of the initial empirical antibacterial therapy must take into account recent changes of bacterial epidemiology in neutropenic patients. Indeed, gram-negative bacteria have been reported to cause an increasing number of infections in neutropenic patients since the early 2000s, however a major concern is the emergence of multidrug-resistant bacteria as listed below: - Extended-spectrum β-lactamases (ESBL)-producing Enterobacteriaceae; - Carbapenemase-producing Enterobacteriaceae: - Klebsiella pneumoniae carbapenemases (KPCs)-producing Enterobacteriaceae; - Resistant non-fermenters: *Pseudomonas aeruginosa, Stenotrophomonas maltophilia, Acinetobacter baumannii* methicillin-resistant *Staphylococcus aureus* (MRSA); - Vancomycin-resistant enterococci. According to these observations, international guidelines have developed recommendations for the management of FN. The main treatment questions are the choice of empiric monotherapy (ceftazidime Vs. cefepime Vs. piperacillin-tazobactam Vs. meropenem), the addition of aminoglycosides (mostly amikacin), the use of empiric coverage of MRSA with specific risk factors; the main epidemiological questions are related to the incidence of carbapenemases-producing Enterobacteria and the need of carbapenem-sparing strategies. *The European Conference on Infections in Leukemia (ECIL-4) guidelines* underscores the need to evaluate the following factors to optimize empirical antibiotic treatment (8): - continuous monitoring of local epidemiology and resistance patterns; - risk factors for resistant bacteria, including prior colonization by resistant pathogens, exposure to broad-spectrum antibiotics; - risk factors that might predict a severe clinical course. ECIL guidelines recommend two antibiotic treatment approaches. The *Escalation strategy* should be employed in standard risk patients who do not have a severe presentation and includes an initial empirical monotherapy regimen avoiding carabapenems and combinations; this approach may include an anti-pseudomonal cephalosporin or piperacillin-tazobactam (AI). A *de-escalation strategy* should be reserved to patients with previous infection or colonization with resistant pathogens, patients with complicated presentation (*i.e.* septic shock) or centers with high prevalence of resistant pathogens. The aim of this approach is to provide a broad spectrum antibiotic treatment with a consistent chance to cover even resistant bacteria, followed by a de-escalation to a narrower-spectrum therapy once microbiology data become available. *The Infectious Disease Society of America (IDSA)* guidelines indicate that high-risk patients may benefit from anti-pseudomonal β-lactam agent (AI), while other antimicrobial agents, including aminoglycosides, fluoroquinolones or vancomycin may be added in case of complicated presentations or when the presence of resistant bacteria are suspected or proven (BIII) (9). *The Infectious Diseases Working Party of the German Society of Hematology and Medical Oncology (AGIHO)* recommend the administration of carbepenems or piperacillin/tazobactam as initial treatment for neutropenic patients with sepsis (AIII) (10). A combination treatment with aminoglycosides may considered in patients with severe sepsis or septic shock (BIII) based on the observation that combinations may be associated with a better outcome as compared to monotherapy in this group of high-risk patients. The management of persistently febrile neutropenic patients despite a broad-spectrum antibiotic treatment, represents a challenging issue. Patients with persistent fever must be investigated for causes other than bacterial infections. In this respect invasive fungal infections (IFI) occurring in 10 to 15% of hematologic patients represent an important cause of treatment failure. Several treatment algorithms have been established during the most recent years leading to the development of different strategies, namely empirical, diagnostic-driven and hybrid approach to antifungal diagnostics and therapeutics, or targeted therapy. *Empirical antifungal therapy* is defined as an antifungal treatment given to neutropenic patients affected by fever of unknown origin which does not respond to broad spectrum antibacterials. *Diagnostic-driven therapy* (or pre-emptive therapy) is a strategy guided by clinical signs, radiological imaging or microbiological results of the diagnostic work-up which may be suggestive for an established fungal infection and trigger the initiation of antifungal therapy. The empiric approach include a consistent number of patients treated unnecessarily, while the diagnostic-driven approach has not been formally validated and cannot be set up in centres with limited or no access to radiological and mycological diagnostic tools. On the other hand, we are now aware of the great importance of an early antifungal intervention, especially in view of the dismal outcome of treating patients with a more advanced invasive fungal disease. Keeping in mind these considerations, the *hybrid approach* relies on the high negative predictive value of diagnostics supporting the early initiation of antifungal therapy followed by step down or stopping antifungals in afebrile patients with a negative diagnostic work-up. *Targeted therapy* of IFI is based on the identification of the pathogen which may be difficult or even time consuming in hematologic patients due to the coexistence of comorbidities. The recent introduction of new antifungal agents has expanded the treatment options for hematologic patients with IFI. Among *polyenes*, liposomal amphotericin B (L-AmB) has demonstrated a favourable toxicity profile with a significant reduction of the incidence of nephrotoxicity, along with an extended spectrum of activity including yeasts, aspergillus and zygomycetes. According to these characteristics the major international guidelines recommended L-AmB for the first line treatment of invasive aspergillosis (IA) in some patients. Mold-active *triazoles*, notably voriconazole and isavuconazole, received the highest grading for first line treatment of IA (AI for voriconazole and AII for isavuconazole respectively). *Echinocandins* include caspofungin, micafungin and anidulafungin which are frequently used for the treatment of invasive candidiasis, but only caspofungin is approved for salvage treatment of IA, although all have *in vitro* activity. There is no consensus for combination antifungal treatment of IA, which is sometimes used in case of severe infections. *Conclusions:* FN remains a major complication of patients treated with chemotherapy and those who received an allogeneic HSCT. An accurate diagnostic-up as well as an appropriate antibiotic administration is mandatory to prevent a clinical deterioration, especially if we consider the recent emergence of multi-resistant strains. In this respect, it is of paramount importance a careful assessment of local bacterial ecology and the evaluation of bacterial history of the patient. It should be emphasized that the successful treatment of hematologic patients with

FN may only arise from the close collaboration of all parties involved including clinicians, microbiologists and radiologists.

References

1. van der Velden WJF, Herbers AHE, Netea MG, Blijlevens NMA. Mucosal barrier injury, fever and infection in neutropenic patients with cancer: introducing the paradigm febrile mucositis. Br J Haematol 2014, 167, 441-452.
2. Klastersky J, Paesmans M, Rubenstein EB et al. The multinational association for supportive care in cancer risk index: a multinational scoring system for identifying low-risk febrile neutropenic cancer patients. J Clin Oncol 2000;18:3038-3051.
3. de Souza Viana L, Serufo JC, da Costa Rocha MO et al. Performance of a modified MASCC index score for identifying low-risk febrile neutropenic cancer patients. Support Care Cancer 2008; 16:841-846.
4. Apostolopoulou E, Raftopoulos V, Terzis K and Elefsiniotis I. Infection Probability Score, APACHE II and KARNOFSKY scoring systems as predictors of bloodstream infection onset in hematology oncology patients. BMC Infectious Diseases 2010, 10:135.
5. Duarte RF, Sánchez-Ortega I, Cuesta I et al. Serum Galactomannan–Based Early Detection of Invasive Aspergillosis in Hematology Patients Receiving Effective Antimold Prophylaxis. Clin Infect Dis. 2014;59:1696-1702.
6. Bergeron A, Porcher RL, Sulahian A et al. The strategy for the diagnosis of invasive pulmonary aspergillosis should depend on both the underlying condition and the leukocyte count of patients with hematologic malignancies. Blood. 2012;119:1831-1837.
7. Legrand M, Max A, Peigne V et al. Survival in neutropenic patients with severe sepsis or septic shock. Crit Care Med 2012,40:43-49.
8. Averbuch D, Coedonnier C, Livermore DM et al. Targeted therapy against multiresistan bacteria in leukemic and hematopoietic stem cell transplant recipients: guidelines of the 4th European Conference on Infections in Leukemia (ECIL-4, 2011). Hematol 2013;98:1836-1847.
9. Freifeld AG, Bow EJ, Sepkowitz KA et al. Clinical Practice Guideline for the Use of Antimicrobial Agents in Neutropenic Patients with Cancer: 2010 Update by the Infectious Diseases Society of America. Clinical Infectious Diseases 2011;52:427–431.
10. Penack O, Becker C, Buchheidt D et al. Management of sepsis in neutropenic patients: 2014 updated guidelines from the Infectious Diseases Working Party of the German Society of Hematology and Medical Oncology (AGIHO). Ann Hematol 2014; 93:1083-1095.

ACQUIRED HEMOPHILIA: FROM DIAGNOSIS TO NOVEL THERAPEUTIC ADVANCES

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Abstract: Autoantibodies against FVIII (acquired Hemophilia A,AHA) are responsible for a rare but often severe and life-threatening bleeding disorder, often developing in association with underlying conditions (malignancy, autoimmune diseases, drugs, post-partum) but also apparently idiopathic in a significant proportion of cases. The disorder is particularly frequent in elderly and treatment and prognosis are influenced by the presence of co-morbidities. Because of the rarity of this disorder and significant challenges in its diagnosis and costs of treatment, patients with AHA should be managed at specialized centres with expertise in bleeding disorders. A considerable amount of new information about epidemiology and management of AHA has become recently available and predictive parameters of response to treatment have been identified. *Introduction:* Autoantibodies inhibiting the activity of clotting factors (acquired inhibitors) can develop in patients with autoimmune or malignant disorders, but also in subjects without apparent underlying conditions. The large majority (>90%) of these inhibitors are directed against factor VIII (FVIII), causing a clinical and laboratory phenotype resembling inherited hemophilia A (Acquired haemophilia A, AHA). Typically, these antibodies are IgG polyclonal immunoglobulins directed against several functional epitopes of FVIII, with complex kinetics of inactivation (second order or exponential) with rapid initial inactivation, followed by a slower phase or a period of equilibrium in which the factor activity can still be measured. *Epidemiology of AHA:* AHA is a rare disease, with an incidence in the general population of about 1.5 case per million persons/year, but its frequency increases with age and more than 80% of the patients are 65 years old or over. No gender difference is evident, apart from the cases occurring post-partum (about 1:350,000 deliveries). In half of cases no apparent clinical conditions or drugs are evident, while tumors, autoimmune diseases, pregnancy (particularly the puerperium), the use of some drugs (e.g, antibiotics, interferon, clopidogrel), and dermatological diseases have been reported in about 40% of cases (Table 1).

Table 1. Prevalence of idiopathic and secondary acquired haemophilia A reported in 4 large studies.

	Delgado 2003 (n=234)	Collins 2007 (UKHCDO; n=172)	Knoebl 2012 (EACH2; n=501)	Borg 2013 (SACHA; n=82)
Idiopathic	58%	53%	52%	55%
Neoplastic disorders/ Autoimmune diseases	27%	32%	25%	37%
Pregnancy	15%	2%	8%	7%

EACH2: European Acquired Haemophilia Registry; SACHA: Surveillance des Auto antiCorps au cours de l'Hémophilie Acquise (prospective French registry); UKHCDO: United Kingdom Haemophilia Centre Directors Organization.

Clinical presentation and diagnosis: Bleeding is the cause of diagnosis in around 90% of cases, but the severity of the initial bleeding does not predict the severity of subsequent hemorrhagic manifestations or outcome. As a consequence, all patients with AHA are at a high risk of unpredictable severe, sometimes fatal bleeding, particularly if diagnosis is delayed. An acquired anti-FVIII inhibitor should be suspected in the presence of unexpected spontaneous bleeding or after minor trauma, invasive procedures or surgical interventions in patients without a previous personal or family history of bleeding. Vast ecchymoses and subcutaneous haematomas are mostly observed and may be so severe as to cause important anemia. Epistaxis, gum bleeding, gastrointestinal bleeding and muscle hematoma are relatively frequent. Unlike in congenital hemophilia, hemarthroses are rare. Retroperitoneal bleeding occurs in 20% of cases and can be fatal. The severity of bleeding, together with delays in diagnosis, the advanced age of patients, associated disorders and inadequate management are all concurrent causes of the reported high rate of mortality (up to 20-30% in recent series). Isolated prolonged activated partial thromboplastin time (APTT), not corrected by the addition of normal plasma (mixing test) is the diagnostic laboratory hallmark for the diagnosis of AHA. The APTT of mixtures of the patient's plasma and normal plasma must be determined before and after incubation at 37°C for at least 2 hours, because the inactivation of FVIII by autoantibodies is time- and temperature-dependent. The presence of heparin is documented by a prolonged thrombin time with a normal reptilase time. Lupus anticoagulant (LA) must be excluded by specific tests, such as the diluted Russell viper venom time (dRVVT) and tests at low phospholipid concentrations, *e.g.* the kaolin clotting time (KCT) and silica clotting time (SCT). The diagnosis of inhibitor is then confirmed by a specific assay of the factor and titration of the inhibitor by the Nijmegen modification of the Bethesda method. Figure 1 is an algorithm for the diagnosis of AHA. Because of the complexity of laboratory evaluation, the laboratory diagnosis of patients with suspected AHA must be made/confirmed in specialised coagulation laboratories working in close collaboration with centres with expertise in the diagnosis and management of patients with congenital haemophilia and other inherited bleeding disorders. *Treatment and prevention of bleeding:* The presence of autoantibodies against FVIII carries a high risk of bleeding which prompts immediate treatment aimed at eradicating the inhibitor. If bleeding is present, immediate evaluation and appropriate therapy is urgently required. Not infrequently the initial evaluation occurs outside specialized centers and delays may occur in appropriate diagnosis and treatment. Thus, it is strongly recommended that patients with acquired inhibitors of clotting factors are cared for in specialised centres for the diagnosis and treatment of hemophilia. Bypassing agents (recombinant activated factor VII [rFVIIa] and activated prothrombin complex concentrate [APCC]) are the first-line treatments of bleeding episodes in patients with AHA. Both types of bypassing agents have been demonstrated to be effective and no comparative studies able to demonstrate the superiority of one product over the other are available. APCC (FEIBA®, Baxter, Deerfield, IL, USA) is a plasma-derived product containing activated prothrombin complex factors (II, VII, IX, X). In reported series of AHA as first-line treatment, with a dose of 75 U/kg every 8-12 hours and different number of infusions to control a bleeding episode, clinical efficacy was around 80-90%. Recombinant FVIIa (NovoSeven®, Novo Nordisk, Bagsvaerd, Denmark) is free of the risk of transmission of blood-borne pathogens and quick to be

administered. Its efficacy in effective control of bleeding in AHA as first-line treatment is 90-95% of reported case series. The median dose administered was 90 µg/kg, but large variability in dose, number of infusions, and duration of treatment have been reported. In the EACH2 Registry, rFVIIa and APCC had the same success rate of controlling bleeding (92% *vs* 93%). The main drawback with these agents is represented by insensitivity to usual laboratory monitoring. Since no modifications of APTT or FVIII level are achieved with these products, evaluation of the efficacy of treatment is based only on clinical ground and surrogate parameters (*e.g.*, hemoglobin level, transfusion requirement…). Furthermore, since costs are not negligible, appropriate duration of treatment must be considered by physician experts with these disorders to avoid over or under-treatment. The main concern related to the use of these bypassing agents remains the risk of thrombosis which is substantially similar between the two bypassing agents, but higher than in patients with congenital hemophilia, probably because of the older age and the presence of cardiovascular and thrombotic risk factors. The initial dose of rFVIIa should be 90-120 µg/kg, followed by additional infusions 2-3 hours apart on clinical ground, while that of APCC should be 50-100 U/Kg every 8-12 hours, and the maximum daily dose of 200 U/kg should not be exceeded. In the case of clear inefficacy of one or other of the agents, a switch to the alternative agent should be considered and the change should be performed early, in order to prevent persistent bleeding leading to disabling sequelae or life-threatening situations.

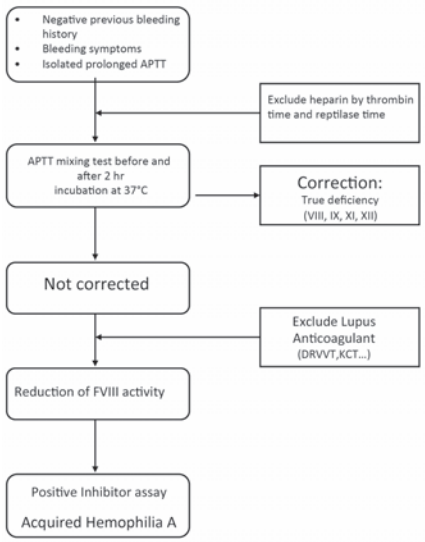

Figure 1. Algorithm for the laboratory diagnosis of acquired haemophilia A.

Alternative treatments, aimed at increasing the levels of circulating FVIII, include DDAVP and FVIII concentrates. DDAVP can be used in patients with anti-FVIII autoantibodies and measurable FVIII levels, without inducing an anamnestic response, but response and its duration is unpredictable. In patients with a low-titre inhibitor (<5 BU/mL), replacement treatment with FVIII can be considered at doses able to neutralise the inhibitor and increase the level of circulating FVIII. The usually adopted formula for calculating the neutralising dose is strongly limited by the inaccuracy of laboratory methods for determining the inhibitor level. The level of FVIII should, therefore, be controlled 15 to 30 minutes after infusion. FVIII concentrates derived from porcine plasma were, in the past, used successfully in patients with low cross-reactivity, but they are no longer available because of concerns with Parvovirus B19 infection. Very recently, however, a recombinant B-domain deleted FVIII of porcine origin, OBI-1 , with the indication for use in patients with AHA has been approved in USA. An initial dose of 200 U/Kg and subsequent adjustments to maintain FVIII target levels, irrespective of inhibitor titre and anti-porcine FVIII cross-reactivity, OBI-1 was effective in the control of bleeding episodes in 86% of cases, but cross-reactivity was also observed. This product is under evaluation by the European Medicines Agency (EMA) for the same indication. *Inhibitor eradication:* If possible, the removal or successful management of conditions which could have triggered the development of the autoantibody (*e.g.* cancer, drugs, autoimmune diseases) is mandatory. Eradication is usually attempted with prednisone, cyclophosphamide, azathioprine, vincristine, cyclosporine, and anti-CD20 monoclonal antibody (rituximab), administered as single therapies or in various combinations. Prospective, controlled clinical trials to evaluate the efficacy of the different treatments are not available, partly because of the possible spontaneous remissions (paediatric cases, pregnancy- and drug-related cases). Prognostic factors predicting success of eradication therapy are a low-titre inhibitor and a short period between appearance of the inhibitor and the start of immunosuppressive therapy. A recent prospective German study identified a low titer at diagnosis with measurable FVIII (FVIII>1 U/dL) as prognostic factors predicting response to corticosteroids. In some cases, the autoantibody may disappear spontaneously, a phenomenon commonly observed if the inhibitor is pregnancy- or drug-related. Different strategies can, therefore, be adopted in different types and subgroups of patients. Steroids (prednisone 1-2 mg/kg/die for 4-6 weeks) as monotherapy or in association with cyclophosphamide (1-2 mg/kg/die for a maximum of 5 weeks) are the most commonly used first-line therapeutic strategies, with 70-80% of cases of successful inhibitor eradication. The only prospective, randomised study dates back to 1993 and involved 31 patients treated with prednisone monotherapy at a dose of 1 mg/kg/die for 3 weeks. If the inhibitor was not eradicated, the patients were randomised to receive, the same dose of prednisone or prednisone+cyclophosphamide (2 mg/kg/die) or cyclophosphamide monotherapy, all for 6 weeks,. About one-third of the patients successfully responded to the initial treatment with the steroid, while about 50% of the resistant patients also failed to respond to the subsequent treatment including cyclophosphamide. No difference was seen in the rates of inhibitor eradication between the patients in the two different treatment arms. A meta-analysis conducted by Delgado *et al.* on 20 studies, showed that cyclophosphamide was superior to prednisone in terms of eradication of the inhibitor, but without improvement of overall survival. This finding was attributed to the greater toxicity of cyclophosphamide and, in particular, to the increased mortality caused by infections. However, a more recent meta-analysis of 32 non-randomised studies showed that patients receiving combined schedules had a lower probability of persistent inhibitor and a lower risk of mortality. These data were confirmed by the EACH2 Registry, with a higher percentage of inhibitor eradication compared to treatment with steroids alone (80% *versus* 58%), but again without differences in survival. In conclusion, currently available data suggest that the association of steroids and cyclophosphamide is more likely to achieve stable eradication of the inhibitor, but with greater toxicity due to cyclophosphamide. For this reason, cyclophosphamide and other cytotoxic agents must be used cautiously, especially in elder patients. The approach to patients with pregnancy-related AHA also requires particular care because, as above mentioned, spontaneous remission of the inhibitor is possible and these patients may be treated with corticosterois alone to speed up remission, without using alkylating agents in fertile women. Furthermore, although recurrence of the inhibitor in subsequent pregnancies is rare, this possibility must be considered. It should not be overlooked that the inhibitor in the mother can influence the level of FVIII in the foetus at the time of delivery. Interesting results have been reported with the use of cyclosporine, although the experience is still not sufficient to define its real advantages. In contrast, there is solid evidence on the efficacy of anti-CD20 monoclonal antibody (rituximab), in particular in patients with relatively low-titre inhibitors resistant to other treatments. In a literature review of 65 patients treated with rituximab in association with various other immunosuppressive agents, the efficacy was greater than 90% although the lack of a control group did not allow possible biases to be excluded. Similar success rates were reported in a study in which 42 patients treated with rituximab were compared to 44 patients treated with steroids+cyclophosphamide. In the EACH2 Registry, 30 of 51 patients (59%) treated with a therapeutic regimen that included rituximab achieved stable inhibitor eradication. However, the efficacy was greater when rituximab was associated with other immunosuppressive agents, resulting in success rate similar to that observed in patients treated with steroids and cyclophosphamide (64% *versus* 70%, respectively). Rituximab has also been shown to be effective in women with post-partum inhibitor, even if the data currently available do not indicate that the monoclonal antibody produced

better results than other treatments. Rituximab should, therefore, be used as a second-line treatment in patients not responsive to treatment with steroids or a combination of steroids+cyclophosphamide and in the case of contraindications to the use of other immunosuppressive drugs. At variance with what is observed in congenital hemophilia with inhibitors, immune tolerance induction regimens have rarely been used in the treatment of AHA and the data available are not sufficient to determine whether the administration of FVIII does contribute to increasing the efficacy of immunosuppressive therapy. Side effects may occur during treatment with immunosuppressive drugs. Infections, sometimes fatal, neutropenia and diabetes mellitus are the most common adverse events, but hypertension, osteoporosis, psychosis, and cataract development following steroid use in elderly patients can also occur. Successful eradication is defined as inhibitor titre 'negative' (<0.6 BU/mL), with normal FVIII levels (>70%) once treatment is discontinued. The risk of inhibitor recurrence ranges between 10% and 20%. Prolonged follow up is necessary to determine whether inhibitors have been stably eradicated and, once the FVIII levels have been normalised, monitoring must be continued at least monthly for the first 6 months and subsequently every 3 months, depending also on the evolution of clinical conditions associated with inhibitor development. *Conclusions:* Patients with AHA must be managed by specialised Centres for the care of hemophilia and other bleeding disorders, with expertise in the treatment and laboratory monitoring of patients with inhibitors. Diagnosis should be as quick as possible because of high risk of life-threatening bleeding. Invasive procedures must be avoided in patients with a suspected acquired inhibitor until the diagnosis has been clarified. Bypassing agents (APCC or rFVIIa) are the first-line treatment for bleeding and have drastically modified the prognosis in these patients. Immunosuppressive treatment must be started as soon as possible, ideally immediately after the diagnosis has been made. The first-line treatment is oral prednisone at a daily dose of 1-2 mg/kg either alone or in combination with oral cyclophosphamide at a daily dose of 1-2 mg/kg. The use of cyclophosphamide and other alkylating agents should be avoided in fertile women. Rituximab (375 mg/m2 once a week for four doses overall) may be indicated as first-line therapy in patients with contraindications to the use of standard immunosuppressive drugs. Rituximab, alone or in combination with immunosuppressive drugs, is the main component of second-line treatment in the case of lack of response to first-line treatment within 8-12 weeks. A persistent undetectable inhibitor (<0.6 UB/mL) with normal plasma levels of FVIII (>70%) is the criterion for the definition of complete response to eradicating therapy. Once remission of the inhibitor has been achieved, patients must continue to be monitored for at least 12 months, because there is a significant risk of recurrence.

References

1. Baudo F, Collins P, Huth-Kuhne A, et al; EACH2 registry contributors. Management of bleeding in acquired hemophilia A: results from the European Acquired Haemophilia (EACH2) Registry. Blood 2012; 120: 39-46.
2. Borg JY, Guillet B, Le Cam-Duchez V, et al; SACHA Study Group. Outcome of acquired haemophilia in France: the prospective SACHA (Surveillance des Auto antiCorps au cours de l'Hemophilie Acquise) registry. Haemophilia. 2013; 19: 564-70.
3. Collins P, Hirsch S, Baglin TP, et al.; UK Haemophilia Centre Doctors' Organisation. Acquired haemophilia A in the United Kingdom: a 2-year national surveillance study by the United Kingdom Haemophilia Centre Doctors' Organisation. Blood 2007; 109: 1870-7.
4. Collins PW, Baudo F, Knoebl P, et al; EACH2 registry collaborators. Immunosuppression for acquired hemophilia A: results of the European Acquired Haemophilia (EACH) registry. Blood 2012; 120: 47-55.
5. Collins P, Chalmers E, Hart D, et al; United Kingdom Haemophilia Centre Doctors' Organization. Diagnosis and management of acquired coagulation inhibitors: a guideline from UKHCDO. Br J Haematol 2013; 162: 758-73.
6. Delgado J, Jimenez-Yuste V, Hernandez-Navarro F, Villar A. Acquired haemophilia: review and meta-analysis focused on therapy and prognostic factors. Br J Haematol 2003; 121: 21- 35.
7. Franchini M, Mannucci PM. Acquired haemophilia: a 2013 update. Thromb Haemost 2013; 110: 1114-20.
8. Knoebl P, Marco P, Baudo F, et al; EACH2 Registry Contributors. Demographic and clinical data in acquired hemophilia A: results from the European acquired hemophilia (EACH2) registry J Thromb Hemost 2012; 10: 622-31.
9. Kruse-Jarres R, St-Louis J, Greist A, et al. Efficacy and safety of OBI-1, an antihaemophilic factor VIII (recombinant), porcine sequence, in subjects with acquired haemophilia A. Haemophilia 2015;21:162-70.
10. Tengborn L, Baudo F, Huth-Kühne A, et al; EACH2 registry contributors. Pregnancy-associated acquired haemophilia A: results from the European Acquired Haemophilia (EACH2) registry. BJOG 2012; 119: 1529-37.

GRAM-POSITIVE INFECTIONS: NEW AND OLD PATHOGENS IN HAEMATOLOGICAL PATIENT

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Although the epidemiological scenario in haematological patients recently showed a progressive increase of Gram-negative (GN) infections, particularly multiresistant (Mikulska 2014), and although mortality due to GP infections is generally lower than GN and polymicrobial infections, Gram-positive (GP) infections are still a major problem in this subset of patients, particularly during uncontrolled underlying haematological disease. Many factors contribute to the high incidence of this kind of infections, such as high frequency of mucositis, frequent use of central venous catheter (CVC) and widespread use of fluoroquinolones (Fq), which were considered all responsible for the predominance of GP infections by the end of the 1980s until the beginning of 2000s (2). Moreover, antimicrobial resistance is a worrisome phenomenon also in GP bacteria among haematological patients, together with the increase/emergence of some GP strains, often understimated, such ad *C. difficile,* anaerobes and *Nocardia* spp. *Epidemiology of more frequent GP infections in haematological patients*: Staphylococci, streptococci and enterococci predominate in the GP epidemiological scenario in haematological patients. More rarely, some opportunistic GP pathogens may affect immunosuppressed haematological patients. A recent revision of the literature (1) showed that Coagulase-Negative Staphylococci (CoNS) represent the vast majority of GP bacteria isolated from bloodstream infections (BSI) in haematological patients, as they represent about 25% (range 5-60%) of all BSI. Prolonged neutropenia and use of CVC were reported as risk factors, as well as Fq prophylaxis, which has been generally associated with a reduction of GN BSI (2). On the contrary, incidence of *S. aureus* BSI is markedly lower than CoNS in haematological patients, with a median frequency of 6% (range 0-20%). These proportions seem to be confirmed also in more recent studies (3). Viridans streptococci are much more frequent than other types of streptococci and accounted for 5% of BSI (range 0-16%) (1). They may be responsible for serious complications (ARDS and septic shock) in up to 30% of cases, with consequent high mortality (up to 80% in complicated cases). Oral mucositis is generally considered the main risk factor for streptococcal infections; however, high doses of Ara-C, diarrhoea, use of proton pump inhibitors and gut decontamination were also identified as predisposing factors in a prospective french study (4). Generally, enterococci represent less than 10% of BSI in haematological patients (1-2, 5), although their incidence seems to be increased when Fq prophylaxis is not used, as reported in a recent epidemiological study (Gudiol). Once again, gastrointestinal mucositis is the main risk factor for enterococcal BSI. Enterococci tipically affect severely ill patients and often are associated to polymicrobial BSI. Among less frequent GP, *Leuconostoc* spp, *Rothia* (formerly *Stomatococcus*) *mucilaginosa* and *Corinebacterium jeikeium* are harmless commensals which may become virulent during immunosuppression causing bactaeremic episodes, often CVC-related. *Rhodococcus equi* may be responsible for suppurative pneumonitis, with abscess, effusion and empyema. *Bacillus cereus* is a GP rod which causes severe infections in neutropenic patients, including pneumonitis, meningitis and necrotizing fascitis. *Listeria monocytogenes* is also a GP rod and may also cause severe sepsis and meningitis in immunosuppressed patients, with a particular predilection for individuals who have an impairment of T-cell mediated immunity. It is acquired by humans primarily through consumption of contaminated food. Besides, between 1% and 10% of the population is a faecal carrier of *L. monocytogenes*. Mortality ranges between 20-30%. *Emerging antimicrobial resistance*: During the last years, the proportion of *methicillin resistant* (MR) stapylococci has progressively increased. This phenomenon is more evident for CoNS with respect to *S. aureus*. Indeed, in many studies carried out in Haematology wards, median MR accounts for about 80% of all CoNS, whereas the percentage of MR was lower, about 56% (range 18-100%) among *S. aureus* (1). Decreased sensibility to glycopeptides (vancomycin-intermediate *S. aureus*) has also been described, as well as selective resistance to teicoplanin. Strictly related to MR, *Fq resistance* is growing up, as a consequence of widespread use of Fq. Many studied demonstrated high incidence of MR staphylococci in patients receiving Fq (3, 6). Moreover, Fq resistance has become frequent also in streptococci and enterococci. *Penicillin-resistant* streptococci are reported in up to 30-40% of cases; as a result of the increased resistance of these microorganisms to various β-lactam agents, it is recommended to include vancomycin in their initial empirical antimicrobial regimens for neutropenic febrile patients with

severe mucositis. The emergence of *vancomycin-resistant* enterococci (VRE) (observed in *E. faecium* more frequently than *E. faecalis*), has been described as a growing and worrisome phenomenon, particularly in the United States (7), with percentage of rectal colonization up to 40% and consequent increased risk of VRE BSI with high mortality. European studies described a more limited phenomenon, as the percentage of colonized patients rarely exceeded 10%. Antibiotic pressure, particularly third-generation cephalosporin and vancomycin, was responsible for VRE emergence. *New antibiotics active agains multiresistant GP bacteria*: Despite a rapidly diminishing antibiotic pipeline over time, at the beginning of 2000s, some new antibiotics active against multiresistant GP bacteria have been commercialized. Some of them have become part of routine therapy, such as tigecycline, the first drug approved in the class of glycylcyclines, linezolid, a synthetic oxazolidonone, and daptomycin, a cyclic lipopeptide. Quinupristin-dalfopristin, a streptogramin, is also active on multiresistant GP bacteria, but it is less used for the dose-limiting adverse effects. Very recently new antimicrobials became available for the treatment of multiresistant GP bacteria. The new cephalosporines ceftaroline and ceftobiprole show activity against MRSA. Dalbavancin, telavancyn and oritavancin, all belonging to the class of lypoglicopeptides, are active also on VRE and have excellent activity on skin and soft tissue infections, as well as tedizolid, a new oxazolidonone. Table 1 summarizes the new treatment options for multiresistant GP bacteria. *C. difficile* infection: In a recent epidemiological study conducted in Germany, incidence of *C. difficile* infection (CDI) was 11.8% in acute myeloid leukaemia patients and 13.1% in stem cell transplantation (SCT) recipients (8). Haematological cancer and SCT patients are particularly predisposed to CDI, because of their immunosuppressed state and the high rate of antibiotic pressure. Increased use of Fq seems to be associated with the emergence of hyper-virulent strains of *C. difficile*. Severe CDI (severe colitis or a complicated course of disease, with significant systemic toxin effects and shock, resulting in need for ICU admission, colectomy or death) occurs in 8% of cases whereas recurrent CDI refers to about 20-25% of cases. Together with older age, immunosuppression is still a risk factor. Proton pumps inhibitors, often used in haematological patients, are also associated with recurrent CDI. Concerning the treatment of CDI, oral antibiotic treatment is indicated, with metronidazole for mild or moderate CDI and vancomycin for more severe cases. Both metronidazole and vancomycin treatments are associated with disruption of the commensal microbiota, predisposing to emergence of VRE clones and to recurrence of CDI. On the other hand, fidaxomycin, the first member of a new class of antibiotics called macrocycles, seems to have minimal effect on the constituents of the normal colonic microflora, thus preventing recurrence. New approaches for recurrent CDI include faecal microbiota transplantation and immunotherapy with specific anti-toxin antibodies to *C. difficile*. *GP anaerobes bacteria*: Anaerobes represent less than 5% of all BSI in neutropenic patients (9). Despite its low incidence, anaerobe BSI are often severe and life threatening infections, with high mortality. Mucositis is the predisposing factor for bacterial translocation through the intestinal wall. Up to 50% of anaerobes BSI are polymicrobial, often associated with *E. coli* or *Enterococcus* spp. *Clostridium* spp, most commonly *C. perfringens* and, to a lesser extent, *C. tertium* and *C. septicum* are the most frequent anaerobic GP infections. They have the ability to release neurotoxic exotoxins and histotoxins, leading to gas gangrene, widespread necrotizing soft tissue infection, and death. Management of the infection begins with broad-spectrum antibiotics, but early and aggressive drainage and meticulous debridement constitute the mainstay of treatment. Together with other pathogens, anaerobes are often involved in the pathogenesis of neutropenic enterocolitis (NEC). Neutropenic patients with fever and abdominal symptoms (cramping, pain, distention, diarrhoea, gatrointestinal bleeding), should undergo evaluation of the abdomen for bowel wall thickening of >4 mm, the hallmark of NEC. Complications include bacteraemia, often polymicrobial, haemorrhage, and bowel wall perforation/abscess formation. Broad-spectrum antibiotic therapy for NEC should always include anti-anaerobes agents. *Nocardia* spp infections*: Nocardia* spp are GP aerobic bacilli contaminating soil, water and dust. Although uncommon, disseminated infections are severe and life-threatening and affect mainly immunodeficient patients. Typical localizations are lung, but central nervous system (CNS), soft tissue, blood and lymph nodes are also involved in disseminated nocardiosis. Due to the rarity of the disease and to the relative difficulty and slowness of cultural growth, the diagnosis of *Nocardia* spp infection may be underestimated. Differential diagnoses include bacterial lung abscess, aspergillosis or other invasive mould infections, actinomycosis, tubercolosis and other malignancies. The recent introduction in clinical practice of new, highly immunosuppressive therapeutic agents also in the non-transplant setting, may be responsible of an increased incidence of nocardiosis observed during the last years. Deficiency of cell-mediated immunity seems to be the main predisposing factor for developing nocardiosis among different patients' categories. Of note, mortality due to nocardiosis ranges between 7 and 44%; disseminated infections, with nocardemia and brain abscesses, are responsible for an even higher risk of death. A recent retrospective study conducted within the SEIFEM Group (10) revealed that lymphoproliferative disorders, prolonged steroid treatment, lymphopenia and active haematological disease were the conditions considered as predisposing factors for developing *Nocardia* spp infections. Therefore, although rare, nocardiosis should be considered in the differential diagnosis of pulmonary and CNS lesions among haematological patients. *Conclusions*: GP bacteria still represent a relevant proportion of infections among haematological patients. Although mortality is generally lower than GN infections, some strains, such as viridans streptococci and anarobes, may be responsible for fatal syndromes. Emerging antibiotic resistance is also associated with increased morbidity and mortality and requires active surveillance program. Rare and opportunistic GP bacteria may affect heavily immunosuppressed patients, with impaired T-cell mediated toxicity, and may rapidly evolve to unfavourable outcome. Accurate knowledge of local epidemiology and of the patient's medical history may guide the choice of appropriate empiric treatment, which is, in turn, the best predictor of good prognosis.

Table 1. Principal antibiotics actives against multiresistant GP bacteria.

Drug	Class	Route of administration	Multiresistant GP target	Notes
Vancomycin	Glycopeptides	IV	MRSA Pen-res streptococci	"slow" bactericidal
Daptomycin Dalbavancin Telavancin Oritavancin	Lypoglycopeptides	IV	MRSA Pen-res streptococci VRE	Bactericidal; Not pneumonia (daptomycin)
Quinupristin-dalfopristin	Streptogramines	IV	MRSA Pen-res streptococci VRE (*E. faecium*)	Bactericidal; Dose-limiting adverse effects
Quinupristin-dalfopristin	Streptogramines	IV	MRSA Pen-res streptococci VRE (*E. faecium*)	Bactericidal; Dose-limiting adverse effects
Linezolid Tedizolid	Oxazolidonone	IV or OS	MRSA Pen-res streptococci VRE	Bacteriostatic, caution with BSI
Ceftaroline Ceftobiprole	Cefalosporins	IV	MRSA Pen-res streptococci	Bactericidal

Pen-res: peicillin-resistant

References

1. Mikulska M, Viscoli C, Orasch C, Livermore DM, Averbuch D, Cordonnier C, Akova M. Aetiology and resistance in bacteraemias among adult and paediatric haematology and cancer patients. J Infect. 2014 Apr;68(4):321-31.
2. Ramphal R. Changes in the etiology of bacteremia in febrile neutropenic patients and the susceptibilities of the currently isolated pathogens. Clin Infect Dis. 2004 Jul 15;39 Suppl 1:S25-31.
3. Gudiol C, Bodro M, Simonetti A, Tubau F, González-Barca E, Cisnal M, et al. Changing aetiology, clinical features, antimicrobial resistance, and outcomes of bloodstream infection in neutropenic cancer patients. Clin Microbiol Infect. 2013 May;19(5):474-9.

4. Cordonnier C, Buzyn A, Leverger G, Herbrecht R, Hunault M, Leclercq R, Bastuji-Garin S. Epidemiology and risk factors for gram-positive coccal infections in neutropenia: toward a more targeted antibiotic strategy. Clin Infect Dis. 2003 Jan 15;36(2):149-58.
5. Klastersky J, Ameye L, Maertens J, Georgala A, Muanza F, Aoun M, et al. Bacteraemia in febrile neutropenic cancer patients. Int J Antimicrob Agents. 2007 Nov;30 Suppl 1:S51-9.
6. Cattaneo C, Quaresmini G, Casari S, Capucci MA, Micheletti M, Borlenghi E, et al. Recent changes in bacterial epidemiology and the emergence of fluoroquinolone-resistant Escherichia coli among patients with haematological malignancies: results of a prospective study on 823 patients at a single institution. J Antimicrob Chemother. 2008 Mar;61(3):721-8.
7. Patel R. Clinical impact of vancomycin-resistant enterococci. J Antimicrob Chemother. 2003 Jun;51 Suppl 3:iii13-21.
8. Vehreschild MJ, Weitershagen D, Biehl LM, Tacke D, Waldschmidt D, Töx U, et al. Clostridium difficile infection in patients with acute myelogenous leukemia and in patients undergoing allogeneic stem cell transplantation: epidemiology and risk factor analysis. Biol Blood Marrow Transplant. 2014 Jun;20(6):823-8.
9. Mathur P, Chaudhry R, Kumar L, Kapil A, Dhawan B. A study of bacteremia in febrile neutropenic patients at a tertiary-care hospital with special reference to anaerobes. Med Oncol. 2002;19(4):267-72.
10. Cattaneo C, Antoniazzi F, Caira M, Castagnola C, Delia M, Tumbarello M, et al. Nocardia spp infections among hematological patients: results of a retrospective multicenter study. Int J Infect Dis. 2013 Aug;17(8):e610-4.

BIOLOGIC BASES OF ACUTE LYMPHOBLASTIC LEUKEMIA AND IDENTIFICATION OF NOVEL THERAPUTIC TARGETS

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Abstract: Acute lymphoblastic leukemia (ALL) is a neoplastic malignancy of B- or T-lymphoblasts which requires a rapid and accurate diagnostic process to support an optimal risk-oriented therapy and thus increase the curability rate. The recognition of novel subsets has been made possible by the introduction of high-troughput technologies and is pivotal not ony for a better patients stratification, but also for the definition of novel molecular lesions which might be regarded as therapeutic targets. The use of tyrosine kinase inhibitors (TKI) in patients with Ph+ ALL represents a successful example of targeted therapy. Other subsets that might benefit from the integration with specifc therapies are represented by the so-called BCR/ABL-like cases; furthermore, it is clearly emerging that the JAK/STAT and RAS pathways are often deregulated in several cases. In T-ALL, a plethora of alterations affecting *NOTCH1*, the JAK/STAT and the PI3K/AKT pathway have been identified; moreover, the recognition of Early T-cell precursor (ETP) cases has permitted to recognize cases with peculiar biological features and, possibly, a worse outcome. Altogether, these findings, on one hand, help identifying the molecular origin of ALL, and on the other pave the way for novel strategies. *Introduction:* Acute lymphobastic leukemia (ALL) is a malignant disorder that originates from hemopoietic precursors of B-cell (80-85%) or T-cell (20-25%) derivation: the acquisition of a series of genetic aberrations leads to an impaired maturation, with an arrest in the differentiation process and an abnormal proliferation. As a consequence, the accumulation of leukemic cells occurs in both the bone marrow, where it suppresses the physiologic hemopoiesis, as well as in extra-medullary sites. ALL is the most common neoplasm in childhood, with the highest peak of incidence in children comprised between 2 and 5 years, whereas it is rather rare in adulthood, although a second peak can be recorded in the elderly. Beyond the different incidence of the disease and possibly the initiation causes, outcome varies profoundly between children and adults. In fact, to date, the majority of children can be considered curable, while the prognosis of adults is still extremely poor, with only 40% of individuals free of leukemia in the long term. While a set of genetic lesions with prognostic value and therapeutic implications are well-recognized since a long-time, for a large fraction of patients a causal genomic lesion has not been fully identified and/or characterized. Consequently, there is a need to improve the molecular dissection of subtypes, thus identifying genetic alterations that might predict the risk of treatment failure and might be useful in developing novel and targeted therapies. *Conventional cytogenetic/genetic risk groups:* Several genetic lesions have been identified over time. Among the good prognosis aberrations, it is worth mentioning del(12p) or t(12p)/t(12;21)(p13;q22) in B-lineage ALL, and t(10;14)(q24;q11) in T-ALL. These abnormalities are relatively rare in adults compared with childhood ALL. Aberrations associated with an intermediate-risk comprise the normal diploid subset plus cases with hyperdiploidy and several other recurrent or random chromosomal abnormalities. Other aberrations, *i.e.* those with isolated trisomy 21, trisomy 8, and perhaps del(6q) and t(1;19)(q23;p13)/E2A-PBX1 may constitute an intermediate-high risk group. Other recently identified aberrations in the intermediate high-risk group are represented by iAMP21 and IGH rearrangements, including *CRLF2*. Finally, patients with t(9;22)(q34;q11) or BCR-ABL1 rearrangements or a positive FISH test (Ph+ ALL), t(4;11)(q21;q23) or MLL rearrangements at 11q23, monosomy 7, hypodiploidy/low hypodiploidy (and the strictly related near triploid group) fall into the poor-risk cytogenetic category, with an overall disease-free survival (DFS) rate of about 25%, or 10% in the case of Ph+ ALL prior to the introduction of tyrosine kinase inhibitors (TKI). Ph+ ALL constitute the most frequent abnormality in the adult/elderly; the role of secondary chromosome abnormalities, beyond *IKZF1* deletion (see below), in addition to t(9;22)(q34;q11) is still under investigation. The management and outcome of Ph+ ALL have dramatically changed since the introduction of tyrosine kinase inhibitors (TKI): the use of TKIs represents the most successful example of a targeted approach, since in these patients, previuosly regarded as those with the most dismal prognosis, it is now possible to achieve hematological (and in some cases cytogenetic/molecular) remissions in >90% cases, and survival has greatly imporved, also in elderly patients. *New genetics and genomics in ALL:* The integration of results of several techniques, *i.e.* gene expression profiling (GEP), SNP array analysis, and currently next-generation sequencing (NGS), have permitted a better definition of the molecular scenario of ALL and the identification of a constellation of novel mutations that might be regarded as therapeutic targets. *B-lineage ALL: IKZF1*, encoding for the transcription factor Ikaros, is frequently disrupted in Ph+ ALL (80% of cases). *IKZF1* deletions, that can be different in size, are predictors of poor outcome in Ph+ ALL, as well as in non-Ph+ ALL. Deregulated overexpression of *CRLF2* (CRLF2), found exclusively in 5-10% B-ALL cases without known molecular rearrangements is usually sustained by two types of aberrations: a rearrangement that involves *CRLF2* and the Ig heavy chain locus (IGH@-CRLF2) or an interstitial PAR1 deletion that juxtaposes intron 1 of *P2RY8* to the coding region of *CRLF2* itself. More rarely, *CRLF2* mutations can be detected. -CRLF2 is frequently documented together with *IKZF1* deletion in Ph-negative ALL patients and with *JAK* mutations (*JAK1* or *JAK2*) or *IL7R* mutations; furthermore, they are identified in roughly 50% of children with Down syndrome. Although some contrasting results have been reported, its presence correlates with an overall poor outcome. By the integration of genome-wide technologies, the "BCR/ABL-like" subgroup has been suggested/identified in both the adult and pediatric populations and it accounts for about 15% of B-ALL cases. This subgroup is characterized by a gene expression signature that is similar to that of BCR/ABL+ patients, frequent detection of *IKZF1* deletions and *CRLF2* rearrangements, JAK members mutations and a dismal outcome. NGS has revealed the presence of mutations and/or rearrangements activating tyrosine kinases, i.e *IGH-CRLF2*, *NUP214-ABL1* rearrangements, in-frame fusions of *EBF1-PDGFRB*, *BCR-JAK2* or *STRN3-JAK2* and cryptic *IGH-EPOR* rearrangements. The recognition of this subgroup is of relevance, for 2 main reasons: 1) prognosis in these patients is usually poor, although it has been recently shown, in childhood cases, that MRD-based risk-directed therapy, including transplant procedures, might overcome the dismal outcome; 2) given the plethora of alterations affecting several tyrosine kinase and their downstream targets, it is plausible that the use of TKIs and/or mTOR inhibitors might be of benefit in these patients. However, the recognition of these cases relies mostly on gene expression profiling, which is not routinely performed in all centers, and by the fact that there is not a recurrent common lesion underlying the signature identified. Current efforts are ongoing to easily and rapidly identify these cases. Hypodiploid ALL, regarded as a poor prognosis group, has been extensively evaluated in pediatric ALL: NGS proved that lesions involving receptor tyrosine kinases and RAS signaling (*i.e. NRAS*, *KRAS*, *FLT3* and *NF1*) can be detected in up to 70% of near haploid cases, whereas low hypodiploid cases are characterized by lesions involving members of the Ikaros family, particularly *IKZF2*, and by *TP53* disruptions, that can be identified in 91.2% of these cases. In adult ALL, these cases are characterized by nonrandom chromosomal losses and the *CDKN2A/B* locus deletion as sole recurrent abnormality; as already reported in children, these cases frequently harbor *TP53* mutations. *TP53* disruption has been also recently evaluated in childhood and adult ALL. In children this is detected in

6.4% and 11.1% of relapsed B-ALL and T-ALL cases, and, in a smaller minority of cases, also at diagnosis. A correlation with poorer outcome has been shown. In adults, *TP53* mutations are identified at diagnosis in 8.2% of cases (11.1% T-ALL and 6.4% B-ALL), and are preferentially identified in cases without molecular aberrations, where they are detected in 12.5% of cases, and are associated with refractoriness to chemotherapy. Other lesions identified by NGS in B-lineage ALL, are represented by mutations in *CREBBP* and its paralogue, *EP300* (p300), which were identified in the relapse samples and appear to be more frequent in hyperdiploid relapsed cases. Similarly, *NT5C2* mutations, which confer increased enzymatic activity on the NT5C2 protein, which normally dephosphorylates nucleoside analogs, such as mercaptopurine, used in consolidation and maintenance therapy, have been described at relapse. Results are summarized in Table 1. *T-lineage ALL:* In T-ALL, well-recognized aberrations include the T-cell receptor (TCR) gene rearrangements, chromosomal deletions, and focal gene deletions. Moreover, chromosomal rearrangements can also lead to in-frame fusion genes encoding chimeric proteins with oncogenic properties such as *PICALM-MLLT10*, *NUP214-ABL1* fusion formed on episomes, *EML-ABL1*, *SET-NUP214* fusion and *MLL* gene rearrangements with numerous different partners. The prognostic significance of these lesions is uncertain. Furthermore, the ETP subgroup and/or myeloid-like subgroup emerged as a grey zone between AML and T-ALL by applying genome-wide technologies. Initially, the reported incidence of this subgroup was established at around 10% of T-ALL cases; however, with the better recognition of these cases, its frequency is likely to be higher. Immunophenotype is characterized by an early T-cell phenotype and co-expression of at least one myeloid marker, while at the transcriptional level they have a stem-cell like profile with overexpression of myeloid transcription factors (including *CEBPA*, *CEBPB*, *CEBPD*), and a set of micro-RNAs (miR-221, miR-222 and miR-223). NGS has highlighted the presence of mutations in the *ETV6* gene, as well as in genes often altered in acute myeloid leukemia (*IDH1*, *IDH2*, *DNMT3A*, *FLT3* and *NRAS*); interestingly, *FLT3* mutations can be detected in more than 30% of cases. Finally, these cases rarely harbor *NOTCH1* mutations. Overall, prognosis is poor in these cases, though their outcome seems improved by the current risk-adapted approaches. A large set of mutations (Table 2) has been identified in T-ALL by re-sequencing and NGS: they include *NOTCH1*, *FBW7*, *BCL11B*, *JAK1*, *PTPN2*, *IL7R* and *PHF6*, beyond those identified in ETPs; some of them have recognized prognostic significance, whereas for others further studies are required. In fact, *NOTCH1* and/or *FBW7* mutations, which occur in more than 60% and about 20% of cases, respectively, are usually associated with a favorable outcome. In the light of this, a prognostic model has been recently proposed, defining as low-risk patients those who harbor *NOTCH1* and *FBW7* mutations, and as high risk those without these mutations or harboring lesions involving *RAS/PTEN*. At variance, *JAK1* mutations, which increase JAK activity and alter proliferation and survival have been associated with chemotherapy refractoriness and should be considered as poor prognostic markers. Finally, another group of mutations/lesions is possibly involved in leukemogenesis, but their prognostic impact is either unknown or absent. They include: 1) *BCL11B* lesions, which can induce a developmental arrest and aberrant self-renewal activity; 2) *PTPN2* - a negative regulator of tyrosine kinases-, mutations, often detected in TLX1 overexpressing cases, NUP214-ABL+ patients and *JAK1* mutated cases; 3) mutations in *IL7R*, that lead to constitutive JAK1 and JAK3 activation and enhancement of cell cycle progression; 4) *PHF6* mutations; 5) mutations in *PTPRC*, encoding the protein tyrosine phosphatase CD45, usually detected in combination with activating mutations of *IL7R*, *JAK1* or *LCK*, and associated with downregulation of CD45 expression; 6) mutations in *CNOT3*, presumed to be a tumor suppressor; 7) mutations of *RPL5* and *RPL10*, which impair ribosomal activity. Lastly, similarly to what is observed in relapsed B-ALL, *NT5C2* mutations have been idientified. *Concluding remarks:* The use of avant-garde molecular techniques is permitting to unravel novel subgroups in ALL - a disease with a complex genomic background - which is pivotal for a better prognostic stratification. Importantly, sevel compounds are currently available against the most recurrent druggable lesions. The most frequent involve tyrosine kinases, members of JAK/STAT pathway, RAS pathway members and of mTOR pathway: several compounds (Table 3), thus their use, possibly within prospectve clinical trials, might indeed be beneficial for such patients.

Table 1. Summary of novel lesion identified in B-lineage ALL.

	Gene/s involved	Functional consequences	Frequency		Clinical relevance
			Children	Adults	
Genomic lesions					
Focal deletions; rarely mutations	*IKZF1*, 7p13-p11.1	Deregulation of lymphoid differentiation	15%; >80% BCR-ABL pos; ~30% HR BCR-ABL-	7%; > 80% BCR-ABL+	Poor outcome
Rearrangement; interstitial Par1 deletion; mutations	*CRLF2*, Xp22.3; Yp11.3	Together with JAK mutations, constitutive JAK-STAT activation	5-10%;>50 DS-ALL	5-10%	Poor outcome
Mutations	*JAK1*, 1p32.3-p31.3 *JAK2*, 9p24	Constitutive JAK-STAT activation	~10% HR-BCR-ABL+; 18%–35% DS-ALL	-	Associated with *CRLF2*, *IKZF1*, poor outcome
Focal deletions; mutations	*CREBBP*, 16p13.3, *EP300*, 22q13.2	Impaired histone acetylation and transcriptional regulation	18% of relapsed ALL		Increased incidence at relapse; association with glucocorticoid resistance.
Focal deletions; mutations	*NT5C2*, 10q24.32	Increased dephosphorylation of nucleoside analogs	10% of relapsed ALL (also in T-ALL)		Identified only at relapse
Intrachromosomal amplification of chr 21	*RUNX1*, 21q22.3	Multiple copies of the RUNX1 gene; possible secondary event	2%	-	Poor outcome
TP53 disruption	*TP53*, 17p13.1	Mutations and/or deletions	90% hypodiploid ALL 6-11% relapsed childhood ALL (also in T-ALL)	8% of ALL at onset of disease (also in T-ALL)	Poor outcome
Novel subgroups					
BCR/ABL-like	Causal gene not known Possible: *IGH@CRLF2*, *NUP214 - ABL1*, *EBF1-PDGFRB*, *BCR-JAK2*, *STRN3-JAK2*, *IGH@-EPOR*, *Δ-CRLF2* *IKZF1* deletion	BCR/ABL-like signature	17%	20%	Possibly poor outcome

HR: high-risk; DS-ALL: Down syndrome ALL

Table 2. Gene mutations in T-ALL.

Gene/s involved	Gene position	Functional consequences	Frequency		Clinical relevance
			Children	Adults	
NOTCH1	9q34.3	Impairment of differentiation of and proliferation	60-70%		Overall favorable outcome
FBW7	4q31.3	Arrest of differentiation, and aberrant self renewal activity	~10%	~10-20%	Usually evaluated in combination with *NOTCH1*
BCL11B	14q32.2	Loss of cell proliferation control	9%	-	Not defined
JAK1	1p32.3-p31.3	Cytokine growth independence, resistance to dexamethasone-induced apoptosis, JAK signaling activation	2%	7-18%	Unfavorable outcome
PTPN2	18p11.3-p11.2	Negative regulator of tyrosine kinases	6%	-	No impact
IL7R	5p13	Lymphoid development	6%	-	No impact
PHF6	Xq26.3	Putative tumor suppressor	5-16%	18-38%	No impact
ETV6 *IDH1* *IDH2* *DNMT3A* *FLT3* *NRAS* *JAK3* *IKZF1*	12p13 2q33.3 15q26.1 2q33.3 13q12 1p13.2 19p13.1 7p13-p11.1	Various, including: signaling, developmental arrest, histone modification	Detected in ETP leukemia		Unfavorable, as per subgroup of the disease
CNOT3	19q13.4	Presumed tumor suppressor	-	8%	Not known
RPL5 and *RPL10*	1p22.1 Xq28	Ribosomal activity impairment	8%	-	Not known
NT5C2	10q24.32	Increased dephosphorylation of nucleoside analogs	19% of relapsed ALL		Identified only at relapse

Table 3. Molecular lesions and potential compounds.

Lesion	Gene/s involved	Drug
t(9;22)(q34;q11)/*BCR-ABL1*	*BCR/ABL1*	First, second or third generation TKI
11q23/*MLL* gene rearrangement	*MLL*, *DOTL1*, *FLT3*	Epigenetic therapy (eg, DNA methyltransferase inhibitor, histone methyltransferase inhibitor, histone deacetylace inhibitor), possibly FLT3-inhibitors
Hypodiploidy	TP53, RAS/RTK/PI3K pathways	MEK inhibitors, PI3K inhibitors
Hyperdiploidy	RAS pathway	MEK inhibitors
BCR/ABL-like	*IGH@CRLF2*, *NUP214 - ABL1*, *EBF1-PDGFRB*, *BCR-JAK2*, *STRN3-JAK2*, *IGH@-EPOR*, *Δ-CRLF2*	TKI, depending on the type of fusion
CRLF2 rearrangements	*CRLF2*	JAK inhibitors mTOR inhibitors
JAK1 or *JAK2* mutations	*JAK1 or JAK2*	JAK inhibitors
CREBBP mutation	*CREBBP*	Histone deacetylase inhibitors

References

1. Bassan R, Hoelzer D. Modern therapy of acute lymphoblastic leukemia. J Clin Oncol. 2011;29:532-543.
2. Pullarkat V, Slovak ML, Kopecky KJ, Forman SJ, Appelbaum FR. Impact of cytogenetics on the outcome of adult acute lymphoblastic leukemia: results of Southwest Oncology Group 9400 study. Blood. 2008;111:2563-2572.

3. Martinelli G, Iacobucci I, Storlazzi CT, et al. IKZF1 (Ikaros) deletions in BCRABL1-positive acute lymphoblastic leukemia are associated with short disease-free survival and high rate of cumulative incidence of relapse: a GIMEMA AL WP report. J Clin Oncol. 2009; 27:5202-5207.
4. Mullighan CG, Su X, Zhang J, et al: Children's Oncology Group: Deletion of IKZF1 and prognosis in acute lymphoblastic leukemia. N Engl J Med. 2009; 360:470-480.
5. Hunger SP, Mullighan CG. Redefining ALL classification: toward detecting high-risk ALL and implementing precision medicine. Blood. 2015 Jun 25;125:3977-3987.
6. Moorman A. New and emerging prognostic and predictive genetic biomarkers in B-cell precursor acute lymphoblastic leukemia. Hematology Education: the education program for the annual congress of the European Hematology Association. 2015;9:7-16.
7. Coustan-Smith E, Mullighan CG, Onciu M, et al. Early T-cell precursor leukaemia: a subtype of very high-risk acute lymphoblastic leukaemia. Lancet Oncol 2009;10:147-156.
8. Trinquand A, Tanguy-Schmidt A, Ben Abdelali R, et al. Toward a NOTCH1/FBXW7/RAS/PTEN-based oncogenetic risk classification of adult T-cell acute lymphoblastic leukemia: a Group for Research in Adult Acute Lymphoblastic Leukemia study. J Clin Oncol. 2013;31:4333-4342.
9. Roberts KG, Li Y, Payne-Turner D, et al. Targetable kinase-activating lesions in Ph-like acute lymphoblastic leukemia. N Engl J Med 2014;371:1005-1015.
10. Roberts KG, Pei D, Campana D, et al. Outcomes of children with BCR-ABL1–like acute lymphoblastic leukemia treated with risk-directed therapy based on the levels of minimal residual disease.

ALLOGENEIC STEM CEL TRANSPLANTATION FOR LYMPHOPROLIFERATIVE SYNDROMES IN THE NOVEL AGENTS ERA.

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During the last years great progress has been made in treating patients with lymphoproliferative disorders. One the most significant improvements was achieved in chronic lymphocytic leukemia (CLL) where novel agents such as new Monoclonal Antibodies (Ofatumumab and Obinotuzimab) and mainly BCR signaling inhibitors have dramatically changed our therapeutic approaches.1 Indeed, these agents were able to obtain really interesting and apparently longer responses among relapse refractory CLL patients, including fludarabine refractory and patients harboring del17p13/TP53 mutation.2-3 Nevertheless, recent studies have reported that patients with complex caryotyped and/or TP53 gene mutation or deletion have a higher risk to develop distinct mutations able to confer ibrutinib resistance.4 Probably, due to this reason, these patients have been reported to be characterized by inferior outcome and survival compared to others undergoing Ibrutinib treatment.5 Despite a higher proportion of patients are sensible to BTK and/or PK3 inhibitor therapies, complete remission (CR) and minimal residual disease negativity are rarely or never achieved.1 Nowadays, few data are actually available about the efficacy of combinations between novel inhibitory agents and standard chemo-immunotherapy regiments such as FCR or R-Bendamustine among young and fit CLL patients. Allogeneic stem cell transplantation (HSCT) represents the only potentially curative approach in CLL.6-7 For many years HSCT has been considered the treatment of choice for patients with high risk CLL defined as: 1) refractory to purine analogs, 2) short response [<24 months] to chemo-immunotherapy (*i.e.* FCR or R-B), and/or 3) presence of del[17p]/TP53mutations. indeed, HSCT is able to overcome all these poor prognostic factors without its efficacy been affected by novel recurrent mutations (NOTCH and SF3B1).8-9 The CLL relapse risk after HSCT is influenced by many clinical and biological features, with the most relevant one being represented by the disease status before transplant. Actually no data have yet demonstrated or suggested novel agents superiority over HSCT among high risk CLL patients. Novel agents may offer an interesting opportunity to improve the response rate and quality before HSCT among young and fit patients and for this reason they should be integrated in a complex therapeutic approach before HSCT consolidation (Figure 1). The connection between HSCT and novel agents may also be extended to the immune recovery following transplant, where drugs, such as Ibrutinib, seemed to be able to modulate the immune activity decreasing GVHD incidence without affecting disease control.10 In conclusion, HSCT actually remains the optimal approach for high risk CLL and the only therapeutic strategy able to achieve disease eradication. Future improvement in CLL biology knowledge and novel drugs combinations may be of great help overcoming the poor prognosis of high risk CLL patients, reducing, and eventually overcoming, the HSCT practice.

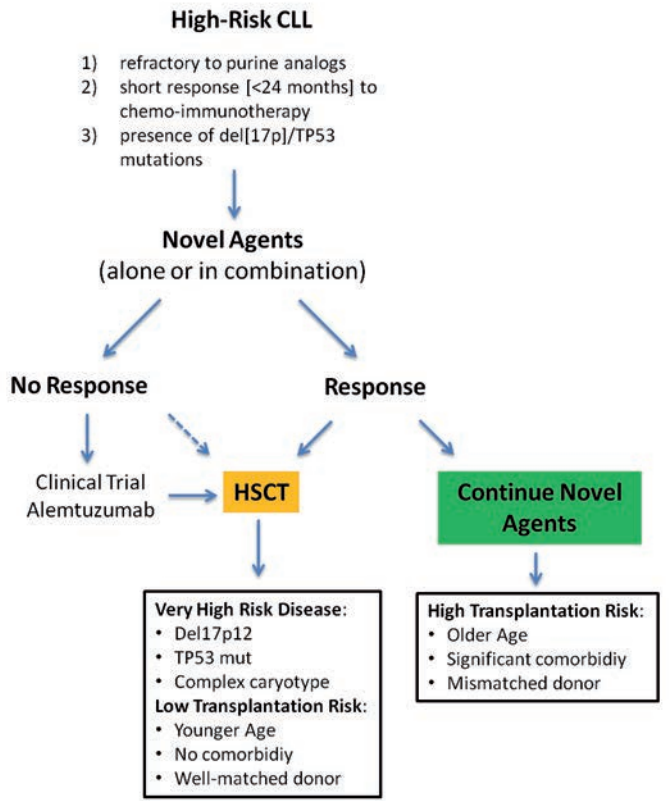

Figure 1. Decision tree for High Risk-CLL. (NA, novel agents).[7]

References

1. Brown JR, Porter DL, O'Brien SM. Novel treatments for chronic lymphocytic leukemia and moving forward. Am Soc Clin Oncol Educ Book. 2014;e317-25.
2. Farooqui MZ, Valdez J, Martyr S et al. Ibrutinib for previously untreated and relapsed or refractory chronic lymphocytic leukaemia with TP53 aberrations: a phase 2, single- arm trial. Lancet Oncol. 2015;16(2):169-76.
3. Stephens DM, Ruppert AS, Jones JA et al. Impact of targeted therapy on outcome of chronic lymphocytic leukemia patients with relapsed del (17p13.1) karyotype at a single center. Leukemia. 2014;28(6):1365-1368.
4. Woyach JA, Furman RR, Liu TM et al. Resistance mechanisms for the Bruton's tyrosine kinase inhibitor ibrutinib. N Engl J Med. 2014;370(24):2286-94.
5. Byrd JC, Furman RR, Coutre SE et al. Three-year follow-up of treatment-naïve and previously treated patients with CLL and SLL receiving single-agent ibrutinib. Blood. 2015;125(16):2497-506.
6. Dreger P, Corradini P, Kimby E et al. Chronic Leukemia Working Party of the EBMT. Indications for allogeneic stem cell transplantation in chronic lymphocytic leukemia: the EBMT transplant consensus. Leukemia. 2007;21(1):12-17.
7. Dreger P, Schetelig J, Andersen N et al. Managing high-risk CLL during transition to a new treatment era: stem cell transplantation or novel agents‡; European Research Initiative on CLL (ERIC) and the European Society for Blood and Marrow Transplantation (EBMT). Blood. 2014;124(26):3841-9.
8. Herth I, Dietrich S, Benner A et al. The impact of allogeneic stem cell transplantation on the natural course of poor-risk chronic lymphocytic leukemia as defined by the EBMT consensus criteria: a retrospective donor *versus* no donor comparison. Ann Oncol. 2014;25(1):200-6.
9. Dreger P, Schnaiter A, Zenz T et al. TP53, SF3B1, and NOTCH1 mutations and outcome of allotransplantation for chronic lymphocytic leukemia: six-year follow-up of the GCLLSG CLL3X trial. Blood. 2013;121(16):3284-8.
10. Dubovsky JA, Flynn R, Du J et al. Ibrutinib treatment ameliorates murine chronic graft- *versus*-host disease. J Clin Invest. 2014;124(11):4867-76.

EMERGING CLINICAL COMPLICATION IN SICKLE CELL DISEASE

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Introduction: Sickle cell disease (SCD) is a hereditary red cell disorder related to a point mutation in the β-globin gene that results in the synthesis of the pathological hemoglobin S (HbS). SCD is a chronic and invalidating disorder, associated with still high mortality. Epidemiological studies suggest an increasing global burden of SCD between 2010 and 2050, making SCD an emerging problem of public health with limited therapeutic options. In fact, the years lived with disability (YLDs)

for hemoglobinopathies and SCD are estimated to be 10,197, which is a dramatic observation since the YLDs for cardiovascular disorders are 21,985. The disability-adjusted life years (DALYs) used to measure the disease burden for hemoglobinopathies and SCD is estimated to be 15,640, which is impressive compared to the DALYs for diabetes that is 75,000 (1, 2). The two main clinical manifestations of SCD are chronic hemolytic anemia and acute vaso-occlusive crisis (VOCs). The generation of VOCs results from a complex and partially known series of factors such as the interaction among different cell types, including red blood cells (RBCs), abnormally activated endothelial cells, leukocytes and plasma factors. SCD chronic hemolytic anemia results in saturation of physiological protective systems involved in binding free hemoglobin (Hb) (haptoglobin; Hp) or free heme (hemopexin; Hpx). As a result, free-Hb and free-heme are present in high levels in the peripheral circulation and leads to nitric oxide (NO) binding, highly pro-oxidant/pro-inflammatory environment that promotes vascular instability and dysfunction (3). Thus, vascular dysfunction is a key element in the pathogenesis of acute and chronic clinical manifestations of SCD, representing a relevant frontier of investigation. The therapeutic tools in SCD are still limited to transfusion regimes and/or hydroxycarbamide (HU) or bone marrow transplantation in highly selected SCD patients. *Sickle cell disease: a monogenic disorder but a multiorgan disease:* Recent studies on mortality and morbidity of SCD patients have shown an amelioration of survival for SCD pediatric population related to the introduction of screening strategies and antibiotic profilaxis. Whereas, the shape of curves for mortality of young-adult SCD patients seems unchanged, indicating the persistence of high mortality for SCD subjects in the second and third decades of their life (4). Young-adults with SCD have generally higher medical resources utilization compared to pediatric population both as number of hospitalization and as emergency department visits. This might be related to different factors such as (i) the increase age related clinical complications due to SCD; (ii) the inadequate care for adult patients with SCD; (iii) the lack of adherence to iron chelation therapy in chronically transfused patients; (iv) the lack of adherence to HU treatment. In addition, acute VOCs are still a life-threatening complication in young-adult patients with SCD and are responsible for 33% deaths of relative healthy adult SCD patients. Chronic organ complications related to SCD are responsible for 18% of deaths in young-adult SCD patients. A recent study on causes for early death in young adult patients with SCD has identified the following risks factors: (i) Hb levels independently from HU treatment;(ii) total leukocyte count (in patients off HU); (iii) glomerulal filtration (GFR); (iv) NT-pro BNP; and (v) soluble VCAM-1 levels (5). It is of note that pain frequency, hospitalization rate and narcotic use are also associated with patient survival, supporting the role of sickle cell related chronic complication and patients survival. Based on literature revision, in adult SCD patients the main chronic clinical complications might be divided into two groups: - *Clinical complications related to SCD* such as pulmonary hypertension, kidney disease evolving towards chronic kidney failure, sickle cell bone disease, cardiovascular disease and cerebrovascular disease; - *Clinical complications related to therapeutic options for SCD* such as iron overload or alloimmunization in chronic transfusion programs. 1. Clinical complications related to SCD: In SCD patients, pulmonary hypertension (PH) is defined as mean pulmonary artery pressure (PAP) above 25 mmHg and a measurable tricuspid regurgitation (TRV) over 2.5 m/sec. Different studies have identified PH in 6-10% of adult SCD subjects (6, 7). Although PH seems not to be a major cause of death in adult SCD patients, PH is a severe invalidating complication of SCD. The major risk factors for development of PH are: (i) low Hct, increased retics and hemolysis (*i.e.*: LDH, bilirubin); (ii) low creatinine clearance; (iii) reduced lung capacity and (iv) liver disease. Additional risks facts are also sleep apnea syndrome and thromboembolic disease. Early diagnosis and close follow-up are key elements in prognosis of PH in SCD patients. Treatment of PH in SCD is based on pharmacologic tools used in PH of other origins. However, special attention should be devoted to their adverse side effects that are peculiar of SCD such as pain in the site of infusion during prostacyclin treatment, increased rate of VOCs during therapy with sindenafil; decreased Hb levels in SCD patients treated with endothelin-1 receptor antagonist (Bosentan). The results of clinical trials with these molecules in SCD patients will be discussed. Another severe emerging chronic organ complication of SCD is kidney disease related to sickle cell anemia (8, 9). Advance renal failure has been observed in 4.2% of SCD patients homozygous for sickle Hb (SS) with a median age of 23.1 years; and in 2.4% of patients with SC anemia at a median age of 49.1 years. Non-selective proteinuria is early detectable in almost 50% of SCD subjects aged between 36 and 45 years. The appearance of albuminuria is generally associated with increased tricuspidal velocity, history of stroke or of acute chest syndrome and cholelytiasis, suggesting a possible connection with chronic hemolytic phenotype. A recent analysis on a large cohort of SCD patients has identified hemoglobinuria being associated with progression of chronic renal disease in SCD subjects, supporting a role of chronic hemolysis in development of sickle cell related kidney disease. Clinical studies with ACE-inhibitors and angiotensin receptor blockers in SCD will be discussed. Renal transplantation represents an interesting therapeutic option for SCD patients with terminal renal disease. A study on survival of transplanted SCD patients compares a SCD population treated between 1988 and 1999 (old era) with another treated between 2000 and 2011 (present era). The Authors found a better survival of SCD patients from the present era compared to the old one, with a survival profile similar to that of patients transplanted for diabetes related end-stage renal disease. *2. Clinical complications related to therapeutic options for SCD:* Transfusion of packed red cells is still a powerful therapeutic tool to treat both acute and chronic complication of SCD. Blood transfusion regimes in SCD are mainly represented by simple transfusion or automatized exchange (EEX) or manual exchange. During their lifetime SCD patients might be treated with one or more of these transfusion modalities. Thus, the transfusion history of SCD patients is generally complex and sometimes it is difficult to calculate the amount of patient iron exposure. In addition, the degree of iron overload due to transfusion approaches depends also on the transfusion modality and on the target Hb S levels to be reached. In fact, a simple transfusion with 30% HbS target induces a iron accumulation of 0.42 mg/Kg/d; whereas, EEX with <50% Hb S target promotes a iron accumulation of 0.057 mg/Kg/d (10). This means that different iron chelation intensity and regimes are required in patients undergoing to either simple transfusion or EEX. The introduction of chronic transfusion treatment in children with SCD to prevent stroke based on TCD screening, has significantly increased the proportion of SCD patients requiring iron chelation therapy. Recent studies have shown that SCD patients rapidly develop liver fibrosis in the absence of an adequate iron chelation program. A multicentric study comparing chronic transfused patients with either SCD or thalassemia major (TM) has shown that transfused SCD patients are more frequently hospitalized and have an higher death rate compared to TM patients exposed to a similar transfusion related iron overload. It is of note that the early death in chronic transfused SCD patients has been linked with a delay in beginning iron chelation therapy compared to those who survived. In SCD, iron distribution is largely affected by the biocomplexity of SCD, which is characterized by chronic inflammation, vasculopathy and chronic hemolysis. In chronically transfused SCD patients, iron compartimentalization is characterized by iron accumulation in mainly Kuppfer cells and in hepatocytes. Liver iron accumulation shows positive correlation with patient transfusion history but not with soluble makers of iron homeostasis. In fact, in transfused SCD patients, serum ferritin levels shows a rapid rise up to 1500-2000 ng/mL, then slowing down even if there are evidences of iron overload. The general approach in clinical management of iron-overload in SCD patients requires a combination of parameters such as LIC ≥7 mg iron/gr DW and serum ferritin >1000 ng/mL. Three different iron chelators (deferoxamine, deferasirox and deferipone) are actually available to treat SCD patients with iron-overload. The oral chelators seem to be more effective in SCD patients compared to deferoxamine mainly due to the route of administration and the patient compliance. No major side effects have been observed in SCD patients treated with deferasirox and HU. Particular attention should be devoted to renal function in SCD patients under deferasirox treatment, even if there are not evidences of progressive renal dysfunction during deferasirox administration (11). In a monocentric study, deferipone has been shown to ameliorate the survival of SCD patients under chronic transfusion treatment, in agreement with previous studies using other iron chelators in SCD (12). The different transfusion regimes required to control chronic complications of SCD might be associated with the development of delayed hemolytic transfusion reaction (DHTR), a life-threatening complication (13). DHTR is defined by the acute drop of Hb levels with acute hemolysis and hemoglobinuria following 2-3 to 15 days after blood transfusion. In SCD, DHTR is generally associated with symptoms of acute VOCs or of acute chest syndrome (ACS). The mechanism(s) responsible for DHTR is still largely

unknown although allo- and auto-antibodies have been reported in few DHTR. Clinical management of DHTR is mainly based on (i) limitation of red cell transfusion when Hb levels are<5 g/dL and (ii) modulation of immune response through Ig infusion combined with prednisone or rituximab. We will discuss the clinical management of SCD patients with previous DHTR to prevent new DHTR events in case of exposition to new transfusion of red blood cells. *Conclusions:* In conclusion, SCD is a monogenic disorder but a multiorgan disease linked to its high biocomplexity. The life with disease of young-adult SCD patients largely impact their quality of life and the level of sickness, making SCD an invalidating chronic disease, which requires a holistic approach and the availability of multidisciplinary comprehensive sickle cell centers.

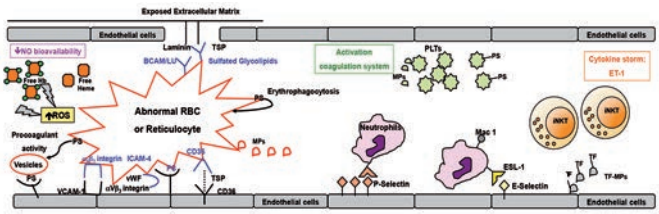

Figure 1. Schematic diagram of the mechanisms involved in the pathogenesis of acute sickle cell related vaso-occlusive events. These involve the adherence of sickle red blood cells (RBCs) or reticulocytes and neutrophils to the abnormally activated endothelial cells, with the participation of activated and phosphatidyl- Serine (PS)-rich platelets (PLTs), activation of the coagulation system, and activation of a cytokine storm. PS: Phosphatidyl-Serine; TSP: thrombospondine; vWF: von Willebrand factor; BCAM/LU: Lutheran blood group protein; ICAM-4: Landstein-Weiner (LW) blood group glycoprotein; MPs: microparticles; Mac1: β2 integrins (αMβ2 or CD11b/CD18); ESL-1: neutrophil E-selectin ligand -1; Hb: hemoglobin; ROS: reactive oxygen species; iNKT: invariant natural killer T cells; ET-1: endothelin-1; NO: nitric oxide (modified from De Franceschi L *et al.* Seminars in Thrombosis, 37: 266; 2011).

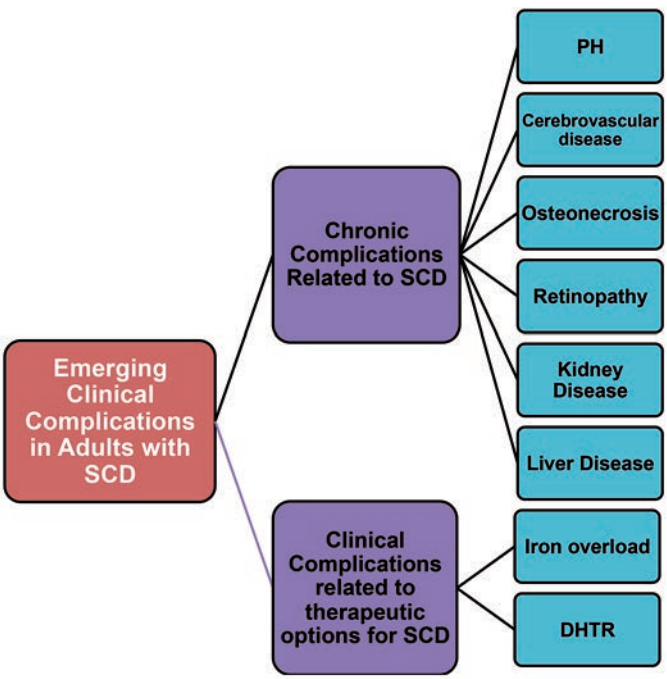

Figure 2. Tree chart of emerging clinical complications in adult subjects with sickle cell disease (SCD); PH: pulmonary hypertension; DHTR: delayed hemolytic transfusion reaction.

References

1. Murray CJ, Vos T, Lozano R, Naghavi M, Flaxman AD, Michaud C, et al. Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet. 2012;380(9859):2197-223.
2. Modell B, Darlison M. Global epidemiology of haemoglobin disorders and derived service indicators. Bulletin of the World Health Organization. 2008;86(6):480-7.
3. De Franceschi L, Cappellini MD, Olivieri O. Thrombosis and sickle cell disease. Seminars in thrombosis and hemostasis. 2011;37(3):226-36.
4. Yusuf HR, Lloyd-Puryear MA, Grant AM, Parker CS, Creary MS, Atrash HK. Sickle cell disease: the need for a public health agenda. American journal of preventive medicine. 2011;41(6 Suppl 4):S376-83.
5. Elmariah H, Garrett ME, De Castro LM, Jonassaint JC, Ataga KI, Eckman JR, et al. Factors associated with survival in a contemporary adult sickle cell disease cohort. American journal of hematology. 2014;89(5):530-5.
6. Bunn HF, Nathan DG, Dover GJ, Hebbel RP, Platt OS, Rosse WF, et al. Pulmonary hypertension and nitric oxide depletion in sickle cell disease. Blood. 2010;116(5):687-92.
7. Rees DC, Williams TN, Gladwin MT. Sickle-cell disease. Lancet. 2010;376(9757):2018-31.
8. Ataga KI, Derebail VK, Archer DR. The glomerulopathy of sickle cell disease. American journal of hematology. 2014;89(9):907-14.
9. Sharpe CC, Thein SL. How I treat renal complications in sickle cell disease. Blood. 2014;123(24):3720-6.
10. Porter J, Garbowski M. Consequences and management of iron overload in sickle cell disease. Hematology / the Education Program of the American Society of Hematology American Society of Hematology Education Program. 2013;2013:447-56.
11. Vichinsky E, Bernaudin F, Forni GL, Gardner R, Hassell K, Heeney MM, et al. Long-term safety and efficacy of deferasirox (Exjade) for up to 5 years in transfusional iron-overloaded patients with sickle cell disease. British journal of haematology. 2011;154(3):387-97.
12. Calvaruso G, Vitrano A, Di Maggio R, Ballas S, Steinberg MH, Rigano P, et al. Deferiprone *versus* deferoxamine in sickle cell disease: results from a 5-year long-term Italian multi-center randomized clinical trial. Blood cells, molecules & diseases. 2014;53(4):265-71.
13. Vidler JB, Gardner K, Amenyah K, Mijovic A, Thein SL. Delayed haemolytic transfusion reaction in adults with sickle cell disease: a 5-year experience. British journal of haematology. 2015;169(5):746-53.

LABORATORY INVESTIGATION FOR INHERITED THROMBOPHILIA: INDICATIONS AND PITFALLS

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Introduction: Venous thromboembolism (VTE) susceptibility genes are present in 5 to 10% of the general population and in at least 40% of patients with VTE [1]. An association with VTE has been firmly established for antithrombin (AT), protein C (PC), and protein S (PS) deficiencies, as well as for factor V Leiden (FVL) and prothrombin (PT) 20210A [1]. Accordingly, the search of the aforementioned inherited abnormalities is the only panel recommended for the laboratory investigation of inherited thrombophilias. However, many experts consider testing for thrombophilia to be of little utility in the clinical management of the large majority of patients with VTE [2,3]. The association of inherited thrombophilia with arterial thrombosis or obstetric complications has been reported to be weaker and equivocal, such that laboratory investigation in this setting is generally not warranted or should be conducted only in selected patients [4,5]. Despite such limitations, testing for inherited thrombophilia is common in clinical practice. The reason for testing for inherited thrombophilia is VTE in 42% of evaluated patients, arterial thrombosis in 15-23%, and an obstetric complication in 13-17%. Asymptomatic individuals account for 12-16% of testing because there is a known history of thrombophilia in a relative or there is a positive family history of VTE [6,7]. Despite the unanimous recommendation against indiscriminate screening [1-3], a number of women are tested prior to the prescription of oral contraceptives or hormone replacement therapy or before planning a pregnancy; in a survey conducted in a tertiary hospital, 15% of the young women tested for FVL were referred before prescribing oral contraception [8]. However, the main reason for testing is laboratory investigation in patients with VTE and their relatives, accounting for more than half of all tests performed. *Risk grading of inherited thrombophilia:* Family history of VTE is per se a strong risk factor for VTE in the general population [9,10]. Therefore, regardless of the presence of other known risk factors, family studies are the most-appropriate tool to investigate the risk and incidence of VTE in carriers of thrombophilia *versus* noncarriers. These studies, conducted among individuals with a shared genetic background, consistently indicate that a risk gradient exists. The risk is higher in those with AT, PC, or PS deficiencies, homozygous FVL or PT20210A, or multiple abnormalities (severe thrombophilia) than in heterozygotes for FVL or PT20210A (mild thrombophilia) [1]. Carriers of AT, PC, or PS deficiencies have a fourfold to 30-fold increased risk of VTE compared with noncarriers. The highest incidence of 0.9–4.0 per 100 person-years is observed in carriers of AT deficiency [1] Patients with type II heparin

binding site defects are the exception, with only a 5–10% probability of developing VTE [1]. On the other hand, carriers of mild thrombophilic traits have a twofold to sevenfold increased risk of VTE, with a much lower incidence of events than those with severe thrombophilia (0.14–0.67 per 100 person-years for FVL; 0.05–0.42 per 100 person-years for PT20210A) [1]. Moreover, in retrospective and prospective family studies of patients with inherited AT, PC, or PS deficiencies, approximately half of VTE events were associated with a concomitant acquired risk factor. During exposure to acquired risk factors, the incidence of VTE in patients with AT, PC, or PS deficiencies is estimated to be as high as 1.2–8.1 per 100 person-years, whereas in mild thrombophilia with exposure to acquired risk factors the incidence of VTE is much lower, ranging from 0.2 to 2.3 per 100 person-years [3]. Finally, although inherited thrombophilia is typically associated with the occurrence of VTE in young people, advanced age is a prominent risk factor for mild thrombophilia [11]. *Testing for thrombophilia in asymptomatic individuals and consequences for primary prophylaxis:* VTE is a common complex (multifactorial) disease, being the resultant of gene-gene and gene-environment interaction. Unfortunately, a simple model due to the presence or the absence of two dichotomous factors (high-risk allele and exposure to an environmental risk factor) is not reliable in most of the cases. This is due to incomplete clinical penetrance of genotypes, since not all carriers develop VTE during life, and to variable expressivity of severity and age of onset of the disease; moreover, the onset of disease is modulated also by gene-gene interactions, in the large majority of cases still obscure, and by multiple effects of various environmental risk factors, acting on the genotype by additive or multiplicative way. The above limitations render so far of little or null clinical utility indiscriminate genetic testing of populations for VTE-susceptibility genes and unlikely to compete for resources with other medical interventions. *Disadvantages of thrombophilia screening:* Unrestricted thrombophilia screening in asymptomatic individuals in the general population who are exposed to situations that increase the risk of VTE, such as oral contraceptive use, during pregnancy, or major orthopaedic surgery is not justified. The reason is not because screening is not cost-effective [12] (the cost of genetic testing will decrease substantially in the next few years), but mainly because thrombophilia is a risk factor, not a disease, and many carriers, particularly of gain-of-function mutations, remain asymptomatic for their lifetime. Another drawback of unrestricted thrombophilia screening is that individuals labelled as 'carriers' could experience insurance discrimination and emotional upset owing to an overestimated perception of genetic risk, while receiving no real benefit in terms of health care. On the other hand, a negative result in thrombophilia screening does not exclude inherited abnormalities that are currently unknown, and should not provide false reassurance for patients or clinicians. Furthermore, counselling for carriers of thrombophilic abnormalities should emphasize that, despite an increased relative risk of VTE with respect to the age-adjusted incidence in the general population, the annual incidence of VTE among these individuals rarely exceeds 1% [1]. Moreover, counselling and information given to patients should be based on the absolute, rather than the relative, risk of VTE. *Benefits of familial thrombophilia screening:* Targeted screening in the siblings of the index patients with VTE is obviously more fruitful than in the general population, with a diagnostic yield of 50%, being such traits genetically dominant. The main theoretical argument in favour of screening asymptomatic relatives of patients with inherited thrombophilia is the possibility of identifying a need for primary anticoagulant prophylaxis during situations that increase the risk of VTE, such as low-risk surgery, pregnancy, and the puerperium period, and are not routinely covered by prophylaxis (Table 1). Some data exist to support this approach. In a retrospective study of 238 individuals with AT, PC, or PS deficiencies, the incidence of VTE during 121 pregnancies and 89 surgical interventions without antithrombotic prophylaxis before diagnosis of thrombophilia was overall 29.2% [13] By contrast, short-term VTE prophylaxis administered to asymptomatic relatives during risk-enhancing situations after diagnosis of thrombophilia failed in preventing VTE in 12.5% of individuals [14] This benefit of anticoagulant prophylaxis was confirmed in prospective cohorts of patients with the same deficiencies. Sanson *et al.* reported that the incidence of VTE during risk-enhancing situations was 16.7% without and 4.5% with antithrombotic prophylaxis [15]. In another study, the annual incidence of VTE secondary to the exposure to acquired risk factors was 0.84% before and 0.58% after diagnosis of thrombophilia [16]. Among asymptomatic heterozygotes for FVL, the incidence of VTE during risk-enhancing situations was 27.1% without and 9.1% with prophylaxis. [17]. On the other hand, in a prospective family study, no VTE events occurred in PT20210A heterozygotes exposed to risk-enhancing situations, independent of low molecular weight heparin prophylaxis [18]. On the whole, screening for thrombophilia in asymptomatic relatives of patients with severe thrombophilia seems to be useful. As regards mild thrombophilia, strong evidence exists in favour of screening among relatives of FVL carriers with VTE, whereas the evidence is weaker for screening relatives of PT20210A carriers with VTE. The risk of VTE has been consistently reported to be higher in asymptomatic carriers of mild thrombophilia who have a family history of VTE, than in those with no family history [19,20]. In family studies of severe thrombophilia, up to 60% of individuals with a relative who has an AT, PC, or PS deficiency develop VTE [1]. On the other hand, PC or PS deficiency have not been associated with thrombotic risk in population-based studies [1]. As regards AT deficiency, in a large cohort of healthy blood donors, all the individuals with rare type I deficiency (prevalence 2.1 in 10,000 individuals) had a family history of VTE, whereas those with the more-common type II heparin binding site mild defect (prevalence 14.5 in 10,000 individuals) did not [21]. In conclusion, laboratory screening for thrombophilia is warranted in families with deficiency of AT, PC, or PS deficiencies and in the relatives of individuals with mild thrombophilia and history of VTE. *Testing for thrombophilia in patients with venous thromboembolism and consequences for secondary antithrombotic prophylaxis:* After a first episode of VTE, the duration of secondary prophylaxis with VKA or direct oral anticoagulants (such as dabigatran, rivaroxaban, apixaban or edoxaban) should be decided balancing the risk of hemorrhagic complications with that of a novel VTE. The cumulative rate of recurrence is as high as 40% within 10 years from the first VTE, being lower in patients with VTE associated with transient risk factors and maximal in those with unprovoked VTE [22]. With this background, many studies have addressed the role of inherited thrombophilia in predicting the likelihood of VTE recurrence. For heterozygous FVL or PT20210A these studies were summarized in three meta-analyses that concluded for an approximately 1.5-fold increased risk for either mutation [1]. Such a modestly increased risk of recurrent VTE contrasts with a higher risk for severe thrombophilia. In the frame of retrospective studies, the risk of recurrent VTE in patients with AT deficiency was 1.9 to 2.6-fold increased, and the annual incidence /100 person-years was 10 for AT, 6 for PC and 8.4 for PS deficiency [1]. Finally, a systematic review showed that homozygous FVL was associated with a 2.6-fold increased risk of recurrent VTE [23]. On the whole, given the low absolute number of recurrent VTE events among carriers of mild thrombophilia, laboratory screening is of little usefulness in the clinical management of the large majority of patients with VTE. However, it cannot be overlooked that severe thrombophilia is detected in the non-trivial proportion of at least 10% of all VTE cases, and that these patients are at a much higher risk of VTE recurrence [1]. On the issue of laboratory screening after VTE there are in the literature contrasting guidelines and recommendations, recently reviewed [2]. All in all, screening seems appropriate in patients who develop VTE at a young age and/or in those with unprovoked events, mainly in order to acquire knowledge on the pathomechanism of VTE rather than to tailor therapeutic strategies. Further, diagnosis of severe thrombophilia can give important information on the optimal duration of anticoagulant treatment (Table1) [2]. This information is even more crucial for patients with thrombosis at life-endangering sites, because international guidelines recommend indefinite anticoagulation in the presence of persistent risk factors (*e.g.*, severe thrombophilia) particularly for cerebral [24] and portal vein thrombosis [25].

Table 1. Antithrombotic management of thrombophilia (*modified from reference 1*).

Type of thrombophilia	Cause of thrombophilia	Primary prophylaxis	Acute treatment	Secondary prophylaxis
Severe	Deficiencies in antithrombin, protein C, or protein S; any homozygous abnormality; multiple abnormalities	Screening asymptomatic relatives of carriers of severe thrombophilia; antithrombotic prophylaxis during exposure to risk-enhancing situations (surgery, confinement to bed, plaster cast); counselling about risk associated with oestrogen–progestogen treatment; antithrombotic prophylaxis in pregnancy and puerperium	Special measures for antithrombin or protein C deficiency in case of life-endangering VTE event	Indefinite duration of anticoagulant treatment after an unprovoked or life-endangering VTE event
Mild	Heterozygous FVL; heterozygous PT20210A	Screening asymptomatic relatives of patients with family history of VTE; antithrombotic prophylaxis during exposure to risk-enhancing situations (surgery, confinement to bed, plaster cast); counselling about risk associated with oestrogen–progestogen treatment; antithrombotic prophylaxis in pregnancy if additional risk factors are present; antithrombotic prophylaxis in puerperium	Standard antithrombotic treatment	Standard duration of antithrombotic treatment

References

1. Martinelli I, De Stefano V, Mannucci PM. Inherited risk factors for venous thromboembolism. Nat Rev Cardiol 2014;11:140-156.
2. De Stefano V, Rossi E. Testing for inherited thrombophilia and consequences for antithrombotic prophylaxis in patients with venous thromboembolism and their relatives. A review of the Guidelines from Scientific Societies and Working Groups. Thromb Haemost 2013;110:697-705.
3. Middeldorp S, van Hylckama Vlieg A. Does thrombophilia testing help in the clinical management of patients? Br J Haematol 2008; 143: 321-335.
4. Lussana F, Dentali F, Abbate R, et al.; Italian Society for Haemostasis and Thrombosis. Screening for thrombophilia and antithrombotic prophylaxis in pregnancy: Guidelines of the Italian Society for Haemostasis and Thrombosis (SISET). Thromb Res 2009; 124: e19-25.
5. de Moerloose P, Boehlen F. Inherited thrombophilia in arterial disease: a selective review. Semin Hematol 2007; 44: 106-113.
6. Coppens M, van Mourik JA, Eckmann CM, Büller HR, Middeldorp S. Current practise of testing for inherited thrombophilia. J Thromb Haemost 2007; 5: 1979-81.
7. Laberge AM, Psaty BM, Hindorff LA, et al. Use of Factor V Leiden genetic testing in practice and impact on management. Genet Med 2009; 11: 750-756.
8. Gartner V, Weber M, Eichinger S. The emotional impact of genetic testing and aspects of counseling prior to prescription of OC. Contraception 2008; 78: 392-398.
9. Zöller B, Li X, Sundquist J, et al. Parental history and venous thromboembolism: a nationwide study of age-specific and sex-specific familial risks in Sweden. J Thromb Haemost 2011; 9: 64–70.
10. Sørensen HT, Riis AH, Diaz LJ, et al. Familial risk of venous thromboembolism: a nationwide cohort study. J Thromb Haemost 2011; 9: 320–324.
11. De Stefano V, Rossi E, Paciaroni K, et al. Different circumstances of the first venous thromboembolism among younger or older heterozygous carriers of the G20210A polymorphism in the prothrombin gene. Haematologica 2003; 88: 61–66.
12. Wu O, Robertson L, Twaddle S, et al.; Thrombosis: Risk and Economic Assessment of Thrombophilia Screening (TREATS) Study. Screening for thrombophilia in high-risk situations: a meta-analysis and cost-effectiveness analysis. Br J Haematol 2005; 131: 80-90.
13. De Stefano V, Leone G, Mastrangelo S, et al. Thrombosis during pregnancy and surgery in patients with congenital deficiency of antithrombin III, protein C, protein S. Thromb Haemost 1994; 71: 799-800.
14. De Stefano V, Leone G, Mastrangelo S, et al. Clinical manifestations and management of inherited thrombophilia: retrospective analysis and follow-up after diagnosis of 238 patients with congenital deficiency of antithrombin III, protein C, protein S. Thromb Haemost 1994; 72: 352-358.
15. Sanson BJ, Simioni P, TormeneD, et al. The incidence of venous thromboembolism in asymptomatic carriers of a deficiency of antithrombin, protein C, or protein S: a prospective cohort study. Blood 1999; 94: 3702-6.
16. Mahmoodi BK, Brouwer JL, Ten Kate MK, et al. A prospective cohort study on the absolute risks of venous thromboembolism and predictive value of screening asymptomatic relatives of patients with hereditary deficiencies of protein S, protein C or antithrombin. J Thromb Haemost 2010; 8: 1193-200.
17. Simioni P, Tormene D, Prandoni P, et al. Incidence of venous thromboembolism in asymptomatic family members who are carriers of factor V Leiden: a prospective cohort study. Blood 2002; 99: 1938-1942.
18. Coppens M, van de Poel MH, Bank I, et al. A prospective cohort study on the absolute incidence of venous thromboembolism and arterial cardiovascular disease in asymptomatic carriers of the prothrombin 20210A mutation. Blood 2006; 108: 2604-2607.
19. Rossi E, Ciminello A, Za T, et al. In families with inherited thrombophilia the risk of venous thromboembolism is dependent on the clinical phenotype of the proband. Thromb Haemost 2011; 106: 646–654.
20. Bucciarelli P, De Stefano V, Passamonti SM, et al. Influence of proband's characteristics on the risk of venous thromboembolism in relatives with factor V Leiden or prothrombin G20210A polymorphisms. Blood 2013; 122: 2555-2561.
21. Tait RC, Walker ID, Perry DJ, et al. Prevalence of antithrombin deficiency in the healthy population. Br J Haematol 1994; 87: 106-112.
22. Prandoni P, Noventa F, Ghirarduzzi A, et al. The risk of recurrent venous thromboembolism after discontinuing anticoagulation in patients with acute proximal deep vein thrombosis pulmonary embolism. A prospective cohort study in 1,626 patients. Haematologica 2007; 92: 199-205.
23. Segal JB, Brotman DJ, Necochea AJ, et al. Predictive value of factor V Leiden and prothrombin G20210A in adults with venous thromboembolism and in family members of those with a mutation: a systematic review. JAMA 2009; 301: 2472-2485.
24. Einhäupl K, Stam J, Bousser MG, et al.; European Federation of Neurological Societies. EFNS guideline on the treatment of cerebral venous and sinus thrombosis in adult patients. Eur J Neurol 2010; 17: 1229-1235.
25. DeLeve LD, Valla DC, Garcia-Tsao G; American Association for the Study Liver Diseases. Vascular disorders of the liver. Hepatology 2009; 49: 1729-1764.

SHOULD CYTOREDUCTIVE TREATMENT BE PERFORMED BEFORE TRANSPLANTATION IN PATIENTS WITH HIGH-RISK MYELODYSPLASTIC SYNDROME?

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The only potentially curative treatment for patients with myelodysplastic syndrome (MDS) is allogeneic hematopoietic stem cell transplantation (allo-HSCT). However, the effectiveness of this approach is limited by considerable morbidity and mortality. The introduction of reduced-intensity conditioning regimens has resulted in a significant reduction in transplantation-related mortality, leading to a rapidly growing number of transplantations in elderly patients. Despite these recent advances, the long-term survival rate is currently about 30%. In MDS patients receiving allo-HSCT, disease relapse represents a leading cause of transplantation failure, especially in those cases with an advanced disease stage (ie, intermediate-2 and high International Prognostic Scoring System risk). The issue of performing cytoreductive chemotherapy before allo-HSCT in these patients to reduce the risk of disease relapse is a matter of debate. Significant concerns about chemotherapy, such as that used to treat acute myeloid leukemia, include the low response rate and the risk of longlasting myelosuppression and organ toxicities. It should be considered, in addition, that there is no definitive evidence of a survival benefit associated with administering chemotherapy before allo-HSCT in patients with MDS. The only randomized study, from the European Group for Blood and Marrow Transplantation, had to be stopped because of slow recruitment, whereas retrospective single-center studies showed no conclusive results, with additional selection bias as a result of the impossibility of accounting for patient dropout (*i.e.*, patients who received induction chemotherapy but never received allo-HSCT because of death or toxicity). The availability of hypomethylating agents, including 5-azacitidine and decitabine, has changed the landscape of MDS treatment. Azacitidine results in hematologic improvements in approximately 25% to 50% of cases and complete response in 10% to 20%, with prolonged survival, compared with supportive care alone in high-risk MDS, with a good toxicity profile, compared with induction chemotherapy. Although hypomethylating agents can induce hematological and cytogenetic responses, these therapies do not appear to eradicate MDS clones, and recent data suggest that even in patients ages 60 to 70 years and with intermediate-2 or high international prognostic scoring system risk, transplantation offers a survival benefit with respect to nontransplantation procedures. The use of hypomethylating agents is, therefore, increasing as a bridge to more definitive therapy, as a part of a comprehensive strategy to prevent relapse after allo-HSCT in MDS patients with advanced disease. The mechanism by which hypomethylating agents exert an antitumor effect in MDS remains not completely understood. Inhibition of DNA methyltransferases results in hypomethylation and, consequently, might result in reactivation of tumor suppressor genes, terminal differentiation, and apoptosis of neoplastic cells, with reduction of tumor burden before allo-HSCT. In addition, treatment with hypomethylating agents seems to affect T cell mediated and innate immunity. Several studies have evaluated the role of hypomethylating agents given before transplantation, although very few were conducted prospectively. Overall, these investigations showed similar post-transplantation outcomes for patients receiving hypomethylating agents *versus* those receiving remission-induction chemotherapy, without significant treatment-related toxicity. Moreover, in some cases, an improved outcome was reported for patients who underwent transplantation in complete remission compared with those with active disease at the time of allo-HSCT. In the absence of data from prospective trials on patients with MDS who are candidates for allo-HSCT, the decision to perform a cytoreductive treatment should be made on an individual basis, accounting for clinical considerations with respect to each specific patient. As the rate of complete remission is generally higher with induction chemotherapy compared with the rate for hypomethylating agents, that strategy might still be the best option in selected medically fit patients with immediate availability of a suitable donor. On the other hand, hypomethylating agents could be considered mainly for older patients (including those with comorbidity) who are at risk of losing eligibility for a transplantation procedure as a result of treatment-related toxicity and as a bridging strategy to allo-HSCT in subjects where no donor has yet been identified. Finally, azacitidine and decitabine may be active in patients with a complex karyotype, for whom conventional chemotherapy invariably fails.

References

1. Della Porta MG, Alessandrino EP, Bacigalupo A, et al. Predictive factors for the outcome of allogeneic transplantation in patients with MDS stratified according to the revised IPSS-R. Blood. 2014;123:2333-2342.
2. Alessandrino EP, Della Porta MG, Pascutto C, et al. Should cytoreductive treatment

be performed before transplantation in patients with high-risk myelodysplastic syndrome? J Clin Oncol. 2013;31:2761-2762.
3. Fenaux P, Mufti GJ, Hellstrom-Lindberg E, et al. Efficacy of azacitidine compared with that of conventional care regimens in the treatment of higher-risk myelodysplastic syndromes: A randomised, open-label, phase III study. Lancet Oncol. 2009;10:223-232.
4. Koreth J, Pidala J, Perez WS, et al. Role of reduced-intensity conditioning allogeneic hematopoietic stem-cell transplantation in older patients with *de novo* myelodysplastic syndromes: an international collaborative decision analysis. J Clin Oncol. 2013;31:2662-2670.
5. Damaj G, Duhamel A, Robin M, et al. Impact of azacitidine before allogeneic stem-cell transplantation for myelodysplastic syndromes: A study by the Societe Francaise de Greffe de Moelle et de TherapieCellulaire and the Groupe-Francophone des Myelodysplasies. J Clin Oncol. 2012;30:4533-4540.

TREATMENT OF ADULT LANGERHANS CELL HISTIOCYTOSIS

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Introduction: Langherans cell histiocitosis (LCH) is a rare, heterogeneous and potentially debilitating disease [1], characterized by accumulation of mononuclear phagocytes positive for CD1a and CD207. LCH can affect virtually all organs and tissues. Highest incidence is observed among children (male/female ratio close to 1) with 2-10 cases per million, whereas in adults LCH affects only one or two cases per million and it is thus considered an orphan disease [1]. The clinical presentation of LCH is extremely heterogeneous varying from single benign lesions which can be treated with minor surgery to aggressive life-threatening disease affecting multiple organs and systems. LCH can affect a single system being unifocal or multifocal (SS-m) (when multiple sites in a single tissue or organ are involved). Less frequently LCH can be a multisystem (MS) disease involving multiple organs and tissues. Due to its rarity and lack of prospective randomized trials there is no specific therapy for adult LCH and treatment schedules have been derived so far from pediatric protocols. Moreover due to the extreme variability of clinical presentations and disease sites the treatments options vary from watchful waiting to aggressive multiagent chemotherapy approaches. In general chemotherapy is reserved to patients with SS-m or MS disease [2]. Since more than 25 years, the back bone of pediatric chemotherapeutic protocols is the combination of vinblastine and steroids, followed by therapy consolidation [2]. This therapeutic strategy is unlikely to be successful in adults, as the only prospective randomized trial evaluating the efficacy of vinblastine/prednisone regimen in adults (LCHA1 trial) was prematurely closed for unacceptable toxicities (vinblastine related neurotoxicity and detrimental effects of prolonged steroid therapy) [2]. In conclusion, available data do not support the use of pediatric regimens in adult LCH, and although alternative approaches have been tested no consensus on the best frontline treatment strategy in adult LCH has been reached yet. In this review we discuss recent progresses in the understanding of the pathogenesis of LCH and our management strategies and therapeutic algorithm in the context of the current knowledge, focusing on adult LCH. *History and Pathogenesis:* In recent years many progresses have been made in the understanding of LCH pathogenesis. Although historically LCH has been classified as a dendritic disorder arising from epidermal dendritic cells and its neoplastic nature has been questioned, it is now clear that LCH is indeed a neoplastic disorder, with recent scientific evidence supporting a myeloid origin [reviewed in 3]. Recently mutations of the *BRAF* oncogene resulting in constitutive activation of the mitogen-activated protein kinase (MAPK) pathway have been discovered by next generation sequencing studies [4], and this discovery could have important implications for the treatment of this disease. First described in early 1900s as Hand-Schüller- Christian disease after the observation of children with a syndrome consisting in bone, mucosal involvement and diabetes insipidus, it was also known as Letterer-Siwe disease (after a report of newborns with hepatomegaly and bone marrow involvement). A syndrome characterized by spontaneously resolving skin lesions in children was later known as Hashimoto-Pritzker syndrome (1970s). Another name commonly used in the past for LCH was "eosinophilic granuloma", referring to the histopathological appearance of LCH lesions. In the 1950s a common origin for these different clinical conditions was first suggested by Lichtenstein under the name of histiocytosis X, where "X" was indicating unknown cell of origin. In the 1970s cytoplasmic CD207 (langerin) positive granules (Birbeck granules) were discovered in LCH lesions, and therefore it was thought that LCH derived from epidermal Langerhans cells, (the only cells known to express Birbeck granules at that time) [3,5]. Very recently this concept has been challenged by multiple studies indicating a probable origin from myeloid precursors as a consequence of misguided myeloid differentiation. Moreover, given that less than 8% of the cells in LCH lesions are Langerhans cells (LC), for long time an inflammatory origin for LCH has been hypothesized, (similarly to what initially postulated for Hodgkin lymphoma, for example). Now it is clear that LCs are monoclonal, and the recently demonstrated occurrence of somatic mutations in different components of the MAPK pathway strongly argues in favor of a neoplastic origin, in which tumor microenvironment and cytokine –chemokine modulation likely plays a major role. BRAF-V600E mutations inducing constitutive activation of the downstream kinases MEK and ERK, were in fact found in over 50% of LCH lesions. Moreover in the remaining cases mutations involving different components of the same pathway were found, such as *MAP2K1* (coding for MEK1) in 30-50% of cases, but also *ARAF* and *ERBB3*. This genomic alterations result in constitutive ERK activation, which can be detected in almost 100% of cases by immunohistochemistry [reviewed in 3]. *Treatment:* As stated above, the back bone of pediatric chemotherapeutic protocols is the combination of vinblastine and steroids, followed by therapy consolidation [2]. Given that early response emerged as an important prognostic predictor, efforts have been made to intensify the induction therapy by adding etoposide (in the LCH II trial) [6] or methotrexate (in the LCH III study) [7] to the standard vinblastine based regimen. These efforts were especially directed to treatment of patients with involvement of risk organs [(RO) liver, spleen, bone marrow)], which is considered a negative prognostic factor. Nevertheless the results of these approaches were not deemed satisfactory, as no major differences in the final outcome were observed [6]. On the other hand the results of the LCH III trial suggested that milder induction but longer therapy duration could significantly improve the outcome by reducing recurrences [7]. Risk adapted therapy as per the LCH III trial in children resulted in 5 year 30 to 40% reactivation rates, and 84 to 99% overall survival depending on RO+ *vs* RO- and the randomization arm [7]. Although with the 3 LCH trials substantial progresses have been made in the treatment of pediatric LCH, this therapeutic strategy is unlikely to be successful in adults, as the only prospective randomized trial evaluating the efficacy of vinblastine/prednisone regimen in adults (LCHA1 trial) was prematurely closed for unacceptable toxicities (vinblastine related neurotoxicity and detrimental effects of prolonged steroid therapy) [2]. Moreover recent studies seem to indicate that this approach might have lower efficacy in adults compared to children, suggesting that adult and pediatric LCH could harbor different biological characteristics [8]. In conclusion, available data do not support the use of pediatric regimens in adult LCH, indicating that the concept of mild induction followed by maintenance therapy probably cannot be translated to adults. Alternative approaches tested in adult LCH include nucleoside analogs such as cytarabine, cladribine (2-CDA) or clofarabine [8,9] but no consensus on the best frontline treatment strategy has been reached yet. In this scenario, in 2010 we first presented the results of our therapeutic strategy using multiagent chemotherapy according to the MACOP-B (Methotrexate, Doxorubicin, Cyclophosphamide, Vincristine, Bleomycin, Prednisone) regimen in 7 adult patients with SS-m or MS LCH [10]. The treatment algorithm is depicted in Figure 1. We demonstrated high activity in terms of overall response rate (ORR 100%) and long-term disease control (4 of 7 patients achieved long lasting complete responses), despite the lack of maintenance therapy and an overall treatment duration of only 3 months. We recently updated these data considering 11 patients treated in a 20-year period, confirming high efficacy and long lasting responses with 82% overall survival (OS) and 64% progression free survival (PFS) after 6.7 years of follow-up: detailed data will be presented during the 45th Italian Society of Hematology (SIE) meeting. These results were obtained with a treatment strategy which is very different from the one exploited in the LCH trials, demonstrating that long term complete responses (CR) and cure can be achieved with therapy intensification, and without treatment consolidation. Moreover these data compare favorably with the results of purine analogs, widely used in adults given the toxicity of pediatric protocols. Based on these data, MACOP-B is currently the first line therapy for adult patients affected by SS-m or MS LCH at our Institution. *Initial Staging:* Given that LCH may affect virtually every

tissue or organ the initial staging must be very accurate and extensive. Moreover initial staging is crucial for proper risk stratification and treatment strategies may vary widely depending on disease characteristics (from simple surgical removal of single lesions, to multiagent systemic chemotherapy). Given that in adults LCH frequently affects bone, lungs, spleen and liver all patients should be initially staged with physical examination, total body computed tomography (CT) scan, complete scheletal X-ray, bone scan, and bone marrow biopsy. Our data strongly support the use of fluorodeoxyglucose positron emission tomography (FDG-PET) for initial staging as in some cases FDG-PET is able to detect additional disease sites, leading to possible changes in therapeutic strategies. Since central nervous system (CNS) disease with functional impairment of pituitary gland is a possibility, all patients should undergo complete endocrinological assessment and brain magnetic resonance imaging (MRI). In case of involvement of the temporal bone or auditory canal a baseline hearing evaluation is recommended. Complete blood cell count and hepatic and renal functions assessment is also recommended. In case of lung LCH, high resolution (HR) CT scan and functional pulmonary studies should be performed. *Response evaluation:* Restaging should be done on the basis of initial sites of disease. In general we restage patients undergoing MACOP-B chemotherapy at week 6 and after week 12, with CT and PET scan. CR is defined as no evidence of active disease with regression of signs and symptoms at physical examination and imaging studies. For bone lesions different criteria should be applied: bone lesions should be restaged by using CT, MRI and PET scan. Restaging of bone lesions can be difficult, since complete reconstitution of the bone can take as long as 2 years: in this case we consider as complete responders those patients who achieve disappearance of contrast enhancement at CT, MRI and PET scan. Specific LCH forms such as pulmonary LCH should be re-evaluated with HR-CT scan and lung function tests. Follow-up is repeated every 3 months during first year, then every 6 months from 2nd to 5th year, then annually. *Specific clinical cases: Case 1- Pulmonary LCH:* A 28-year old woman with a past history of heavy cigarette smoking was initially diagnosed with LCH involving the lung and the occipital bone in 1997. After initial successful therapy with vinblastine and etoposide, she relapsed in 2011 at the age of 42, with radiologic evidence of new lesions in the absence of symptoms. The patients started close follow-up together with smoking cessation, achieving disease stability, with no need of systemic therapy. *Case 2- Multisystem LCH successfully treated with MACOP-B:* A 23-year old woman was diagnosed with MS-LCH involving lymphnodes, and bone (skull and spine). She underwent MACOP-B chemotherapy for 12 courses achieving a CR. After 13 years from initial diagnosis the patient is still in CR, with no permanent consequences. *Case 3- Multisystem therapy refractory LCH successfully treated with autologous stem cell transplant:* A 23-year old man with a history of cigarette smoking presents with mild dyspnea, and diffuse bone pain. After complete diagnostic workup he is diagnosed with MS-LCH involving lungs and bone, with multiple bone lesions of the spine and lower limbs. He undergoes MACOP-B chemotherapy (plus smoking cessation) for 12 courses achieving initial CR, but shortly after the end the treatment course the disease relapses with new bone and lung lesions. After unsuccessful radiotherapy, the patient is treated with high dose chemotherapy and autologous stem cell transplantation (ASCT) achieving a CR. After 7 years from ASCT the patients is well and disease-free. *Case 4- The value of FDG-PET for initial staging of LCH:* A 26-year old woman presented with bone pain at the thoracic spine. MRI showed a unique lesion of T4 with narrowing of the spinal canal and posterior bulging. CT-guided biopsy indicated LCH, indicating a single system unifocal case. At this point the patient was referred to our center. FDG-PET scan demonstrated an additional lesion of T10, indicating a multifocal disease, and thus changing the therapeutic approach. Given the good performance status and the young age we treated this woman with MACOP-B for 12 courses. The patient achieved a CR and is still disease free after 6 years from the completion of therapy. *Conclusions:* In this brief review we outlined the most relevant aspects of LCH, from biology and pathogenesis, to clinical management strategies. Adult LCH, which is the main focus of this review, is a highly heterogeneous disease. As briefly reported in the clinical cases presented here, the natural history of the disease may vary from self-limited lesions involving a single organ or tissue, to aggressive life-threatening disease requiring systemic therapy and bone marrow transplantation. Therefore accurate and extensive diagnostic workup and staging are mandatory, as treatment strategies and protocols may vary widely depending on the extension of the disease. For example isolated and asymptomatic pulmonary LCH can be controlled with smoking cessation, whereas systemic therapies are required for MS-LCH cases. Table 1 summarizes our clinical approach to adult LCH. Regarding the management of adult LCH, the application of pediatric protocols is generally difficult due poor tolerance, and in fact the LCHA1 trial was closed prematurely due to unacceptable toxicity [2]. Multiple studies recently reported suboptimal efficacy of pediatric approaches in adults [2,8]. Our therapeutic strategy for adult patients affected by MS or SS-m-LCH is the short course MACOP-B chemotherapy regimen. Long-term results of our study confirm that a substantial fraction of patients achieve long term CR and are cured with this approach, without the need of therapy consolidation. Recent advances in the understating of the biology of LCH, such as the discovery of recurrent somatic mutations of druggable targets belonging to the MAPK pathway (BRAF and MAP2K1), will likely have major treatment implications in the next future.

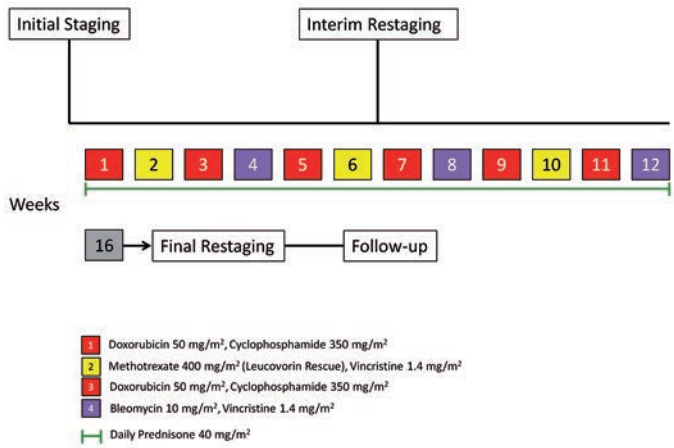

Figure 1. MACOP-B chemotherapy treatment algorithm. Treatment schedule and timing of staging procedures.

Table 1. Clinical management of adult LCH

Disease sites or staging	Specific Diagnostic procedures	Treatment
Single system unifocal	PET scan should be used to rule out multifocal disease.	Surgical removal of single lesions Radiotherapy in selected cases
Single system multifocal	Standard plus specific procedures, depending on sites involved	Chemotherapy (MACOP-B in our institution)
Multisystem	Standard plus specific procedures	Chemotherapy (MACOP-B in our institution)
Specific sites		
CNS (Sella Turcica-Pituitary gland)	Brain MRI; complete endocrinological evaluation	Chemotherapy (MACOP-B in our institution)
Lung	HR-CT scan and pulmonary function tests should be performed	If only site consider smoking cessation and wait and see
Temporal bone/auditory canal	MRI; Baseline hearing assessment	Depends on the final staging

References

1. Stockschlaeder M, Sucker C. Adult Langerhans cell histiocytosis. Eur J Hematol 2006; 76: 363-368.

2. Girschikofsky M, Arico M, Castillo D, et al. Management of adult patients with Langerhans cell histiocytosis: recommendations from an expert panel on behalf of Euro-Histio-Net. Orphanet J Rare Dis. 2013;8:72.
3. Berres ML, Merad M, Allen CE. Progress in understanding the pathogenesis of Langerhans cell histiocytosis: back to Histiocytosis X? Br J Haematol. 2015;169:3-13.
4. Badalian-Very G, Vergilio JA, Degar BA, et al. Recurrent BRAF mutations in Langerhans cell histiocytosis. Blood. 2010;116:1919-23.
5. Lampert F. Langerhans cell histiocytosis. Historical perspectives. Hematol Oncol Clin North Am.;12:213-9.
6. Gadner H, Grois N, Pötschger U, et al. Improved outcome in multisystem Langerhans cell histiocytosis is associated with therapy intensification. Blood. 2008; 111:2556-62.
7. Gadner H, Minkov M, Grois N, et al. Therapy prolongation improves outcome in multisystem Langerhans cell histiocytosis. Blood. 2013;121:5006-14.
8. Maia RC, de Rezende LM, Robaina M, Apa A, Klumb CE. Langerhans cell histiocytosis: Differences and similarities in long-term outcome of paediatric and adult patients at a single institutional centre. Hematology. 2015;20:83-92.
9. Cantu MA, Lupo PJ, Bilgi M, Hicks MJ, Allen CE, McClain KL. Optimal therapy for adults with Langerhans cell histiocytosis bone lesions. PLoS One. 2012;7:e43257.
10. Derenzini E, Fina MP, Stefoni V, et al. MACOP-B regimen in the treatment of adult Langerhans cell histiocytosis: experience on seven patients. Ann Oncol. 2010;21:1173-8.

SMOLDERING MYELOMA: WALKING THROUGH RISK FACTORS AND TENTATIVE CLASSIFICATION SYSTEMS

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The history of Smoldering Myeloma (SMM) started when Kyle and Greipp described six myeloma patients in whom the percentage of plasma cells in the bone marrow (BMPC) and level of M protein were higher than those seen in monoclonal gammapathy of uncertain significance (MGUS), but without lytic bone lesions, anemia, or hypercalcemia and maintained this status without any specific therapy for more than five years [1]. At the same time, Alexanian *et al.* described 20 patients with low tumor mass disease who were asymptomatic, with an hemoglobin level greater than 10 g/dL, and with not more than 3 lytic bone lesions or compression fractures or recurrent infection. These patients were defined as having an indolent MM [2]. Since then, the two terms of *smoldering* and *indolent* myeloma were variably used in an undefined manner until 2003 when the IMWG defined SMM as BMPC ≥10% and/or M protein level ≥30 g/L and lack of organ damage (CRAB—hypercalcemia, renal failure, anemia, and bone lesions) [3]. SMM accounts for about 15% of all the patients with newly diagnosed MM [4] and the risk of progression to symptomatic MM is higher compared to MGUS patients (10% per year *versus* 1% per year, respectively) [5, 6]. Although it is a well clinically defined entity, SMM is a biologically heterogeneous disease that includes patients with a premalignant condition, as MGUS, and patients who will develop clinical symptoms and end-organ damage within the first two years from diagnosis. Therefore, after the diagnosis of SMM, it is necessary to evaluate the risk of progression to symptomatic disease. Unfortunately, at this time, there is no single clinical or molecular feature that can reliably distinguish patients with SMM who have only premalignant plasma cells from those with a clonal malignant disease. However, several studies (the majority retrospective) described different parameters that can predict the risk of progression to symptomatic MM. *The Level and the Type of Serum M Protein Concentration*: the Mayo Clinic study underlined the relevance of size and type of the serum M-protein as a significant risk factor for progression in MM. The median time to progression (TTP) in patients with a component ≥4g/dL was 18 months *vs* 75 months in patients with a lower serum M protein; the median TTP was significantly shorter in patients with IgA *versus* IgG M-protein (27 *vs* 75 months, respectively, p=0.004). *Percentage of Bone Marrow Plasma Cells*: Kyle reported that the median TTP was 117, 26 and 21 months for patients with BMPC <20%, 20-50% and >50% respectively (P<0.001). On this basis, Kyle *et al.* divided patients with SMM into three prognostic groups by the percentage of BMPC and level of serum M protein. Group 1was defined by BMPC ≥10% and serum M protein ≥3 g/dL with a median TTP of 2 years. Group 2 included patients with BMPC ≥10% but serum M protein <3 g/dL, with a TTP of 8 years. Group 3: serum M protein ≥3 g/dL but BMPC <10% and a TTP of 19 years. Similarly, Kastritis *et al.* reported a median TTP of 19 months for patients with BMPC ≥10% and serum M protein ≥3 g/dL *vs* 73 months for patients with BMPC ≥10% but serum M protein <3 g/dL [7]. A recent study indicates that the risk of progression is extremely high (approximately 90% at 2 years) when the BMPC is ≥60%, and these patients are now considered as MM. It should be underlined that the amount of BMPC is evaluated on either the bone marrow aspirate or biopsy examination, and in case of discrepancies the higher of the two values should be used [8]. *Immunoparesis:* Kyle *et al.* reported that in SMM, immunoparesis (suppression of one or more uninvolved immunoglobulins) was a significant risk factor for progression. The median TTP was 159 months for patients without immunoparesis, 89 months in those with a reduction of only one isotype, and 32 months in patients with reduction in two isotypes of uninvolved immunoglobulins. The Spanish group reported similar findings, showing a not reached median of TTP in patients with normal immunoglobulins *versus* 31 months in those patients carrying one or more reduced uninvolved immunoglobulins [9]. *Immunophenotyping:* The same Spanish study [9] found that 60% of patients with SMM have an aberrant immunophenotype (defined by the absence of CD19 and/or CD45 expression, decreased expression of CD38, and overexpression of CD56) similar to MM, where >95% of PCs are aberrant and only <5% of the detected PCs are normal. The risk of progression in patients with aberrant phenotype was significantly higher compared to those who had a lower rate of aberrancy with a median TTP of 34 months *vs* not reached respectively. On these basis, Pèrez-Persona *et al.* defined a risk of progression to active MM at 5 years of 4%, 46%, and 72%, for patients with none, 1, or 2 risk factors respectively (aPCs/BMPC ≥95% and immunoparesis). *Serum-Free Light-Chain Ratio:* Dispenzieri and colleagues studied 273 patients with SMM and demonstrated that an involved/uninvolved free-lite chain (FLC) ratio of ≥8 was a significant risk factor for progression. Median TTP was 30 months in patients with an involved/uninvolved FLC ratio of ≥8 *vs* 110 months for patients with FLC ratio less than 8. The 2 years risk of progression was approximately 40% in patients with an involved/uninvolved FLC ratio of ≥8. Therefore, the FLC ratio was included in a risk-stratification model based on the following risk factors: BMPC ≥10%; serum M protein ≥3 g/dL; and FLC ratio >8. The 5-year progression rates were 25%, 51%, and 76%, in the presence of one, two, or three risk factors respectively [10]. When the involved/uninvolved FLC ratio rises to ≥100, the median TTP is only 15 months, and the 2 year risk of progression approaches 80%. Therefore, this can be considered as a biomarker of early progression and such patients are now considered as MM [11]. *Circulating Peripheral Blood Plasma Cells (PBPC).* Bianchi *et al.* have shown that patients with high circulating PBPC have a higher risk to progress to active disease within 2 years compared with patients without high circulating PC (71 *versus* 25%, respectively, P=0.001). However, the detection of circulating PC is still not standardized and difficult to reproduce [12]. *Genetic Abnormalities* Neben *et al.* described the impact of chromosomal aberrations in patients with SMM and found that the presence of del (17p13), t(4;14), +1q21 and hyperdiploidy predicted shorter TTP [13]. The same conclusion was reached by Rajkumar *et al.*, who reported that a median TTP was not reached in patients without detectable abnormalities (low-risk), while patients with t(4;14) and/or del(17p) were defined as high risk SMM with a significantly shorter median TTP (24 months) compared with patients with trisomies (intermediate-risk), or other cytogenetic abnormalities including t(11;14) (standard-risk) [14]. Based on a cohort of 331 patients with MGUS and SMM, Dhodapkar and colleagues identified a gene expression profiling (GEP70-gene signature) signature as an independent predictor of the risk of progression to MM [15]. Very recently, a study conducted at the University of Arkansas has identified four genes that can predict high risk of progression from smoldering to symptomatic MM [16]. *Imaging:* Bone disease detectable by magnetic resonance imaging (MRI) are able to predict TTP. Moulopoulos *et al.* first demonstrated that in patients with asymptomatic myeloma TTP was 16 months for patients with abnormal MRI *versus* 43 months for those with normal MRI. In addition, median TTP was shorter in patients with focal lesions (6 months) compared with those who had diffuse (16 months) or variegated pattern (22 months) [17]. Kastritis *et al.* confirmed that an abnormal marrow signal of MRI of the spine in a patient with SMM is associated with a significant factor for progression to symptomatic myeloma (median 15 months). Similar findings have been found in the Dhodapkar's study. In a recent study of 149 patients with SMM, using whole-body MRI, Hillengass and al. detected focal lesions in 28% of patients and they found that 15% of patients had more than one focal lesion on wb-MRI imaging.

The median TTP in such patients was 13 months, and the 2-year progression rate was 70% [18]. These patients should no longer considered as SMM but as MM according to the current IMWG criteria. In conclusion, for each newly diagnosed SMM patient, it is necessary to identify the risk of progression. So far, the Mayo Clinic and the Spanish models have been used and validated in prospective trials. However, the two models do not overlap and there are many patients that are differently classified according to the two models. In addition, many other models have been generated in the last years although all of them need to be validated [19] (Table 1). In any case, the probability of each SMM patient to evolve in MM should be defined by taking into account all the available data rather than defining the risk according to a fixed model. In general, according to the available models and to the aforementioned risk factors, SMM patient can be divided in three categories of risk of progression [19]: a) *low risk:* these patients have a probability of progression at 5 years of 8% and should be followed similarly to MGUS patients. b) *intermediate risk:* with a risk of progression of 42% at 5 years. They represent the true SMM patients and should be followed every 6 months. c) *high risk:* half of these patients will progress to MM within 2 years from diagnosis and the key question is whether they should be treated at diagnosis or at progression. Some interventional trials are ongoing in this group of patients and nowadays there are no clear indications on the correct choice. In this uncertainty, clinicians are invited to include these patients in clinical prospective trials in order to better understand the natural course of the disease and the real survival benefits of an early treatment.

Table 1. Risk models for SMM.[19]

Risk model	Risk of progression to MM	
Mayo Clinic		Median TTP
- ≥ 10% clonal PCBM infiltration	1 risk factor	10 years
- ≥ 3 g/dL of serum M-protein	2 risk factors	5 years
- serum FLC ratio between <0.125 or >8	3 risk factors	1.9 years
Spanish myeloma		Median TTP
- ≥ 95% of aberrant PCs by MFC	No risk factors	NR
- immunoparesis	1 risk factor	6 years
	2 risk factors	1.9 years
Heidelberg		3 years TTP
- Tumor mass using the Mayo Model	T-mass low + CA low risk	15%
- t(4;14), del 17p or + 1q	T-mass low + CA high risk	42%
	T-mass high + CA low risk	64%
	T-mass high + CA high risk	55%
SWOG		2 years TTP
- Serum M-protein ≥ 2 g/dL	No risk factors	30%
- Involved FLC > 25 mg/dL	1 risk factor	29%
- GEP risk score > - 0.26	≥ 2 risk factors	71%
Penn		2 years TTP
- ≥ 40% clonal PCBM infiltration	No risk factors	16%
- sFLC ratio ≥ 50	1 risk factor	44%
- Albumin ≤ 3.5 mg/dL	≥ 2 risk factors	81%
Japanese		2 years TTP
- Beta 2-microglobulin ≥ 2.5 mg/L	2 risk factors	67.5%
- M-protein increment rate > 1 mg/dL/day		
Czech & Heidelberg		2 years TTP
- immunoparesis	No risk factors	5.3%
- serum M-protein ≥ 2.3 g/dL	1 risk factor	7.5%
- involved/uninvolved s FLC > 30	2 risk factors	44.8%
	3 risk factors	81.3%
Barcellona		2 years TTP
- evolving pattern = 2 points	0 points	2.4%
- serum M-protein ≥ 3 g/dL = 1 point	1 point	31%
- immunoparesis = 1 point	2 points	52%
	3 points	80%

References

1. Kyle RA, Greipp PR. Smoldering multiple myeloma. N Engl J Med 1980; 302: 1347-1349.
2. Alexanian R. Localized and indolent myeloma. Blood 1980; 56: 521-525.
3. Kyle R, Gertz M, Witzig T et al. Review of 1027 patients with newly diagnosed multiple myeloma. Mayo Clin Proc 2003; 78: 21 - 33.
4. Kristinsson SY, Holmberg E, Blimark C. Treatment for high-risk smoldering myeloma. N Engl J Med 2013; 369: 1762-1763.
5. Kyle RA, Remstein ED, Therneau TM et al. Clinical course and prognosis of smoldering (asymptomatic) multiple myeloma. N Engl J Med 2007; 356: 2582-2590.
6. Kyle RA, Therneau TM, Rajkumar SV et al. A long-term study of prognosis in monoclonal gammopathy of undetermined significance. N Engl J Med 2002; 346: 564-569.
7. Kastritis E, Terpos E, Moulopoulos L et al. Extensive bone marrow infiltration and abnormal free light chain ratio identifies patients with asymptomatic myeloma at high risk for progression to symptomatic disease. Leukemia 2013; 27: 947-953.
8. Rajkumar SV, Dimopoulos MA, Palumbo A et al. International Myeloma Working Group updated criteria for the diagnosis of multiple myeloma. Lancet Oncol 2014; 15: e538-548.
9. Perez-Persona E, Vidriales MB, Mateo G et al. New criteria to identify risk of progression in monoclonal gammopathy of uncertain significance and smoldering multiple myeloma based on multiparameter flow cytometry analysis of bone marrow plasma cells. Blood 2007; 110: 2586-2592.
10. Dispenzieri A, Kyle RA, Katzmann JA et al. Immunoglobulin free light chain ratio is an independent risk factor for progression of smoldering (asymptomatic) multiple myeloma. Blood 2008; 111: 785-789.
11. Larsen JT, Kumar SK, Dispenzieri A et al. Serum free light chain ratio as a biomarker for high-risk smoldering multiple myeloma. Leukemia 2013; 27: 941-946.
12. Bianchi G, Kyle RA, Larson DR et al. High levels of peripheral blood circulating plasma cells as a specific risk factor for progression of smoldering multiple myeloma. Leukemia 2013; 27: 680-685.
13. Neben K, Jauch A, Hielscher T et al. Progression in smoldering myeloma is independently determined by the chromosomal abnormalities del(17p), t(4;14), gain 1q, hyperdiploidy, and tumor load. J Clin Oncol 2013; 31: 4325-4332.
14. Rajkumar SV, Gupta V, Fonseca R et al. Impact of primary molecular cytogenetic abnormalities and risk of progression in smoldering multiple myeloma. Leukemia 2013; 27: 1738-1744.
15. Dhodapkar MV, Sexton R, Waheed S et al. Clinical, genomic, and imaging predictors of myeloma progression from asymptomatic monoclonal gammopathies (SWOG S0120). Blood 2014; 123: 78-85.
16. Khan R, Dhodapkar M, Rosenthal A et al. Four genes predict high risk of progression from smoldering to symptomatic multiple myeloma (SWOG S0120). Haematologica 2015.
17. Moulopoulos LA, Dimopoulos MA, Smith TL et al. Prognostic significance of magnetic resonance imaging in patients with asymptomatic multiple myeloma. J Clin Oncol 1995; 13: 251-256.
18. Hillengass J, Fechtner K, Weber MA et al. Prognostic significance of focal lesions in whole-body magnetic resonance imaging in patients with asymptomatic multiple myeloma. J Clin Oncol 2010; 28: 1606-1610.
19. Mateos MV, San Miguel J. V. Smoldering multiple myeloma. Hematol Oncol 2015; 33 Suppl 1: 33-37.

GENE THERAPY FOR THALASSEMIA: STATE OF THE ART

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Severe β-thalassemia (β-thal) is a form of congenital anemia caused by reduced or absent production of the β-globin chains of the adult hemoglobin (HbA), a tetramer made of two α and two β-globin chains. It is a heterogeneous disorder and more than 200 mutations in the β globin gene locus have been described causing a B-thal phenotype (Higgs *et al.*, 2012), characterized by a variety of clinical manifestations. B-thal patients' bone marrow contains 5-6 times the number of erythroid precursors than normal donors', with a number of apoptotic cells at the differentiative stage of polychromatic or ortochromatic erythroblast about 15 times greater. The reason of this condition might depend on the fact that the reduced or absent β-globin chain synthesis leads to an excess and imbalance of α-chains which, in turn, is responsible of two phenomena closely related: the increased peripheral destruction of circulating red blood cells (hemolytic anemia) and the death of erythroid precursors within the bone marrow (ineffective erytropoiesis or intramedullary hemolysis). Patients affected by B-thal present severe anaemia in the first years of life, stigmata of chronic haemolysis, hepatosplenomegaly, skeletal abnormalities due to rapid expansion of erythroid bone marrow and complications related to the iron overload such as cardiopathies, hepatic dysfunction and endocrine disorders. Treatment of B-thal is essentially supportive. Patients require lifelong transfusions combined with iron chelation therapy to reduce hemosiderosis that is ultimately fatal if not continuously treated. Optimal clinical management, based on regular blood transfusions and iron chelation therapy, have greatly improved the survival and quality of life of β-thalassemia major patients converting a previously fatal disease into a chronic, progressive disease with a life expectancy into adulthood. Indeed, B-thal remains a challenge in developing areas where children have poor access to safe blood products and iron chelat-

ing drugs, resulting in a life expectancy below 20 years of age (Modell and Darlison, 2008). At present, the only curative approach is represented by allogeneic hematopoietic stem cell transplantation (HSCT) and recent recommendation from the European Society for Blood and Marrow Transplantation (EBMT) Inborn Error and EBMT Paediatric Working Parties (Angelucci *et al.*, 2014) indicate this therapeutic option for young patients. Possible transplant sources are an HLA identical sibling, a matched unrelated donor (MUD), a mismatched family donor (haplo) or an unrelated umbilical cord blood (UCB) unit, the latter two still considered experimental options. However, it should be considered that the probability to find a matched sibling donor (in the EU population) is <25% while the probability to find a matched donor (10/10) is about 25%. Thus, about one half of the patients' population will not have access to a well-matched donor for allogeneic transplant. *Ex vivo* gene transfer of the β-globin gene into autologous HSCs of B-thal patients and transplantation of these genetically-modified HSC potentially represents a cure applicable to all patients regardless of donor availability and free from transplant related immunological complications such as graft rejection and GVHD. The evidence that as little as 15-20% donor chimerism can cure B-thal patients from transfusion dependence provides the rationale for the development of a gene therapy approach (Andreani *et al.*, 2011). Gene therapy for B-thal, as an alternative cure to allogeneic HSCT, is based on the autologous transplantation of hematopoietic stem cells (HSCs) engineered by lentiviral vectors (LV) expressing a transcriptionally regulated human β-globin gene. The development and large scale production of clinical grade LV, and the optimization of gene transfer protocols in human CD34+ cells have progressed this field to the pioneering clinical trials in France and in USA. The first clinical trial using a LV to treat patients with β-thalassemia was started in 2007 in France (LG001 Study) (Cavazzana-Calvo *et al.*, 2010). More recently, two other trials have been approved in U.S.A (NTC01639690, sponsor MSKCC and NTC01745120, sponsor Bluebird Bio). The trials sponsored by Bluebird Bio are ongoing in France (HGB-205 Study) and in USA (Northstar Study). Very recently, communication at scientific meetings reported promising results of early engraftment, hemoglobin expression and transfusion independence in patients treated in Bluebird Bio trials. Our contribution to this field was devoted to the clinical development of a safe gene therapy approach, based on high-titer globin vector GLOBE, new source of HSCs and an innovative clinical protocol favoring efficient engraftment of corrected cells with reduced toxicity. We have developed the GLOBE LV, able to express therapeutic levels of β-globin under the transcriptional control of a minimal β-globin promoter regulated by a locus control region (LCR), containing sequences from the HS2 and HS3 elements. We have demonstrated that GLOBE LV is able to provide long-term correction with an *in vivo* selection of genetically-corrected erythroid cells in a severe B-thal mouse model (Miccio *et al.*, PNAS 2008 and PLoSOne 2011), and to restore the normal erythropoiesis by transduced CD34$^+$ cells from B-thal patients (Roselli *et al.*, EMBO MolMed 2010; Milsom and Williams, 2010). Starting from these proofs of efficacy of gene therapy in thalassemic mice and in hematopoietic cells from patients, we moved towards the clinical development by assessing the risk/benefit ratio prior to administration in humans, in comprehensive *in vivo* pre-clinical studies. Evaluating the biosafety of gene therapy medicinal products following EMA and ICH guidelines, in the GLPs (good laboratory practices) framework, provided results of scientific significance within regulatory standards, paving the way towards future market registration. GLP main studies of toxicology and tumorigenicity (in thalassemic mice) and biodistribution (in NSG mice) proved safety and efficacy of gene therapy with GLOBE vector. The clinical trial proposal was anticipated for Scientific Advice to EMA and approved by institutional ethical committee and Italian regulatory authorities (AIFA/ISS). The clinical study TIGET BTHAL (NCT02453477) will be presented.

References

Higgs, D.R., J.D. Engel, and G. Stamatoyannopoulos. 2012. Thalassaemia. Lancet 379:373-383.

Modell, B., and M. Darlison. 2008. Global epidemiology of haemoglobin disorders and derived service indicators. Bull World Health Organ 86:480-487.

Angelucci, E., S. Matthes-Martin, D. Baronciani, F. Bernaudin, S. Bonanomi, M.D. Cappellini, J.H. Dalle, P. Di Bartolomeo, C.D. de Heredia, R. Dickerhoff, C. Giardini, E. Gluckman, A.A. Hussein, N. Kamani, M. Minkov, F. Locatelli, V. Rocha, P. Sedlacek, F. Smiers, I. Thuret, I. Yaniv, M. Cavazzana, and C. Peters. 2014. Hematopoietic stem cell transplantation in thalassemia major and sickle cell disease: indications and management recommendations from an international expert panel. Haematologica 99:811-820.

Andreani M, Testi M, Gaziev J, Condello R, Bontadini A, Tazzari PL, Ricci F, De Felice L, Agostini F, Fraboni D, Ferrari G, Battarra M, Troiano M, Sodani P, Lucarelli G. 2011. Quantitatively different red cell/nucleated cell chimerism in patients with long-term, persistent hematopoietic mixed chimerism after bone marrow transplantation for thalassemia major or sickle cell disease. Haematologica 96:128-33.

Cavazzana-Calvo, M., E. Payen, O. Negre, G. Wang, K. Hehir, F. Fusil, J. Down, M. Denaro, T. Brady, K. Westerman, R. Cavallesco, B. Gillet-Legrand, L. Caccavelli, R. Sgarra, L. Maouche-Chretien, F. Bernaudin, R. Girot, R. Dorazio, G.J. Mulder, A. Polack, A. Bank, J. Soulier, J. Larghero, N. Kabbara, B. Dalle, B. Gourmel, G. Socie, S. Chretien, N. Cartier, P. Aubourg, A. Fischer, K. Cornetta, F. Galacteros, Y. Beuzard, E. Gluckman, F. Bushman, S. Hacein-Bey-Abina, and P. Leboulch. 2010. Transfusion independence and HMGA2 activation after gene therapy of human beta-thalassaemia. Nature 467:318-322

Miccio, A., R. Cesari, F. Lotti, C. Rossi, F. Sanvito, M. Ponzoni, S.J. Routledge, C.M. Chow, M.N. Antoniou, and G. Ferrari. 2008. *In vivo* selection of genetically modified erythroblastic progenitors leads to long-term correction of beta-thalassemia. Proc Natl Acad Sci U S A 105:10547-10552.

Miccio A., Poletti V., Tiboni F., Rossi C., Antonelli A., Fulvio Mavilio F. and Ferrari G. 2011. The GATA1-HS2 enhancer allows persistent and position-independent expression of a beta-globin transgene. PLoS ONE 6 (12):e27955

Roselli, E.A., R. Mezzadra, M.C. Frittoli, G. Maruggi, E. Biral, F. Mavilio, F. Mastropietro, A. Amato, G. Tonon, C. Refaldi, M.D. Cappellini, M. Andreani, G. Lucarelli, M.G. Roncarolo, S. Marktel, and G. Ferrari. 2010. Correction of beta-thalassemia major by gene transfer in haematopoietic progenitors of pediatric patients. EMBO Mol Med 2:315-328.

PLATELET INDICES. CONTRIBUTION TO THE CLINICAL DIAGNOSIS OF THROMBOCYITOPENIA

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Since the discovery of the platelet population by Giulio Bizzozzero (1882), a great amount of studies have been carried out regarding megakariocytopoiesis, platelet morphology and thrombocyte count; more recently, researchers focused their attention on RNA, especially related to the different stages of cell maturation and functionality. In the 1960s, in fact, platelets were shown to be active cells, containing mRNA formed during thrombocytopoiesis, presenting protein-synthetic activity. Nowadays, by the new technologies applied in studying RNA and proteins expression patterns, platelets have been shown to play a fundamental role in haemostasis as well as in intercellular communication, in inflammatory response, in modulation of immunological activity (1). The continuous research on platelet structure and the latest discoveries about Ribonucleic Acid testify the above-mentioned functions; nevertheless, for many years the main aim of scientific research has been to improve platelet counting because of the impact on clinical problems due to errors in their number evaluation, especially in thrombocytopenic patients. In fact, the difficulties presented in studying platelet cluster are related to their intrinsic cellular characteristics (morphology, volumes, absence of nucleus), and, in practice, they more or less affect all methods available (Indirect counting: 1912, Fonio and 1932, Demasheck; Direct counting: 1923, Rees-Ecker using a direct light microscope and 1953, Breker-Conkire using a phase contrast microscope). Since the 60's a steady development of counting with semi-automatic methods on plasma rich in platelets (PRP) occurred; later the test became completely automatic and on whole blood. The high level of technological performance obtained in counting methods (impedance, optical scattering and fluorescence) and the possibility to obtain integrated and sophisticated instrumental "synchretisms" have given an important contribution towards more precise and efficient platelet counts, leading to a re-definition, by mean of guidelines of the threshold value of prophylactic transfusional therapies in order to reduce the risks (transfusional reactions, alloimmunization, viral infections) and the cost involved. In Edinburgh, 1997, (Consensus Conference on Platelet Trasfusion), on the basis of a revision of publication during the '90s (in particular, Gmur *et al.*, 1991, and Rebulla *et al.*, 1997), the Italian Group for the study of Hematological Disease in the Adult (GIMEMA), established to decrease the threshold for prophylactic platelets transfusion in stable patients at 10.000/microliter and to restrict higher thresholds only to specific situations (20.000/microliter if patient treated with heparin, or suffering from coagulopathies or undergoing bone marrow biopsy; 30,000/microliter for catheter insertion by central venous access; 50,000/microliter for minor surgery). In 2001, the American Society of Clinical Oncology guidelines also suggested a threshold that

could be even lower than 10,000/microlitre in some patients with solid tumors except patients with aggressive chemotherapy for bladder neoplasia or in cases of necrotic tumors. In 2003, finally, the British Committee for Standards in Hematology guidelines allowed to decrease the prophylactic platelets threshold from 10,000/microliter to 5,000/microliter in presence of clinical risk of platelet refractoriness from alloimmunization (grade B, level IIa). To meet the advanced clinical demands, the technological synchretisms for platelet counting offer increased accuracy and, further, various additional parameters about platelets. These data can provide basic information about the morphology, functionality, hemostatic balance and may result of some clinical utility in thrombocytopenia's classification.

The Platelet Indices can be described as shown below:

- parameters common to all technologies of platelet counters. In this case, the meaning of the data are similar, also if supplied by different technology;
- instrumental-linked data. These platelet parameters are not provided by all the technologies, although they can indicate similar functions.

A) Platelet parameters common to all counting technologies:

The mean platelet volume (MPV) represents the mean of the platelet volumes and, along with their number, defines the platelet mass. It is expressed in femtoliters (fl). MPV shows a non-linear, inverse relationship with the platelet count. Its use has been evaluated in the study of the origin of cases of thrombocytopenia ethiopathogenesis, in the assessment of the risk both hemorrhagic or thrombotic one. Platelet Distribution Width (PDW) is the variation coefficient of platelet volume distribution, an index of anisocytosis. In some cases it is more efficient in revealing anomalies in volume, especially in myeloproliferative diseases and myelodisplasic syndromes, while it seems to be less useful in hemorrhagic events. The plateletcrit (PCT%; PLTxMPV) expresses the platelet mass per blood volume unit and represents the active hemostatic mass. It is considered an index of hemorrhagic risk.

B) Instrumental-linked parameters:

These include:

The percentage of Large PLTs, referring to their total, with the achronym P-LCR, expressing increased turnover.

The concentrations of small and large-sized platelets, PLTs, PLTL, detected by monoclonal antibody CD61 and CD41. The larger ones are the first elements attending at the hemostatic process in great hemorrhages. Their number is constant in normal subjects and they are known to be the most sensitive and active in reply to aggregating drugs.

The Mean Platelet Component (MPC) measures in g/l the whole concentration of the internal components, whose width of distribution is indicated by the achronym PCDW. It is indicated as a screening test for the platelet activation process in patients with thrombotic or hemorrhagic risk. An inverse correlation is known with the state of platelet activation, involved in the pathogenesis of several diseases (coronary disease, Alzheimer, diabetes, =preeclampsia).

The Mean Platelet Mass (MPM) (pg) is the relationship between the platelet volume and the mean platelet component; its distribution width is defined with the achronym PMDW.

The fraction of immature PLT is called IPF or r-PT according to the instruments used; it is obtained in a fluorescent optical test in which fluorescent staining bind the platelet RNA.

The current use of these parameters in the laboratories is suggested by several clinical studies. In these reported studies, there was evaluated the trend of the instrumental data with different congenital and acquired pathologies in order to demonstrate possible advantages in diagnosis, follow up, and thrombocytopenia's prognosis.

The parameters most studied and used for thrombocytopenia are MPV and IPF.

MPV: The evaluation of MPV is useful for clinical and therapeutic follow up of thrombocytopenia with different and multi-factorial origin. This index decreases in marrow deficiency and aplasia, in hypersplenism and during/after chemotherapy, in vitamin B12 and/or folic acid deficit, in acute leukemia and reactive thrombocytosis. MPV is used to differentiate a thrombocytopenia when due to increased peripheral destruction or to decreased bone marrow production. In the context of acquired pathologies, this parameter increases in myeloproliferative syndromes, splenectomy, cerebral and post myocardial infarction, hypertension, hyperlipidemia, diabetes, preeclampsia and sepsis. If compared to the small platelets, those with a high mean volume give the most efficient hemostatic response as they present a higher prothrombotic material amount, increased expression of GPIIb-IIIa receptors and enhanced response to the ADP aggregation pattern. Some authors (Kaito *et al.*, 2005) have shown that the slower the bone marrow production results, the lower MPV value is, and higher indices are found in patients with autoimmune thrombocytopenia and more efficient megakaryopoiesis compared to patients with aplastic anemia. Other authors (Numbenjapon *et al.*, 2008) suggest a cut-off to distinguish thrombocytopenia of central origin from those of peripheral ones: MPV>7.9 in the hyperproduction from peripheral destruction and <7.9 hyperproduction due to bone marrow damage (2). MPV was also used to classify the different types of congenital thrombocytopenia, becoming a part of the diagnostic algorithms in hereditary pathologies. Although congenital thrombocytopenia is rare, its frequency is probably underestimated because of the diagnostic difficulties, especially in patients with a negative family history (Balduini L. *ET AL.*,2003)(3).

IPF: Recently introduced, IPF holds a large clinical interest, mostly in the evaluation of thrombocytopenia pathogenesis. Case of reduced bone marrow production, the parameter is normal or decreased; when due to increased destruction/consumption it results increased. Many studies show that also in other different pathologies, such as Thrombocytopenic Thrombotic Purpura or Idiopathic Thrombocytopenic Purpura (ITP), the IPF raises and an inverse correlation with the PLT count reflects the degree of thrombocytopoiesis. In some studies, this pattern was analyzed related to the pathology stage. Adly *et al.* defined an IPF cut-off (9.4% for a diagnosis of ITP, with sensitivity 88% and specificity 85.7%) studying 41 children with Autoimmune Thrombocytopenic Purpura, compared with 14 patients undergoing chemotherapy for hematological diseases and healthy control subjects). In particular, higher IPF values are found in chronic ITPs compared to acute forms, so as it is high in active forms compared to the remission ones and in non-responders therapy compared to responders. Substantially, this parameter can be considered a good prognostic marker and useful for therapeutic monitoring (4). In addition, it can indicate a megakaryopoiesis recovery avoiding to perform an invasive and potentially dangerous handling as bone-marrow biopsy, unnecessary especially if the case history and clinical picture are typical of Acute PTI (Grade B recommendation) and the isolated thrombocytopenia is the only alteration in the peripheral blood cell count (4). In cases of hepatopathies, IPF appears to be particularly useful in proving thrombocytopenia of multifactorial origin. In this clinical scenario, platelets recovery can be predicted without a bone marrow biopsy, using different and more complex analytical pathways, as the full picture is a result depending on the growth factor synthesis linked to the liver failure degree, on the amount of bone marrow dysfunction for toxic effect of etiological agents (alcohol, viruses), as well as on possible concomitant hypersplenism or essential factors deficit (B12, Folic acid) (5). There is a great interest about IPF as a marker of post-transplant recovery. This parameter seems to be the earliest in increasing, followed by the IRF (Immature Reticulocyte Fraction), PLT and ANC (Absolute Neutrophil Count). It has recently been described that, in the autologous transplant, IPF cut-off value=5.3% predicts a platelet recovery within two days, with a positive prediction value=0.93 (6). Some authors highlighted a possible use of the IPF in hematological diseases. The evaluation of this parameter in the context of onco-hematological pathologies is still under study and verification because of the well-known co-existence of a wide morphological variability of the platelet population; other studies suggest instead a prognostic value in myelodysplastic syndromes. From a general morphologic point of view, most patients with high IPF values present signs of dysmegakaryopoiesis associated with marked peripheral platelet anisocytosis; as to the karyotype study, cases with a worse prognosis can show chromosome anomalies, including monosomy 7 and complex abnormalities with 7 or 5q- involvement (7). In premature newborn with low weight, thrombocytopenia is one of the commonest hematological disorders observed in neonatal intensive care, effecting up to 35% of newborns in hospital; these prevalence data increase for those with a low weight. In most cases it is a form of "consumption" in a reduced capacity of the compensatory response by the bone marrow, due to thrombopoietin low levels and to a consequent reduced megakaryopoiesis. In this kind of thrombocytopenia, various etiologies can be found: the commonest are infections, drugs, DIC, sepsis, necrotic enterocolitis, delayed intrauterine growth-usually associated with neutropenia, premature birth, perinatal asphyxia, T.T.P. and congenital HUS. In these infants, the IPF can be monitored as an early marker of platelet recovery, allowing the number of transfusions to be reduced (8). Recent studies showed the

possibility to use IPF in screening macro thrombocytopenia, a congenital pathology characterized by the presence of large platelets (more than 20% of platelets have a diameter>4 microns) and thrombocytopenia (<150, 000/microliter) and differentiating it from other forms of macro thrombocytopenia. IPF reaches values till five times greater (48.6 +/- 1.9%) in patients with congenital pathology and twice greater in the other types of macro thrombocytopenia, when compared to subjects with immune thrombocytopenia (9). Finally, an interesting IPF clinical contribution was provided about studying donors blood for platelet transfusion: in presence of high basic values of the parameter, the therapy came out more efficient and affected by fewer transfusion complications. *Conclusions:* Despite all what above expressed about the usefulness of platelet parameters, their validity is trial because of the marked variability and the difficulty to standardize their detection. The main current issues are still represented by the remarkable instrumental and method links, critical features about pre-analytic phase (time, temperature) and, particularly, the effects/interferences due to the anticoagulant used in blood sample collecting. MPV is a specific instrument and depends markedly on the anticoagulant used. The EDTA induces a platelet swelling changing their volume (and therefore shape), but also affecting the internal refraction index, which leads to values variable by time, sometimes in an opposite direction according to the different analytical technique used, (impedance/ optical). Various studies have shown that the variations in the MPV caused by EDTA are quite unpredictable, with artifact oscillations varying from 2% to 50%. The use of alternative anticoagulants is mostly limited. Impedance methods usually have a threshold limit greater than the size of platelets between 24 and 36 fl, so that the calculation of the MPV could exclude the large platelets depending on the instruments. The optical systems cause a MPV underestimation by diluting the cytoplasmic components, as shown by the light scatter. Because of this extreme variability, MPV as a clinical tool is recommended to be observed, experimented and used under laboratory control. Also IPF presents limitations linked to its instrument technical link. Actually, GdSE-SIMeL recommendations (2002) (10) about platelet indices can still be considered valid. The use of platelet parameters is suggested to create standardized and shared comments, since the technical characteristics necessary for a recognized and universal use are not present and the possibilities to harmonize individual results at the moment seem to be few. Not included in the report, they can nonetheless be a tool of specific advice dedicated to the clinician by laboratory's hematologist and/or be included in articulated comments. Laboratory personnel must know the technical value, the possible clinical meaning of these instrumental parameters and the patient history in order to suggest if, when and how these data can be really useful to the clinician, till to propose their inclusion in standardized diagnostic pathways and prognostic procedures. Finally we must not forget the vital role that microscopic observation has at the beginning of diagnostic procedure. A peripheral blood smear can allow a diagnosis of exclusion and identification of artifacts (pseudo-thrombocytopenia, …), evaluation of platelets in the morphology (staining, volume, anisocytosis, …), single or combining defects of all the blood cells suggesting clonal or systemic pathological processes. From consistency of this first observation point, the evaluation of appropriate diagnostic insights and the interpretative synthesis of a specialist, the laboratory can provide great value information for diagnosis, prevention and treatment of illness.

References

1. Rowley JW, Schwertz H, Weyrich AS.Platelet mRNA: the meaning behind the message. Curr Opin Hematol. 2012 Sep;19(5):385-91.
2. Numbenjapon T, Mahapo N, Pornvipavee R, Sriswasdi C, Mongkonsritragoon W, Leelasiri A, Prayoonwiwat W. A prospective evaluation of normal mean platelet volume in discriminating hyperdestructive thrombocytopenia from hypoproductive thrombocytopenia. Int J Lab Hematol. 2008 Oct;30(5):408-14.
3. Balduini CL, Cattaneo M, Fabris F, Gresele P, Iolascon A, Pulcinelli FM, Savoia A; Italian Gruppo di Studio delle Piastrine.Inherited thrombocytopenias: a proposed diagnostic algorithm from the Italian Gruppo di Studio delle Piastrine.Haematologica. 2003 May;88(5):582-92.
4. Adly AA1, Ragab IA, Ismail EA, Farahat MM. Evaluation of the immature platelet fraction in the diagnosis and prognosis of childhood immune thrombocytopenia. Platelets. 2014 Oct 28:1-6.
5. Zucker ML1, Hagedorn CH, Murphy CA, Stanley S, Reid KJ, Skikne BS. Mechanism of thrombocytopenia in chronic hepatitis C as evaluated by the immature platelet fraction. Int J Lab Hematol. 2012 Oct;34(5):525-32. doi: 10.1111/j.1751-553X.2012.01429.x. Epub 2012 Jun 18.
6. Noreen van der Linden, Lieke J.J. Klinkenberg, Steven J.R. Meex, Erik A.M. Beckers, Norbert C.J. de Wit, Lenneke Prinzen. Immature platelet fraction measured on the Sysmex XN hemocytometer predicts thrombopoietic recovery after autologous stem cell transplantation. European Journal of Haematology 93 (150–156).
7. Sugimori N, Kondo Y, Shibayama M et al. (2009) Aberrant in- crease in the immature platelet fraction in patients with myelodysplastic syndrome: a marker of karyotypic abnormalities associated with poor prognosis. Eur J Haematol 82:54–60.
8. Yuko S, Takeda T, Hirota A, Hisaeda Y, Amakata S, Nakao A, Kawakami T. Examination of the percentage of immature platelet fraction in term and preterm infants at birth. J Clin Neonatol. 2013 Oct;2(4):173-8.
9. Miyazaki K, Koike Y, Kunishima S, Ishii R, Danbara M, Horie R, Yatomi Y, Higashihara M. Immature platelet fraction measurement is influenced by platelet size and is a useful parameter for discrimination of macro thrombocytopenia. Hematology. 2015 Jun 2
10. Cappelletti, B. Biasioli, P. Bulian, M. Buttarello, A. Cenci, G. Da Rin, P. Doretto, V. Miconi, L. Pasini, C. Piccinini, G. Pigoli, E. Piva Linee guida per il referto ematologico Riv Med Lab-JLM, Vol. 3, N. 2-S1, 2002.

GLI INDICI PIASTRINICI:

CONTRIBUTO ALLA DIAGNOSTICA CLINICA DELLE PIASTRINOPENIE

Domande

A. Quale è la soglia profilattica delle piastrine nel rischio clinico di refrattarietà piastrinica da alloimmunizzazione decisa nell'anno 2003 dalle linee guida del British Committee for Standard in Hematology?

1) 10.000/microlitro
2) 50.000/microlitro
3) 5.000/microlitro
4) 20.000/microlitro

B. Quali sono gli indici piastrinici comuni a tutte le metodologie di lettura?

1) MPV, MPC, IPF
2) MPV, PDW, PCT
3) PMDW, PLTI, PCT
4) PDW, P-LCR, MPM

C. Quali sono le limitazioni degli indici piastrinici?

1) Sono strumento specifici
2) Sono crono labili
3) Nono sono standardizzati
4) Tutte le precedenti

D. Refertazioni degli indici piastrinici secondo GdSE-SIMeL?

1) Possono essere refertati
2) Possono essere interpretati e integrati, in commenti articolati
3) Possono essere refertati con indicazione della tecnologia di lettura
4) Tutte le precedenti

SECONDARY LEUKEMIAS AFTER CHILDHOOD CANCER. THE AIEOP EXPERIENCE

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As a result of the ever-increasing success rates achieved in recent decades in paediatric oncology, an increasing number of children and adolescents have successfully overcome their cancer experience and have reached or are entering adulthood. However, "cure" may come to a cost, in particular in children when anticancer treatments are given to growing individuals. According to a British population based study[1], childhood cancer survivors (CCS) have an almost 11 fold increased risk of death standardized mortality ratio (SMR) 10.7 (95% CI 10.3-11.1) as compared to their age and gender peers; and second primary cancers are the second most frequent cause of death after recurrence of the first cancer with a SMR ranging between 7.3 (95% CI: 6.7-8.0) to 15.2 (95% CI: 13.9 to 16.6). Subsequent primary malignant neoplasms (SMN) are defined as any distinct cancer developing after the occurrence of the first one. According to the population based Nordic study[2], CCS have a 3.3 (95% CI 3.1-3.5) fold increased risk of developing a SMN compared to the general population, and the incidence increases with the ageing of the population. A similar North American cohort study[3] reported a 30 year 7.9% cumulative incidence of subsequent neoplasms for a 6 fold increased risk (SIR=6.0, 95% CI=5.5 to 6.4) as compared to the general population.

Two main groups of SMN can be identified mainly based on treatment exposure: i) Solid malignant neoplasms that are mainly radiation-related, and represent about 80% of the total SMN burden. They are characterized by a long interval since first diagnosis usually exceeding 10 years; ii) Treatment-related myelodysplasia and acute myeloid leukaemia that represent the remaining 20% of SMN cases. Usually, these leukemias have a shorter latency occurring within the first 5 to 15 years after therapeutic exposure, and are often associated with alkylating agents and/or topoisomerase II inhibitors (epipodophyllotoxins) as part of the previous treatment protocol. Few SMN have also been reported in subjects not exposed to alkylating agents/epipodophyllotoxins nor to radiotherapy. Genetic predisposition may also increase the risk of SMN. However, inherited gene changes are quite uncommon and affect <10% of people diagnosed with childhood cancer. Secondary leukaemias can be further differentiated in two categories related to the previous exposure: alkylating agents or topoisomerase II inhibitors. - Cases secondary to alkylating agents exposure: i) typically develop 4-10 years after exposure, ii) in most cases are preceded by myelodysplasia and/or cytopenia; iii) are frequently characterized by abnormalities involving chromosomes 5 (–5/del[5q]) and 7 (–7/ del[7q]). - Cases secondary to topoisomerase II inhibitors: i) have a short latency period usually within 3-4 years since exposure; ii) are not preceded by myelodysplastic phase; iii) are often associated with balanced translocations involving chromosome bands 11q23 or 21q22. The schedule of epipodophyllotoxins (rather than cumulative dose) seems to increase the risk of topoisomerase II inhibitor associated AML; in fact in children receiving the drug weekly or twice weekly have 12.3 cumulative risk of developing the leukaemia while the figure is of 1.6% for those treated every two weeks[4]. The long term risk of developing treatment related leukaemia is not high, approaching 2% at 15 years after conventional therapy. Sub-groups of patients as those with acute lymphoblastic leukemia (ALL) treated with epipodophyllotoxins containing regimens, and those with high risk Ewing sarcoma treated with high doses of alkylating agents and topoisomerase II inhibitors, have higher cumulative risk (between 4 and 10% at 6 years).[3-6] The inter-individual variability in risk of developing an SMN for any given exposure to potentially carcinogenic treatments suggests a role for genetic variation in individual susceptibility. Mutations in high-penetrance genes that lead to serious genetic diseases *e.g.*, Li-Fraumeni syndrome, and Fanconi anaemia, may have a role in this, but the attributable risk is expected to be very small because of the extremely low prevalence of genes such as those implicated in Li-Fraumeni syndrome. More SMN cases may be explained by interindividual variability due to common polymorphisms in low penetrance genes that regulate metabolism of drugs into active agents, or those responsible for DNA repair. A particular group of "secondary leukaemias" is that observed associated to subjects affected by Langerhans Cell Histiocytosis (LCH). It is already known that among LCH patients malignancies occur to a rate higher than expected if they would occur by chance alone [6]. With regards to LCH associated leukaemias, both AML and ALL cases have been reported. Most cases are AML occurring following treatment with epipodophyllotoxins and/or alkylating agents. However, also several cases of association between LCH and ALL have also been reported. In this case, ALL (in most cases of T lineage) can either precede, be concurrent, or follow the LCH diagnosis. The majority of cases were documented within +/- 3 years from LCH diagnosis. A clonal origin of the two diseases can be speculated. *The Italian experience:* Since 1980 the Italian Association of Paediatric Haematology and Oncology (AIEOP) set up a retrospective and prospective registry to collect information on all children (age 0-21) with cancer that, independently from the further disease evolution, have reached the elective end of therapies (the off therapy status). At the last update, the Off-Therapy Registry (OTR) had collected information on 13,485 subjects (55. 8% males, 44.2% females). All tumour types are represented with ALL representing the larger group (37.6%) followed by Hodgkin disease (9.4%), neuroblastoma (8.8%), central nervous system tumours (8.6%), Wilms tumour (8.1%) and non-Hodgkin lymphomas (7.9%). The median age at diagnosis was 5.2 years (IQR 2.8 – 9.6), and the median age at follow-up was 26.9 years (IQR 19.8-34.3), for a total of 175,761 person years at risk. Among these subjects 534 SMN were observed, of these 465 (87.1%) were solid tumours and only 69 (12.9%) were secondary leukaemias. Overall, the cumulative risk (CR) of secondary leukaemias was low (0.8% at 25 years (95%CI 0.62-1.05), with no further events occurring thereafter; on the contrary, the CR for solid malignant neoplasms was 7.5% (95% CI 6.7-8.3) at 25 years and continued to increase up to 24.6% (95%CI 19.5-30.8) after 40 years since diagnosis. Secondary leukaemias were more likely to occur in survivors exposed to alkylating agents and/or epipodophyllotoxins compared to those not exposed to these agents (0.9% vs. 0.1% P<0.0001). As expected (Figure 1) treatment related leukaemia cases observed in subjects exposed to epipodophyllotoxins only occurred with a shorter interval, mostly within the first 5 years since diagnosis CR 2.61 (95% CI: 0.98-6.83) at 25 years since diagnosis, while those occurring after AA only had longer latency period (3-25 years) and a CR of 1.45(95% CI: 0.96-2.18).

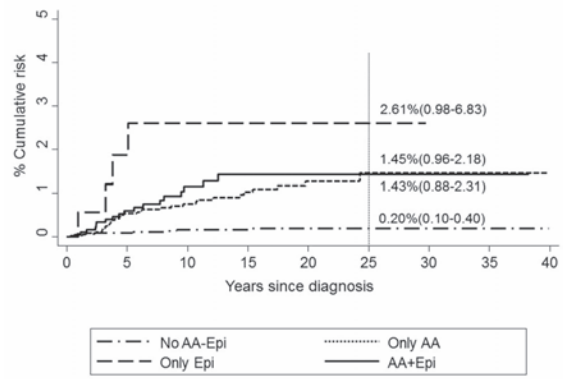

Figure 1. Cumulative risk of developing treatment related secondary leukaemia by type of treatment exposure among childhood cancer survivors enrolled in the Italian OTR Registry.

Subjects treated both with AA and epipodophyllotoxins had an intermediate pattern of occurrence CR 1.43 (95% CI: 0.88-2.31), while the CR was 0.2 (95% CI: 0.1-0.4) among subjects exposed neither to AA nor epipodophyllotoxins (P<0.0001). Standardized incidence ratios (SIRs) of secondary leukaemias were calculated using standard cohort techniques. The SIR was defined as the ratio of the observed over the expected (O/E) number of leukaemias. To derive the expected number of leukaemias used in the calculation of the SIR, person-years for each sex-specific, age-specific (5-year bands), and calendar year–specific (5-year bands) stratum were multiplied by the corresponding incidence rate for the population of Italy and then summed across the strata. SIRs were stratified by the following factors: sex, type of childhood cancer and type of treatment. The confidence interval of SIR were calculated according to a model with a Poisson error distribution and the log of the expected number of leukaemias as offset was employed. Compared to age and gender peers from the Italian general population, CCS had a 9.86 fold (95% CI 7.79-12.49) increased risk of developing a treatment related leukaemia (O/E: 69/6.9).

Table. Standardized Incidence rate of treatment-related secondary leukemias, by different risk factors among childhood cancer survivors enrolled in the Italian OTR Registry.

	Observed	pyr	Expected	SIR	95% CI
overall	69	175761.1	6.99	9.86	7.79-12.49
Gender					
F	38	78828.73	2.85	13.35	9.71-18.35
M	31	96932.39	4.15	7.47	5.26-10.63
Type of first cancer					
ALL	11	75672.88	2.92	3.76	2.08-6.79
HD	10	19743.59	0.69	14.49	7.79-26.93
NHL	10	13861.73	0.52	19.27	10.37-35.82
Bone and soft tissue sarcomas	9	11831.3	0.47	19.089	9.93-36.69
NB	8	13571.64	0.65	12.35	6.17-24.69
CNS tumors	8	11181.67	0.47	17.21	8.60-34.41
AnLL	5	7356.942	0.30	16.86	7.01-40.49
WT	4	15353.83	0.64	6.25	2.35-16.66
Other tumors	4	7187.551	0.34	11.71	4.39-31.21
Chemotherapy					
AA/Epi	56	75244.23	3.10	18.04	13.88-23.45
No AA/Epi	8	88480.37	3.31	2.41	1.21-4.83
Only Epi	4	2041.3	0.09	44.49	16.69-118.54
AA + Epi	18	19681.56	0.86	20.83	13.12-33.06
Only AA	34	53241.9	2.14	15.90	11.35-22.24
No AA-Epi	8	88480.37	3.31	2.41	1.21-4.83

Table 1 also reports the SIR stratified by gender, type of first primary, and treatment exposure. Treatment with epipodophyllotoxins alone had the highest SIR 44.49 (95% CI: 16.69-118.54) although based on few events and a small sample size as shown by the wide 95% CI. Also combined treatment with epipodophyllotoxins and alkylating agents (SIR 20.83) and with AA alone (SIR 15.89) increased the risk of treatment related leukaemia. Subjects not exposed to these drugs had only a 2 fold risk of secondary leukaemias. Survival after treatment related leukaemia was poor with only 22 (31.9%) subjects still alive at the last follow-up. The cumulative probability of survival was 30.7(95% CI 20.1-41.9) after 10 years since diagnosis of the secondary leukaemia.

References

1. Reulen, R. C., Frobisher, C., Winter, D. L., Kelly, J., Lancashire, E. R., Stiller, C. A., et al. Long- term risks of subsequent primary neoplasms among survivors of childhood cancer. JAMA, 2011 (305), 2311–2319.
2. Olsen, J. H., Moller, T., Anderson, H., Langmark, F., Sankila, R., Tryggvadottir, L., et al. (2009). Lifelong cancer incidence in 47,697 patients treated for childhood cancer in the Nordic countries. J Nat Cancer Inst, 2009; 101 , 806–813.
3. Friedman, D. L., Whitton, J., Leisenring, W., Mertens, A. C., Hammond, S., Stovall, M., et al. Subsequent neoplasms in 5-year survivors of childhood cancer: The Childhood Cancer Survivor Study. J Nat Cancer Inst, 2010;102, 1083–1095
4. Pui CH, Ribeiro RC, Hancock ML, , Rivera, G. K., Evans, W. E., Raimondi, S. C., et al. Acute myeloid leukaemia in children treated with epipodophyllotoxins for acute lymphoblastic leukaemia. N Engl J Med. 1991;325(24):1682-7.
5. Bhatia, S., Krailo, M. D., Chen, Z., Burden, L., Askin, F. B., Dickman, P. S., et al. Therapy-related myelodysplasia and acute myeloid leukaemia after Ewing sarcoma and primitive neuroectodermal tumor of bone: A report from the Children's Oncology Group. Blood, 2007; 109:46–51.
6. Smith, M. A., Rubinstein, L., Anderson, J. R., Arthur, D., Catalano, P. J., Freidlin, B., et al. Secondary leukaemia or myelodysplastic syndrome after treatment with epipodophyllotoxins. J Clinl Oncol, 1999; 17: 569–577.
7. Haupt R, Nanduri V, Egeler M. Late effects of Langerhans cell histiocytosis and its association with malignancy. In: Weitzman SW, Egeler RM, editors. Histiocytic Disorders of Children and Adults. Cambridge press, 2005. p. 272-292.

KILLER IMMUNOGLOBULIN LIKE RECEPTORS AS PROGNOSTIC FACTOR IN CHRONIC MYELOID LEUKEMIA AND HODGKIN'S LYMPHOMA

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It is important to find innovative tools and/or biomarkers able to represent prognostic and predictive factors in patients suffering of classic Hodgkin lymphoma (cHL) or chronic myeloid leukemia (CML). Natural Killer (NK) cells are crucial components of the innate immune system for the recognition of virus-infected or neoplastic cells[1]. Their activity is regulated by several receptor families, including killer cell immunoglobulin-like receptors (KIRs) which recognize human leukocyte antigen (HLA) class I molecules expressed on target cells.[2] NK cells express different combinations of the inhibitory and activating KIR genes.[3] The KIR gene family has been divided into two groups of haplotypes, according to gene content: KIR A haplotypes, encoding for inhibitory genes, with higher allelic polymorphism, and KIR B haplotypes, containing various combinations of both activating and inhibitory KIR genes, with less allelic polymorphism.[5,6] KIR A haplotypes seem to provide better immune surveillance against viral infections and tumor cells, whereas KIR B haplotypes appear to have a favorable role during pregnancy.[7] KIR receptors recognize 4 mutually exclusive epitopes carried by HLA-A, -B and -C Class I molecules: C1, C2, Bw4 and A3/A11.[7,8] The diversity in gene content of KIR haplotypes and the extensive polymorphism of HLA and KIR genes determine extremely variable immunogenetic profiles among individuals.[3] We investigated KIR and HLA gene frequencies, KIR haplotypes and KIR-ligand combinations in a cohort of cLH patients and CML patients. Patients were stratified into two groups according to homozygosity for KIR A haplotype (KIR genotype AA), heterozygosity or homozygosity for KIR B haplotype (KIR genotypes AB and BB, referred together as KIR genotype Bx). *KIRs and CML:* In a previous report, we found that homozygosity for KIR A haplotype was significantly associated to achievement of deep molecular response ($MR^{4.5}$).[9] We investigated whether KIR genes and/or KIR haplotypes could represent predictive immune biomarkers of stable treatment free remission (TFR) in CML and which factors were related to relapse/non relapse after stopping tyrosine kinase inhibitors (TKIs) treatment. The distribution of KIR and HLA gene frequencies, KIR and HLA genotypes and KIR-ligand combinations were investigated in 22 chronic-phase CML patients who discontinued TKI treatment after achieving $MR^{4.5}$. All patients had been treated with TKI therapy for more than 2 years and had achieved $MR^{4.5}$ for at least 18 months. All patients achieved $MR^{4.5}$ after a mean of 24 months (range 3-93) and discontinued TKI treatment at a median of 72 months from the start of treatment (range 23-105). The median follow-up after discontinuation was 30 months (range 18-60). The 36-month probability of treatment-free remission (TFR) was 58.7%. The majority of relapses occurred within 8 months of interrupting therapy. TFR was significantly higher in patients not carrying the inhibitory KIR2DL2 receptor (81.4% *vs* 36.4%, p=0.039) (Figure 1). There was also a trend toward better TFR in patients homozygous for KIR A haplotype (80% *vs* 41.7%). Younger age, male sex and lower duration of treatment were significantly associated with relapse. All relapsed patients regained $UMR^{4.5}$ after restarting treatment with the same TKI. *KIRs and cHL:* There are still no strong immunobiomarkers capable of discriminating between cHL patients requiring less or more intensive treatment and the availability of such biomarkers would make it possible to maximize the benefit of treatment and at the same time to minimise treatment-related toxicity. We investigated the frequencies of KIRs and their ligands in a cohort of 80 cHL patients (35 males/45 females, mean age 33 years) and 121 healthy controls.[12] We also evaluated whether specific KIR genes or KIR haplotypes could be related to the achievement of negative interim Positron Emission Tomography (PET-2) and progression-free survival (PFS). In the management of cHL, PET is the standard procedure for disease staging. Interim PET after 2 cycles of treatment with adriamycin, bleomycin, vinblastine and dacarbazine (ABVD) is currently used in clinical practice as a strong predictor of response to therapy.[13] The 5-year overall survival (OS) and PFS were 91.9 and 79.4%, respectively. In multivariate analysis, homozygosity for KIR haplotype A was the only significant predictive factor of interim PET-2 negativity (p=0.035). Also PFS was higher, albeit not statistically significant, in patients homozygous for KIR haplotype A (87% *vs* 76.6%). *Conclusions:* There are currently no available prognostic immunebiomarkers capable of distinguishing cHL patients requiring more or less intensive treatment and/or identifying CML patients who could benefit from TKI discontinuation. Increasing evidences suggest that NK cells and their KIR receptors play a key role in controlling the leukemic growth. Further studies of NK cell function will be required to explore the possibility of increasing the number and activity of NK cell in combination with chemotherapy treatment.

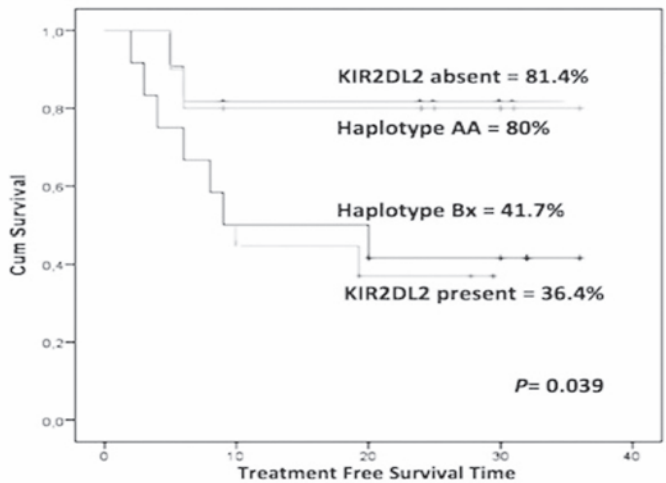

Figure 1. Treatment Free Remission cumulative probability in 22 CML patients according to KIR haplotype.

References

1. Smyth MJ, Hayakawa Y, Takeda K, Yagita H. New aspects of natural-killer-cell surveillance and therapy of cancer. Nat Rev Cancer. 2002; 2:850–61.
2. Purdy AK, Campbell KS. Natural killer cells and cancer: regulation by the killer cell Ig-like receptors (KIR). Cancer Biol The . 2009;8:2211-2220.12.
3. Bashirova AA, Martin MP, McVicar DW, Carrington M. The killer immunoglobu-

lin-like receptor gene cluster: tuning the genome for defense. Annu Rev Genomics Hum Genet. 2006;7:277-300.
4. Middleton D & Gonzalez F. The extensive polymorphism of KIR genes. Immunology. 2010;129:8-9.
5. Hsu KC, Chida S, Geraghty DE, Dupont B. The killer cell immunoglobulin-like receptor (KIR) genomic region: gene-order, haplotypes and allelic polymorphism. Immunol Rev. 2002;190:40-52.
6. Bontadini A, Testi M, Cuccia MC, et al. Distribution of killer cell immunoglobulin-like receptors genes in the Italian Caucasian population. J Transl Med. 2006;4:44.
7. Parham P, Moffett A. Variable NK cell receptors and their MHC class I ligands in immunity, reproduction and human evolution. Nat Rev Immunol. 2013;13:133-44.
8. O'Connor GM, Guinan KJ, Cunningham RT, et al. Functional polymorphism of the KIR3DL1/S1 receptor on human NK cells. J Immunol. 2007;178:235-241.
9. La Nasa, G., Caocci, G., Littera, R. Homozygosity for killer immunoglobin-like receptor haplotype A predicts complete molecular response to treatment with tyrosine kinase inhibitors in chronic myeloid leukemia patients. Experimental Hematology. 2013, 41, 424-431.
10. Mori S., Vagge E., le Coutre P. The Risk of Relapse in CML Patients Who Discontinued imatinib Can Be Predicted Based on Patients Age and the Results of dPCR Analysis. Blood. 2014, 124:813.
11. Diefenbach C, Advani R. Customized targeted therapy in Hodgkin lymphoma: hype or hope? Hematol Oncol Clin North Am. 2014;28:105-22.
12. Caocci G, Littera R, Oppi S. Homozygosity for Killer Immunoglobin-like Receptor (KIR) Haplotype a Is Associated to Negative Interim Positron Emission Tomography (PET) and a Favourable Outcome in Hodgkin Lymphoma. 56th Annual Meeting of the American Society of Hematology (ASH), San Francisco,(CA), USA. Blood 2014 124:136.
13. Gallamini A, Barrington SF, Biggi A. The predictive role of interim Positron Emission Tomography on Hodgkin lymphoma treatment outcome is confirmed using the 5-point scale interpretation criteria. Haematologica. 2014; 99:1107-13.

IMMUNE MECHANISMS OF DISEASE PROGRESSION IN MGUS: LESSONS FROM V 9V 2 T CELLS

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It is now well-recognized that Multiple Myeloma (MM) is universally preceded by Monoclonal Gammopathy of Undetermined Significance (MGUS)(1,2). However, only 1% of MGUS individuals progresses to overt MM per year (3), even if MGUS plasma cells already share many of the cytogenetic abnormalities harbored by myeloma cells in MM patients with progressive disease. MM is a paradigm disease in which the "seed and soil" theory formulated by Paget more than 120 years ago finds a full confirmation. The bone marrow (BM) is where clonal plasma cells initiate their challenge and where the local microenvironment and immune system face the challenge. The BM is a multi-faceted microenvironment populated by a great variety of cells of hematopoietic and non-hematopoietic origin whose interactions are finely tuned by membrane-bound proteins, cytokines, growth factors, and extracellular matrix components (4). Under physiological conditions, the BM microenvironment is set to accommodate less than 5% of polyclonal plasma cells. In MGUS, a clonal plasma cell population initiates to locally accumulate as a consequence of growth and survival advantages conferred by randomly occurred genetic alterations. Not being a physiological situation, the local homeostasis is perturbed and a variety of bystander cells are alerted and checkpoints activated to hold in check the expanding plasma cell clone. Even if many mechanisms remain unexplored, most of MGUS individuals succeed to hold in check the emerging plasma cell population, whereas a very minority fails to do that (approx 1% per year)(3). Several laboratories have dedicated a great effort to understand the microenvironment-dependent mechanisms involved in the transition from MGUS to MM (4,5). Our laboratory has a long-lasting interest in the role played by innate and adaptive immunity. The current dogma is that the immune system in MGUS individuals recognizes and hold in check clonal plasma cells, but this profitable activity is vanished in MM patients. The assumption is based on several reports showing the occurrence of natural antibodies against tumor-associated antigens (TAA) and the presence of TAA-reactive cytotoxic T cells in MGUS, but not in MM (6,7). Unconventional T cells such as NKT cells have also been reported to be fit in MGUS and become unfit in MM (8). Concurrently to the appropriate anti-tumor immune surveillance exerted by effector cells, there are reports indicating that suppressor cells such as regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSC) are increased and outbalance effector cells in MM patients compared with MGUS individuals. Thus, fading anti-tumor immune responses are claimed to play a major role in MGUS to MM evolution. However, the lack of longitudinal studies does not allow to conclude whether immune failure precedes or it is rather a consequence of disease progression. The difficulty in the interpretation is also due to the bias that most studies have interrogated the immune competence of MGUS and MM patients in the peripheral blood (PB) rather than the BM. Recently, we have initiated to challenge the dogma that anti-tumor immune surveillance is fully preserved in the BM of MGUS individuals. We have shown that Tregs are steadily rooted in the BM of MGUS individuals and MM patients without any difference in terms of number, phenotype, and function (9). Likewise, we have recently shown that the frequency of PD-L1+ MDSC is similar in MGUS and MM patients at diagnosis (10). Further evidence that the BM microenvironment is already immunologically compromised in MGUS is provided by the analysis of V 9V 2 T cells. These are non-conventional T cells halfway between innate and adaptive immunity with a natural inclination to react against malignant B cells, including myeloma cells (11). We have found that the BM microenvironment in MM significantly hampers the ability of BM V 9V 2 T cells to react against their natural ligands including those generated by zoledronic acid (ZA)(12,13) V 9V 2 T-cell dysfunction is an early event that can be already detected in individuals with MGUS and not fully reverted even when MM patients achieve clinical remission. BM V 9V 2 T cells are PD-1+ in MGUS, MM at diagnosis and remain PD-1+ in MM in remission. These data may explain why ZA administration in high-risk MGUS and smoldering myeloma decreased the frequency of bone lesions, but not the transition rate to MM (14). The crucial role played by the BM microenvironment in the suppression of anti-myeloma immune responses is highlighted by the observation in individual patients that V 9V 2 T cells are dysfunctional only in the BM, but not in the PB (11). This explains why the clinical trials that have used ZA or other antigens to intentionally activate V 9V 2 T cells *in vivo or ex vivo* have fallen short of clinical expectations even when patients were selected based on the *in vitro* reactivity of PB V 9V 2 T cells (15,16). In conclusion, our results challenge the dogma that MGUS individuals are fully immune competent and provide the rationale to explore immune checkpoint neutralization and Vg9Vd2 T-cell activation as non-genotoxic approaches to boost anti-myeloma immune surveillance in high-risk MGUS.

References

1. Landgren O, Kyle RA, Pfeiffer RM, et al.Monoclonal gammopathy of undetermined significance (MGUS) consistently precedes multiple myeloma: a prospective study. Blood.2009;113(22):5412-5417;
2. Weiss BM, Abadie J, Verma P, Howard RS, Kuehl WM. A monoclonal gammopathy precedes multiple myeloma in most patients. Blood. 2009; 113(22):5418-5422.
3. Kyle RA, San-Miguel JF, Mateos MV, Rajkumar SV. Monoclonal gammopathy of undetermined significance and smoldering multiple myeloma. Hematol Oncol Clin North Am. 2014 Oct;28(5):775-90
4. Kawano Y, Moschetta M, Manier S, Glavey S, Görgün GT, Roccaro AM, Anderson KC,Ghobrial IM. Targeting the bone marrow microenvironment in multiple myeloma. Immunol Rev. 2015 Jan;263(1):160-72
5. Bianchi G, Munshi NC. Pathogenesis beyond the cancer clone(s) in multiple myeloma. Blood. 2015 May 14;125(20):3049-58
6. Spisek R, Kukreja A, Chen LC, Matthews P, Mazumder A, Vesole D, Jagannath S, Zebroski HA, Simpson AJ, Ritter G, Durie B, Crowley J, Shaughnessy JD Jr, Scanlan MJ, Gure AO, Barlogie B, Dhodapkar MV. Frequent and specific immunity to the embryonal stem cell-associated antigen SOX2 in patients with monoclonal gammopathy. J Exp Med. 2007 Apr 16;204(4):831-40.
7. Blotta S, Tassone P, Prabhala RH, Tagliaferri P, Cervi D, Amin S, Jakubikova J, Tai YT, Podar K, Mitsiades CS, Zullo A, Franco B, Anderson KC, Munshi NC. Identification of novel antigens with induced immune response in monoclonal gammopathy of undetermined significance. Blood. 2009 Oct 8;114(15):3276-84.
8. Dhodapkar MV, Geller MD, Chang DH, Shimizu K, Fujii S, Dhodapkar KM, Krasovsky J. A reversible defect in natural killer T cell function characterizes the progression of premalignant to malignant multiple myeloma. J Exp Med. 2003 Jun 16;197(12):1667-76.
9. Foglietta M, Castella B, Mariani S, Coscia M, Godio L, Ferracini R, Ruggeri M, Muccio V, Omedé P, Palumbo A, Boccadoro M, Massaia M. The bone marrow of myeloma patients is steadily inhabited by a normal-sized pool of functional regulatory T cells irrespectiveof the disease status. Haematologica. 2014 Oct;99(10):1605-10.
10. Castella B, Foglietta M, Sciancalepore P, Rigoni M, Coscia M, Griggio V, Vitale C, Ferracini R, Saraci E, Omede' P, Riganti C, Palumbo A, Boccadoro M, Massaia M. Anergic bone marrow V 9V 2 T cells as early and long-lasting markers of PD-1-targetable microenvironment-induced immune suppression in human myeloma. OncoImmunology, 2015, in press
11. Castella B, Vitale C, Coscia M, Massaia M. V 9V 2 T cell-based immunotherapy in hematological malignancies: from bench to bedside. Cell Mol Life Sci. 2011; 540 68(14):2419-32;
12. Gober HJ, Kistowska M, Angman L, Jeno P, Mori L, De Libero G. Human T cell

receptor gammadelta cells recognize endogenous mevalonate metabolites in tumor cells. J. Exp. Med. 2003; 197:163-168;
13. Roelofs AJ, Thompson K, Gordon S, Rogers MJ. Molecular mechanisms of action of bisphosphonates:current status. Clin. Cancer Res. 2006; 12:6222s-6230s;
14. Musto P, Petrucci MT, Bringhen S, Guglielmelli T, Caravita T, Bongarzoni V, Andriani A, D'Arena G, Balleari E, Pietrantuono G et al. GIMEMA (Italian Group for Adult Hematologic Diseases)/Multiple Myeloma Working Party and the Italian Myeloma Network. A multicenter, randomized clinical trial comparing zoledronic acid *versus* observation in patients with asymptomatic myeloma. Cancer. 2008; 113(7):1588-95;
15. Wilhelm M, Kunzmann V, Eckstein S, Reimer P, Weissinger F, Ruediger T, Tony HP. Gammadelta T cells for immune therapy of patients with lymphoid malignancies. Blood. 2003; 102(1):200-6;
16. Abe Y, Muto M, Nieda M, Nakagawa Y, Nicol A, Kaneko T, Goto S, Yokokawa K, Suzuki K. Clinical and immunological evaluation of zoledronate-activated Vgamma9gammadelta T-cell-based immunotherapy for patients with multiple myeloma. Exp Hematol. 2009; 37(8):956-68;

ROLE AND CONTRIBUTION OF THE LABORATORY IN THE DIFFERENTIAL DIAGNOSIS OF THROMBOCYTOPENIA IN PREGNANCY

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Thrombocytopenia (platelet count <150x10^9/L) affects 6% to 11% of all pregnant women and, after anemia, is the second most common hematologic disorder in pregnancy. Thrombocytopenia in pregnancy may result from a variety of causes (Table 1), ranging from benign disorders such as Gestational (incidental) thrombocytopenia, the most common cause of thrombocytopenia in pregnant women, accounting for approximately 75% of all cases is usually a mild thrombocytopenia with a platelet count often over 70x10^9/L and not associated with adverse outcomes either of mother or fetus. Such women don't need therapy and because there is no diagnostic testing available for gestational thrombocytopenia, this disorder is a diagnosis of exclusion. Thrombocytopenia can also be associated with several diseases, either pregnancy-related or not, such as preeclampsia and HELLP syndrome (haemolysis, elevated liver enzymes, low platelet count), which represents about 18% of cases, and idiopathic thrombocytopenic purpura (ITP), which is found in about 5% of cases. Some rare conditions, such as thrombotic thrombocytopenic purpura (TTP), haemolytic uremic syndrome (HUS), disseminated intravascular coagulation (DIC) and others account for about 2% of the total. It may be a diagnostic and management problem and sometimes part of a more complex disorder with significant morbidity and may be life-threatening. The Differential diagnosis between these disorder and gestational thrombocytopenia may be problematic, sometimes impossible, in the absence of previous data like pre-pregnancy platelet counts or a previous history of ITP and as usual, situations in real life are often more complicated than in guidelines recommendation.

Table 1. Causes of maternal thrombocytopenia in pregnancy.

Pregnancy specific conditions	
Gestational (incidental) thrombocytopenia	~75% cases, commonest cause of thrombocytopenia in pregnancy
Hypertensive disorders including pre-eclampsia	Thrombocytopenia occurs in 50% cases of pre-eclampsia
HELLP syndrome	0,5–0,9% pregnancies
Acute fatty liver of pregnancy	1:7000–1:20 000 pregnancies
Pregnancy associated conditions (not specific but increased association)	
Thrombotic Thrombocytopenic Purpura	1 in 25000 Pregnancy
Disseminated intravascular coagulation	In HELLP syndrome: 20% all cases 84% severe cases
Haemolitic Uremic Syndrome	1 in 25000 Pregnancy associated cases
Not pregnancy associated	
Spurious	0-1% general population
Immune thrombocytopenia	0.1-1.0/ 1000 pregnancies
Hereditary	Rare
Marrow disease	Very rare
Viral infection	Common temporary cause ; screen for CMV, EBV, HIV Hepatitis
Folate/vitamin B12 deficiency	All rare
Drug/ipersplenism	All rare
HELLP: Haemolysis, Elevated liver enzyme, Low Platelets – CMV: Citomegalovirus- EBV: Epstein Barr virus- HIV: human immunodeficiency virus	

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Laboratory tests give relevant information regarding the type, the condition and the grading of the disease and depend on the stage of pregnancy. Complete blood count (CBC), Reticulocyte count and a careful review of the peripheral blood smear remains the main diagnostic procedure. liver function tests (bilirubin, albumin, total protein, transferases, and alkaline phosphatase), Viral screening (HIV, hepatitis C virus, hepatitis B virus) are always recommended. Screening for coagulation abnormalities (prothrombin time, activated partial thromboplastin time, fibrinogen, fibrin split products), haemolysis screen, type IIB von Willebrand disease testing, direct antiglobulin (Coombs) test, autoimmune profile (antinuclear antibody, antiphospholipid antibodies and lupus anticoagulant, renal and thyroid function tests, H. pylori testing and quantitative immunoglobulins are done if laboratory data, history, and physical examination suggest the thrombocytopenia may be secondary and/or if clinically indicated. The key initial assessment by CBC, reticulocyte count and a blood film review can confirm the presence of a genuine thrombocytopenia and may contribute to exclude quickly the presence of microangiopathy. The evolution of technology and automation in the laboratory of Hematology of the last two decades offered a great contribution. The technical performance has been improved in terms of precision and accuracy, such as in the platelets counting by introducing new physical principles for cellular analysis. Moreover, the newly automated blood cell analyzers allowed the use of well known parameters, such as reticulocyte and nucleated red blood cells (NRBCs) counts, improving their diagnostic performance. Last generation technologies also enable the introduction of new hematological parameters as fragmented red blood cells (FRBCs) and immature platelets fraction (IPF) that allows a rapid and careful management of thrombocytopenia in pregnancy, can contribute to the differential diagnosis of different forms of thrombocytopenia and may help a better selection of the samples that need review of the peripheral blood smear. At the state of the art, it is very important to focus the particular and specific aspects of the laboratory contribute to improve the diagnostic pathway of the disease and the monitoring of pregnant women suffering from thrombocytopenia. *Platelet counts:* Current blood cells counters assure high accuracy and high precision even for low platelet count. Different thresholds in platelet counts have been proposed; lowering the thresholds for diagnosis thrombocytopenia in pregnancy to a platelet count of <116x10^9/L has been suggested given the excellent maternal and fetal outcomes reported with isolated mild thrombocytopenia that develops late in pregnancy. Other authors argue that the limit may drop to <100x10^9/L or when the δ check compared to the previous control exceeds the limits of inter-individual biological variability. The degree of thrombocytopenia may be helpful as it is generally accepted that a platelet count <70 to 75x10^9/L would exclude gestational thrombocytopenia. On the other hand, not all patients with severe ITP have severe thrombocytopenia, so platelet count of >70x10^9/L does not exclude ITP. In asymptomatic, non pregnant patient with ITP, a platelet count <20x10^9/L is the threshold below which treatment is recommended. Similarly during pregnancy there isn't a "safe" level but guidelines suggest a similar threshold of 20 to 30x10^9/L for treatment of pregnant women with ITP and a target level of >50x10^9/L in preparation for delivery and level of >70 to 100x10^9/L are recommended if regional analgesia/anesthesia is desired or required. *Fragmented red blood cells (FRBCs) and schistocyte:* Nowadays only two analyzers manufacturers offer the possibility of direct count of FRBCs. In both analyzers, FRBCs are identified only on the basis of size and hemoglobin content, independent of their shape; therefore, other particles such as small red blood cells or even membrane themselves fragments can be included in the count. In consideration of their high negative predictive value, the automated methods can be is extremely useful in all cases in which there is a suspicion of Microangiopathic hemolytic anemia (TTP, HUS or HELLP) even if the platelet count is within normal limits. Anyway, these data don't exclude a microscopic review to confirm the schistocyte presence supporting a positive results. Finally we have to stress the idea that the schistocyte description is a closely morphological concept. *Reticulated platelet and immature platelet fraction (IPF):* Newly released platelets are more reactive than mature platelets and contain an higher amount of RNA, they were called reticulated platelets. The number of reticulated platelets is related to thrombopoiesis, increasing with increased production and decreasing when production declines. This parameter not available on all automated blood cell counter has several potential clinical applications particularly in diagnosis and monitoring thrombocytopenias. The increase in reticulated platelets is an early indicator of platelet destruction in pregnant women with immune thrombocytopenic or thrombotic thrombocytopenic purpura. An IPF value of 7.7% was reported as the best threshold in the diagnosis of immune thrombocytopenic purpura. Some papers report that IPF analysis and evaluation demonstrates a difference in thrombopoiesis between normotensive and preeclamptic pregnancies. All the above reported considerations suggest that laboratories performing hemato-

logic diagnostics require to have dedicated, skilled and updated personnel, with a deep knowledge of both technical and clinical aspects. Despite the essential role of automation in the modern hematology laboratory, microscopic control of pathologic samples remains essential and sometimes diagnostic itself. Moreover, knowledge of the limits of the instrumentation and/or the pre-analytic, analytic and post-analytic process is the main aspect for the correct interpretation of results.

References

Myers B. Diagnosis and management of maternal thrombocytopenia in pregnancy British Journal of Haematology, 2012, 158, 3–15

Gernsheimer T. Andra H.J. and Stasi R. How I treat thrombocytopenia in pregnancy Blood 2013 121: 38-47

Rajasekhar A., Gernsheimer T., Stasi R., Andra H.J. 2013 Clinical Practice Guide on Thrombocytopenia in Pregnancy. Haematology.org

McCrae K.R. Thrombocytopenia in Pregnancy Hematology 2010

Pourrat O., Pierre F, Que faire en pratique à la découverte d'une thrombopénie en cours de grossesse? La Revue de médecine interne 29 (2008) 808–814

Buttarello M. Plebani M. Automated Blood Cell Counts State of the Art Am J Clin Pathol 2008;130:104-116

Briggs C., Kunka S.,Hart D.,Oguni S, and Machin S.J. Assessment of an immature platelet fraction (IPF) in peripheral thrombocytopenia British Journal of Haematology, 2004; 126, 93–99

Zini G, d'Onofrio G, Briggs C, Erber W, Jou JM, Lee SH, McFadden S, Vives-Corrons JL, Yutaka N, Lesesve JF; ICSH recommendations for identification, diagnostic value, and quantitation of schistocytes. Int J Lab Hematol. 2012 Apr;34(2):107-16.

NOVEL ERYTHROPOIESIS STIMULATING AGENTS IN THALASSEMIA

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β-thalassemia is the most prevalent genetic disease in humans worldwide. Around 80–90 million people (~1.5% of the global population) are carriers, with approximately 60,000 symptomatic individuals born annually. The annual incidence is estimated at 1 in 100,000 individuals worldwide, and 1 in 10,000 in the European Union. β-thalassemia is caused by mutations in the β-globin gene: nearly 200 different mutations have been described in patients with β-thalassemia. All the mutations either prevent or reduce the synthesis of β-globin chains. Reduced biosynthesis of the β-globin subunit of hemoglobin-A (HbA) is the biochemical hallmark of β-thalassemia. Complete absence of expression of HbA results in the most severe form of β-thalassemia. γ-chain synthesis is partially reactivated so that the patient produces relatively large quantities of fetal hemoglobin (HbF); however, these γ-chains are quantitatively insufficient to replace β-chain production. The degree of imbalance between α- and β-globin production correlates with the severity of disease. Free α-globin accumulates, and unpaired α-chains aggregate and precipitate to form inclusion bodies, which cause oxidative damage to the membrane of the red blood cell, and destruction of immature developing erythroblasts within the bone marrow (*i.e.* ineffective erythopoiesis; IE). Both intramedullary death of red-cell precursors through arrest of the G1 phase of the cell cycle and increased intramedullary apoptosis of late erythroblasts after the polychromatophilic stage have been demonstrated. Consequently, relatively few erythroid precursors undergoing maturation in the bone marrow survive long enough to be released into the bloodstream as erythrocytes. Their survival and proliferation is erythropoietin (EPO) dependent on persistent phosphorylation of Janus kinase-2 (Jak2). Other factors such as growth differentiation factor11 (GDF11) have regulatory properties in these mechanisms. Moreover, many of the red blood cells that are released from the bone marrow are either damaged and removed by the spleen or hemolyzed in the circulation. These pathologic processes result in anemia and poor tissue oxygenation, causing the kidneys to produce more EPO, and further stimulating ineffective intramedullary and extramedullary marrow production. IE also results in inhibition of hepcidin synthesis, by factors derived from the expanded erythron, leading to increased iron absorption. Here we describe novel erythropoiesis stimulating agents used in patients with thalassemia syndromes. Use of erythropoiesis stimulating agents (such as EPO) is rational but maybe harmful because it exacerbates extra-medullary erythropoiesis and iron hyper-absorption. A more suitable strategy is to ameliorate the effectiveness of erythropoiesis; the possible main benefits of this approach are improved anemia, reduced spleen and extra-medullary expansion, and increased serum hepcidin levels. Different strategies are used to optimize erythropoiesis: (i) reduction in globin chain imbalance via HbF induction; (ii) Jak2 inhibition; (iii) modulation of GDF11 via activin receptor traps; (iv) iron restriction; and (v) manipulation of molecular chaperones. In non-transfusion-dependent thalassemias (NTDT), increasing Hb levels results in improved quality of life (QoL). In transfusion-dependent thalassemias (TDT), the aim is to decrease the transfusion requirement (very important in regions where blood is still not safe), thus resulting in lower demand for chelation, fewer morbidities, and lower health care costs. *Erythropoiesis stimulating agents (ESAs):* Erythropoietin, a hormone released mainly in the kidneys in response to hypoxia, enhances proliferation, differentiation, and maturation of erythroid progenitors in bone marrow. When EPO binds to its receptor (a class-1 cytokine expressed on the surface of the earliest erythroid progenitors), Jak2 is phosphorylated and in turn phosphorylates and activates the signal transducer and activator of transcription-5 (STAT5). Upon activation, STAT5 migrates to the nucleus and activates genes crucial for the proliferation and differentiation of erythroid progenitors. In β-thalassaemia, EPO levels are low relative to the degree of anemia, giving rise to the hypothesis that exogenous administration of EPO could improve anemia. In a variety of clinical trials with EPO dosed at up to 3 g/dL in small cohorts of patients, variable increments in Hb levels were documented in patients with NTDT. EPO combined with hydroxyurea has also been evaluated in trials. Darbopoietin α – a long-acting EPO – was shown substantially to increase Hb levels in patients with HbE-β-thalassemia (1). The effect of treatment on risk of expansion of extra-medullary erythropoiesis or splenomegaly is still unclear due to short follow-up periods in clinical trials. *Improving the effectiveness of erythropoiesis via reduction of globin chain imbalance:* Clinical observations have suggested that patients who express high levels of HbF – particulary those with deletions that result in hereditary persistence of HbF – have a relatively benign clinical course, with mild anemia and often are transfusion independent. HbF-inducing agents increase the production of γ-globin, which binds to α-chains to produce HbF thereby decreasing globin chain imbalance and thus reduces IE. Several classes of drugs induce HbF through mechanisms that result in transcriptional reactivation of γ-globin gene expression. Response to these agents is not universal, and mechanisms of action are still not entirely clear, but it is likely to involve multiple pathways including acceleration of erythroid maturation and differentiation. They include the following. 1. Chemotherapeutic agents: Hydroxyurea (HU) acts as potent inhibitor of ribonucleotide reductase, causing cell cycle arrest in the S phase. This encourages differentiation at the expense of proliferation with consequently less HbF switching. Following early case reports documenting hematological improvements in β-thalassemia patients treated with HU, several studies have evaluated its efficacy and safety in this patient population. These were primarily small single-arm trials or retrospective cohort studies. Reported elevations in HbF level from baseline were highly variable, ranging between 1% and 90%, and averaging 20%. An association between the degree of HbF increase and improved hematological outcomes was noted in some studies, but not in others. Combined therapy with EPO+HU is reported to mitigate the effects of excessive erythron expansion by promoting HbF (2). 2. Hypomethylating agents: Decitabine and 5-azacytidine act as histone deacetylase (HDAC) and DNA methyl transferase (DNMT) inhibitors that increase levels of HbF. A few small studies have demonstrated Hb increases of >1.5gr/dl. 3. Short-chain fatty-acid derivatives (SCFADs): These alter the chromatin structure to increase accessibility to transcription factors to γ-globin genes, such as inhibition of histone deacetylase (HDAC) activity. Continuous high dose intravenous (IV) arginine butyrate (500 mg/kg/day) for 2–3 weeks was reported to increase the levels of HbF and Hb; unfortunately these results were not achieved in the long-term. It has been suggested that the loss of response to butyrates in the long term may be a result of their antiproliferative effects on the bone marrow. Oral sodium phenylbutyrate (SPB) requires large doses and has a strong musty odor that limits its use. Oral sodium 2,2-dimethylbutyrate also has shown poor results. 4. Synthetic glutamic derivatives: Thalidomide, and its derivatives pomalidomide and lenalidomide modulate erythroid proliferation and maturation. *In vitro* culture models have suggested that thalidomide, a drug known for its immunomodulating and anti-angiogenic properties, induces γ-globin gene expression and increases the proliferation of erythroid cells. Two case reports reported that thalidomide therapy at 75–100 mg/kg per day caused a progressive and rapid increase in total Hb and HbF levels in patients with β-thalassemia. More recently it was shown that rapamycin, a lypophilic

macrolide used for the prevention of acute rejection in renal transplant recipients, could be a good candidate for inducing HbF production. *In vitro* studies documented an increase in γ-globin mRNA expression in 15 patients with sickle cell disease and 14 with β-thalassemia, and a corresponding increase in HbF (3). *Improvement of effectiveness of erythropoiesis via Jak2 inhibition:* Jak2 is an important signaling molecule that regulates proliferation, differentiation, and survival of erythroid progenitor cells in response to EPO. In both murine models and patients with β-thalassemia, erythroid precursors express elevated levels of the active (phosphorylated) form of Jak2 (pJak2), and other downstream signaling molecules that promote proliferation and inhibit differentiation of erythroid progenitor cells. Based on the potential role of Jak2 in IE and iron metabolism, Jak2 inhibitors may be a promising treatment for β-thalassemia. Murine models of β-thalassemia have demonstrated that Jak2 inhibitors can affect IE and decrease spleen size. Results from clinical studies in patients with myelofibrosis suggest that Jak inhibitors may be an effective treatment option with a tolerable safety profile. In a phase 1–2 study, the Jak2 inhibitor INCB018424 was shown to rapidly reduce splenomegaly in patients with myelofibrosis and palpable spleens. A Phase IIa study with ruxolitinib (INC424; Jakavi) is ongoing in thalassaemia syndromes (NCT02049450). *Improvement of effectiveness of erythropoiesis via modulation of GDF11:* GDF11 is a member of the bone morphogenetic protein (BMP) family and of the TGF-β superfamily. It is also a ligand of the activin receptor-II trap ligands A and IIB (ActRIIA and ActRIIB). These molecules form complexes with additional receptors that regulate gene expression primarily by activating the SMAD2/3 subfamily of intracellular effectors. Ligand traps are molecules that inhibit signaling by binding ligands and sequestering them away from their receptors. ActRIIA and ActRIIB are recognized by several ligands, including GDF11, and have been involved in a variety of physiological functions, including bone homeostasis and age-related bone loss. The trap ligand ACE-011(sotatercept) was made by fusing the extracellular domain of ActRIIA to the Fc domain of human immunoglobulin G1 (IgG1) and was originally developed to improve bone mineral density (BMD) in menopausal women (4). In a phase I clinical trial in postmenopausal women with osteoporosis, ACE-011 increased BMD and interestingly (and unexpectedly) increased Hb values (4).Further investigations into ACE-011 and another trap ligand targeting ActRIIB (ACE-536, luspatercept), in mouse models of myelodysplastic syndromes (MDS) and β-thalassemia showed significant improvement of the anemia (5–7). It has been suggested that the mechanism of action of these drugs In both of these disorders is mediated by targeting GDF11. This decreases Smad2/3 activation in erythroid progenitors, and ultimately improves erythroid maturation and RBC production (5–7). Studies have demonstrated that GDF11 is overexpressed in the spleen and erythroid cells of thalassaemic animals (and in the serum of patients with thalassaemia) and its inhibition in mouse models of anemia with ineffective erythropoiesis restores normal erythropoietic differentiation and alleviates anemia. Over-expression of GDF11 may be responsible for the ineffective erythropoiesis associated with β-thalassaemia. GDF11 itself also inhibits erythroid maturation in mice *in vivo* and *ex vivo*, while expression of GDF11 and ActRIIB in erythroid precursors decreased progressively with maturation, suggesting an inhibitory role for GDF11 in late-stage erythroid differentiation. Furthermore, inactivation of GDF11decreased oxidative stress, α-globin membrane precipitates in RBCs, and also corrected the abnormal ratio of immature/mature erythroblasts by inducing apoptosis of immature erythroblasts through the Fas–Fas ligand pathway. Clinical trials with these agents are ongoing and thus far have shown amelioration of anemia in patients with NTDT and suggest that the need for transfusion in patients with β-thalassemia major might be reduced. A Phase-2a dose-escalation of ACE-011 (sotatercept) has been conducted in patients with TDT (defined as a requirement for ≥2 units RBCs/30 days for ≥6 months prior to enrolment) and NTDT (a requirement for ≤4 units RBCs during the 6 months period prior to enrolment). Sotatercept's long plasma half-life (approximately 23 days) dosing can be given 3-weekly. Patients initially received ACE-011 subcutaneously at 0.1 mg/kg once every 3 weeks and escalation to 1.0 mg/kg is ongoing. Efficacy is assessed by Hb increase from baseline for patients with NTDT and reduction in the need for RBC transfusion for TDT patients. To date, 46 patients have been enrolled (30TDT/16NTDT). Preliminary results (Cappellini MD, Oral Communication, European Hematology Association, 2015) show that, compared with the 0.1 mg/Kg cohort, more patients with NTDT in the sotatercept 0.3, 0.5 and 0.75 mg/Kg cohorts achieved maximum Hb increases of ≥1 g/dL and ≥2 g/dL during the first 9 weeks, and more patients with TDT in the sotatercept 0.3, 0.5 and 0.75 mg/Kg cohorts achieved a reduction of transfusion burden of ≥20% . A significant improvement in RBC morphology was also noted in patients with NTDT. Tolerability was generally good: adverse events included: grade 3 bone pain in one patient with TDT who had a history of osteoporosis, grade 2 phlebitis in a patient with NTDT, and grade 3 ventricular extrasystoles in a patient with a history of ventricular extrasystoles at baseline. ACE-356 (luspatercept) is the subject of a similar Phase 2a dose-finding trial is also ongoing with in patients with TDT (defined as ≥4 units RBCs/8 weeks prior to baseline confirmed over 6 months) and NTDT (defined as a requirement for <4 units RBCs/8 weeks prior to baseline, and hemoglobin level <10 g/dL). Doses were administered subcutaneously every 3 weeks. Sequential cohorts (n=6 patients per cohort) received doses of 0.2, 0.4, 0.6, 0.8, 1.0, 1.25 mg/kg. Preliminary data (Piga A, Oral Communication, European Hematology Association, 2015) in 39 patients (25 NTDT/14 TDT) have been reported. 38% of patients with NTDT who received ≥0.8 mg/Kg of luspatercept experienced sustained increases in total hemoglobin in a 16-week study. Ten patients with TDT who were each treated for ≥12 weeks experienced >40% reduction in transfusion burden. A trend for reduction in liver iron concentration (LIC) was observed in the majority of patients with NTD and TDT, with and without iron chelation therapy. Rapid healing was observed in three of three patients with leg ulcers. Luspatercept has a favorable safety profile: the most frequent related adverse events included bone pain, headache and myalgia. A Phase 3, pivotal, controlled, study of luspatercept in patients with β-thalassemia is planned. *Improvement of effectiveness of erythropoiesis via iron restriction:* Hepcidin is the hormone that controls iron absorption. It is synthesized in the liver and secreted into the bloodstream where it targets ferroportin (FPN), the only known iron exporter. Upon binding of FPN, this protein is internalized and degraded, preventing iron egress. Hepcidin synthesis is controlled by Tf-sat and iron storage, inflammation and erythropoiesis' demand. FPN is expressed mainly on enterocytes, macrophages, and hepatocytes. Together, the hepcidin in the bloodstream and FPN on the cellular membranes control iron absorption in the duodenum, iron recycling in the reticuloendothelial system and iron storage in the liver. $Hbb^{th3/+}$ mice over-expressing hepcidin showed decreased organ iron content. Furthermore, decreased iron absorption was associated with decreased transferrin-saturation (Tf-sat) which, in turn, decreased erythroid iron intake, heme synthesis, formation of insoluble membrane-bound globins, and reactive oxygen species (ROS). Altogether, moderate overexpression of hepcidin ameliorated iron overload and also increased the lifespan of RBC, reversed IE and splenomegaly, and increased total hemoglobin levels. *Hepcidin agonist*: Minihepcidins (MH) are short peptide mimetics (9 amino acids long) that are sufficient to induce FPN degradation in reporter cells. *In vivo*, these compounds lowered serum iron levels and were efficacious in ameliorating the iron overload in animals affected by Hfe- and Hamp-related hemochromatosis (8). *Increasing hepcidin*: Matriptase-2, or transmembrane protease serine 6 (TMPRSS6), is a transmembrane serine protease that attenuates hepcidin expression. Patients and mice with mutations in this gene are affected by iron-refractory iron deficiency anemia (IRIDA). Interestingly, lack of Tmprss6 in $Hbb^{th3/+}$ mice significantly improved iron overload and anemia, corroborating the hypothesis that increased hepcidin activity could be beneficial in this disorder (9). *Transferrin treatment:* It has been shown that administration of apotransferrin normalized labile plasma iron concentrations and RBC survival, increased hemoglobin production, and decreased reticulocytosis, EPO synthesis and splenomegaly. There is some clinical evidence that large doses of purified human apotransferrin given to patients with restricted erythropoiesis and raised free plasma iron (NTBI), provide effective binding of this fraction by apotransferrin as well as prevention of full transferring saturation. This study is more interesting as a proof of principle, however, than as a therapeutic modality. Recently, chelation with deferiprone has been evaluated along with other approaches to iron restriction in animal models. TMPRSS6 inhibitors, apotransferrin and MH can be improved by the concurrent use of iron chelators that accelerate the removal of iron from the liver (10–11). The potential for chelation therapy, alone or in combination with other interventions, to improve IE in thalassaemias has not been fully assessed. *Improvement of effectiveness of erythropoiesis via manipulation of molecular chaperones:* Alteration of protein homeostasis networks by pharmacologic manipulation of chaperone machinery and protein degradation pathways represents

a novel target for decreasing IE. Normal human erythroid maturation requires a transient activation of caspase-3 in the later stages of maturation. Chaperone heat shock protein 70 (HSP70) is constitutively expressed in erythroblasts and, at later stages of maturation, translocates into the nucleus and co-localizes with GATA-1, protecting it from caspase-3 cleavage. This ubiquitous chaperone participates in the refolding of proteins denatured by cytoplasmic stress, thus preventing their aggregation and thus playing an anti-apoptotic role. During the maturation of human TDT erythroblasts, HSP70 interacts directly with free α-globin chains. As a consequence, HSP70 is sequestrated in the cytoplasm and GATA-1 is no longer protected. This results in arrest of end-stage maturation and apoptosis. Transduction of a nuclear-targeted HSP70 mutant or a caspase-3-uncleavable GATA-1 mutant restores terminal maturation of β-thalassemic erythroblasts, which may provide a rationale for the development of targeted therapies for β-thalassemia (12).

References

1. Singer ST, Vichinsky EP, Sweeters N, Rachmilewitz E. Darbepoetin alpha for the treatment of anaemia in alpha- or beta- thalassaemia intermedia syndromes. British journal of haematology. 2011 Jul;154(2):281-4.
2. Musallam KM, Taher AT, Cappellini MD, Sankaran VG. Clinical experience with fetal hemoglobin induction therapy in patients with b-thalassemia. Blood. 2013 Mar 21; 121(12):2199-212.
3. Pecoraro A, Troia A, Calzolari R, Scazzone C, Rigano P, Martorana A, Sacco M, Maggio A, Marzo RD. Efficacy of Rapamycin as Inducer of Hb F in Primary Erythroid Cultures from Sickle Cell Disease and Beta-thalassemia Patients Hemoglobin. 2015 May 27:1-5.
4. Raje N, Vallet S. Sotatercept, a soluble activin receptor type 2A IgG-Fc fusion protein for the treatment of anemia and bone loss. Curr Opin Mol Ther. 2010;12(5):586-597.
5. Suragani RN, Cawley SM, Li R, et al. Modified activin receptor IIB ligand trap mitigates ineffective erythropoiesis and disease complications in murine beta-thalassemia. Blood. 2014;123(25):3864-3872.
6. Dussiot M, Maciel TT, Fricot A, et al. An activin receptor IIA ligand trap corrects ineffective erythropoiesis in beta-thalassemia. Nat Med. 2014;20(4):398-407.
7. Suragani RN, Cadena SM, Cawley SM, et al. Transforming growth factor-beta superfamily ligand trap ACE-536 corrects anemia by promoting late-stage erythropoiesis. Nat Med. 2014;20(4):408-414.
8. Ramos E, Ruchala P, Goodnough JB, et al. Minihepcidins prevent iron overload in a hepcidin-deficient mouse model of severe hemochromatosis. Blood. 2012;120(18): 3829-3836
9. Nai A, Pagani A, Mandelli G, et al. Deletion of TMPRSS6 attenuates the phenotype in a mouse model of beta-thalassemia. Blood. 2012;119(21):5021-5029.
10. Schmidt PJ, Racie T, Westerman M, Fitzgerald K, Butler JS, Fleming MD. Combination therapy with a Tmprss6 RNAi-therapeutic and the oral iron chelator deferiprone additively diminishes secondary iron overload in a mouse model of beta-thalassemia intermedia. Am J Hematol. 2015 Jan 2. [Epub ahead of print]
11. Casu C, Aghajan M, Oikonomidau R, Guo S, Monia BP, Rivella S. Combination of Tmprss6-ASO and the Iron Chelator Deferiprone Improves Erythropoiesis and Reduces Iron Overload in a Mouse Model of Beta-Thalassemia. Blood. 2014; 124(21):abs. 4024.
12. Arlet JB, Ribeil JA, Guillem F, Negre O, Hazoume A, Marcion G, et al. HSP70 sequestration by free alpha-globin promotes ineffective erythropoiesis in beta-thalassaemia. Nature. 2014 Oct 9;514(7521):242-6.

MODERN THERAPY OF ADULT ALL

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Modern intensive induction chemotherapy allows most adult patients with acute lymphoblastic leukemia (ALL) to achieve a complete hematologic remission (CR). However, only 40% of patients survive five years or more. Allogeneic hematopoietic stem cell transplantation (alloHSCT) is an effective post-remission therapy in patients with ALL, but its remarkable curative potential is often counterbalanced by a high incidence of post-transplant complications, which lead to a high non-relapse mortality (NRM). The uncertain results in evaluating the true benefit of the allogeneic transplant in HR patients has been highlighted by several meta-analyses performed over the past few years and the age at transplant turned out to be the most important prognostic factor. Indeed, a true survival benefit is evident only for patients <35 years of age because of the higher absolute risk of NRM for older patients. These results highlight the fact that in adult ALL, classic prognostic factors have a limited accuracy since a significant proportion of SR patients (up to 40% to 50%), treated with standard chemotherapy, will eventually relapse, and conversely, 20% to 25% of HR patients will not relapse, even in the absence of an alloHSCT. In addition, chronic graft-*versus*-host disease (GvHD) with a related poor quality of life represents an additional severe concern, so that the optimal timing and use of this treatment modality remains an issue of debate. Thus, it is crucial to identify patients who have high chances of cure with standard therapy and those for whom alloHSCT is the only possible post-remission therapy. In this regard, a risk-adapted strategy, using clinical and/or biological features, such as age, white cell count, time to obtain CR, disease immunophenotype, cytogenetics, and molecular abnormalities, may help in selecting patients at highest risk for relapse, who may benefit from alloHSCT. Evidence is now growing that the evaluation of minimal residual disease (MRD) can further improve the prognostic accuracy in defining ALL risk classes. Overall, these data provide evidence for introducing MRD analysis for the identification of patients at HR of relapse who may benefit from early transplantation, despite a morphologic remission and the absence of clinico-biological risk factors. Conversely MRD analysis identifies a group of patients who are sensitive to chemotherapy, achieve a non-detectable level of MRD, and then probably do not need transplantation, as consolidation therapy also when risk factors are present. The most relevant and urgent challenges into future investigations about MRD are: A) to determine the most predictive point time for the measurement of MRD and its threshold during and after treatment; B) to identify the best strategy in patients with high MRD, before allogeneic transplantation, such as the need of further therapy to reduce tumor load before transplant; C) to demonstrate the utility and efficacy of tapering or withholding the immunosuppressive therapy or of using donor lymphocyte infusion (DLI) in patients in molecular relapse after transplant; D) to demonstrate the efficacy of an MRD-guided choice in avoiding the alloHSCT in HR patients with no detectable MRD. A specific subgroup of patients remains that of Philadelphia-positive ALL for whom the introduction of tyrosine kinase inhibitors pre and post alloHSCT have remarkably improved the clinical outcomes. However, in the absence of specifically designed clinical trials, alloHSCT remains the most effective post-remission therapy.

IMMUNOTHERAPY OF ACUTE LEUKEMIAS

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Although allogeneic stem cell transplantation represents a fantastic example of immunotherapy of hematological malignancies, the dream to cure leukemias by specific immunologic approaches has remained elusive for many years. After the first reports showing the ability of donor lymphocyte infusions (DLI) to induce hematological remission in chronic myelogenous leukemia (CML) patients relapsed after transplantation, the use of DLI has also become common for other diseases, but the results have been usually modest. Unmanipulated or minimally manipulated donor T cells have been shown to be effective in a minority of patients with the main limit of inducing a severe graft-*versus*-host disease in many cases. For this reasons alternative approaches have been developed and brought about a huge research effort that led to the development of leukemia-specific T-cell therapy aiming at the direct recognition of leukemia-specific rather than minor histocompatibility antigens. A great scientific interest was raised by the demonstration that leukemia-reactive cytotoxic T lymphocytes lines (CTL), generated from human leukocyte antigen (HLA)-identical donors were indeed able to completely eradicate *in vivo* an accelerated phase of CML. Similarly, a great excitement was raised when CTLs were generated *in vitro* against minor histocompatibility antigen and were adoptively transferred to patients relapsing after transplantation. Nonetheless, the clinical feasibility of such an approach has proven to be quite problematic and the results of the subsequent clinical studies remarkably disappointing because CTL lines could be generated only in some cases and only a minority of patients could be actually treated with GMP (good manufacturing practice)-compliant cells. To overcome many of the problems related to the use of CTL cell lines, the use of naturally cytotoxic effector cells has been intensively investigated. Following pilot experiences, the safety, feasibility and engraftment of haploidentical NK cell infusions after an immunosuppressive regimen has been tested in children and adults with AML. Non hematologic toxicity was very limited, with no GvHD. All in all, best responses were observed in patients with molecular relapse, underlying the possibility of implementing this strategy only in conditions of minimal residual disease. Cytokine-induced killer (CIK) cells have also been considered for passive transfer in relapsed

leukemia patients. CIK cells are T effector memory CD8 T lymphocytes that have acquired NK-like cytotoxicity in culture. Infusions is always well tolerated and no acute or late infusion-related reactions were recorded. Despite the T cell ontogeny of these cells, acute GvHD is rare and usually of limited stage and promising results have been reported by our group as well as by others and many clinical trials are still ongoing. However, during the past few years the field of immunotherapy of hematologic malignancies has changed dramatically as the consequence of two main technological developments. The first one has come from the possibility of genetically modifying unselected peripheral blood T cells with the use chimeric antigen receptors (CARs). CARs are artificial T-cell receptors composed by an extracellular antigen-binding domain derived from the fusion of the variable regions of the heavy and light chains of immunoglobulins, and an intracellular T cell-activating domain, usually the CD3z chain of the T-cell receptor complex. Thus, CAR-redirected T cells are able to specifically recognize and kill tumor cells, as they combine the antigen-binding properties typical of an antibody molecule with a T cell-triggering domain. This innovative CAR strategy stands out because of the impressive results achieved in recent clinical trials performed using autologous or donor-derived, allogeneic T lymphocytes. A second, biotechnology breakthrough has led to the development of a new class of bi-specific antibodies able to bind simultaneously T cells and the leukemic target. The prototype of these antibodies is blinatumomab the first member of T-cell engaging, bispecific single chain (BiTE) antibodies (engaging T cells for re-directed lysis of CD19+ target cells) that showed very encouraging results in relapsed or refractory ALL patients. Treatment with this molecule allowed to achieve a robust and durable response even when disease recurrence occurred after allogeneic transplantation. All in all, immunotherapy of acute leukemias is not anymore a dream but rather a fascinating clinical reality.

LYMPHOMA IN THE ELDERLY

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In population-based studies the median age of patients with lymphoma ranges between 65 and 70 years and in most histological subtypes of lymphoma the frequency increases with age. The histological subtypes of lymphoma predominantly occurring over the age of 60 are diffuse large B cell lymphoma (DLBCL), marginal zone lymphoma (MZL) and follicular lymphoma (FL). Together they account for more than 80% of case. Thus lymphoma can be considered mainly a disease of the elderly and indeed in a recent epidemiological survey exactly two third of lymphomas were diagnosed after the age of 60 (*Lymphoma incidence, survival and prevalence 2004-2014: sub-type analyses from the UK's Haematological Malignancy Research Network. Smith A, Crouch S, Lax S, Li J, Painter D, Howell D, Patmore R, Jack A, Roman E. Br J Cancer. 2015 Apr 28;112(9):1575-841).* Until recently the number of elderly patients enrolled in clinical trials was largely underrepresented. However, over the last decade, very effective drugs with a better toxicity profile have become available and the prognostic outlook has so much improved that the attention of investigators toward the specific problems of aged patients with lymphoma has markedly increased and clinical studies, specifically designed to address the many issues of lymphoma treatment in older persons, have been launched. While the clinical presentation and the natural history of the disease is similar in most subtypes of lymphoma in older compared to younger patients, the management approach may substantially differ according to age and to patient's status. Indeed the different histological subtypes of lymphoma markedly differ in their aggressiveness and in the possibility of being controlled or cured, and since potential treatments also markedly differ in their toxicity profile, the best treatment choice for elderly lymphoma patients should always consider at least three aspects: a) the host-related factors, b) the clinical and pathological characteristics of the specific lymphoma subtype when it occurs in the elderly, c) the goal of available treatments, as well as their efficacy and tolerability. Host-related factors include not only age, but also the comorbidities and the overall "fitness" status. *(Specific geriatrics parameters have been proven to significantly impact on prognosis and should be objectively analysed to optimize treatment outcome. Tucci A, Ferrari S, Bottelli C, et al. A comprehensive geriatric assessment is more effective than clinical judgment to identify elderly diffuse large cell lymphoma patients who benefit from aggressive therapy. Cancer 2009;115:4547–4553).* This is particularly important in aggressive lymphomas. *Aggressive lymphoma:* In aggressive histologies, like the most frequent DLBCL, the possibility of effectively controlling the disease, requires the obtainment of the complete eradication of lymphoma. DLBCL cannot be controlled for prolonged time if a sustained complete remission cannot be obtained, which is in many cases synonimous of disease cure. However, the treatments required to achieve this goal are not always tolerable by aged patients. In this setting the integration of geriatric concepts and tools with the hematological know-how is probably the best way to achieve the most effective compromise between the characteristics of the single geriatric patient and the toxicity of the treatment needed for its specific type of lymphoma. In DLBCL the treatment paradigm has been for many years the use of anthracyclin at full doses. Dose intensity had been demonstrated as essential for achieving disease control *(Doxorubicin-based chemotherapy for diffuse large B-cell lymphoma in elderly patients: comparison of treatment outcomes between young and elderly patients and the significance of doxorubicin dosage.Lee KW, Kim DY, Yun T, Kim DW, Kim TY, Yoon SS, Heo DS, Bang YJ, Park S, Kim BK, Kim NK. Cancer. 2003 Dec 15;98(12):2651-6).* Age over 60 was an adverse prognostic factor but patients over 60 receiving adequate anthracyclin dose intensity had the same outcome than younger. However, full dose anthracyclin has been considered too toxic for many elderly people and many attempts at obtaining a better balance between efficacy and toxicity compared to classical CHOP failed until rituximab, with its markedly better toxicity profile, became available. To date R-CHOP has become the gold standard at any age, if tolerated. Most interestingly, while in the absence of rituximab CHOP14 was better than CHOP21 (*Prognostic significance of maximum tumour (bulk) diameter in young patients with good-prognosis diffuse large-B-cell lymphoma treated with CHOP-like chemotherapy with or without rituximab: an exploratory analysis of the MabThera International Trial Group (MInT) study Pfreundschuh M, Ho AD, Cavallin-Stahl E, Wolf M, Pettengell R, Vasova I, Belch A, Walewski J, Zinzani PL, Mingrone W, Kvaloy S, Shpilberg O, Jaeger U, Hansen M, Corrado C, Scheliga A, Loeffler M, Kuhnt E; MabThera International Trial (MInT) Group. Lancet Oncol. 2008 May;9(5):435-44)* , with rituximab no differences could be demonstrated between R-CHOP 14 and R-CHOP21 at any age (*Rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisolone in patients with newly diagnosed diffuse large B-cell non-Hodgkin lymphoma: a phase 3 comparison of dose intensification with 14-day versus 21-day cycles Cunningham D, Hawkes EA, Jack A, Qian W, Smith P, Mouncey P, Pocock C, Ardeshna KM, Radford JA, McMillan A, Davies J, Turner D, Kruger A, Johnson P, Gambell J, Linch D. Lancet. 2013 May 25;381(9880):1817-26).* Furthermore a number of recent phase 2 and randomized studies with reduced intensity chemotherapy and rituximab showed results similar to those obtained with full dose R-CHOP and in patients aged >80, full-dose anthracyclin had a negative impact on survival whereas rituximab use was a favorable prognostic factor. *(Comparative effectiveness of anthracycline-containing chemotherapy in United States veterans age 80 and older with diffuse large B-cell lymphoma Carson KR, Riedell P, Lynch R, Nabhan C, Wildes TM, Liu W, Ganti A, Roop R, Sanfilippo KM, O'Brian K, Liu J, Bartlett NL, Cashen A, Wagner-Johnston N, Fehniger TA, Colditz GA. J Geriatr Oncol. 2015 May;6(3):211-8).* Therefore, the concept is emerging that rituximab has increased the overall efficacy of treatment at a point, that the old dogma of the need of full dose anthracyclin for achieving cure may be revised and that in the near future even unfit patients with DLBCL may enjoy long term cure when we will be able to adapt our treatments at best to their characteristics. Indeed, in the studies of the Fondazione Italiana Linfomi, patients are now prospectively classified as fit, unfit or frail according to a simple algorythm which includes geriatric parameters and a comorbidity score and prospective trials are being conducted specifically in each fitness category. In addition, by correctly identifying patients that, in spite of age, are otherwise very fit, even the addition of new non-cytotoxic agents, *e.g.* lenalidomide, to R-CHOP has been tested and proved to be feasible, tolerated and to improve efficacy, particularly in the ABC genetically subtype of DLBCL occurring more frequently in the elderly. The rare patients with very aggressive histologies, like Burkitt lymphoma, cannot be controlled and are not curable at any age, even with anthracyclin-containing therapy. High-dose methotrexate and cytarabine regimens are necessary, together with a precise timing of administration and optimal supportive care in hospitalized patients. Elderly subjects are generally not considered suitable to receive such regimens. However there are some experiences showing that a proportion of elderly patients can be effectively cured even with high-dose

programs. In the study recently reported by the German group based on HD-methotrexate and HD-cytarabine *(Improved outcome of adult Burkitt lymphoma/leukemia with rituximab and chemotherapy: report of a large prospective multicenter trial Hoelzer D, Walewski J, Döhner H, Viardot A, Hiddemann W, Spiekermann K, Serve H, Dührsen U, Hüttmann A, Thiel E, Dengler J, Kneba M, Schaich M, Schmidt-Wolf IG, Beck J, Hertenstein B, Reichle A, Domanska-Czyz K, Fietkau R, Horst HA, Rieder H, Schwartz S, Burmeister T, Gökbuget N; German Multicenter Study Group for Adult Acute Lymphoblastic Leukemia.Blood. 2014 Dec 18;124(26):3870-9)* , there were approximately 25% of patients aged over 55 who received a dose-adjusted regimen. Age was clearly an adverse prognostic factor but even patients older than 70 had a plateau in the survival curve at more than 40% which is synonymous of disease cure. Such outcome was clearly superior to what could have been obtained using palliative care only or even CHOP. The selection criteria in this study was the clinical judgement and we need the integration of geriatric-based criteria for this specific setting. The NILG group has used the same treatment program confirming its feasibility and efficacy in a multiinstitutional setting and showing that performance status (PS) according to ECOG was useful in predicting the success of treatment. Indeed patients aged >60 but with a good PS (ECOG 0-1) had a very satisfactory 57% overall survival rate and 70.5% disease-free survival rate. *Indolent lymphoma:* On the other hand, follicular lymphoma (FL), the most frequent "low-grade" histology lymphoma, has an indolent disease course which does not depend an age itself. Its prognosis has markedly improved by the introduction of immunotherapy and further improvement may occur with the use of combinations of non-cytotoxic immunomodulatory agents (5). Median survival of patients is projected to exceed ten or even fifteen years. The treatment paradigm in elderly FL may be not much different from FL occurring in younger patients, with an emphasis on treatment control for prolonged periods using low toxicity treatment programs, rather than on trying to achieve lymphoma eradication. As an example, in patients with "low tumor burden" FL, the wait and see policy can be applied to the elderly as well and may be even more effective than in younger patients. In a randomized trial comparing chlorambucil treatment at diagnosis with a "wait and see" policy, the proportion of asymptomatic patients who did not need to be treated after ten years of observation was highest over the age of 70. However older patients with progressive disease should start treatment even if they remain asymptomatic to avoid the subsequent development of conditions potentially complicating the start of treatment. In elderly patients with "high tumor burden" FL in need of treatment, the most effective chemoimmunotherapy programs, which represent the treatment of choice at any age, can indeed be tolerated even by the oldest patients, with the possible exception of R-CHOP, which however does not confer an overall survival advantage compared to other anthracyclin free regimens. Even the new treatments, like the R-squared chemotherapy-free schema associating rituximab with lenalidomide, should have a toxicity profile allowing its use also in older patients, although this will need to be confirmed in larger patient's groups. Second-line treatments in indolent lymphoma also do not substantially differ between younger and older patients, except for the use of high-dose therapy with autologous stem cell support as consolidation which can be proposed only to younger patients responsive to salvage treatments. In elderly patients, even when unfit according to geriatric parameters, the use of radioimmunotherapy is advocated by most treatment guidelines, as an elective option given its very favourable cost-benefit profile. Hodgkin lymphoma (HL) occurs in approximately 20% of cases at an age older than 65 (2) but its biological and clinical characteristics in the elderly markedly differ from those of HL occurring in younger persons. Discrepancies include male predominance, very uncommon mediastinal involvement, higher frequency of B symptoms, advanced stage, and mixed cellularity histology, and association with EBV, which occurs in more than 50% of cases *(Klimm B, Diehl V, Engert A. Hodgkin's lymphoma in the elderly: a different disease in patients over 60. Oncology. 2007;21:982-90)*. With standard ABVD, the lower than 50% reported 5-year event-free and overall survival rates in elderly HL are significantly inferior to those achieved in the adult population *(Evens AM, Hong F, Gordon LI, Fisher RI, Bartlett NL, Connors JM, Gascoyne RD, Wagner H, Gospodarowicz M, Cheson BD, Stiff PJ, Advani R, Miller TP, Hoppe RT, Kahl BS, Horning SJ. The efficacy and tolerability of adriamycin, bleomycin, vinblastine, dacarbazine and Stanford V in older Hodgkin lymphoma patients: a comprehensive analysis from the North American intergroup trial E2496. Br J Haematol. 2013;161:76-86*) The differences are so marked that HL in the elderly may be considered biologically distinct from its counterpart in the young. Therefore the treatment paradigm may be different from the very successful one used in younger patients. Tolerability of treatment is a further problem which is more important in HL than in other lymphomas. A potentially fatal pulmonary toxicity can occur in as high as 24-46% of cases. Even radiation therapy is less well tolerated. Acute toxicity from RT is far more pronounced in elderly patients when extended-fields are used. A number of treatment programs specifically designed for the elderly have been tested, but overall they did not achieve superior results than ABVD, which remain the standard for fit patients. Among adverse prognostic factors, age >70, comorbidities, and the loss of one activity of daily living were identified, suggesting that a specific geriatric assessment may help selecting HL patients, particularly among the oldest ones, where a different treatment approach should be investigated. Future developments will also require a better biological characterization of the disease, the use of response-adapted strategies, including treatment intensity downgrading after interim PET, the use of less toxic chemotherapy and of new agents, *e.g.* brentuximab and even of immune checkpoint modifiers, which hold promise to better couple antitumor efficacy with tolerability. Finally a better biological characterization of the disease is mandatory. The very distinct behavior of HL according to age raises the more general question, which applies to all subtypes of lymphoma, of the interplay between age-related factors and the ability of the host to fight against tumor. A number of adverse prognostic factors are more prevalent in older people and concur in worsening the outcome. As an example, in DLBCL, the cell of origin is more frequently of the ABC genetic subtype, and cytogenetic complexity is higher as demonstrated by the higher median age of double-hit DLBCL, which carry a worse prognosis based on still poorly defined biological factors. Weather immunosenescence plays a role in such aspects is a matter of debate and should be analysed in detail in future studies.

IMMUNE ACQUIRED THROMBOCYTOPENIA, INHERITED THROMBOCYTOPENIAS AND INHERITED PLATELET FUNCTION DISORDERS IN PREGNANCY

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IMMUNE ACQUIRED THROMBOCYTOPENIA IN PREGNANCY: Immune ThrombocytoPenia (ITP) is an acquired immune disorder, characterized by a transient, persistent or chronic decrease of blood platelet count and an increased risk of bleeding complications (1). Chronic ITP in adult patients has an incidence of 58-66 new cases per million per year, affecting mainly women of childbearing age (female: male, 3:1) (2). Consequently, could be not rare for hematologists to be asked to manage pregnant women with a pre-existing or "*de novo*" ITP. A unique feature of ITP in pregnancy is that physician should treat, at the same time, both the mother (differential diagnosis with other causes of thrombocytopenia; influence of pregnancy on ITP; need of therapy) and the fetus (assessment of the risk of neonatal thrombocytopenia; modality of delivery to minimize the hemorrhagic risk; need of a specific treatment after partum). DIAGNOSTIC WORK-UP: Platelet counts tend to fall during pregnancy, mainly in the third trimester (3); thus, a separate normal range for late-pregnant and non-pregnant women should be calculated. Thrombocytopenia in pregnancy may occur as an isolated abnormality, such as *gestational thrombocytopenia* (GT), a benign disorder characterized by a mild decrease of platelet count, occurring most commonly in the third trimester in healthy women, with normal platelet values before and after pregnancy, no maternal hemorrhage or neonatal thrombocytopenia; *ITP*; *drug-induced thrombocytopenia*; *type IIB von Willebrand disease*; *one of the many rare congenital thrombocytopenias*. Furthermore, the decrease of platelet count may be due to pregnancy-specific syndromes, such as pre-eclampsia, HELLP syndrome (a rare disorder characterized by hemolytic anemia, elevated liver enzymes and thrombocytopenia), acute fatty liver or to non-pregnancy-specific syndromes, such as thrombotic thrombocytopenic purpura, systemic lupus erythematosus, intravascular disseminated coagulation, viral infections, primary and secondary bone marrow dysfunctions. An accurate estimate of the frequency of these causes was calculated in a large, cross-sectional study (4). A total of 1.027 thrombocytopenic mothers (platelet count £150x10^9/L) among 15.471 (platelet count performed before delivery), was identified with a frequency of 6.6% (CI 95%: 6.2-7). The most common cause was GT (756 mothers,

73% of cases, 4.8% absolute frequency); thrombocytopenia associated to the hypertensive disorders (216 mothers, 21% of cases, 1.4% absolute frequency); ITP mothers (31 mothers, 3% of cases, 0.25% absolute frequency); thrombocytopenia associated to other hematological and medical diseases, such as aplastic anemia or acute leukemia (13 cases). Table 1 summarizes some clinical characteristics. However, it should be considered that the real frequency of GT could be only estimated using a specific normal range for pregnant women, which was not the case in this study. A physician managing a pregnant woman with thrombocytopenia (anamnestic or concomitant) will have to deal with two main clinical presentations. First, the patient has a prior history of ITP, either with a stable, chronic thrombocytopenia, or with a normal platelet count (*e.g.* after splenectomy). In both instances, no diagnostic investigation is warranted, but neonatal thrombocytopenia may occur, because of the presence of maternal anti-platelet antibodies that can cross the placental barrier and enter the fetal circulation. In the second type of presentation, the mother does not refer any previous history of ITP but has a low platelet count. If her platelet count is substantially decreased (<70-80x10^9/L) a diagnostic investigation is needed, to differentiate ITP from the other causes of pregnancy-related thrombocytopenias (Table 1).

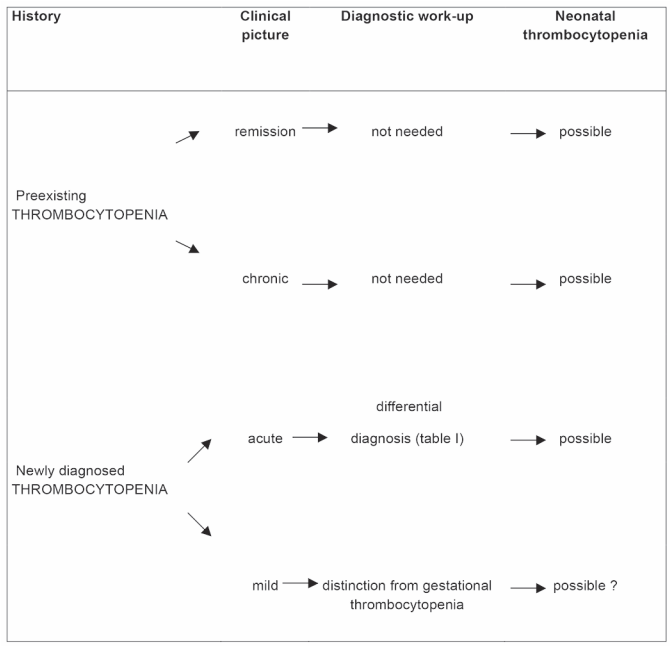
Table 1. Differential diagnosis of thrombocytopenias in pregnancy.

Cause of thrombocytopenia	Time of the most common onset	Grade of thrombocytopenia	Biochemical abnormalities	Clinical symptoms
Gestational	III trimester	mild	no	no
ITP	I-II trimester	mild to severe	no	bleeding in severe cases
Eclampsia	III trimester	mild to severe	DIC[4]) proteinuria	hypertension
HELLP[1]	III trimester	mild to severe	DIC, hemolytic anemia ↑ AST/ALT	no or complex presentation
TTP[2]	II trimester	mild	hemolytic anemia	fever, CNS[5]
HUS[3]	Post - partum	mild	hemolytic anemia	fever, renal failure
AFL[6]	III trimester	mild	DIC, hemolytic anemia, hypoglycemia	complex presentation

[1] = Hemolytic anemia, elevated liver enzyme, low platelet count; [2] = thrombotic thrombocytopenic purpura; [3] Hemolytic uremic syndrome; [4]= disseminated intravascular coagulation; [5]= involvement of central nervous system; [6]= acute fatty liver.

Anyway , also in case of mild platelet count value is mild (>80-100x10^9/L), ITP cannot be excluded with certainty and neonatal thrombocytopenia might occur, so that the newborns should, for safety reasons, be considered as if they were born by a mother with ITP· (see Table 2 for a summary). PROGNOSTIC ISSUES: In the pregnant ITP patient, the fetus as well as the mother may be affected by thrombocytopenia. Consequently, it is important to evaluate the prognosis (morbidity and mortality) of both of them. Unfortunately, only few studies have addressed these clinical outcomes, in term of hemostatic complications, in ITP obstetric patients. A retrospective analysis of symptoms and the need of treatment during pregnancy and at delivery, based on clinical records of 92 women with ITP (123 children delivered in 119 pregnancies), was published by the Mc Master University group (4). Information was available for 116 pregnancies, showing that in 76 women (65.5%) hemorrhage was not present; in 15 (12.9%) and 21 (18.1%) cases the women had mild and moderate bleeding symptoms; in only 4 pregnancies a severe bleeding was observed (2 hematuria, 1 hematoma, 1 gastrointestinal hemorrhage). In 37 out of 119 pregnancies (31.1%) women required treatment (steroids and/or immune globulins) to raise their platelet counts. 98 (82.4%) deliveries were vaginal and 21 (17.6%) were by caesarian section (the mean platelet count was not statistically significant between the two groups). Hemorrhagic complications at partum were uncommon (4 women with a blood loss of at least 1000 mL) without need of red blood cells transfusion. In the post - partum, 2 women experienced prolonged bleeding, but they did not require blood products. With the caveat of a probable referral bias, one could conclude that maternal bleeding can occur in ITP, but it is uncommon and the pregnancy does not have an unfavorable impact on mother's disease. As to the neonatal outcome, several issues remain controversial. The real incidence of thrombocytopenia has not been clearly established, with estimates ranging from 16% to 56%. The platelet count in the fetus and the newborn is the most important risk factor influencing the outcome of the neonate; however, there is no consensus on the level of platelet count required to define severe thrombocytopenia. The incidence of low platelet counts reported by different authors ranges between 4% and 15%, but differences concerning the definition of severe thrombocytopenia (<20, <30, <50x10^9/L) make it difficult to critically assess its clinical impact. The disparity between results can be explained, at least partly, by differences at the timing of the platelet count reported. In neonatal autoimmune thrombocytopenia the platelet nadir usually occurs 3 to 5 days after delivery. In a recent review on 119 pregnancies (4) an 10.1% incidence of platelet count <50x10^9/L in cord blood was reported, but the follow-up of these neonates showed that 16.6% of them reached a nadir platelet count <50x10^9/L at any time during the first two weeks of life. Regarding the occurrence of significant bleeding complications, mainly intracranial hemorrhage, previous studies reported a high perinatal mortality (12%-21%), but currently there is general consensus that the incidence is low. Although reports up to 4% have been communicated, most authors agree that the incidence is less than 1%. Several studies have attempted to define whether maternal characteristics could predict the platelet level of the newborns. No significant correlations were found with maternal platelet count, history of prior splenectomy or the level of maternal platelet antibody. The only, reliable predictor of neonatal thrombocytopenia could be a previous history of severe neonatal thrombocytopenia and, perhaps, the severity of the disease in the mother.

Table 2. Different clinical pictures and diagnostic work-up of ITP in pregnancy.



MANAGEMENT: This issue was recently reviewed by Gernsheimer *et al.* (7). No treatment is required during pregnancy until delivery in asymptomatic patients with a platelet count >30x10^9/L. If a specific therapy is needed for hemorrhage or low platelet count, corticosteroids (*e.g.* oral prednisone, starting with low dosage, 15-25 mg/day and adjusting the schedule to maintain a safe platelet count) or, in case of no response or intolerance, use of intravenous immune globulins, IVIg (1-2 g/Kg body weight in 2 doses) represent the first choice, as in non – pregnant women. Rarely a second-line treatment is required, because

a not adequate response, but safety consideration could restrict the available options. Azathioprine, cyclosporine A, high-dose methylprednisolone, thrombopoietin – receptor agonists were used during pregnancy, in ITP and non – ITP conditions, without significant toxicity, but given a very limited experience, their use should be considered only in case of high risk conditions (*e.g.* mucosal hemorrhage and very low platelet count). Rituximab in pregnancy cannot be recommended because it potential for crossing placenta, whereas splenectomy could be performed during the second trimester, in selected cases. Most authors agree that there is no evidence that caesarean section is a safer option for the thrombocytopenic neonate as compared to vaginal delivery. However, most evidence supporting this conclusion arises from retrospective studies, since only a few prospective studies have been published. Recent guidelines agree that ITP is not an indication for caesarean section, basing the delivery modality on obstetric consideration but avoiding any traumatic procedure on the fetus, such as use of forceps, vacuum extraction, fetal scalp samples. Uncomplicated vaginal delivery has been reported with platelet count <20x10^9/L; however, given the risk of conversion to surgery, a platelet count at least of 50x10^9/L should be achieved pre-partum, with steroids administration, use of IVIg or infusion of platelet concentrate in refractory cases. To safely perform neuraxial analgesia, a platelet count >80x10^9/L should be obtained, but local practice may differ, taking into account that a validated platelet threshold has not been evidence-based demonstrated. After partum, a newborn platelet count should be obtained; an elective treatment of the severely thrombocytopenic neonate (<30x10^9/L) with IVIg (1 g/kg), alone or combined with steroids is recommended; in case of bleeding complications platelet transfusions should be considered as adjunctive therapy. In all newborns with a platelet count <50x10^9/L, a cranial ultrasound should be requested, even if asymptomatic. Moreover, in thrombocytopenic neonate, intramuscular injections should be avoided, and platelet count should be assayed 2 and 5 days after birth, because its expected nadir. A spontaneous rise of the platelet count is usually expected within 7-10 days from partum, but in rare instance, thrombocytopenia may last for several weeks. Recently, transfer of antiplatelet antibodies from ITP mothers by breastfeeding was demonstrated in neonates with persistent thrombocytopenia (more than 4 months from birth) (8) It is to underline that no evidence-based guidelines for treatment of iso-immune thrombocytopenic newborns have been established so far.INHERITED THROMBOCYTOPENIAS IN PREGNANCY: Inherited thrombocytopenias (IT) are a rare, heterogeneous group of syndromic and non-syndromic diseases, usually, but not always, transmitted in an autosomal dominant fashion, characterized by a thrombocytopenia as an outstanding and constant feature, with different grading of severity. A platelet defect function is present in almost all the type of IT, usually non – specific, apart some rare exceptions (*e.g.* Bernand-Soulier Syndrome). A diagnostic algorithm was proposed by the Italian Gruppo di Studio delle Piastrine, with a classification based on platelet size (small, normal, large platelet) and presence or absence of clinical features (syndromic and non-syndromic forms) (9). Recently, the European Hematology Association Scientific Working Group on Thrombocytopenias and Platelet Function Disorders published a large, retrospective multicenter study on pregnancy outcomes in IT mothers, providing very interesting and new data in these rare conditions (10). One hundred and eight-one women with 13 different types of IT (diagnosis confirmed by genetic analysis), with a total of 339 pregnancies, were evaluated. Spontaneous bleeding diathesis before pregnancy were graded using 5-grades WHO bleeding scale; post-partum hemorrhages were classified as "excessive bleeding requiring blood transfusion" (EBBT) in case of need of platelet concentrate and/or red blood cell transfusions to treat bleeding episodes and "all excessive bleeding" (AEB) with inclusion also of patients with hemorrhages judged larger than normal blood loss. The results are briefly summarized in Table 3. Miscarriages was reported in 34 pregnancies, 10.1%, a rate quite similar to that observed in general population. 156/258 newborns evaluated were affected by IT. Cutaneous bleeding were observed in 5 neonates, and fatal cerebral hemorrhages were recorded in 2 newborns from 2 MYH9 – related disease mothers, with severe (12 and 16x10^9/L) thrombocytopenia.116/303 births were caesarian section. EBBT occurred in 20 cases (6.8%) and AEB in 42 cases (14.2%) of deliveries (a rate higher than in general population, 3%-7%), without fatalities. A significant correlation between previous history of bleeding (WHO >2) and base-line platelet count (platelet cut-off at delivery of 50x10^9/L with ROC analysis) and EBBT e AEB was found. Bleeding risk at delivery seems higher in IT populations, for both mothers and newborns. Use of platelet concentrate in mothers with history of previous hemorrhages could be considered to reach a pre-partum platelet count >50x10^9/L. After partum, a newborn cord platelet count should be obtained, and severe thrombocytopenic patients investigated with cerebral ultrasound. Platelet transfusion in such neonates should be also considered.

Table 3. IT characteristics and post-partum outcomes (modified from Noris P. *et al.*,[10])

Disorder	N° women	N° pregnancies/miscarriages	Median (25%-75% percentile) platelet count before pregnancy (x 10^9/L)	N° (%) EEBT[1]	N° (%) AEB[2]	N° affected newborns with bleeding/babies evaluated
MYH9 related disease	98	185/21	40 (23-64)	13 (8.3)	24 (15.3)	6/94
ANKRD26	23	48/6	54 (30-84)	3 (7.1)	5 (11.9)	0/23
Biallelic BSS[3]	13	22/1	50 (23-92)	1 (4.7)	3 (14.2)	0/3
Monoallelic BSS[3]	24	42/3	86 (77-111)	1 (2.5)	5 (12.8)	1/18
ACTN1	9	18/1	77 (65-94)	0	0	0/11
FMD/AML[4]	4	9/2	116 (93-138)	1 (14.2)	1 (14.2)	0/3
ITGB3	3	3/0	78 (65-114)	0	0	0/2
Platelet-type VWD	2	5/0	80 (30-130)	1 (20)	3 (60)	0/0
GPS	1	3/1	65	0	1 (50)	0/0
FLNA	1	1/0	43	0	0	0/0
TUBB1	1	1/0	100	0	0	0/0
VCFS[5]	1	1/0	75	0	0	0/0
CYSC	1	1/0	35	0	0	0/0
TOTAL	181	1/0	57 (30-82)	20 (6.7)	42 (14)	7/156

[1]: EEBT: excessive bleeding requiring blood transfusion; [2]: AEB: all excessive bleeding; [3]: Bernard-Soulier Syndrome; [4]: Familial platelet disorder and predisposition to acute myeloid leukemia; [5]: Velocardiofacial Syndrome.

INHERITED PLATELET FUNCTION DISORDERS IN PREGNANCY: Inherited platelet function disorders (IPFD) are rare diseases affecting primary hemostasis, with a variable severity grading of bleeding manifestations. The diagnosis of IPFD are based on clinical picture, morphology and platelet aggregation *in vitro* investigations, studies of platelet glycoproteins IIb-IIIa by flow cytometry and identification of causative gene mutations, whenever possible. At variance with IT, the platelet count in such patients is almost within normal range. IPFD are often autosomal recessive traits (such as Glanzmann thrombasthenia (GLT), Hermansky-Pudlak Syndrome (HPS) and P2Y12 defect). Studies on management of pregnancy in mothers affected by IPFD are scarce. The disease more investigated is GLT, with some reports suggesting an high bleeding risk at delivery. Again, the European Hematology Association Scientific Working Group on Thrombocytopenias and Platelet Function Disorders published recently a retrospective multicenter study on pregnancy outcomes in some forms of IPFD mothers (11), with the aim to improve knowledge about these rare coagulopathies. Clinical data from 65 pregnancies in 34 women (20 with storage-pool disease, 10 with GLT, 2 with HPS, 1 with P2Y12 defect and 1 with thromboxane A2 receptor deficiency) were collected. Bleeding tendency before pregnancy and hemorrhages after partum were classified as in IT study. Only pregnancies in storage-pool disease and GLT had a number sufficient for statistical analysis (40 and 17, respectively). Concerning storage-pool disease, the rate of miscarriages was similar to that observed in the general population (3/40, 7.5% 95% CI 1.6-20.3), with no case of EBBT, in spite of most mothers did not receive prophylactic platelet transfusion. An excessive bleeding frequency was recorded in GLT pregnancy, with 7/17 EBBT (50%, 95% CI 23-76.9) and need of red blood cell transfusion in 28.6% of GLT deliveries (5% in IT mothers), notwithstanding use of platelet prophylactic transfusions in 11/17 deliveries. This study confirms that severe bleeding can occur in GLT women, and

that prophylactic platelet transfusions have poor effectiveness. Mothers with GLT diagnosis during pregnancy should be referred to obstetric department which could have a full collaboration from well-trained and experienced coagulation and hemostasis center. Given the inheritance modality, the delivery is expected to be safe for babies in the majority of the cases of IPFD.

Bibliography

1. Rodeghiero F, Stasi R, Gernsheimer T, et al. Standardization of terminology, definitions and outcome criteria in immune thrombocytopenic purpura of adults and children: report from an international working group. Blood. 2009;113: 2386-93.
2. Schoonen WM, Kucera G, Coalson J et al. Epidemiology of immune thrombocytopenic purpura in the General Practice Research Database. Br J Haematol 2009; 145: 235-44.
3. Sill PR, Lind T, Walker W. Platelet values during normal pregnancy. Br J Obstet Gynecol 1985; 92: 480-3.
4. Burrows RF, Kelton JG. Fetal thrombocytopenia and its relation to maternal thrombocytopenia. N Engl J Med 1993; 329: 1463-6.
5. Webert KE, Mittal R, Sigouin C et al. A retrospective 11-year analysis of obstetric patients with idiopathic thrombocytopenic purpura. Blood 2003; 102: 4306-11.
6. Cines DB, Dusak B, Tomaski A et al. Immune thrombocytopenia and pregnancy. N Engl J Med 1982; 306: 826-30.
7. Gernsheimer T, James AH, Stasi R. How I treat thrombocytopenia in pregnancy. Blood 2013; 121: 38-47.
8. Hauschner H, Rosemberg N, Seligsohn et al. Persistent neonatal thrombocytopenia can be caused by IgA antiplatelet antibodies in breast milk of immune thrombocytopenic mothers. Blood 2015; 126: 661-4.
9. Balduini CL, Cattaneo M, Fabris F, et al. Inherited thrombocytopenias : a proposed diagnostic algorithm from the Italian Gruppo di Studio delle Piastrine. Haematologica 2003; 88: 582-92.
10. Noris P, Schlegel N, Klersy C et al. Analysis of 339 pregnancies in 181 women with 13 different forms of inherited thrombocytopenia. Haematologica 2014; 99: 1387-94.
11. Civaschi E, Klersy C, Melazzini F et al. Analysis of 65 pregnancies in 34 women with five different forms of inherited platelet function disorders. Br J Haematol 2015; 170: :559-63.

ANTIMICROBIAL RESISTANT GRAM-NEGATIVE BACTERIA IN FEBRILE NEUTROPENIC PATIENTS WITH CANCER: CURRENT EPIDEMIOLOGY AND CLINICAL IMPACT

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Although in the last decades significant improvements have been achieved in the treatment of hematologic malignancies, different types of infections may occur in hematological patients, in particular during the periods of treatment-induced neutropenia, representing bloodstream infections (BSIs) the most common severe infectious complications observed with a reported prevalence ranging from 11% to 38% and crude mortality rates rising 40% [1]. In recent years, several studies have demonstrated a clear trend in the epidemiology of severe infections, in particular BSIs, showing a shift of prevalence from Gram-positive to Gram-negative bacteria in hematological patients. In particular, in a recent review examining cohort studies focused on infections in cancer (mostly hematological) patients published from 2007 to 2013, overall, Gram-negative bacteria were the most frequent cause of bacterial infections, being recovered in percentages ranging from 24.7% to 75.8% (mean 51.3%)[1]. In addition, in a recent Italian multicenter study, Gram-negatives were the most frequent (25.8%) microorganisms causing bacterial BSIs in patients with hematologic malignancies [2], and mortality rate was significantly higher for patients with BBSI caused by Gram-negative bacteria compared to those with BBSI caused by Gram-positive bacteria (16.9% vs. 5.6%). Furthermore, a worrisome and extensive emergence of antimicrobial-resistance among Gram-negatives strains has been recently highlighted in hematological patients [1-5]. *Current epidemiology of infections caused by Gram-negative bacteria in hematological patients:* Among clinical studies published from 2007 to 2013, *Escherichia coli* represented the Gram-negative bacterial species most frequently isolated in hematological patients, with reported frequencies ranging from 10.1% to 53.6% (mean 32.1%); *Pseudomonas aeruginosa* was the second most common bacterial species with a mean frequency of 20.1%, whereas *Klebsiella pmeumoniae*, *Acinetobacter* spp., and *Enterobacter* spp. were isolated with frequencies ranging from 4.1% to 44.6% (mean 19.5%), from 0% to 33.9% (mean 8.2%), and from 2.2% and 11.6% (mean 4.7%) respectively; noteworthy, *Stenotrophomonas maltophilia* isolation, whose incidence globally ranged from 0 to 16.3% (mean 3.7%), has been reported to be increased from the ninth-most commonly isolated Gram-negative rod in 1986 (2%) to the fifth-most common in 2002 (7%) in patients with cancer [6]. In line with these data, in a recent 4th European Conference on Infections in Leukemia (ECIL) questionnaire survey conducted in 2011 on the aetiology of BBSI episodes that occurred in adult hematological and cancer patients in 39 centres (in 18 countries), *Enterobacteriaceae* represented overall the most frequent bacterial species isolated (30%) [4], and in a recent multicenter Italian survey on bacterial BSI episodes in hematological patients, among Gram-negative bacteria, *Escherichia coli* represented the most frequent species (52.9%), followed by *P. aeruginosa* (18.7%), *Klebsiella pneumoniae* (12.2%), and *Enterobacter cloacae* (7.7%)[2]. *Emerging antibiotic resistance among the most common Gram-negatives in patients with hematological malignancies: Escherichia coli* - In recently (2007-2013) published clinical studies investigating infections in cancer patients, >95% of *E. coli* isolates were susceptible to carbapenems (mean rates of susceptibility 98.2% for imipenem and 96.4% for meropenem), whereas the mean susceptibility rates were 82.8% to piperacilin/tazobactam 68.1% of to cefepime, 46.7% to ceftazidime 74.6% to amikacin, 64.1% to gentamicin, and 47.2% to fluoroquonolones [1]. In a recent Italian multicenter survey on onco-hematological patients with BSIs similar susceptibility rates of *E. coli* isolates were reported for meropenem (98.4%), piperacilin/tazobactam (83.4%), ceftazidime (70%), amikacin (97.9%), and gentamicin (82.9%); however, a significantly lower susceptibility rate to fluoroquinolones (9.6%) was reported [2]. *Klebsiella pneumoniae* - Among *K. pneumoniae* isolates, in published clinical studies on infections in cancer patients, overall, 98.5% were susceptible to meropenem, 71.8% to piperacilin/tazobactam 68.7% to cefepime, 54.7% to ceftazidime, 80.3% to amikacin, 58.7% to gentamicin, and 61.1% to fluoroquonolones [1]. Noteworthy, a worrisome reduction in susceptibility to carbapenems has been recently highlighted [2,7,8]. In particular, Girmemia *et al.* conducted a retrospective survey (January 2010 to July 2013) involving 52 Italian centers to assess the epidemiology and the prognostic factors of carbapenem-resistant K. pneumoniae infections (CRKP) in stem cell transplantation (SCT) recipient, reporting an incidence of 0.4% (from 0.1% in 2010 to 0.7% in 2013) in auto-SCTs and 2% (from 0.4% in 2010 to 2.9% in 2013) in allo-SCTs [8]. In a single Italian hospital hematology department CRKP accounted for 18% of all Gram-negative bacteria causing bloodstream infections in patients with hematological malignancies from January 2009 through December 2012; in this study authors reported a significant increase in the incidence of CRKP among all gram-negative causes of bloodstream infections from 2009–2010 (1.4%) to 2011–2012 (32.1%) ($p<0.0001$) [9]. In another multicenter Italian survey on BSI in onco-hematological patients, a percentage of resistance to carbapenems of 34.9% among *K. pneumoniae* isolates was reported [2]. In this latter epidemiological study, susceptibility rates of K. pneumoniae isolates were slightly lower compared to those previously reported also for the other most used antibiotics: 41.9% to ceftazidime, 30.2% to ciprofloxacin, 58.1% to amikacin, 67.4% to gentamicin, and 44.2% to piperacillin/tazobactam. The spread of CRKP infections could represent at the moment one of the most emerging concern for patients with hematologic malignancies and SCT recipients; in fact, on one hand these patients may be particular vulnerable of these infections, due to treatment-related gastrointestinal mucositis, prolonged hospitalizations and neutropenia; on the other hand, no clinical trials have to this date been performed in order to establish the most effective antibiotic treatment protocols for this "difficult to treat" infections [7]. *Pseudomonas aeruginosa - P. aeruginosa* represents the most frequent cause of severe infections in onco-hematological patients among Gram-negative bacteria after *Enterobacteriaceae* [1,2,4]. Multiple patterns of antibiotic resistance have been reported for *P. aeruginosa* isolates causing infections in hematological patients during the last decade. Overall, susceptibility rates of *P. aeruginosa* isolates have been described as ranging from 24% to 100% for carbapenems, from 61.6% to 100% for piperacilin/tazobactam, from 11% to 100% for amikacin, from 34.9% to 100% for gentamicin , and from 18 to 94% (mean 51.6%) for fluoroquonolones; among anti-pseudomonal cephalosporins mean susceptibility rates of 62.3% and 53.6% have been reported for ceftazidime and cefepime, respectively [1]. Multi-drug resistance by P. aeruginosa isolates causing BSI in hematological patients has been

reported as ranging from 31.4% to 71.1% in four different epidemiological studies [1,2]. More recently, a very low rate of susceptibility to carbapenems (28.8%) among *P. aeruginosa* isolates causing BSI in hematological patients has been reported in an Italian study [2]. Finally, Samosis *et al.* have conducted a cohorts studies to evaluate the characteristics and outcomes of cancer patients with extensively drug-resistant (XDR) *P. aeruginosa* infections in Greece, reporting an incidence of XDR pattern in 24.7% of isolates and presence of hematologic malignancy as independent risk factors for XDR *P. aeruginosa* [5]. Notably, no *P. aeruginosa* isolates has been reported to be resistant to colistin among the above mentioned studies. *Other Gram-negative bacteria:* Among other less frequent Gram-negative bacterial species causing severe infections in patients with hematological malignancies, particular concern has been highlighted on *Acinetobacter* spp. and *Stenotrophomonas maltophilia*. Variable antimicrobial susceptibility patterns, including multidrug-resistant, have been reported for *Acinetobacter* spp.; notably, a percentage of 6% of *A. calcoaceticus-baumannii complex* bloodstream isolates of neutropenic patients have been reported to resistant to all antimicrobials in one study [1]. With regard to *S. maltophilia,* rates of resistance to trimethoprim-sulfamethoxazole (TMP/SMX), which is considered the drug of choice for such infections, have been reported to be as high as 30%–40%) among isolates recovered in cancer patients [6]. *Conclusions:* Severe infections caused by antibiotic resistant Gram-negative bacteria have been associated with high rates of treatment failure, mortality, and hospital costs in nosocomial settings, including among onco-hematological patients [1,7,10]. Increased incidence of MDR Gram-negative bacterial species has been recently highlighted to cause severe infections among these latter patients, compared to previous periods. Although this is in line with the increasing frequency of MDR Gram-negative strains causing infections in general population, all well known risk factors for infections in patients with haematological malignancies, and in particular the severe and prolonged post-chemotherapy neutropenia, particularly predispose these patients to severe infections, including those caused by MDR Gram-negatives [10]. In addition, no well established empiric neither etiologic antimicrobial treatment protocols have been approved to this date for severe infections caused by MDR Gram-negative bacterial strains (*e.g.* CRKP), also for onco-hematological patients. For these reasons, sound knowledge of the local distribution of pathogens and their susceptibility patterns and judicious use of antibiotics and control measures to prevent the development and spread of antibiotic resistant Gram-negative bacteria are necessary and could improve the efficacy of therapeutic treatment protocols for patients suffering from hematological malignancies.

References

1. Trecarichi EM, Tumbarello M. Antimicrobial-resistant Gram-negative bacteria in febrile neutropenic patients with cancer: current epidemiology and clinical impact. Curr Opin Infect Dis. 2014;27:200-10.
2. Trecarichi EM, Pagano L, Candoni A, Pastore D, Cattaneo C, Fanci R, Nosari A, Caira M, Spadea A, Busca A, Vianelli N, Tumbarello M; HeMABIS Registry—SEIFEM Group, Italy. Current epidemiology and antimicrobial resistance data for bacterial bloodstream infections in patients with hematologic malignancies: an Italian multicentre prospective survey. Clin Microbiol Infect. 2015; 21:337-43.
3. Montassier E, Batard E, Gastinne T, Potel G, de La Cochetière MF. Recent changes in bacteremia in patients with cancer: a systematic review of epidemiology and antibiotic resistance. Eur J Clin Microbiol Infect Dis. 2013; 32:841-50.
4. Mikulska M, Viscoli C, Orasch C, Livermore DM, Averbuch D, Cordonnier C, Akova M; Fourth European Conference on Infections in Leukemia Group (ECIL-4), a joint venture of EBMT, EORTC, ICHS, ELN and ESGICH/ESCMID. Aetiology and resistance in bacteraemias among adult and paediatric haematology and cancer patients. J Infect. 2014; 68:321-31.
5. Samonis G, Vardakas KZ, Kofteridis DP, Dimopoulou D, Andrianaki AM, Chatzinikolaou I, Katsanevaki E, Maraki S, Falagas ME. Characteristics, risk factors and outcomes of adult cancer patients with extensively drug-resistant Pseudomonas aeruginosa infections. Infection. 2014; 42:721-8.
6. Safdar A, Rolston KV. Stenotrophomonas maltophilia: changing spectrum of a serious bacterial pathogen in patients with cancer. Clin Infect Dis 2007; 45:1602–9.
7. Satlin MJ, Jenkins SG, Walsh TJ. The global challenge of carbapenem-resistant Enterobacteriaceae in transplant recipients and patients with hematologic malignancies. Clin Infect Dis. 2014; 58:1274-83.
8. Girmenia C, Rossolini GM, Piciocchi A, Bertaina A, Pisapia G, Pastore D, Sica S, Severino A, Cudillo L, Ciceri F, Scimè R, Lombardini L, Viscoli C, Rambaldi A; Gruppo Italiano Trapianto Midollo Osseo (GITMO); Gruppo Italiano Trapianto Midollo Osseo GITMO. Infections by carbapenem-resistant Klebsiella pneumoniae in SCT recipients: a nationwide retrospective survey from Italy. Bone Marrow Transplant. 2015 Feb;50(2):282-8.
9. Pagano L, Caira M, Trecarichi EM, Spanu T, Di Blasi R, Sica S, Sanguinetti M, Tumbarello M. Carbapenemase-producing Klebsiella pneumoniae and hematologic malignancies. Emerg Infect Dis. 2014; 20:1235-6.
10. Ruhnke M, Arnold R, Gastmeier P. Infection control issues in patients with haematological malignancies in the era of multidrug-resistant bacteria. Lancet Oncol. 2014; 15:e606-19.

GENETIC AND BIOLOGIC CHARACTERISTICS OF SECONDARY LEUKEMIAS

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Therapy-related myeloid neoplasms (t-MN) include acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS) occurring in patients treated with radiotherapy and/or chemotherapy for a previous cancer or autoimmune diseases. They were first recognized as a distinct entity in 2001. In the subsequent 2008 revision of the WHO classification, t-MN have been included in the "Acute myeloid leukemias and related precursor neoplasms" group (1). One of the main characteristics of t-MN is the high incidence of abnormal karyotypes, which accounted for 64% of 212 t-MN enrolled in the Italian Registry on Secondary Leukemias (2), indicating that genomic instability at the chromosomal level plays a significant role in t-MN. Complex karyotype with >3 abnormalities was the most frequent finding in our patient series and accounted for 38% of abnormal karyotypes, while isolated chromosome 7 deletions were present in 18% of cases. Balanced translocations were present in 6%, while an additional 9% of the cases were t-APL (acute promyelocytic leukemia). Chromosome 5 abnormalities are also very frequent in t-MN. In the University of Chicago's series including 386 cases of t-MN, 79 patients (20%) had abnormalities of chromosome 5, 95 (25%) abnormalities of chromosome 7, and 85 patients (22%) had both chromosome 5 and 7 alterations (3). -5/del(5q) were often associated with complex karyotypes, characterized by trisomy 8, as well as loss of 12p, 13q, 16q22, 17p (TP53 locus), chromosome 18, and 20q (3). Unlike abnormal karyotypes, somatic mutations are rare in t-MN. The most frequent mutations recently identified in *de novo* AML and MDS, including those affecting epigenetic regulators, spliceosome machinery and SETBP1, were rare in t-MN, with the exception of SRSF2 (4-5). These data have been confirmed by large-scale next generation sequencing (NGS) studies, which found significant differences between *de novo* AML, secondary AML (s-AML) and t-MN. Lindsley *et al*. (6), performing a target mutational analysis of 82 genes, recently reported differences in the profile of s-AML, when compared to *de novo* AML, and better defined the mutation pattern in t-MN. In s-AML, defined as a transformation of an antecedent diagnosis of MDS or a myeloproliferative neoplasm (MPN), there was a large prevalence (>95%) of SRSF2, SF3B1, U2AF1, ZRSR2, ASXL1, EZH2, BCOR and STAG2 mutations (defined "secondary-type" mutations). NPM1 mutations, MLL/11q23 and CBF alterations (defined "*de novo*-type" alterations) were under-represented (Figure 1A). On the other hand, in 101 t-MN patients, the authors found 33% "secondary-type" mutations, 23% TP53 mutations and 47% "*de novo*" or "pan-AML" alterations (defined as not belonging to "secondary-type" or "*de novo*-type"). In this line, they could not define a mutational profile characteristic of t-MN patients, or associated to the different prior therapies (Figure 1B). In AML of the elderly clinically defined as *de novo* AML, the presence of *de novo*/pan-AML mutations led to the identification of a prognostically favorable patient group, compared to patients with TP53 or secondary-type mutations, which also suggested evolution from a previous MDS. Ok *et al*. (7), studied consecutive bone marrow samples of 498 patients, including 70 t-MN (28 MDS and 42 AML) and 428 *de novo* MDS/AML samples (147 MDS and 281 AML) using a modified-TruSeq Amplicon Cancer Panel (Illumina), covering mutation hotspots of 53 genes. Overall, the frequency of mutations was similar in t-MN (41/70, 58.6%) and in *de novo* MDS/AML (243/428, 56.8%). In t-MN, the mutation frequency was significantly higher in t-AML (30/42, 71.4%), compared to t-MDS (11/28, 39.3%). The exception were TP53 mutations, whose prevalence was similar in t-MDS (35.7%) and in t-AML (33.3%), and significantly higher than *de novo* MDS (17.7%) and AML (12.8%). PTPN11 was more frequently mutated in t-AML (11.9%) than *de novo* AML (2.1%). NPM1 and FLT3 mutations were rare in t-AML *vs de novo* AML (2.5% *vs* 16.4% and 7.1% *vs* 21.7%, respectively). Taken together, these data show that TP53 mutations remain the principal molecular marker of t-MN. FLT3 and NPM1 muta-

tions are characteristic of *de novo* AML. Until recently, the prevalent hypothesis was that TP53 mutations would be somatically acquired as a result of DNA injury due to the primary cancer therapy, and that TP53-mutated patients were at higher risk to develop t-MN due to inefficient DNA repair. The recent publication by Wong *et al*. (8) highlighted a new scenario. The number of somatic single nucleotide variants (SNV) was similar in t-MN samples (n=22), *de novo* AML (n=199) and *de novo* MDS (n=150), suggesting that cytotoxic therapy does not induce genome-wide DNA damage. On the other hand, TP53 mutations were found at very low frequencies in 7 TP53-mutated t-MN patients in the bone marrow harvested at the time of primary cancer diagnosis, before any chemotherapy. The authors were able to analyze samples collected 3-8 years before t-MN diagnosis using a modified NGS protocol allowing to detect variant allele frequencies of 0.009%.

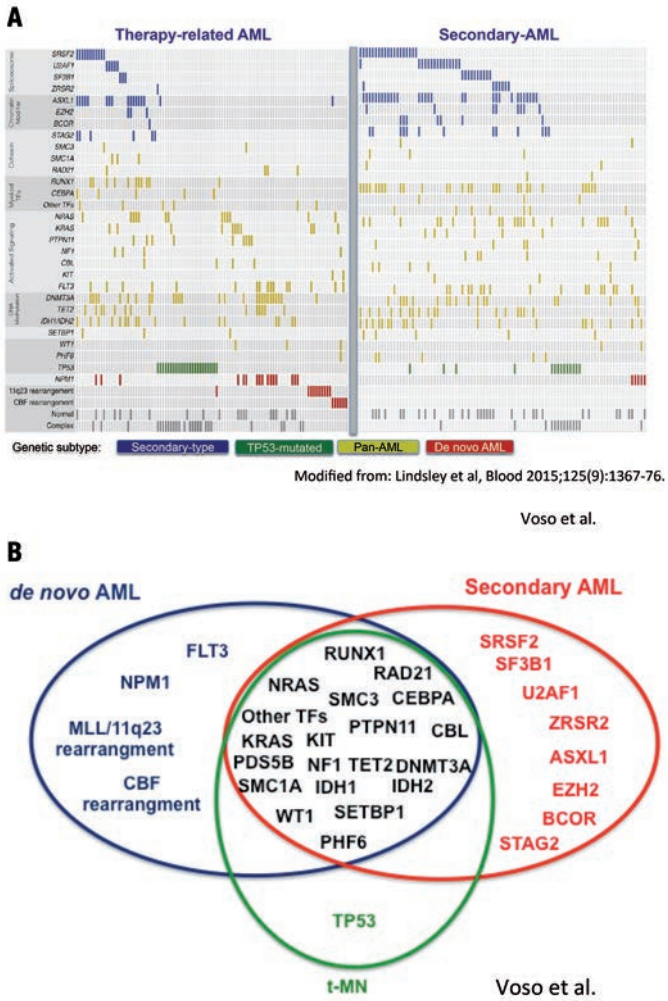

Figure 1. The distribution of mutations of spliceosome genes, chromatin modifiers, cohesins, myeloid transcription factors, activated signaling pathway, DNA methylation enzymes, is shown according to the type of AML: therapy-related, *versus* secondary to MDS or MPN (A). A mutational profile characteristic of t-AML could not be identified. Genetic ontogeny groups could be identified and are marked with different colors (a detailed description of these data can be found in Refererence 6). B shows mutations prevalent (but not exclusive) in different AML subtypes.

The specific TP53 mutation identified in the t-MN sample could be found in 4 of 7 patients. They were unable to identify any TP53 mutation in the pre-treatment samples of the remaining 3 cases, but it is unclear whether the mutations were present at a level below the limit of the assay or were really absent. These results, also in light of the recent reports by Jaiswal *et al.*, and Genovese *et al.* (9, 10) on the increased frequency of somatic mutations acquired in elderly, otherwise apparently healthy individuals, suggest that cytotoxic therapy does not directly induce TP53 mutations. Rather, these data suggest a model in which hematopoietic stem cells carrying age-related TP53 mutations are more resistant to chemotherapy than un-mutated cells, and may acquire a clonal advantage. This suggests that clonal evolution might be seen at least in part as "clonal selection". "Early" mutations may further contribute to the typical genetic instability of t-MN, the frequent cytogenetic abnormalities and poor response to chemotherapy. In addition to TP53 mutations, Wong *et al.* reported that one patient acquired after chemotherapy a del(5q) and a del(7q), another patient a ETV6 mutation, and a third patient NUP98, TET2 and KRAS mutations (8). We have recently identified a t-MN patient carrier of a TP53 Y220C mutation, who had a previous history of APL. During the process of clonal selection, probably under the pressure of the cytotoxic treatment given for APL, the mutated allele frequency increased from the time of APL diagnosis (when the TP53 mutation was undetectable) to 2% during follow-up and finally to 6% at the time of t-MN diagnosis (unpublished observations). Only the further improvement and diffusion of NGS technologies will help to identify mutations at very low levels at the time of primary malignancy, and may indicate at this time point t-MN "susceptible" patients, who will require more intensive follow-up schedules. The pattern of incidence of t-MN has been changing in the last years due to changes in the treatment approaches in different malignancies (11). Nowadays, only less than 2% of patients treated with cytotoxic drugs and/or radiotherapy develop a t-MN. Individual susceptibility to development of t-MN related to single nucleotide polymorphisms (SNP) of genes implicated in detoxification, DNA repair, and apoptosis pathways has been hypothesized. In particular, enzymes involved in phase I and II detoxification pathways play an important role in the response to chemotherapeutic agents, adjusting the concentration of active drug metabolites. On the same line, DNA repair enzymes play a fundamental role in the control of DNA synthesis, in the presence of antimetabolites like fludarabin and azathioprine, and in the repair of damaged DNA, due to alkylating agents (intrastrand / interstrand DNA crosslinks), topoisomerase II inhibitors (DNA double-strand breaks) and radiotherapy (single/double strand damage). Highly damaged cells should undergo apoptosis, but badly repaired cells may survive and originate malignant clones. Single nucleotide polymorphisms in genes belonging to DNA-repair pathways, alone or in combination, may alter the normal death pathways, favor chromosomal instability, severe failure of cell functions and t-MN clone formation. Along this line, several authors have reported a correlation between some SNPs and t-MN risk. Among these, SNP in detoxification enzymes as NQO1, CYP3A4, GSTM1, GSTP1 and GSTT1 and DNA repair enzymes as RAD51 and XRCC3 have been reported as potential risk factors for t-MN development. We have recently identified a lower frequency of the -21Arg variant of the apoptotic regulator BCL2L10 in t-MN patients than in controls, which may translate into a reduced risk of developing t-MN in carriers of this SNP (8). Although these results appear promising, the validation of these findings will require confirmatory studies in wider t-MN patient cohorts prospectively collected, and appropriate controls. In particular, the choice of controls is critical and should include patients with the same primary malignancy, receiving the same cytotoxic treatment, and not developing a t-MN at a comparable follow-up. Most t-MN patient series are clinically heterogeneous, especially for the types of primary malignancy and their treatment. The most frequent primary malignancies remain lymphoproliferative diseases among hematological malignancies, and breast cancer among solid tumors (2, 11). In our registry, patients previously treated with radiotherapy alone experienced a longer latency time to t-MN development when compared to chemotherapy or the combination of chemotherapy and radiotherapy (mean, 11.2 vs. 7.1 years, p=0.0005)(2). The long latency time associated to radiotherapy and the biological similarity of t-MN after radiotherapy to *de novo* MDS/AML question the real "therapy-related" nature of these MDS/AML. Moreover, the addition of topoisomerase-II inhibitors to alkylating agents was associated with a shorter latency time when compared to alkylating agents alone (median, 6 vs. 8.4 years, P=0.02)(2). Similar data had been previously reported in the context of Hodgkin lymphoma, where the relative risk of developing therapy-related breast cancer and t-MN and latency time strongly depended on the type of cytotoxic therapy. These findings further underline the necessity to collect patient populations homogeneous for primary malignancy and treatment (type, dose and latency) in order to obtain reproducible data on the potential role of SNPs in t-MN development. This same concept is more difficult to apply in the case of germline SNV, where the very low frequency of allelic variants (<1%) does not allow to achieve sufficient statistical power for

susceptibility studies. NGS enabled large screening of germline SNV, and facilitated the evaluation of pathways of interest in large cohorts of patients. Using a "target enrichment sequencing" approach, we have recently studied the frequency of germline SNV in the Fanconi anemia (FA) pathway in 37 patients with a t-MN (13). Fanconi anemia is a childhood syndrome characterized by chromosomal instability, developmental abnormalities, aplastic anemia and cancer predisposition. It is associated to mutations in the Fanconi pathway, comprising 16 DNA repair genes. Our study showed a relatively high frequency of FA germline SNV in t-MN patients (16%), but the prevalence was similar to that observed in *de novo* AML (12.5%). Furthermore there were no differences between t-MN secondary to lymphoproliferative diseases and those secondary to solid tumors. Our data do not clarify whether carriers of FA germline SNV have increased susceptibility to the DNA damaging action of cytotoxic therapy for primary tumors, or possibly to environmental carcinogens. The functional role of these variants should be tested to better define their role as t-MN predisposing factors. In addition to genetic alterations, SNPs, SN*Vs* and somatic mutations, changes in the methylation profile may play a pivotal role in therapy-related leukemogenesis. Alterations of methylation pattern in cancer are based on a two-step model. The first step is associated to global hypomethylation of the genome, whereas the second implicates the selective hypermethylation of specific genes, such as tumor-suppressor genes. On the other hand, Maegawa *et al*. (14), starting from a mouse model of MDS, and exporting their results to human samples, reported interesting differences in the global DNA methylation profile of *de novo* MDS patients as compared to s-AML progression. They found 41 genes differentially methylated according to age, in young *versus* old mice, *versus* MDS, and in the transition from MDS to AML. Twenty-seven of 41 genes identified in the mouse model also had an orthologous gene in the human genome. Of these, 14 genes showed a drift in methylation levels associated with age, whereas 21 genes showed a tendency towards hypermethylation in the evolution from MDS to s-AML. Eleven genes were involved in both processes. Changes of global methylation in t-MN have not been defined yet, but may follow similar patterns. Using a next generation bisulfite sequencing approach, we recently studied the global methylation profile of 20 patients, including 5 *de novo* MDS, 5 AML arising after MPN, 5 t-MN after cytotoxic treatment of Hodgkin Lymphoma (HL) and 5 t-MN after breast cancer (BC) (15). t-MN were indeed epigenetically distinct from *de novo* MDS and from post-MPN AML. Main methylation differences affected the "self-renewal" and "differentiation of hematopoietic stem and cancer cells" pathways. Moreover, the epigenetic footprint of t-MN samples was strongly dependent on the type of primary malignancy (HL *vs* BC). In conclusion, t-MN are heterogeneous diseases, whose characteristics are strongly associated to individual patient features, which likely increase the susceptibility to develop the first malignancy and/or the t-MN. This soil favors secondary events due to the cytotoxic treatment, which then may drive therapy-related leukemogenesis.

References

1. Vardiman JW, Thiele J, Arber DA, et al. The 2008 revision of the WHO classification of myeloid neoplasms and acute leukemia: rationale and important changes. Blood 2009; 114: 937–51.
2. Fianchi L, Pagano L, Piciocchi A, et al. Characteristics and outcome of therapy-related myeloid neoplasms: Report from the Italian network on secondary leukemias. Am J Hematol 2015; 90: E80-5.
3. Qian Z1, Joslin JM, Tennant TR, et al. Cytogenetic and genetic pathways in therapy-related acute myeloid leukemia. Chem Biol Interact 2010; 184: 50-7.
4. Voso MT, Fabiani E, Fianchi L, et al. Mutations of epigenetic regulators and of the spliceosome machinery in therapy-related myeloid neoplasms and in acute leukemias evolved from chronic myeloproliferative diseases. Leukemia 2013; 27: 982-5.
5. Fabiani E, Falconi G, Fianchi L, et al. SETBP1 mutations in 106 patients with therapy-related myeloid neoplasms. Haematologica 2014; 99: e152-3.
6. Lindsley RC, Mar BG, Mazzola E, et al. Acute myeloid leukemia ontogeny is defined by distinct somatic mutations. Blood 2015; 125: 1367-76.
7. Ok CY, Patel KP, Garcia-Manero G, et al. Mutational profiling of therapy-related myelodysplastic syndromes and acute myeloid leukemia by next generation sequencing, a comparison with *de novo* diseases. Leuk Res 2015; 39: 348-54.
8. Wong TN, Ramsingh G, Young AL, et al. Role of TP53 mutations in the origin and evolution of therapy-related acute myeloid leukaemia. Nature 2015; 518: 552-5.
9. Jaiswal S, Fontanillas P, Flannick J, et al. Age-related clonal hematopoiesis associated with adverse outcomes. N Engl J Med 2014; 371: 2488-98.
10. Genovese G, Kähler AK, Handsaker RE, et al. Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. N Engl J Med 2014; 371: 2477-87.
11. Morton LM, Dores GM, Tucker MA, et al. Evolving risk of therapy-related acute myeloid leukemia following cancer chemotherapy among adults in the United States, 1975-2008. Blood 2013; 121: 2996-3004.
12. Fabiani E, Fianchi L, Falconi G, et al. The BCL2L10 Leu21Arg variant and risk of therapy-related myeloid neoplasms and *de novo* myelodysplastic syndromes. Leuk Lymphoma 2014; 55: 1538-43.
13. Voso MT, Fabiani E, Zang Z, et al. Fanconi anemia gene variants in therapy-related myeloid neoplasms. Blood Cancer J 2015; 5: e323.
14. Maegawa S, Gough SM, Watanabe-Okochi N, et al. Age-related epigenetic drift in the pathogenesis of MDS and AML. Genome Res 2014; 24: 580-91.
15. Figueroa M, Sotzen J, Fabiani E, et al. Distinct epigenetic abnormalities distinguish therapy-related myeloid neoplasms (t-MN) following Hodgkin lymphoma (HL) vs. breast cancer (BC). Leuk Res 2013, S26–S27.

DIAGNOSTIC AND PROGNOSTIC EVALUATION IN MDS: WHAT IS MANDATORY AND WHAT IS REDUNDANT?

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Myelodysplastic syndromes (MDS) are a heterogeneous group of clonal myeloid malignancies characterized by dysplastic and ineffective hematopoiesis, variable rates of leukemic evolution and, accordingly, different survival outcome. The incidence of MDS increases with age, varying from 0.4 cases per 100,000 under the age of 30 to almost 40 cases per 100,000 individuals over the age of 65. Diagnosis and classification of MDS might be challenging and specific criteria together with a correct approach are needed to establish a correct diagnosis and to distinguish MDS from reactive forms of cytopenia and other myeloid disorders. Clinical suspicion of MDS usually arises from blood count abnormalities, with variable combinations of anemia, neutropenia and thrombocytopenia. If MDS is suspected, a complete patient history should be collected including information on previous treatment with chemotherapy, radiotherapy, immunotherapy and occupational exposure to known leukemogenic agents, together with accurate recording on concomitant medications and family history. Other non-malignant causes of anemia need to be excluded and viral, autoimmune and thyroid screening are generally recommended. Diagnosis of MDS largely relies on morphologic evaluation and detection of dysplastic features in peripheral blood (PB) and, most importantly, in bone marrow (BM) specimens. Identification of dysplastic changes in one or more lineages, evaluation of the percentage of blasts in both PB and BM and quantification of ring sideroblasts (RS) are essential for diagnosis and prognostic stratification. The WHO 2001 classification of myeloid disorders defined the minimal diagnostic criteria for MDS, including the presence of unequivocal dysplasia in 10% of the cells of at least one myeloid lineage. Based on the number of myeloid lineages presenting with dysplastic features, the WHO classification has been revised in 2008, introducing the category of refractory cytopenia with unilineage dysplasia (RCUD) including patients with refractory anemia (RA), refractory neutropenia or refractory thrombocytopenia, displaying bone marrow dysplasia in one myeloid lineage and <5% of blasts in bone marrow. The category of multilineage dysplasia with or without ring sideroblasts (RCMD, RCMD-RS), together with the pre-existing entities of RAEB-1 and RAEB-2 have been maintained from the previous WHO classification, with increased emphasis on the percentage of blasts in the peripheral blood. Morphologic examination of BM aspirates is usually adequate for the diagnosis of MDS, especially in cases with hypercellular BM. A bone marrow biopsy is mandatory when the bone marrow is hypocellular, to evaluate fibrosis, quantify CD34+ cells, and exclude other hematologic conditions or myeloftisis. The rate of marrow fibrosis should always be assessed at diagnosis given its negative prognostic impact reported in several studies.[1] Cytogenetic analysis retains a major role in diagnosis, prognosis and treatment choice in MDS. Conventional karyotyping is mandatory in patients with suspected MDS, while FISH is recommended for the identification of selected chromosomal abnormalities in patients who repeatedly failed karyotype evaluation.[1] Recurring chromosomal abnormalities are detected in up to 50-60% of patients with MDS and have an important role in refining MDS diagnosis, disease clonality and risk stratification. The recently published comprehensive cytogenetic scoring system for primary MDS defined cytogenetic analysis as a determinant prognostic factor in MDS, refining risk groups previously identified in the International Prognostic Scoring System (IPSS).[2] Based on the risk of evolution to AML and survival data, 19 specific cytogenetic categories were identified and subsequently grouped into five different prognostic groups, compared to the three

groups present in the IPSS. The category of very good risk including –Y and del(11q) has been introduced, while deletion of 5q, normal karyotype, del(20q), and del(12p) are still classified as good risk abnormalities. Deletion of 7q was found to be associated with better outcome compared to monosomy 7 and therefore reclassified in the intermediate risk category. Double abnormalities were grouped in three prognostic categories based on the chromosome aberrations: double abnormalities affecting 5q were classified as good risk, those affecting -7/7q were included in the poor risk, while all other double abnormalities were classified as intermediate risk. Complex karyotype with more than 3 abnormalities showed worse overall survival (OS) and shorter time to AML evolution as compared to complex karyotype with 3 abnormalities, and was therefore classified in the novel very-poor risk category. All these new cytogenetic data have been used to implement the revision of the IPSS in the revised IPSS (IPSS-R), together with other major changes in the classification of cytopenias.[3] The identification of a specific genetic abnormality is also in tight relationship with disease phenotype. MDS harbouring isolated del(5q) (5q- syndrome) has been classified as a distinct entity in the 2001 WHO classification and its peculiar features in terms of morphology, clinical presentation and outcome have been defined. In this MDS subtype, the introduction of novel effective therapies has conferred to cytogenetic analysis the additional critical role of directing clinical decisions. Lenalidomide is an immunomodulatory drug which is currenly approved for lower-risk, transfusion-dependent del(5q) MDS patients. Lenalidomide efficacy first described in 5q- MDS by Ebert *et al.* in 2006, has been in fact confirmed in patients with low- and intermediate 1-risk del(5q) MDS, being able to induce transfusion independency and complete cytogenetic response in 67% and 45% of patients, respectively.[4] This agent is particularly effective in patients without additional abnormalities other than del(5q), who also achieve higher rates of cytogenetic remission. The longitudinal evaluation of karyotype and FISH are required to assess cytogenetic response to therapy, and in particular to readily detect additional genetic abnormalities during disease follow-up. The role of flow cytometry in the diagnosis and prognosis of MDS is acquiring relevance and international efforts are ongoing to standardize analysis and criteria for MDS diagnosis through flow cytometry. Currently, the detection of multiple aberrations in maturation patterns either in granulocyte, monocyte or erythroid lineage together with the identification of aberrant progenitor cells even in the setting of low blast numbers (<5%) makes flow cytometry a useful tool to distinguish clonal MDS from other non-clonal cytopenias.[1] However, flow cytometry results should be interpreted with caution in MDS and need to be integrated with morphology and cytogenetics reports. More recently, mutational analysis has implemented knowledge on clonal origin and evolution of MDS. At least one somatic mutation in genes involved in RNA splicing (*SF3B1, SRSF2, U2AF1, ZRSR2*), chromatin regulation (*EZH2, ASLX1*) DNA methylation (*DNMT3A, TET2, IDH1-2*), cell cycle and signalling (*TP53, NRAS, KRAS*), transcription regulation (*RUNX1, ETV6, EVI1*), has been identified in almost 70% of MDS cases.[5-7] A recent study reported that 52% of MDS patients with normal karyotype harboured at least one genomic point mutation.[5] Studies in large cohorts of MDS patients employing deep-sequencing techniques have detected a median of 3 mutations per patient with SF3B1, TET2, SRSF2, ASXL1, DNMT3A, and RUNX1 as top mutated genes.[6, 7] Most of these recurrent molecular defects are present also in other myeloid malignancies, especially myeloproliferative neoplasms. In addition, recent studies showed that some of these mutations occur also in normal, otherwise healthy individuals, who are however at higher risk to develop a myeloid neoplasm, including MDS.[8] As a consequence, molecular alterations should not be interpreted as unequivocally diagnostic for MDS, but could be part of the so-called age-related clonal hematopoiesis process (ARCH). The identification of specific molecular aberrations can broaden disease-related information and, in selected cases, improve prognostic assessment, especially in younger patients. Mutations of *TP53* have been described in up to 20% of low-risk del(5q) MDS and in almost 70% of MDS with complex karyotype including -5/5q-. In the context of low-risk MDS with isolated del(5q), the presence of *TP53* mutations has been confirmed to be associated with shorter time to AML evolution and shorter overall survival compared to *TP53* wild-type patients, independent of treatment.[9] In addition, lower response rates to lenalidomide have been reported in *TP53*-mutated cases. Assessment of *TP53* mutations in these patient proves useful in guiding therapeutic choice, and monitoring for treatment response and disease progression, and may potentially indicate earlier use of more aggressive treatment approaches, including allogeneic stem cell transplantation. In intermediate-2 and high risk IPSS categories, *TP53* mutations are strongly associated with complex karyotype, high blast counts and shorter overall survival, also after adjustment for IPSS categories and in the context of hypomethylating treatment.[7]

The RNA splicing machinery has been recently found to be involved in MDS pathogenesis. In particular, the *SF3B1* gene, encoding for a component of the U2 small nuclear ribonucleoprotein, is mutated in 20-28% of cases and is therefore one of the most commonly mutated gene in MDS.[10] The *SF3B1* mutation, similar to the 5q- syndrome, is strongly associated with a specific disease phenotype characterized by the presence of ring sideroblasts, both in the forms of RA-RS and RCMD-RS, with up to 70% mutations in these subsets. In addition, *SF3B1* mutations have been consistently associated with better outcome in patients with low-risk MDS.[10] Recent data indicated that mutant SF3B1 is directly involved in the pathogenesis of ring sideroblasts, by inducing expression of a specific splice variant of SLC25A37 (mitoferrin-1), a crucial iron importer into mitochondria.[11] As *SF3B1*, other molecular markers have shown to impact survival. Among the most studied, *RUNX1*, *TP53* and *ASXL1* have been consistently associated with poor prognostic features and outcome. More recently, the number of mutations per patient also emerged as a negative prognostic factor for survival. Beside being outcome predictors, molecular markers have been associated with response to hypomethylating agents in MDS.[6,7] The presence of *TET2* mutations correlates with a higher probability to respond to azacitidine and decitabine, especially when they are not associated with *ASXL1* mutations.[12] Despite the great advances and information due to the identification of mutations in MDS diagnosis and risk stratification, molecular alterations are still not included in MDS prognostic scores. At least in younger MDS patients, suitable to allogeneic stem cell transplantation, the implementation of these additional tools may improve outcome prediction and strengthen the indication to this still "risky" procedure. The biologic heterogeneity of MDS is paralleled by variable clinical course and outcome, which has raised the necessity of a risk-adapted treatment approach. The introduction of prognostic scores combining several disease-related characteristics has allowed to predict the clinical course of MDS and therefore direct therapeutic decisions. Together with cytogenetic data, the original IPSS, the most widely used prognostic score, identified the combination of blast count and number of cytopenias as the main biologic and clinical variables useful to predict risk of leukemic evolution and survival. The recent revision of IPSS (IPSS-R), has refined cytogenetics, blast count and cytopenias classifications.[3] Cytogenetic risk groups in IPSS-R weighted more on score calculation compared to IPSS and the number of cytogenetic abnormalities included has been more than doubled. According to the WHO, the category of 21-30% of blasts has been eliminated, while the <2% blasts risk category has been introduced. In addition, cytopenias are considered separately in the IPSS-R and their severity is weighted in this scoring system. Other disease-related variables known to retain prognostic significance in MDS are multilineage dysplasia and transfusion requirements, which are included in the WHO prognostic scoring system (WPSS).[1] Factors related to patient general health status including age, comorbidities, performance and cognition status are important in the clinical decision-making process and choice of treatment options. The concept that chronological age might not necessarily be a reliable indicator of patient functional ability has fostered the developement of scores combining clinical variables and comorbidities to assess patient eligibility to disease-modifying therapies and intensive treatment options, like allogeneic stem cell transplantation. More than half of patients with MDS are actually affected by one or more comorbidities at the time of MDS diagnosis, with cardiac disease being the most prevalent. The presence of comorbid conditions appears to impact differently in low- and high-risk MDS patients. While in low-risk MDS, it increases the risk of non-leukemic death, in the high-risk setting they often reduce the eligibility for disease-modifying therapies, especially the intensive approaches. Sorror and colleagues generated the comprehensive Hematopoietic Stem Cell Transplantation Comorbidity Index combining pre-transplant comorbities and found that it strongly predicted HCT-related morbidity and mortality in MDS and AML patients. Other general scores not specific for MDS have been employed in the clinical practice. A dynamic comorbidity index specific for MDS, the MDS-CI, has been developed by an Italian group.[13] By combining the presence of cardiac, pulmonary,

renal, hepatic comorbidities and solid tumor, the authors discriminated three groups of MDS patients previously stratified according to WPSS (WHO prognostic scoring system). Each group was associated with a different risk of non-leukemic death. The MDS-CI resulted therefore useful for refining prediction of life expectancy for MDS patients, especially in those with low-risk disease. A comprehensive approach including disease- and patient-related factors is necessary for the risk stratification of MDS patients, given the high rate of comorbities and the influence of patient-related factors on treatment choice and outcome. In addition, the integration of molecular data in prognostic scores may improve risk assessment and may help to identify patients with a high probability of response to still "challenging therapies", including immunomodulators and hypomethylating agents. Furthermore, the charaterization of patient-specific mutational profile may pave the way to the use of newly discovered inhibitors of specific mutations, such as the IDH2-mut inhibitor AG-221.[14]

References

1. Malcovati L, Hellström-Lindberg E, Bowen D et al. Diagnosis and treatment of primary myelodysplastic syndromes in adults: recommendations from the European LeukemiaNet. Blood 2013; 122: 2943-64.
2. Schanz J, Tüchler H, Solé F, et al. New comprehensive cytogenetic scoring system for primary myelodysplastic syndromes (MDS) and oligoblastic acute myeloid leukemia after MDS derived from an international database merge. J Clin Oncol 2012; 30:820-9.
3. Greenberg PL, Tuechler H, Schanz J, et al. Revised international prognostic scoring system for myelodysplastic syndromes. Blood 2012; 120: 2454-65.
4. Fenaux P, Giagounidis A, Selleslag D, et al. MDS-004 Lenalidomide del5q Study Group. A randomized phase 3 study of lenalidomide *versus* placebo in RBC transfusion-dependent patients with low-/intermediate-1-risk myelodysplastic syndromes with del5q. Blood, 2011; 118:3765-3776.
5. Bejar R, Stevenson K, Abdel-Wahab O, et al. Clinical effect of point mutations in myelodysplastic syndromes. N Engl J Med, 2011; 364: 2496-2506.
6. Papaemmanuil E, Gerstung M, Malcovati L, et al. Clinical and biological implications of driver mutations in myelodysplastic syndromes. Blood, 2013: 122: 3616–3627.
7. Haferlach T, Nagata Y, Grossmann V, et al. Landscape of genetic lesions in 944 patients with myelodysplastic syndromes. Leukemia 2014; 8: 241-7.
8. Xie M, Lu C, Wang J, et al. Age-related mutations associated with clonal hematopoietic expansion and malignancies. Nat Med 2014: 20, 1472–8.
9. Kulasekararaj AG, Smith AE, Mian SA, et al. TP53 mutations in myelodysplastic syndrome are strongly correlated with aberrations of chromosome 5, and correlate with adverse prognosis. Br J Haematol 2013; 160: 660-72.
10. Malcovati L, Karimi M, Papaemmanuil, et al. SF3B1 mutation identifies a distinct subset of myelodysplastic syndrome with ring sideroblasts. Blood. 2015 May 8.
11. Visconte V, Avishai N, Mahfouz R, et al. Distinct iron architecture in SF3B1-mutant myelodysplastic syndrome patients is linked to an SLC25A37 splice variant with a retained intron. Leukemia 2015; 29: 188-95.
12. Bejar R, Lord A, Stevenson K, et al. TET2 mutations predict response to hypomethylating agents in myelodysplastic syndrome patients. Blood 2014; 124: 2705-12.
13. Della Porta MG, Malcovati L, Strupp C, et al. Risk stratification based on both disease status and extra-hematologic comorbidities in patients with myelodysplastic syndrome. Haematologica 2011; 96:441-9.
14. Stein EM, Altman JK, Collins R, et al. AG-221, an Oral, Selective, First-in-Class, Potent Inhibitor of the IDH2 Mutant Metabolic Enzyme, Induces Durable Remissions in a Phase I Study in Patients with IDH2 Mutation Positive Advanced Hematologic Malignancies. ASH abstract, 2014.

THERAPEUTIC PERSPECTIVES IN SMOLDERING MULTIPLE MYELOMA

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Smoldering multiple myeloma (SMM) is an asymptomatic clonal plasma cell disorder defined by the presence of a serum monoclonal component (MC) of ≥3 g/dL and/or of more than 10% clonal bone marrow plasma cells (BMPCs), with no evidence of end-organ damage (CRAB criteria: hypercalcemia, renal failure, anemia or lytic bone lesions or other myeloma-defining events, MDE) (1). The annual progression rate into active multiple myeloma (MM) is 10% per year for the first five years, the cumulative probability of progression is 73% at 15 years (1). However, SMM is biologically and clinically highly heterogeneous, with some patients behaving like those with monoclonal gammopathy of undetermined significance (MGUS), with a very low rate of progression, and thus carrying a biological pre-malignancy, and a subset with biological malignancy, with a high risk of developing clinical symptoms within the first years after diagnosis (2). Therefore, the identification of predictors of progression into MM is of great importance. Different prognostic models have been proposed to predict the risk of progression, based either on conventional serologic parameters (size and isotype of M component and its progressive increase, free light chains, FLCs ratio, immunoparesis) or on the percentage and immunophenotype of BMPCs, presence of circulating PCs, or on the presence of cytogenetic and molecular abnormalities in PCs or on the presence of focal lesions (FLs) at MRI (2). Unfortunately, no single pathological or molecular feature can be used to distinguish SMM patients who are certainly developing a malignant disease. In light of the advances in laboratory and imaging techniques, displaying higher sensitivity and capability to detect early damage, diagnostic criteria were recently updated by the International Myeloma Working Group (IMWG) (3). In particular, two major changes were established, the first one defining, on the basis of the results of robust studies, those patients carrying at least one out of three biomarkers of progression (*i.e.* clonal BMPCs >60%, sFLC ratio ≥100 and >1 FL at MRI), with approximately 80% of risk to develop an end organ damage within 2 years from diagnosis, as active MM, deserving the start of treatment. The second major improvement was the definition of bone lesions within myeloma defining events not only on the appearance of sites of bone destruction on skeletal radiography, but also on the presence of osteolytic lesions on CT or PET/CT, basing on the well recognized higher sensitivity of newer imaging techniques over WBXR, with more lesions detected and in an earlier phase (4). The current standard practice for patients diagnosed with SMM is to observe them without therapy, as a watch-and-wait strategy. However, despite the IMWG has revised the diagnostic criteria for MM, and a subset of patients with early malignancy is now considered MM and treated as such (3), SMM still includes a high-risk subgroup, with an 50% risk of progression within 2 years. The rationale for observation as the standard of care for SMM over the years has been the lack of clear data from randomized trials of an overall survival (OS) or quality-of-life benefit with early therapy, the toxicity of therapy in an asymptomatic patient population, and the fact that some patients can be free of progression for many years without any therapy. There is also a concern that early therapy may increase the risk of selecting resistant clones. We therefore need to accurately identify patients who are most likely to benefit from intervention. Although there are still no laboratory methods to definitively differentiate clonal premalignancy (biological MGUS) from clonal malignancy (biological MM), we now have several biomarkers that help us to identify the patients with SMM who are at the greatest risk of progression. Moreover, results from one clinical trial showing a difference in progression free survival (PFS) and OS in SMM patients treated with novel agents are available, allowing us to believe that high-risk patients need to be considered for clinical trials testing early therapy. There are 2 major ideas for therapeutic intervention: the first is prevention of progression and the second is definitive therapy, to try to aim at the deepest level of response, with the hope that all subclones are eradicated at this early disease state and cure can be achieved. The first attempts to examine the hypothesis of early intervention were conducted in the 1990s, with 3 small trials comparing early therapy with melphalan plus prednisone *vs* observation and melphalan plus prednisone treatment at the time of progression. (5,6). These studies did not demonstrate a survival advantage, although they were not adequately powered to make definitive conclusions. Following, several studies tested bisphosphonates (pamidronate or zoledronate) in SMM (including 2 randomized controlled studies) and again they did not show improvement in OS or time to progression, but did demonstrate fewer skeletal-related events. Thalidomide was the next agent to be tested in this patient population in 2 small phase II studies e one phase III randomized trial. However, therapy was limited by the development of neuropathy in most patients. In one trial thalidomide was combined with pamidronate. However, a reduction in dose of thalidomide due to adverse events was needed in 86% of patients within the first 2 years. In the phase III randomized trial, Witzig and colleagues compared thalidomide plus zoledronic acid *vs* zoledronic acid alone in 68 patients with SMM. TTP was superior with thalidomide plus zoledronic acid *vs* zoledronic acid alone (median TTP, 2.4 *vs* 1.2 years, respectively; P =.02). Partial response or better was seen in 37% *vs* 0%, respectively (P <.001). However, there were no significant differences in TTP to symptomatic MM (4.3 *vs* 3.3 years, respectively) or OS (5-year survival, 74% *vs* 73%, respectively) (7). The most interesting study in SMM, that has revive interest in therapeutic intervention in this patient population, came from the PETHEMA group, using lenalidomide and dexamethasone in comparison with observation in an open label randomized trial in 120 patients with high-risk SMM, classified on the basis of Spanish

criteria (*i.e.* bone marrow plasma cells, presence of aberrant phenotype and immunoparesis) (8). Patients treated with lenalidomide and dexamethasone had a superior 3-year survival without progression to symptomatic disease (77% *vs* 30%; P <.001) and a superior 3-year OS (94% *vs* 80%; P =.03) from the time of registration. This study showed for the first time that OS of high-risk SMM patients can be improved by effective early treatment. Although the Spanish study results are of importance, there are some limitations that affect generalizability. First of all the definition of high-risk SMM, with a high proportion of patients who progressed from SMM to MM within the first 6 months that were diagnosed as having MM due to lytic bone lesions, and it is possible that, with routine MRI or PET-CT studies, these patients could have been identified at baseline as active MM. Second, we also need to determine whether patients, identified as high risk using different criteria than those used in the Spanish trial, would benefit in a similar manner from therapy. Third, some have argued that waiting for end-organ damage in the control arm rather than initiating therapy at the time of biological progression (as was done in the treatment arm with the addition of dexamethasone) may have biased the trial in favor of early therapy. However, at the time the Spanish trial was conducted, the standard of care in the control arm was indeed observation until end-organ damage occurs. Lastly, the trial was not designed for regulatory purposes and, therefore, results need to be reproduced by other studies. In line with this, a randomized trial being conducted by the Eastern Cooperative Oncology Group comparing lenalidomide to observation will be of value. Because of these concerns, further studies are needed before implementing therapeutic interventions as standard of care in patients with high-risk SMM. However, this trial has triggered the development of many clinical trials, that are ongoing, to examine the role of therapy in this patient population. Several novel agents are under testing, including 2 or 3 drugs combination therapies with second generation proteasome inhibitors (carfilzomib and lenalidomide), novel immunotherapies such as the Signaling Lymphocytic Activation Molecule family member 7 targeting agent elotuzumab, CD38 targeting antibodies, and programmed cell death-1 targeting antibodies among others, with the goal of achieving the deepest responses and possibly of eradicating the clone. If therapy is chosen, peripheral blood stem cells should be collected for cryopreservation after 4 cycles of therapy. Moreover, studies using both molecular-based (eg, VDJ sequencing) and multiparametric flow cytometry–based MRD detection are needed to compare sensitivity, feasibility, and other important aspects. In conclusion, despite the standard of care for SMM remains observation, there is a high priority to enroll patients with high-risk SMM into clinical trials testing early intervention as, although it remains to be formally tested and proven, it can be speculated that early myeloma is genetically less adverse and, with optimal therapy, some patients can be cured. Ongoing and future trials will help us to find an answer for these important questions.

References

1. Kyle RA, Remstein ED, Therneau TM, et al. Clinical course and prognosis of smoldering (asymptomatic) multiple myeloma. New England Journal of Medicine 2007; 356(25):2582-90.
2. Rajkumar V, Landgren O, Mateos MV. Smoldering multiple myeloma. Blood 2015; 125:3059- 3075.
3. Rajkumar SV, Dimopoulos MA, Palumbo A, et al. International Myeloma Working Group updated criteria for the diagnosis of multiple myeloma. Lancet Oncol 2014; 15(12): 538-48.
4. Zamagni E, Cavo M. the role of imaging techniques in the management of multiple myeloma.Br J Haematol 2012, 159(5): 499-513.
5. Rajkumar V, Landgren O, Mateos MV. Smoldering multiple myeloma. Blood 2015; 125:3059- 3075.
6. Ghobrial IM, Landgren O. How I treat smoldering myeloma. Blood 2014, 124(23):3380-8.
7. Witzig TE., Laumann KE., Lacy MQ. et al. A phase III randomized trial of thalidomide plus zoledronic acid *versus* zoledronic acid alone in patients with asymptomatic multiple myeloma. Leukemia 2013; 27(1): 220-225.
8. Mateos MV, Hernández MT, Giraldo P, et al. Lenalidomide plus dexamethasone for high-risk smoldering multiple myeloma. N Engl J Med 2013, 369(5):438-47.

MORPHOLOGICAL DIAGNOSTIC PREDICTIVITY IN LYMPHOID NEOPLASMS

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Lymphopoiesis[1,2] is a complex process which starts with the first lymphocyte-committed progenitor and continues in the primary and secondary lymphoid organs. The differentiation of stem cells and lymphocyte progenitors is guided in a determining manner by their interaction with stromal cells and with appropriate growth and differentiation factors. Development into T-lymphocytes occurs in the microenvironment of the thymus and of B-lymphocytes in the microenvironment of the bone marrow. Lymphocytes are cells small in size (diameter 8-12 m) with a high nucleocytoplasmic ratio. The cytoplasm is scanty and weakly basophilic (sky blue), homogeneous and clear, without inclusions. The nucleus is round or slightly indented, in a slightly eccentric position; it occupies 90% of the cell. The chromatin is strongly stained and distributes in dense masses or clumps, alternating with irregular paler zones, without a clear demarcation. Sometimes there is a small pale paranuclear zone. This usual morphology of the circulating lymphocyte is, however, subject to considerable variability, in the absence of hematological or lymphoproliferative pathology, following various immunological stimuli, in inflammatory or infective conditions, particularly viral infections (exanthematous disorders, cytomegalovirus, hepatitis), toxoplasmosis, pertussis, allergic reactions and autoimmune disorders. Following an antigenic stimulus activated B cells from germinal centres enter the blood stream by the thoracic duct and thus reach other organs, including the bone marrow. From the morphological point of view activated B cells can have the appearance of large lymphocytes, or plasmacytoid lymphocytes or immunoblasts. The latter are large round or oval cells, with a diameter of the order of 20-30 µm. The cytoplasm is intensely basophilic because of the high content of RNA and sometimes there are a few vacuoles; the nucleus is voluminous, round or oval with delicately interwoven chromatin, in which there are one or more prominent pale blue nucleoli. At this stage of maturation the ribosomes are not yet associated with the endoplasmic reticulum and the cell is thus incapable of antibody synthesis: an incomplete line of arcoplasm often surrounds two thirds of the nucleus. Once they reach peripheral sites, the B immunoblasts divide and mature into plasma cells, which are end stage cells normally incapable of further division, destined to synthesize a single type of antibody to finally expire in 5-14 days. About 5% of immunoblasts from the thoracic duct differentiate into long-living small memory B cells. The intermediate cells between the B immunoblasts and the mature plasma cells are plasmacytoid lymphocytes, cells that have the capacity to secrete immunoglobulin, particularly IgM. In other conditions, such as infectious mononucleosis, morphologically atypical reactive lymphocytes are of T lineage. Chronic lymphoid leukemias are a heterogenous group of disorders arising from neoplastic proliferation of lymphoid cells with mature B or T phenotype and peripheral blood and bone marrow involvement. Present capabilities for assessing these disorders by integrating morphology, immunophenotyping, cytogenetic and molecular studies have resulted in the identification of several subsets with biological and clinical significance.[3-5] In suspected chronic lymphoid leukemias, before proceeding to a detailed cytomorphological evaluation, a simple immunophenotypic analysis should be performed, in order to define their mature B or T cell origin. Then, a detailed morphological, immunophenotypical and cytogenetic/molecular examination will enable the correct identification of the clinicopathological entity (Tables 1-3). As lymphatic leukemias and lymphomas characterized by the same cell type are actually considered a single disease with different clinical presentation or different stage, for chronic lymphoid leukemias WHO group has not proposed a classification separate from that of lymphomas derived from the same cell type.[3] Among the small B-cell lymphoid leukemias different main groups are recognized. These diseases should be differentiated from the leukemic phase of non Hodgkin lymphoma (NHL), arbitrarily defined by the presence in the peripheral blood of more than $5x10^9$/L abnormal lymphoid cells. Leukemic phase is more frequent in low-grade lymphomas, particularly in follicular lymphoma. *B-cell chronic lymphocytic leukemia (CLL).* The diagnosis of CLL requires the presence in the peripheral blood of at least $5x10^9$/L lymphocytes with peculiar phenotype. The morphology of the lymphocytes is usually uniform in any one case. In the typical forms the majority of cells is represented by small lymphocytes, with a diameter slightly greater than that of an erythrocyte, narrow rims of clear cytoplasm, coarsely clumped nuclear chromatin, regular nuclear outline with rarely visible nucleolus. Few cells show irregularities of the nuclear border with

indentations or lobulations. In some cases small numbers of larger lymphocytes, with more abundant cytoplasm, are observed. Broken cells are characteristic but not pathognomonic. The FAB category of CLL, mixed cell type, includes two morphological subtypes: in one there are both small lymphocytes and larger lymphocytes with abundant, clear or slightly basophilic cytoplasm and variably prominent nucleoli; another, called chronic lymphocytic leukemia/ prolymphocytic leukemia (CLL/PLL), presents a percentage of circulating prolymphocytes ranging from 10 to 54%. In a minority of CLL cases, during the course of the disease, it is observed a change in the morphology of leukemic cells in the bone marrow, lymph nodes and other tissues, in association with a more aggressive clinical course. Morphological changes often appear suddenly and are characterized by increased cell size and more immature aspect. Two main types of transformation of the disease may occur: prolymphocytoid change and Richter's syndrome. While the prolymphocyte proliferation usually represents a transformation of the original CLL clone, in cases of Richter's syndrome there is often a modification of the Ig isotype, indicative of the possible emergence of a second malignant clone. CLL needs to be distinguished from monoclonal B lymphocytosis, which is usually an incidental finding; it has fewer circulating clonal cells than CLL and no tissue manifestation of small lymphocytic lymphoma. *B-cell prolymphocytic leukemia (PLL).* The diagnostic feature is the presence of a predominance of prolymphocytes. These cells differ from lymphocytes for the larger size, the lower nuclear/cytoplasmic ratio, the less dense chromatin, but especially for the prominent nucleolus. In blood smears, prolymphocytes are always more than 55%, usually over 70%. *Hairy cell leukemia (HCL).* In most cases the diagnosis of HCL can be made by recognition of specific cytological and histopathological features of the pathological cells in blood and bone marrow. Hairy cells are larger than most normal lymphocytes, with slightly eccentric, ovoid or indented nuclei; the chromatin is rather delicate, and the nucleoli often absent or very small. The cytoplasm, usually abundant and clear, may contain small vacuoles and/or azurophilic granules. The most characteristic cytological feature, from which the name, is the presence of numerous fine and short cytoplasmic projections, with relatively homogeneous distribution around the entire cell circumference. Heavy deposition of reticulin fibers in the bone marrow areas involved by HCL often results in a poor marrow aspirate or a "dry tap". In trephine sections the hairy cell infiltrate may be diffuse or patchy. Abundant clear water cytoplasm or cytoplasm shrinkage separates nuclei by wide margins with a "honeycomb" feature. This finding is useful to differentiate HCL from the bone marrow localizations of other types of low grade lymphoma. The *HCL-variant (HCL-v)* is a very rare disorder, with hematological and clinical characteristics intermediate between HCL and LPL. HCL-v is included, as provisional entity, in the WHO category of splenic B-cell lymphoma/leukemia, unclassifiable. The cells of HCL-v have abundant basophilic cytoplasm with villous projections, moderate condensation of chromatin and a prominent nucleolus. They differ from the classical form for the higher nuclear/cytoplasmic ratio, the central location of the nucleus and the presence of evident nucleolus, and from LPL for the cytoplasmic projections and the less evident nucleolus. The HCL-v phenotype is similar to that of B-PLL. *Plasma cell leukemia (PCL).* At presentation, patients may have large numbers of circulating plasma cells (primary), or this blood picture may arise during the course of multiple myeloma (secondary). There are greater than $2.0x10^9$/L in the peripheral blood and more than 20% plasma cells in the peripheral blood differential white cell count. The circulating cells may have features of lymphoplasmacytic cells, typical plasma cells, or plasmablasts. Plasmablasts have few signs of morphological differentiation. They vary in size and sometimes they are giant, bi- or multinucleated, with hyperbasofilic cytoplasm. Diagnosis may be difficult, especially if the serum monoclonal component is absent. Immunophenotyping is of essential importance. *Splenic lymphoma with villous lymphocytes (SLVL).* The SLVL, synonymous with *splenic marginal zone lymphoma (SMZL)*, is a diffuse low-grade B-cell lymphoma, mainly localized in the spleen, with little or no bone marrow involvement and presence of circulating small villous lymphocytes that may resemble hairy cells. The characteristic cells are larger than the normal small lymphocytes, have a round to oval, sometimes eccentric nucleus, and contain clumped chromatin. A small nucleolus is seen in half of the cases.The cytoplasm, more or less abundant, presents variable basophilia. The main feature is the irregularity of the cytoplasmic membrane, with the presence of short villi often at one pole of some of the cells. The marrow is easily aspirated; in half of the cases it is not involved and there is no fibrosis. Other splenic small B-cell lymphomas which do not fall into any of the other types of B-cell lymphoid neoplasms recognized in the WHO classification, and so called unclassifiable, usually are diagnosed at clinical stage IV, with spleen, bone marrow and peripheral blood involvement and with circulating villous lymphocytes similar to those reported in SMZL. The two best defined of these rare provisional entities are *splenic diffuse red pulp small B-cell lymphoma and HCL-v. Leukemic, follicular lymphoma (FL)***.** The leukemic phase of FL is most often misdiagnosed as CLL. FL is a neoplasm of follicle centre cells (centrocytes and centroblasts). Generally, only small lymphoid cells, corresponding to centrocytes, are present in the peripheral blood. These cells have small size, scanty cytoplasm, sometimes visible only between the walls of a nuclear indentation, clumped and homogeneous chromatin. The peculiar feature is the irregularity of the border of the nuclei that are cleaved and may appear to be dissected. The bone marrow trephine biopsy documents a distinctive focal paratrabecular distribution of the lymphoma infiltrate. *Mantle cell lymphoma/leukemia (MCL).* Mantle cell lymphoma, which is derived from follicular mantle cells, typically affects older adults and, at onset, is often characterized by conspicuous splenomegaly and a frankly leukemic picture. Circulating lymphoid cells are very heterogeneous: some cells look like small lymphocytes; others are more similar to prolymphocytes; at least a portion of the cells have irregular nuclear borders, and are similar to centrocytes. In the blastoid variant, the nuclear chromatin is rather loose and nucleoli are prominent. *Lymphoplasmacytic leukemia (LPL)/Waldenström macroglobulinemia.* LPL is a rare condition characterized by circulating cells with morphological characteristics that are intermediate between lymphocyte and plasma cell, often associated with the presence of an IgM monoclonal band. Waldenström macroglobulinemia is defined as LPL with bone marrow involvement and an IgM monoclonal gammopathy of any concentration often associated with hyperviscosity and/or cryoglobulinemia. The typical cells are larger than normal lymphocytes, and have round to oval nuclei, sometimes eccentric, apparently adhering to the cytoplasmic membrane; the cytoplasm is abundant, light blue, with regular borders. Some cells have frank plasmacytoid aspect, with basophilic cytoplasm and a bright paranuclear area. The incidence of a frankly leukemic phase is low in *diffuse large cell lymphomas*, especially at the onset and bone marrow involvement occurs in 10% of cases. The circulating cells are pleomorphic: medium to large sized, with round, oval, or irregular and convoluted nucleus, coarse chromatin, usually evident nucleoli, variable cytoplasmic basophilia. Leukemic cells often remind monoblasts for their large size, abundant cytoplasm and irregular shape of the nucleus with problems of differential diagnosis with acute leukemias. Small T-cell lymphoid leukemias are morphologically and immunologically heterogeneous. They have post thymic phenotype; however, their antigenic profile often differs from that of normal mature T lymphocytes. Because immunophenotypic methods generally cannot define clonality in T cell processes, molecular studies of DNA should be performed in problematic cases. All small T-cell lymphoid neoplasms may have a leukemic presentation. *T-cell large granular lymphocytic leukemia (T-LGL).* This term is used for cases of persistent lymphocytosis with circulating cells having the morphology of large granular lymphocytes (LGL). This disorder represents only 2% of all cases of CLL. The degree of lymphocytosis is variable; in some cases, it is not very high and tends to remain stable for years, with problems of differential diagnosis with reactive lymphocytosis. Large granular lymphocytes have a characteristic morphology: round, eccentric nucleus with dense chromatin, abundant clear cytoplasm with numerous azurophilic granules of various sizes that by electron microscopy correspond to lysosomal formations or to bundles of parallel tubular arrays. In most cases lymphocytes have features of suppressor/cytotoxic T lymphocytes; in the remainder cases they have the phenotype of natural killer cells, with possible abnormalities. As already mentioned, the course of such forms is usually chronic and indolent; in rare cases, however, the course is aggressive. *Aggressive NK-cell leukemia.* Circulating leukemic cells can show a range of appearances from cells indistinguishable from normal LGL to cells with enlarged, irregularly folded nuclei, open chromatin and distinct nucleoli. The abundant pale or lightly basophilic cytoplasm contains fine or coarse azurophilic granules. *T-cell prolymphocytic leukemia (T-PLL).* T-PLL represents about a

quarter of PLL cases. In most cases prolymphocytes are indistinguishable from B-prolymphocytes; they are medium sized with coarse nuclear chromatin and a single prominent nucleolus. In about half of the cases, however, there is variable nuclear irregularity with folds and convolutions and the cytoplasm is intensely basophilic. In a minority of cases, the cell size is smaller than that of B-prolymphocytes and the nucleolus is less evident. *Adult T-cell leukemia/lymphoma (ATLL)*. It is an aggressive malignancy which is endemic in Japan and in the Caribbean, but also sporadically reported in Western countries, in association with HTLV-I infection. The spectrum of clinical manifestations is broad. The leukocyte count is variable with 10-90% abnormal cells in blood smears. Circulating lymphoid elements are very pleomorphic, from small to large in size with a characteristic lobated nucleus, coarse chromatin and scant cytoplasm. Nucleoli are small or inconspicuous. Sometimes, ATLL is difficult to distinguish from T-PLL and Sezary syndrome. *Sezary syndrome.* Sezary syndrome is the leukemic variant of cutaneous T-cell lymphoma (CTCL), characterized by chronic course and slow evolution and by a triad of diffuse erythroderma, lymphadenopathy, and circulating atypical cells. Large and small variants of Sezary cells have been described, but only the large variant is specific of CTCL. Both variants are characterized by striking nuclear convolutions which may give the nucleus a cerebriform appearance. Also the other types of peripheral T-cell lymphomas may show, though rarely, a leukemic picture at presentation. In particular, in *anaplastic large cell lymphoma*, circulating and bone marrow infiltrating cells may be very large with monstrous appearance.

Table 1. Morphological features of leukemic lymphoid cells.

Cell	Size	Chromatin	Nucleolus	Cytoplasm	Other features
B-lineage					
Small lymphocyte	< 2 RBC	Coarsely clumped	Absent	Scanty	
Prolymphocyte	> 2 RBC	Clumped	One, prominent	Abundant	
Hairy cell	> 2 RBC	Rather loose	Absent or small	Abundant	Cytoplasmic villi
Lymphoplasmacyte	> 2 RBC	Clumped	Absent	Abundant, basophilic	Eccentric nucleus
Plasma cell	> 2 RBC	Coarsely clumped	Absent	Abundant, basophilic	Eccentric nucleus, clear paranuclear area
Centrocyte	< 2 RBC	Clumped, homogeneous	Absent	Very scanty	1-2 nuclear indentations
Centroblast	> 2 RBC	Loose	Multiple, evident	Scanty	
Immunoblast	> 2 RBC	Rather clumped	One, evident	Abundant, basophilic	
Lymphoblast	> 2 RBC	Loose	One or more	Variable	
T-lineage					
Large granular lymphocyte	> 2 RBC	Clumped	Absent	Abundant, clear	Cytoplasmic azurophilic granules
Prolymphocyte	> 2 RBC	Clumped	One, inconspicuous	Scanty	Irregular nuclear border
ATLL cell	> 2 RBC	Clumped	Absent	Rather scanty	Multilobated nucleus
Sezary cell	> 2 RBC	Clumped	Absent	Scanty	Cerebriform nucleus
Lymphoblast	> 2 RBC	Loose	One or more	Variable	Possible convoluted nucleus

RBC: red blood cell
ATLL: adult T-cell leukemia/lymphoma

Table 2. Immunophenotypic, cytogenetic and molecular features of small B-cell lymphoid leukemias.

Marker	CLL	PLL	HCL	PCL	LPL	SLVL	FL	MCL
Igs	weak	strong	strong	absent	var	strong	strong	moderate
Igc	-	-/+	-/+	+	+	-/+	-	-
CD5	+	-/+	-	-	-	-	-	+
CD19,20,24	+	+	+	-	+	+	+	+
FMC7	-	+	+	-	+	+	+	+
CD10	-	-	-	-/+	-	-	+	-
CD25	-	-	+	-	+	-/+	-	-
CD38	-/+	-/+	-/+	+	+	-/+	-/+	-
CD23	+	-/+	-	-	-	-	-/+	-
CD43	+	-	-	-/+	-/+	-	-	+
CD103	-	-	+	-	-	-	-	-
BCL6	-	-	-	-	-	-	+	-
IRF4/MUM1	+ PC	?	-	+	+	-	-/+*	-
Cyclin D1	-	-	+/-	-/+	-	-	-	+
ANXA1	-	-	+	-	-	-	-	-
Cytogenetics	del(13)(q12-14), +12, del(6)(q21), del (11)(q22-23), del(17)(p13)	14q+, +3, +12, del(6q), del(13)(q14), del(17)(p13)	No specific abnormality	t(4;14)(p16;q32), t(11;14)(q13;q32), +3, +7, +9, +11, 13q-	no specific abnormality	+3, +7, +12, t(6;14)(p12;q32), t(9;14)(p13;q32), del7(q22-36)	t(14;18)(q32;q21), t(2;18)(p12;q21), t(18;22)(q21;q11.2) t(3;14)(p27;q32)	t(11;14)(q13;q32), t(11;22)(q13;q11)
Molecular genetics (mutated or dysregulated gene)	*TP53 ATM NOTCH1*	*TP53 C-MYC*	*BRAF-V600E*	*IGH CCND1 CCND3 MAF FGFR3 TP53 RAS MYC*	*MYD88*	*CCND3* (cyclin D3) *PAX5 NOTCH2 MLL2 TP53*	*BCL2 BCL6*	*CCND1* (cyclin D1) *ATM TP53*

+: >90% of cases positive; +/-: >50% of cases positive; -/+: <50% of cases positive; -: <10% of cases positive; var: variable; CLL: chronic lymphocytic leukemia; PLL: prolymphocytic leukemia; HCL: hairy cell leukemia; PCL: plasma cell leukemia; LPL: lymphoplasmacytic leukemia; SLVL: splenic lymphoma with villous lymphocytes; FL: follicular lymphoma; MCL: mantle cell lymphoma; IRF4/MUM1: interferon regulating factor; PC: proliferation centers; *: some grades 3a and 3b; ANXA1: annexin A1.

Table 3. Immunophenotypic, cytogenetic and molecular features of small T-cell lymphoid leukemias.

Marker	LGL	Agg NK	PLL	ATLL	Sezary Syndrome
CD2	+	+	+	+	+
CD3	+	+ c	+	+	+
CD5	-	-	+	+	+
CD7	-	-	+	-	-
CD4	-	-	+	+	+
CD8	+	-/+	-/+	-	-
CD25	-	-	-	+	-
CD56	-	+	-	-	-
CD16	+	-	-	-	-
TIA1	+	+	-	-	-
GrB Per	+	+	-	-	-
Cytogenetics	No specific abnormality	del(6)(q21q25), del(11q)	inv(14)(q11q32), t(14;14)(q11;q32), t(X;14)(q28;q11), +8q, del(12)(p13)	+3, +7, 6q abnormalities	No specific abnormality
Molecular genetics (mutated or dysregulated gene)	*TCRB TCRAD*	EBV-EBER expression	*TCRB TCRG TCL1 MTCP1 ATM TP53*	*TCR* Monoclonal integration of *HTLV-I*	*TCR TP53 p16INK4a*

+: >90% of cases positive; +/-: >50% of cases positive; -/+: <50% of cases positive; -: <10% of cases positive; LGL: large granular lymphocytic leukemia; Agg NK: aggressive NK-cell leukemia; PLL: prolymphocytic leukemia; ATLL: adult T-cell leukemia/lymphoma; c: cytoplasmic only; GrB: granzyme B; Per: perforin; EBER: EB virus early RNA; TCR: T-cell receptor; HTLV-I: human T-cell lymphotropic virus I.

References

1. Greer JP, Arber DA, Glader B, List AF, Means RT, Paraskevas F, Rodgers GM. Wintrobe's Clinical Hematology 13^ Ed. Section 4: The lymphocytes. Lippincott Williams & Wilkins; 2014.
2. d'Onofrio G, Zini G. Morphology of Blood Disorders, 2nd Edition. ISBN: 978-1-118-44260-9, Wiley-Blackwell; 2014.
3. Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J, Vardiman JW, editors. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. Lyon: IARC Press; 2008.
4. Bain B, Clark D, Wilkins W. Bone marrow pathology. 4th ed. Hoboken, New Jersey: Wiley-Blackwell; 2010.
5. Rossi D. Role of MYD88 in lymphoplasmacytic lymphoma diagnosis and pathogenesis. Hematology; 2014; 1:113-118.

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